

Evolution of phenotypic diversity



Ming Liu
Worcester College
University of Oxford

A thesis submitted for the degree of
Doctor of Philosophy
Trinity 2023

Declaration

I declare that this thesis was composed by myself and that the work contained herein is my own except where explicitly stated in the text. The work has not been submitted for any degree or professional qualification except as specified.

Ming Liu, Trinity Term 2023

Acknowledgement

I would like to thank my main supervisor, Stu, who has all the best qualities to be a mentor and a scientist: wise, open-minded, inspiring, relaxed, and generous. Thank you for always making time to chat and review manuscripts, for being enthusiastic about our projects, and for allowing me to include a project that you did not supervise in the thesis. You have demonstrated how to be a great leader and scientist. I will always be grateful and look up to you.

Pandemic has taken up more than half of my DPhil journey and it is for this reason that I would like to give special thanks to Sheng-Feng, my master's degree supervisor. Thank you for spending countless hours online with me discussing manuscripts and literature, and for advising me on the priorities for academic career. Without your guidance and patience on how to formulate papers, I would never have gotten to where I am today.

I am indebted to many great scientists. First of all, I am grateful to my second supervisor, Guy, who showed me how to be an excellent theorist. Thank you for teaching me about game theory and division of labour. Geoff, although not listed as a supervisor, took the time to teach me about inclusive fitness theory. I am very grateful for your wisdom and generosity. To my examiners, Alan and Kevin, thank you for being part of my DPhil and for showing me how to think critically. I would also like to thank Elizabeth Tibbets, Pei-Ju Chen, Alper Mutlu, Melanie Ghoul, Joshua Thomas, and Louis Bell-Roberts, who offered brilliant ideas for collaboration. Further, I appreciate the conceptual input from Ashleigh Griffin, Carlos Botero, Charlie Cornwallis, Daisuke Kyogoku, Hadleigh Frost, John Park, Jonathan Green, Jun Abe, Samuel Gascoigne, Sara Mitri, and Shana Caro; and all kinds of support from advanced research computing, Andrew Wood, Becci James, Craig MacLean, Dustin Rubenstein, Heather Green, Henry Hung, Joe Crook, John Bruce, John Wallcraft, Matteo Tanadini, Paul Buerkner, Pete Ellis, Samuel Levin, Siew-Ann Cheong, Sumali Bajaj, and Xiang-Yi Li Richard.

Many lab mates, Alison, Alper, Anna, Asher, Chiara, Chucky, Chunhui, David, George, Indigo, Jack, James, Josh, Juliet, Laurie, Louis, Maddie, Mati, Meghna, Mel, Miguel, Milo, Ming2, Rory, Rosa, Saran, Simon, Tom, Zheren, and Zohar, created the

lovely working environment which I am very thankful for. I also thank Alex Figueiredo, Andrew Wood, Ben Jenkins, Guy Leonard, Holly Coombes, and Manuel Jara Espejo for making our lives at ZRAB so much more enjoyable with the coffee breaks, cakes, happy hours, football sessions, and pub Thursdays. In addition, I appreciate Oxford University Taiwanese Student Society and Oxford University Photographic Society for providing all kinds of social events. I am deeply grateful to the financial support from the Ministry of Education of Taiwan, Natural Motion, and the European Research Council.

Lastly, I would like to express my gratitude to my family for providing an open environment to explore my passion since my childhood. Another special thank goes to my grandmother, Min Liu-Liao, for teaching me to cook over pandemic and for her endless support for the entire family. I also thank my uncle, Eric Luo, for his financial support, which has freed me from worrying about running out of money. And most importantly, I would like to thank Mika for her company and support throughout our time in Oxford.

This thesis is dedicated to my parents. To my mom, Mei-Chen, for teaching me what it meant to be passionate. And to my dad, Ted, for showing me what it meant to get to the bottom of matters.

Publications and Contributions

The following publications have arisen from this thesis. The main texts of them are presented in Chapters 2-5, whereas their supplementary documents are presented in Appendices 1-4 (†: co-first authorship; *: correspondence).

Chapter 2& Appendix 1

- **Liu, M.**^{*}, Cooper, G.A., West, S.A. (2021). Relatedness and the evolution of mechanisms to divide labour in microorganisms. *Ecology and Evolution* 11(21): 14475-14489. (<https://doi.org/10.1002/ece3.8067>)
- S.A.W., G.A.C., and I conceived the project together. I performed the mathematical analysis and simulation, and wrote the first draft with feedback from S.A.W. and G.A.C. All authors contributed to the final manuscript.

Chapter 3& Appendix 2

- **Liu, M.**, Rubenstein, D.R., Cheong, S.-A., Shen, S.-F.^{*} (2021). Antagonistic effects of long- and short-term environmental variation on species coexistence. *Proceedings of the Royal Society B: Biological Sciences* 288 (1958), 20211491. (<https://doi.org/10.1098/rspb.2021.1491>)
- S.-F.S. and I conceived the project. I carried out the simulation and S.-A.C. derived the analytical approximation (later on removed upon reviewer's request). S.-F.S. and I wrote the first draft, and all authors contributed to the preparation of the manuscript in its final form.

Chapter 4& Appendix 3

- **Liu, M.**^{*}, West, S.A., Wild, G. (2022). The evolution of manipulative cheating. *eLife* 11 (80611). (<https://doi.org/10.7554/eLife.80611>)
- S.A.W., G.W. and I conceived the project together. I ran and analysed the numerical calculation and simulation. G.W. made the analytical derivation. S.A.W. and I wrote the first draft, and all authors edited the paper into its final form.

Chapter 5& Appendix 4

- **Liu, M.**^{*}, Wild, G., West, S.A. (2023). Equilibria and oscillations in cheat-cooperator dynamics. *In press in Evolution Letters* (<https://doi.org/10.1093/evlett/grad032>)
- I conceived the project with feedback from S.A.W. and G.W. I carried out the numerical analysis and simulation, and G.W. made the analytical derivation. S.A.W. and I wrote the first draft, all authors contributed to the manuscript in its final form.

The following manuscript has arisen from this thesis, and is presented in chapter 6.

Chapter 6

- **Liu, M.**^{*}, Cornwallis, C.K., West, S.A. What can climatic dataset tell us about evolution: an analysis on environmental predictabilities and correlations. *In preparation*.
- I conceived the project, performed the time series and statistical analysis, and wrote the first draft with feedback from S.A.W. and C.K.C. All authors contributed to the preparation of the final manuscript.

I contributed to other publications that are included in the appendices 5-8.

Appendix 5

- Tibbetts, E. A.^{*}, **Liu, M.**, Laub, E. C., Shen, S.-F. (2020). Complex signals alter recognition accuracy and conspecific acceptance thresholds. *Philosophical Transactions of the Royal Society B: Biological Sciences* 375(1802), 20200482. (<https://doi.org/10.1098/rstb.2019.0482>)
- I helped to formalise the project concept. I designed and carried out the simulation with feedback from S.-F.S., and contributed to editing the draft into its final form.

Appendix 6

- Cooper, G. A.^{*}, Frost, H., **Liu, M.**, West, S. A. (2021). The evolution of division of labour in structured and unstructured groups. *eLife* 10 (71968).

(<https://doi.org/10.7554/eLife.71968>)

- I conceived the project with all co-authors, gave feedback on the modelling and presentation, and contributed to editing the manuscript into its final form.

Appendix 7

- Cooper, G.A.^{*}, **Liu, M.**, Peña, J., West, S. A. (2022). The evolution of mechanisms to produce phenotypic heterogeneity in microorganisms. *Nature Communications* 13 (195). (<https://doi.org/10.1038/s41467-021-27902-4>)
- I performed some simulation to support numerical results, wrote up the methods and results for those simulation results, and contributed to the preparation of the final manuscript.

Appendix 8

- Lin, Y.-H.[†], Chen, Y.-Y.[†], Rubenstein, D. R., **Liu, M.**, Liu, M., Shen, S.-F.^{*} (2023). Environmental quality mediates the ecological dominance of cooperatively breeding birds. *Ecology Letters* (14226). (<https://doi.org/10.1111/ele.14226>)
- I reran some simulation to check results, annotated the codes, wrote the readme document and model description, uploaded the codes and data. Y.-H.L., S.-F.S. and I together addressed reviewers' comments during the publication process.

Due to the length and scope of the thesis, I did not include the following publications or manuscripts into the thesis:

1. Shen, S.-F.^{*}, Reeve, H. K., Emlen, S. T., **Liu, M.**, Rubenstein, D. R. (2023). Group size and the resolution of insider-outsider conflict in animal societies. *In press in Animal Behaviour*.

I helped with revising the methods, results, and figures after the manuscript was rejected by *American Naturalist*. I also helped to address the reviewer's comments with S.-F.S.

2. Cooper, G.A., **Liu, M.**^{*}, Zhang, Z., Fisher, R.M., West, S.A. (2023). The evolution of predominantly sterile social groups. *Under review in Evolution Letters*.

I reran the analysis, uploaded the codes and data, rewrote the first draft into final draft, and was corresponded for the publication process.

3. Thomas, J. L., Wilson, D. J., **Liu, M.**, Bray, J. E., Griffin, A. S.^{*}, Ghoul, M.^{*} (2023). The evolution & ecology of bacteriocin-mediated competition in natural populations of *Staphylococcus aureus*. *In preparation*.

I wrote time series analysis for the clinical dataset to analyse strain displacement events; I also worked with Josh together to perform the main statistical analyses (phylogenetic Monte Carlo Markov chain logistic regression).

4. **Liu, M.**[†], Wu, S.[†], Hung, T.-M.[†], Colwell, R.K., Liu, M., Mai, J., Jang, Y.-S., Huang, C.-C., Hsu, C.-Y., Wei, C.-H., Tuanmu, M.-N., Huei, Y.S., Tang, Y., Chen, I.-C., Shen, S.-F.^{*} (2023). Benign environment promotes thermal trait diversity. *In preparation*.

I helped conceiving the project and modelling concept, built the eco-evolutionary simulation model, performed hypervolume calculation and other analysis for simulated data, and wrote the first draft with other co-authors.

5. Wu, S.[†], Hung, T.-M.[†], Colwell, R.K., **Liu, M.**, Liu, M., Mai, J., Jang, Y.-S., Huang, C.-C., Hsu, C.-Y., Wei, C.-H., Tuanmu, M.-N., Huei, Y.S., Tang, Y., Chen, I.-C., Shen, S.-F.^{*} (2023). Climate variability and the trade-off between cold and heat tolerance in animals. *In preparation*.

I helped to address the comments from R.K.C., and to formulate the revising plans.

Abstract

Phenotypic diversity, also known as phenotypic heterogeneity, occurs when individuals within a species have different phenotypes. Phenotypic diversity plays a crucial role in adaptation, and understanding how it is maintained is fundamental for comprehending the complexity of life. In this thesis, I use theoretical approaches to investigate different mechanisms that support the selection for variation. Specifically: (1) I model the optimal degree of coordination in the division of labour in a non-clonal population, and predict that communication between individuals would be less frequent in a population with low relatedness; (2) I show that the temporal scale of abiotic environmental fluctuations can play a crucial role in favouring a monomorphic or polymorphic population, that short-term variation selects for diversity, but long-term variation selects against it; (3) I develop and test a new form of cheating strategy that manipulates other social group members and find that it can lead to arms-race-like dynamics, which can help explain the genetic complication found in nature; (4) I construct a general model to incorporate different properties in experimental setups and provide general principles for when the cheater-cooperator ratio remains stable and when oscillations occur; (5) I examine how comparative studies, an important tool for validating theoretical predictions, have quantified abiotic environments and highlight the potential problem with correlating climatic quantities and using environmental predictability. Taken together, this body of work extends our knowledge on phenotypic diversity and identifies several promising research directions related to eco-evolutionary feedbacks.

Contents

1 Introduction	- 1 -
2 Relatedness and the evolution of mechanisms to divide labour in microorganisms	- 20 -
3 Antagonistic effects of long- and short-term environmental variation on species coexistence	- 37 -
4 The evolution of manipulative cheating	- 48 -
5 Equilibria and oscillations in cheat–cooperator dynamics	- 66 -
6 What can climatic dataset tell us about evolution: an analysis on environmental predictabilities and correlations	- 80 -
7 Discussion	- 129 -

Appendix

A1 Supplementary information of chapter 2	- 141 -
A2 Supplementary information of chapter 3	- 159 -
A3 Supplementary information of chapter 4	- 163 -
A4 Supplementary information of chapter 5	- 186 -
A5 Complex signals alter recognition accuracy and conspecific acceptance thresholds	- 206 -
A6 The evolution of division of labour in structured and unstructured groups	- 219 -
A7 The evolution of mechanisms to produce phenotypic heterogeneity in microorganisms	- 237 -
A8 Environmental quality mediates the ecological dominance of cooperatively breeding birds	- 251 -

1

Introduction

Phenotypic diversity, also known as phenotypic variation, phenotypic heterogeneity, and phenotypic polymorphism, is when individuals of the same species perform different behaviours or show different properties across space or time (Ackermann 2015). Phenotypic diversity can be found across all levels of biological organisation: from viruses, bacteria, and other microbes, to insects, birds, and mammals (Huang 1973; Shine 1989; Strassmann *et al.* 2000; Ackermann *et al.* 2008; Smith *et al.* 2008; van Gestel *et al.* 2015a). Its prevalence poses a central question in biology: if there is single optimal strategy for the population, why do not all individuals behave in the same way?

In this thesis, I explored theoretically some of the reasons that phenotypic diversity can be favoured. Because each of these chapters have their own introduction, I only provide a brief overview of some key evolutionary mechanisms that has been proposed to explain phenotypic diversity, namely (1) cheats against cooperation, (2) division of labour, (3) phenotypic specialisation, (4) diversified bet-hedging, (5) alternative mating strategies, and (6) sexual dimorphism. I will start from the universal mechanisms and drives that they share before diving into the details of each mechanism.

1. Universal mechanisms for phenotypic diversity

Natural selection can only select for or against diversity when there is variation in the population. There are two canonical mechanisms to generate variation: mutation and drift. Mutation creates genetic variation, such as the changes in nucleotide sequences, and the mutated individual could express genes at a different level, or even make a different protein that has a modified function (Futuyma & Kirkpatrick 2017). Although most of the mutations are not adaptive, it opens the chance that individuals can be different from each other (Hartl & Clark 2007; Futuyma & Kirkpatrick 2017). Drift creates stochastic changes in gene frequency in the population through changes in

population size, small sampling size, or other processes (Gillespie 2000; Hartl & Clark 2007). Both mechanisms increase the genetic diversity of a population, which could directly induce higher phenotypic diversity.

Beside the two canonical mechanisms, there are some conditional and developmental mechanisms for generating variation, such as alternative splicing and stochastic cell differentiation. Alternative splicing can generate variation when cells randomly splice the transcribed mRNA into smaller pieces that is different between cells. This mechanism is known to be important for cell compatibility between individuals (e.g., major histocompatibility complex) and producing immune cells for antigen-specific defensive response (Trowsdale & Knight 2013; Murphy *et al.* 2022). In addition, during the developmental stage of a multicellular organism, cells might stochastically respond to the same signal peptide due to spatial structure and resulting in different cellular organisation between individuals of the same species (e.g., X-chromosome inactivation) (Heard *et al.* 1997; Alberts *et al.* 2022). Although we have only discussed genetic variation so far, it is important to note that phenotype diversity does not always require genetic variation.

2. Selection forces: direct and indirect fitness benefits

Phenotypes can be favoured because they provide either a direct or indirect fitness benefits (Hamilton 1964a, b). The key difference between the two benefits is whether the benefit is directly increasing the focal individual's own survival or reproductive success, or increasing the fitness of other individuals who share some gene of the focal individual (e.g., non-descendant relatives). An example of direct fitness benefit is bacterial individuals within the same population are specialised to either secrete polymers to facilitate spreading or for rapid replication. The two phenotypes have

complementary ecological functions such that population grows much better when both types are present, and the specialisation is highly repeatable to evolve (van Gestel *et al.* 2015a; Kim *et al.* 2016). An example of indirect fitness benefit is cooperative breeding in birds, where offspring stay in the nest and help their parents to rear further offspring (Jennions & Macdonald 1994; Cockburn 1998, 2006; Shen *et al.* 2017). Because the older and younger siblings are related, helping them would increase the older offspring's fitness indirectly. For instance, empirical observations have found there is more helping when species is monogamous, and when individuals can discriminate kinship and preferentially aiding closer relatives (Hatchwell *et al.* 2001; Russell & Hatchwell 2001; Griffin & West 2003; Cornwallis *et al.* 2009). The concepts of direct and indirect fitness benefits can be used to explain the evolution of phenotypic diversity in populations. The balance between these two types of benefits can provide insights into the selective pressures that drive the evolution of different traits and behaviours in populations.

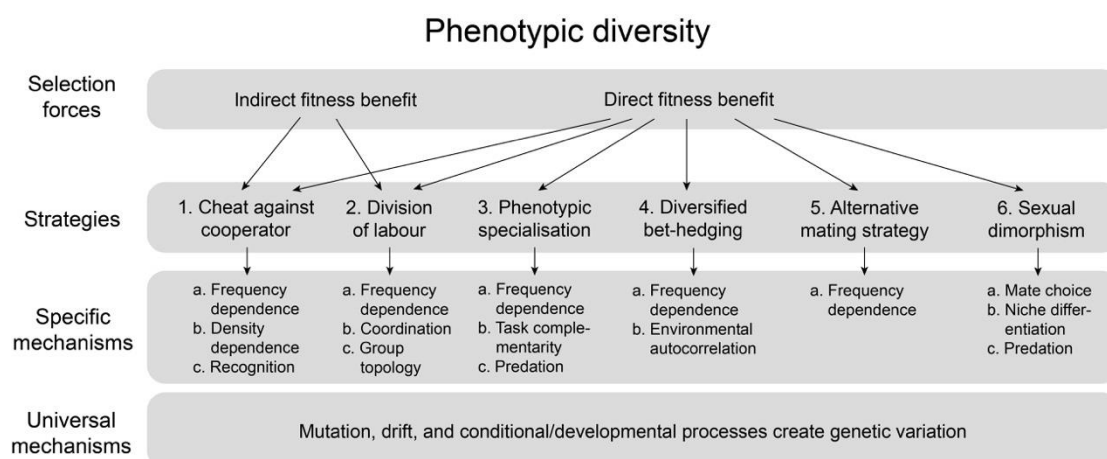


Figure 1. Selection forces, strategies, and evolutionary mechanisms for phenotypic diversity. There are four sections in this figure: selection forces, strategies, specific mechanisms, and universal mechanisms. The selection forces are the drives to get diversity within a species, while the mechanisms are properties that help diversity to be generated or maintained. The arrows here only illustrate the main associations, and some additional cases between selection forces and strategies are discussed in the text of each strategy.

3. Evolutionary strategies

Various evolutionary strategies have been proposed to explain phenotypic diversity. In this section I will review these strategies and link them to their underlying mechanisms. My survey is illustrative, not exhaustive. Specifically, I will explain cheat against cooperation, division of labour, phenotypic specialisation, and diversified bet-hedging in individual subsections (Fig. 1). I have listed alternative mating strategies and sexual dimorphism as the main adaptive strategies related to sex, but since my thesis does not cover the realm of sexual selection, I will only discuss them very briefly.

3.1 Strategy 1: cheat against cooperation

Cooperation takes place when individuals are producing some sharable benefit that can be utilised by not only itself but also its neighbours. Cooperation is widespread and plays a key role in major evolutionary transitions (West *et al.* 2015). However, all cooperative systems suffer from the potential to be exploited by cheats. A cheat occurs when an individual performs a lower level of cooperation, allowing it to pay a smaller or no cost while enjoying the benefit provided by the cooperation of others (Trivers 1971; Ghoul *et al.* 2014). As a result, there is a direct fitness benefit to cheat. Nevertheless, indirect fitness components can also be crucial for cheat-cooperator systems because the degree of cheating can be modulated by relatedness (Frank 1998; Dionisio & Gordo 2006). In other words, to cheat is not the best strategy if all social partners are genetically identical to the focal individual. Thus, the dynamics between cheat and cooperator can take many forms, including fixation, where the cheat or cooperator dominates the population, and coexistence of both (Griffin *et al.* 2004; Diggle *et al.* 2007; Rumbaugh *et al.* 2012; Frensing *et al.* 2013; Pollitt *et al.* 2014; Andersen *et al.* 2015; Kümmerli *et al.* 2015; Mizuuchi *et al.* 2022). Phenotypic diversity emerges when both cheat and cooperator are present within the population.

Three mechanisms are involved in cheat-cooperator co-existence: frequency dependence, density dependence, and recognition. Frequency dependence occurs when the growth rate of cheats or cooperators varies when the proportion of cooperator in the population changes. For example, if all cooperators produce the same amount of public good while the cheats do not produce at all, then the share of public good per individual would depend on the frequency of cooperators within the population (Buckling *et al.* 2007; Ross-Gillespie *et al.* 2007). Density dependence occurs when the growth rate of cheats or cooperators varies with the absolute abundance of cooperators. For instance, if the public goods production is triggered by quorum sensing, that the gene would not be expressed unless there is enough abundance of cooperator, then the growth of cheats would depend on the density of cooperator (Henke & Bassler 2004; Smalley *et al.* 2015). The last mechanism is recognition, which is when the cooperators have some ability to distinguish individuals and direct cooperative behaviour toward some individuals rather than all neighbours. Examples of allorecognition at molecular levels in microbes have been found in *Myxococcus* and *Dictyostelium* (Pathak *et al.* 2013; Dey *et al.* 2016; Hirose *et al.* 2017). Cheats could potentially mimic the signals that cooperators use to recognise other cooperators (Dawkins 1976; Gardner & West 2010).

3.2 Strategy 2: division of labour

Division of labour is an adaptive strategy that individuals are specialised into different tasks cooperatively (West & Cooper 2016). That is, some individuals are specialised to benefit other individuals and specialisation provides indirect benefit to all of the individuals involved. Examples of division of labour can be found across the tree of life. For instance, some individual bacterial cells of *Salmonella* self-lyse to release toxin to help the growth of other cells during infection; slime mould cells differentiate into stalk and spores and the stalk facilitates the spread of spores; heterocysts in

cyanobacteria fix nitrogen to help neighbouring cells grow better; workers in eusocial insect colonies help the queen by providing food and care (Hölldobler & Wilson 1990; Strassmann *et al.* 2000; Ackermann *et al.* 2008; Flores & Herrero 2010). In these examples, some individuals specialised and sacrificed their reproductive potential in order to help other individual and the entire population do better. Additionally, division of labour can also be achieved through mutual specialisation between species, through direct fitness benefit (West & Cooper 2016). An example is plant trade carbon nutrients for phosphorus compounds with their fungal partner (Kiers *et al.* 2011; Wyatt *et al.* 2014).

Mechanisms that facilitate division of labour are frequency dependence, coordination, and group topology. Frequency dependence happens when the fitness of each division depends on the ratio of them within the population: when one division is too common, its fitness could be reduced because of the benefit provided by other division is diluted; when one division is too rare, its fitness could be increased because of a less dilution of benefit from other divisions (Giri *et al.* 2019). As a result, selection would favour the rare division and restore the optimal ratio of the two divisions (Giri *et al.* 2019; Krishna & Laxman 2020). Coordination is when individuals can communicate before developing and specialising to a phenotype. Communication can reduce stochasticity and guarantee a more stable outcome of ratios between divisions, which in turn helps the diversity to be maintained within the population (Cooper *et al.* 2022). Group topology is the connectivity between individuals across space, which is relevant to how the benefits are distributed within a social group. If some individuals have better connectivity than others, they are more likely to become reproductive than becoming sterile helpers (Rueffler *et al.* 2012; Cooper *et al.* 2021).

3.3 Strategy 3: *phenotypic specialisation*

Phenotypic specialisation is when individuals are specialised in a non-cooperative way. Examples of phenotypic specialisation can also be found across a wide range of organisms, from bacteria to mammals. In the bacterium *Bacillus subtilis*, populations are capable of producing two genotypes through highly reversible mutation: (1) producing extracellular protease to help cells spread and (2) fast growing with no protease production, and have complementary ecological functions (Veening *et al.* 2008). Another example is the sentinels in meerkat community, where some individuals stay alert for predators while others are foraging because those individuals are satiated (Clutton-Brock *et al.* 1999; Wright *et al.* 2001). In other words, no specialisation was performed to solely benefit other individuals and self-interest is always involved in phenotypic specialisation (indirect benefit can also be involved in this case). Because the benefit in specialisation always leads to increased fitness for both strains, not the group as a whole, it is different from division of labour and termed as phenotypic specialisation (van Gestel *et al.* 2015b).

Mechanisms relevant to phenotypic specialisation are frequency dependence, task complementarity, and predation. Frequency dependence arises when, similar to division of labour, that individual fitness is affected by the ratio of phenotypes (Kim *et al.* 2016; Dragoš *et al.* 2018). Task complementarity measures the similarity between the performed tasks (Giri *et al.* 2019), if two phenotypes are specialised to very similar tasks, competition is more likely to take place and phenotypic diversity will not be maintained in the population. Predation can bring direct fitness benefits for specialising in predator detection tasks, for example, some individuals can be specialised in increasing birth rate while others can be specialised in decreasing death rate (Jürgens & Matz 2002).

3.4 Strategy 4: diversified bet-hedging

When abiotic environmental conditions are inconsistent over time or space, individuals can be selected for reducing the variance in fitness through not being particularly adapted to any certain environmental condition. The selection on fitness variation can potentially produce a strategy that has a lower (arithmetic) mean fitness across all environments, but still being adaptive against the competing strategy with a higher fitness (Philippi & Seger 1989; Starrfelt & Kokko 2012). The underlying reason is geometric mean fitness, which is multiple single-generation fitnesses being multiplied together. If one of the generations of a strategy has zero fitness because of unfavourable environments, the population goes extinct even if it has a higher arithmetic mean fitness (Philippi & Seger 1989; Starrfelt & Kokko 2012). These interesting adaptations that are seemingly maladaptive are known as evolutionary bet-hedging strategies.

There are two types of bet-hedging strategies: conservative and diversified bet-hedging (Starrfelt & Kokko 2012; Liu *et al.* 2018). The former is producing an unspecialised generalist that is not very well-adapted to all possible environmental conditions but can have some reproductive output across all conditions. Conservative bet-hedging does not involve phenotypic diversity as all individuals in the population are generalists. On the contrary, diversified bet-hedging is producing a mixture of specialists in every single generation, so that there are always some individuals capable of reproducing in all environmental conditions. Examples of conservative bet-hedging include suboptimal timing of flowering in perennial plants to avoid occasional dry years or short growing seasons (Simons & Johnston 2003; Rees *et al.* 2004; Rees *et al.* 2006; Childs *et al.* 2010). Examples of diversified bet-hedging include persister cells in bacterial population, and diversified flowering age of the same genotype of seeds in

some plants (Baskin & Baskin 1979; Kachi & Hirose 1990; Balaban *et al.* 2004; Rees *et al.* 2006; Kümmerli & Frank 2023).

Two mechanisms are involved in diversified bet-hedging: frequency dependence and environmental autocorrelation. Frequency dependence arises in diversified bet-hedging when one phenotype is produced at a proportion less than the probability of encountering the environment where the phenotype is specialised to. Selection will favour genotypes that produce higher ratio of the focal phenotype as it has greater (geometric) mean fitness than the resident population (Starrfelt & Kokko 2012). Environmental autocorrelation is also a crucial mechanism for maintaining phenotypic diversity. When environmental conditions are negatively autocorrelated, diversified bet-hedging would be more favoured as the environment essentially alternates between each condition (Metcalf & Koons 2007; see also Schreiber 2021 for a more ecology-based approach). On the contrary, when environmental conditions are positively autocorrelated, diversified bet-hedging would not be favoured as each environmental condition would last for a long period of time (Metcalf & Koons 2007; Liu *et al.* 2019; Schreiber 2021). Besides these two mechanisms related to direct fitness benefit, there are also cases where indirect fitness benefit can help increase phenotypic diversity as persister cells are predicted to be more abundant when relatedness is higher (Gardner *et al.* 2007).

3.5 Strategy 5&6: alternative mating strategies and sexual dimorphism

Phenotypic diversity can also be favoured by sexual selection. One example is individuals within the same sex can have very distinct mating strategies like sneaking or female-mimicking morphology (alternative mating strategies). One hypothesis for such variation is that poorer quality individuals are making the best of a bad job (Gross

1996; Neff & Svensson 2013). Another example is sexual dimorphism, where males and females have different phenotypes because of mating selection, niche differentiation, or predation (Gilbert & Williamson 1983; Shine 1989; Owens & Hartley 1998). As the theme of sex is not covered in the rest of the thesis, I only summarise some main mechanisms and strategies here.

4. Thesis outline

The topic of phenotypic diversity is a major research theme in evolutionary biology, and recent advances in experimental techniques have enabled us with more abundant and better-quality data, but have also revealed greater complexities of biological systems. I addressed these outstanding research questions in the following chapters:

In chapter 2, I asked how individuals should divide labour in a non-clonal population. Individuals could determine their phenotypes independently through random specialisation, communicate between each other and achieve coordinated specialisation, or some intermediate strategies that is not random nor fully coordinated. Nonclonality is important because many empirical organisms that have division of labour do not have clonal groups, and the extent of nonclonality could help explain the variation in how labour is divided between species. I built a heuristic model and derived both analytical solutions and simulations for the model. In addition, I also made an exhaustive literature collection of division of labour in microbes in this paper.

In chapter 3, I investigated how strains, or populations, can coexist under the presence of (abiotic) environmental fluctuations, such as the temporal changes in temperature. There has been a long debate on how environmental variability would affect species coexistence, with numerous hypotheses proposed, such as intermediate

disturbance hypothesis, limiting similarity hypothesis, and modern coexistence theory. These hypotheses sometime contradict with each other, both theoretically and empirically. We sought a unified solution for these conflicting predictions by explicitly modelling the time scales of environmental fluctuations. Through tracking the eco-evolutionary dynamics in a system of stochastic Lotka-Volterra competition equations, we found new insight on how environmental fluctuations shape the evolution of population coexistence.

In chapter 4, I looked into one possible explanation for why the genetic composition differs between strains of cooperative populations, which is a pattern found across bacteria, slime moulds, and ants. The explanation is there are other co-evolving and manipulative traits that attempt to make other individuals more cooperative, so the social group does not suffer from a group-level cost of cheating. We built a series of multi-trait co-evolving models and derived numeric solutions and simulations for the model. We provide directions for designing experiments to test if these manipulative traits are present in empirical populations.

In chapter 5, I sought a systematic answer of why the dynamic of cheat proportions in populations ranges from fixation, equilibria, to oscillations. All three types of dynamics have been observed in real-world population, but it is unclear which factor is crucial for creating oscillations. We considered four factors that are closely related to experimental evolution set up: periodic population bottlenecks, density dependence, frequency dependence, and stochastic group formation in a set Lotka-Volterra competition equations. We disentangled the essential ingredients for oscillation dynamics, and provided experimental plans for testing two types of oscillations.

In chapter 6, I examined the correlations in meteorological quantities used in past comparative studies, and compared different ways of calculating environmental predictability. I asked how much these quantities can improve statistical models, and what the predictabilities are trying to capture from climatic time series data. Comparative studies have been considered as one of the most powerful tools to test predictions for evolutionary models, and many literatures have focused on the association between climates and evolutionary patterns. We found substantial degrees of correlation and contradicting patterns from different predictabilities. We advocate future studies to be more cautious when using these meteorological measurements.

Finally, I included the papers from a number of projects on which I collaborated, but for which I was not the leading author, in the appendices. These include (1) co-evolving signalling in host-parasite system, (2) division of labour in spatially structured groups, (3) optimal mechanism to divide labour in clonal groups, and (4) mapping theoretical predictions on two types of cooperative breeding benefits to an empirical bird dataset.

References

1. Ackermann, M. (2015). A functional perspective on phenotypic heterogeneity in microorganisms. *Nature Reviews Microbiology*, 13, 497-508.
2. Ackermann, M., Stecher, B., Freed, N.E., Songhet, P., Hardt, W.-D. & Doebeli, M. (2008). Self-destructive cooperation mediated by phenotypic noise. *Nature*, 454, 987-990.
3. Alberts, B., Heald, R., Johnson, A., Morgan, D., Raff, M., Roberts, K. *et al.* (2022). *Molecular Biology of the Cell*. W. W. Norton.
4. Andersen, S.B., Marvig, R.L., Molin, S., Krogh Johansen, H. & Griffin, A.S. (2015). Long-term social dynamics drive loss of function in pathogenic bacteria. *Proceedings of the National Academy of Sciences*, 112, 10756-10761.
5. Balaban, N.Q., Merrin, J., Chait, R., Kowalik, L. & Leibler, S. (2004). Bacterial persistence as a phenotypic switch. *Science*, 305, 1622-1625.
6. Baskin, J.M. & Baskin, C.M. (1979). Studies on the autecology and population biology of the weedy monocarpic perennial, *Pastinaca sativa*. *Journal of Ecology*, 67, 601-610.
7. Buckling, A., Harrison, F., Vos, M., Brockhurst, M.A., Gardner, A., West, S.A. *et al.* (2007). Siderophore-mediated cooperation and virulence in *Pseudomonas aeruginosa*. *FEMS Microbiology Ecology*, 62, 135-141.
8. Childs, D.Z., Metcalf, C.J.E. & Rees, M. (2010). Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. *Proceedings of the Royal Society, Series B: Biological Sciences*, 277, 3055-3064.
9. Clutton-Brock, T.H., O'Riain, M.J., Brotherton, P.N.M., Gaynor, D., Kansky, R., Griffin, A.S. *et al.* (1999). Selfish sentinels in cooperative mammals. *Science*, 284, 1640-1644.
10. Cockburn, A. (1998). Evolution of helping behavior in cooperatively breeding birds. *Annual Review of Ecology and Systematics*, 29, 141-177.
11. Cockburn, A. (2006). Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society, Series B: Biological Sciences*, 273, 1375-1383.
12. Cooper, G.A., Frost, H., Liu, M. & West, S.A. (2021). The evolution of division of labour in structured and unstructured groups. *eLife*, 10, e71968.
13. Cooper, G.A., Liu, M., Peña, J. & West, S.A. (2022). The evolution of mechanisms to produce phenotypic heterogeneity in microorganisms. *Nature Communications*, 13, 195.
14. Cornwallis, C.K., West, S.A. & Griffin, A.S. (2009). Routes to indirect fitness in cooperatively breeding vertebrates: kin discrimination and limited dispersal. *Journal of Evolutionary Biology*, 22, 2445-2457.

15. Dawkins, R. (1976). *The selfish gene*. Oxford University Press New York, New York.
16. Dey, A., Vassallo Christopher, N., Conklin Austin, C., Pathak Darshankumar, T., Troselj, V. & Wall, D. (2016). Sibling rivalry in *Myxococcus xanthus* is mediated by kin recognition and a polyploid prophage. *Journal of Bacteriology*, 198, 994-1004.
17. Diggle, S.P., Griffin, A.S., Campbell, G.S. & West, S.A. (2007). Cooperation and conflict in quorum-sensing bacterial populations. *Nature*, 450, 411-414.
18. Dionisio, F. & Gordo, I. (2006). The tragedy of the commons, the public goods dilemma, and the meaning of rivalry and excludability in evolutionary biology. *Evolutionary Ecology Research*, 8, 321-332.
19. Dragoš, A., Kieseewalter, H., Martin, M., Hsu, C.-Y., Hartmann, R., Wechsler, T. *et al.* (2018). Division of labor during biofilm matrix production. *Current Biology*, 28, 1903-1913.e1905.
20. Flores, E. & Herrero, A. (2010). Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nature Reviews Microbiology*, 8, 39-50.
21. Frank, S.A. (1998). *Foundations of Social Evolution*. Princeton University Press.
22. Frensing, T., Heldt, F.S., Pflugmacher, A., Behrendt, I., Jordan, I., Flockerzi, D. *et al.* (2013). Continuous influenza virus production in cell culture shows a periodic accumulation of defective interfering particles. *PLoS One*, 8, e72288.
23. Futuyma, D.J. & Kirkpatrick, M. (2017). *Evolution*. Fourth edition edn. Sinauer Associates, Inc., Publishers Sunderland, Massachusetts U.S.A., Sunderland, Massachusetts U.S.A.
24. Gardner, A. & West, S.A. (2010). Greenbeards. *Evolution*, 64, 25-38.
25. Gardner, A., West, S.A. & Griffin, A.S. (2007). Is bacterial persistence a social trait? *PLoS One*, 2, e752.
26. Ghoul, M., Griffin, A.S. & West, S.A. (2014). Toward an evolutionary definition of cheating. *Evolution*, 68, 318-331.
27. Gilbert, J.J. & Williamson, C.E. (1983). Sexual dimorphism in zooplankton (copepoda, cladocera, and rotifera). *Annual Review of Ecology and Systematics*, 14, 1-33.
28. Gillespie, J.H. (2000). Genetic drift in an infinite population: the pseudohitchhiking model. *Genetics*, 155, 909-919.
29. Giri, S., Waschina, S., Kaleta, C. & Kost, C. (2019). Defining division of labor in microbial communities. *Journal of Molecular Biology*, 431, 4712-4731.
30. Griffin, A.S. & West, S.A. (2003). Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science*, 302, 634.

31. Griffin, A.S., West, S.A. & Buckling, A. (2004). Cooperation and competition in pathogenic bacteria. *Nature*, 430, 1024-1027.
32. Gross, M.R. (1996). Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology and Evolution*, 11, 92-98.
33. Hamilton, W.D. (1964a). The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, 7, 1-16.
34. Hamilton, W.D. (1964b). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, 7, 17-52.
35. Hartl, D.L. & Clark, A.G. (2007). *Principles of population genetics*. 4 edn. Oxford University Press.
36. Hatchwell, B.J., Ross, D.J., Fowlie, M.K. & McGowan, A. (2001). Kin discrimination in cooperatively breeding long-tailed tits. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268, 885-890.
37. Heard, E., Clerc, P. & Avner, P. (1997). X-chromosome inactivation in mammals. *Annual Review of Genetics*, 31, 571-610.
38. Henke, J.M. & Bassler, B.L. (2004). Bacterial social engagements. *Trends in Cellular Biology*, 14, 648-656.
39. Hirose, S., Chen, G., Kuspa, A. & Shaulsky, G. (2017). The polymorphic proteins TgrB1 and TgrC1 function as a ligand–receptor pair in Dictyostelium allorecognition. *Journal of Cell Science*, 130, 4002-4012.
40. Hölldobler, B. & Wilson, E.O. (1990). *The ants*. Harvard University Press.
41. Huang, A.S. (1973). Defective interfering viruses. *Annual Review of Microbiology*, 27, 101-118.
42. Jennions, M.D. & Macdonald, D.W. (1994). Cooperative breeding in mammals. *Trends in Ecology and Evolution*, 9, 89-93.
43. Jürgens, K. & Matz, C. (2002). Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. *Antonie Van Leeuwenhoek*, 81, 413-434.
44. Kachi, N. & Hirose, T. (1990). Optimal time of seedling emergence in a dune-population of *Oenothera glazioviana*. *Ecological Research*, 5, 143-152.
45. Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E. *et al.* (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, 333, 880-882.
46. Kim, W., Levy, S.B. & Foster, K.R. (2016). Rapid radiation in bacteria leads to a division of labour. *Nature Communications*, 7, 10508.
47. Krishna, S. & Laxman, S. (2020). Emergence of metabolic heterogeneity in cell populations: lessons from budding yeast. In: *Phenotypic Switching* (eds. Levine, H, Jolly, MK, Kulkarni, P & Nanjundiah, V). Academic Press, pp. 335-360.

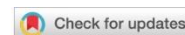
48. Kümmerli, R. & Frank, S.A. (2023). Evolutionary explanations for heterogeneous behavior in clonal bacterial populations. *Trends in Microbiology*, 31, 665-667.
49. Kümmerli, R., Santorelli, L.A., Granato, E.T., Dumas, Z., Dobay, A., Griffin, A.S. *et al.* (2015). Co-evolutionary dynamics between public good producers and cheats in the bacterium *Pseudomonas aeruginosa*. *Journal of Evolutionary Biology*, 28, 2264-2274.
50. Liu, M., Rubenstein, D.R., Cheong, S.-A. & Shen, S.-F. (2018). Multitasking and the evolution of optimal clutch size in fluctuating environments. *Ecology and Evolution*, 8, 8803-8817.
51. Liu, M., Rubenstein, D.R., Liu, W.-C. & Shen, S.-F. (2019). A continuum of biological adaptations to environmental fluctuation. *Proceedings of the Royal Society, Series B: Biological Sciences*, 286, 20191623.
52. Metcalf, C.J.E. & Koons, D.N. (2007). Environmental uncertainty, autocorrelation and the evolution of survival. *Proceedings of the Royal Society, Series B: Biological Sciences*, 274, 2153-2160.
53. Mizuuchi, R., Furubayashi, T. & Ichihashi, N. (2022). Evolutionary transition from a single RNA replicator to a multiple replicator network. *Nature Communications*, 13, 1460.
54. Murphy, K., Weaver, C., Berg, L., Barton, G. & Janeway, C. (2022). *Janeway's immunobiology*. 10th edition edn. W.W. Norton and Company New York, NY, New York, NY.
55. Neff, B.D. & Svensson, E.I. (2013). Polyandry and alternative mating tactics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20120045.
56. Owens, I.P.F. & Hartley, I.R. (1998). Sexual dimorphism in birds: why are there so many different forms of dimorphism? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265, 397-407.
57. Pathak, D.T., Wei, X., Dey, A. & Wall, D. (2013). Molecular recognition by a polymorphic cell surface receptor governs cooperative behaviors in bacteria. *PLoS Genetics*, 9, e1003891.
58. Philippi, T. & Seger, J. (1989). Hedging one's evolutionary bets, revisited. *Trends in Ecology and Evolution*, 4, 41-44.
59. Pollitt, E.J.G., West, S.A., Crusz, S.A., Burton-Chellew, M.N. & Diggle, S.P. (2014). Cooperation, quorum sensing, and evolution of virulence in *Staphylococcus aureus*. *Infection and Immunity*, 82, 1045-1051.
60. Rees, M., Childs, D.Z., Metcalf, Jessica C., Rose, Karen E., Sheppard, Andy W. & Grubb, Peter J. (2006). Seed dormancy and delayed flowering in monocarpic

- plants: selective interactions in a stochastic environment. *The American Naturalist*, 168, E53-E71.
61. Rees, M., Childs, D.Z., Rose, K.E. & Grubb, P.J. (2004). Evolution of size-dependent flowering in a variable environment: partitioning the effects of fluctuating selection. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271, 471-475.
62. Ross-Gillespie, A., Gardner, A., West, Stuart A. & Griffin, Ashleigh S. (2007). Frequency dependence and cooperation: theory and a test with bacteria. *The American Naturalist*, 170, 331-342.
63. Rueffler, C., Hermisson, J. & Wagner, G.P. (2012). Evolution of functional specialization and division of labor. *Proceedings of the National Academy of Sciences*, 109, E326-E335.
64. Rumbaugh, K.P., Trivedi, U., Watters, C., Burton-Chellew, M.N., Diggle, S.P. & West, S.A. (2012). Kin selection, quorum sensing and virulence in pathogenic bacteria. *Proceedings of the Royal Society, Series B: Biological Sciences*, 279, 3584-3588.
65. Russell, A.F. & Hatchwell, B.J. (2001). Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268, 2169-2174.
66. Schreiber, S.J. (2021). Positively and negatively autocorrelated environmental fluctuations have opposing effects on species coexistence. *The American Naturalist*, 197, 405-414.
67. Shen, S.-F., Emlen, S.T., Koenig, W.D. & Rubenstein, D.R. (2017). The ecology of cooperative breeding behaviour. *Ecology Letters*, 20, 708-720.
68. Shine, R. (1989). Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *The Quarterly Review of Biology*, 64, 419-461.
69. Simons, A.M. & Johnston, M.O. (2003). Suboptimal timing of reproduction in *Lobelia inflata* may be a conservative bet-hedging strategy. *Journal of Evolutionary Biology*, 16, 233-243.
70. Smalley, N., E., An, D., Parsek, M., R., Chandler, J., R. & Dandekar, A., A. (2015). Quorum sensing protects *Pseudomonas aeruginosa* against cheating by other species in a laboratory coculture model. *Journal of Bacteriology*, 197, 3154-3159.
71. Smith, C.R., Toth, A.L., Suarez, A.V. & Robinson, G.E. (2008). Genetic and genomic analyses of the division of labour in insect societies. *Nature Reviews Genetics*, 9, 735-748.
72. Starrfelt, J. & Kokko, H. (2012). Bet-hedging- a triple trade-off between means, variances and correlations. *Biology Review (Cambridge)*, 87, 742-755.

73. Strassmann, J.E., Zhu, Y. & Queller, D.C. (2000). Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature*, 408, 965-967.
74. Trivers, R.L. (1971). The evolution of reciprocal altruism. *The Quarterly Review of Biology*, 46, 35-57.
75. Trowsdale, J. & Knight, J.C. (2013). Major histocompatibility complex genomics and human disease. *Annual Review of Genomics and Human Genetics*, 14, 301-323.
76. van Gestel, J., Vlamakis, H. & Kolter, R. (2015a). From cell differentiation to cell collectives: *Bacillus subtilis* uses division of labor to migrate. *PLoS Biology*, 13, e1002141.
77. van Gestel, J., Vlamakis, H. & Kolter, R. (2015b). Division of labor in biofilms: the ecology of cell differentiation. In: *Microbiology Spectrum*, pp. 67-97.
78. Veening, J.-W., Igoshin, O.A., Eijlander, R.T., Nijland, R., Hamoen, L.W. & Kuipers, O.P. (2008). Transient heterogeneity in extracellular protease production by *Bacillus subtilis*. *Molecular Systems Biology*, 4, 184.
79. West, S.A. & Cooper, G.A. (2016). Division of labour in microorganisms: an evolutionary perspective. *Nature Reviews Microbiology*, 14, 716-723.
80. West, S.A., Fisher, R.M., Gardner, A. & Kiers, E.T. (2015). Major evolutionary transitions in individuality. *Proceedings of the National Academy of Sciences*, 112, 10112-10119.
81. Wright, J., Berg, E., De Kort, S.R., Khazin, V. & Maklakov, A.A. (2001). Cooperative sentinel behaviour in the Arabian babbler. *Animal Behaviour*, 62, 973-979.
82. Wyatt, G.A.K., Kiers, E.T., Gardner, A. & West, S.A. (2014). A biological market analysis of the plant-mycorrhizal symbiosis. *Evolution*, 68, 2603-2618.

2

Relatedness and the evolution of mechanisms to divide labour in microorganisms



Received: 8 August 2021 | Accepted: 16 August 2021

DOI: 10.1002/ece3.8067

ORIGINAL RESEARCH

Ecology and Evolution WILEY

Relatedness and the evolution of mechanisms to divide labor in microorganisms

Ming Liu¹ | Stuart Andrew West¹ | Guy Alexander Cooper^{1,2} ¹Department of Zoology, University of Oxford, Oxford, UK²St. John's College, Oxford, UK

Correspondence

Ming Liu, Department of Zoology, University of Oxford, Oxford, OX1 3SZ, UK.
Email: ming.liu@zoo.ox.ac.uk

Funding information

H2020 European Research Council, Grant/Award Number: Advanced Grant 834164

Abstract

Division of labor occurs when cooperating individuals specialize to perform different tasks. In bacteria and other microorganisms, some species divide labor by random specialization, where an individual's role is determined by random fluctuations in biochemical reactions within the cell. Other species divide labor by coordinating across individuals to determine which cells will perform which task, using mechanisms such as between-cell signaling. However, previous theory, examining the evolution of mechanisms to divide labor between reproductives and sterile helpers, has only considered clonal populations, where there is no potential for conflict between individuals. We used a mixture of analytical and simulation models to examine non-clonal populations and found that: (a) intermediate levels of coordination can be favored, between the extreme of no coordination (random) and full coordination; (b) as relatedness decreases, coordinated division of labor is less likely to be favored. Our results can help explain why coordinated division of labor is relatively rare in bacteria, where groups may frequently be nonclonal.

KEYWORDS

cellular differentiation, coordination, division of labor, evolutionary theory, phenotypic noise, random specialization, signaling, social microbes

1 | INTRODUCTION

Division of labor occurs when cooperating individuals specialize to carry out different tasks, to the benefit of all individuals involved (West & Cooper, 2016). Division of labor plays a key role across the tree of life: RNAs or replicators serving different functions form a genome together; cells specialize to perform different functions in multicellular organisms; and multicellular organisms perform different tasks in eusocial societies (Hart & Ratnieks, 2001; Higgs & Lehman, 2015; Hölldobler & Wilson, 1990; Ispolatov et al., 2012;

Levin & West, 2017; Michod, 2005; Oster & Wilson, 1978; Simpson, 2012; Szathmáry & Smith, 1995).

Different species use different mechanisms to divide labor (Ackermann, 2015; Anderson et al., 2008; Schwander et al., 2010; Wahl, 2002; West & Cooper, 2016). In some bacteria species, individual cells specialize into distinct roles randomly and independently of one another by amplifying random fluctuations in the biochemical reactions of each cell (phenotypic noise). For example, whether or not a cell produces and secretes protease in *Bacillus subtilis* is determined randomly (Marlow et al., 2014; Veening, Igoshin, et al., 2008).

Stuart Andrew West and Guy Alexander Cooper contributed equally to this paper.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Ecology and Evolution* published by John Wiley & Sons Ltd.

Ecology and Evolution, 2021;11:14475–14489.

www.ecoevol.org | 14475

Other microbial species use some form of coordination across cells when dividing labor. For example, cyanobacteria cells use signaling molecules to help determine which cells will develop into sterile, nitrogen fixing heterocysts (Herrero et al., 2016; Zhang et al., 2006). Finally, some insect species have a genetic component to division of labor, where an individual's role can depend on its genotype. For example, in some ants, different lineages develop into queens and workers at different rates (Hughes & Boomsma, 2008; Linksvayer, 2006; Schwander et al., 2010; Smith et al., 2008). These mechanisms are not mutually exclusive; for example, the ants using genetic determination also use coordination and signaling to control the proportion of different castes in the colony (Anderson et al., 2008; Schwander et al., 2010; Smith et al., 2008).

We lack evolutionary theory to explain why different species use different mechanisms to divide labor (Wahl, 2002; Cooper et al., 2020). Bacteria and other microorganisms provide a good system for tackling this problem, because both random and coordinated division of labor have evolved in these systems (Cooper et al., 2020). As a first step toward explaining this variation in microbes, Cooper et al. showed that coordination is more likely to be favored when group size is small and cooperation is more essential. Random specialization leads to variance in the proportion of helpers in a group, so some groups will end up with suboptimal proportions of helpers. The expected deviation from the optimal proportions of helpers is greater in smaller groups and leads to a larger fitness cost when cooperation is more essential.

However, this previous theory assumed clonal populations, with no conflict within groups (relatedness, $R = 1$). In contrast to this assumption, many microbial species appear to be in nonclonal populations where social interactions do not just take place with the same lineage (clone-mate; i.e., $R < 1$). Indeed, at the scale of social interactions, such as within bacterial biofilms, populations often contain multiple species, let alone different lineages of the same species (Dragoš et al., 2018; Kim et al., 2016; Schiessl et al., 2019). Evidence for nonclonality ($R < 1$) in microbial social interactions has come from a variety of different experimental and observational studies on cooperation, both within and across species, as well as from the diversity of mechanisms that bacteria have evolved for attacking nonrelatives (Belcher et al., unpublished data; Bruce et al., 2017; Butaitė et al., 2017; Cordero et al., 2012; Fiegna & Velicer, 2005; Fisher et al., 2013; Foster et al., 2002; Gilbert et al., 2007; Granato et al., 2019; Hawlena et al., 2012; Simonet & McNally, 2021).

Theory has already shown that a lower relatedness leads to division of labor being less likely to be favored, or have a lower proportion of helpers (Cooper & West, 2018; Johnstone, 2000; Madgwick et al., 2018). In contrast, we do not know how nonclonality influences selection on the mechanism used to divide labor. For example, does nonclonality lead to a conflict of interest that disrupts coordination, and hence favors random specialization? Given that many cooperative microorganisms are nonclonal ($R < 1$), answering this question could help explain why coordinated specialization has been found relatively rarely in bacteria (Ackermann, 2015; West & Cooper, 2016).

We used a mixture of analytical and simulation approaches to examine the evolution of division of labor in both clonal and nonclonal

populations. We focused on reproductive division of labor, where more cooperative "helpers" gain indirect fitness benefits by the aid they provide to less cooperative "reproductives." Reproductive division of labor has been found in bacteria, algae, fungi, and slime molds; the cooperative traits in this form of division of labor include the following: fruiting body formation; nitrogen fixation; extracellular polysaccharide matrix production; beating flagella formation; programmed cell death; antibiotic production; adopting a tubular mitochondria; triggering an inflammatory response to eliminate competing bacteria; and releasing toxins (Table 1). We modeled cooperation as the production of a "public good" that benefits all the members of the group, because this form of cooperation is common in bacteria and other microbes (West et al., 2006, 2007). We asked whether division of labor was favored, and whether it was favored by random or coordinate specialization. We developed a relatively simple analytical model that compared the extreme cases of fully random and fully (perfectly) coordinated specialization. This model allowed us to examine analytically how different factors would influence selection for coordinated as opposed to random specialization.

We then developed a simulation model that allowed us to relax some assumptions of our analytical model. We allowed individuals to vary on a continuum, from purely random to fully coordinated, so that there could be intermediate levels of coordination. This simulation model allowed us to examine both: how any coordination could initially evolve from no coordination; and whether intermediate levels of coordination could be favored. In both our analytical and simulation models, we examined the influence of group size, the extent to which cooperation was essential, and within-group relatedness.

2 | METHODS AND RESULTS

2.1 | Analytical game theory model

2.1.1 | Population structure and life cycle

We employed a deliberately simple model, focusing on factors of general importance across many microbial species, rather than a specific model for a particular species. We assumed that social groups are formed by I founding cells, each of which spawns a lineage with m cells, giving a final group size of $n = Im$ (Haystack model; Maynard Smith (1964). Hence, the whole-group relatedness is $1/I$ (including self; Pepper (2000).

When the group has reached a size of n , division of labor may occur with individuals becoming either a sterile helper or a pure reproductive. The reproductives then produce a large number of offspring. Although we are interested in all forms of reproductive division of labor, we assume the extreme case of sterile helpers and pure reproductives in the model for mathematical tractability. Across different microbial species, there are examples of reproductive division of labor with sterile helpers, and with helpers that just show reduced reproduction (Table 1). After reproduction, individuals in the current generation all die (nonoverlapping generations),

TABLE 1 Microbial examples of reproductive division of labour, where some individuals specialised to tasks at the cost of their own fitness while the benefit of specialisation is shared by other conspecific individuals. Note that we have not included the examples of non-reproductive division of labour (or mutual division of labour)

Specialisation	Species	Description	References
<i>Group 1: Sterile helpers</i>			
Fruiting body formation	<i>Myxococcus xanthus</i>	Sterile rods+ reproductive spores	Kaiser (2004), Fiegna and Velicer (2005), Kononova et al. (2010), Higgs et al. (2014)
	<i>Dictyostelium discoideum</i>	Sterile stalk+ reproductive spores	Foster et al. (2002), Gilbert et al. (2007), Strassmann and Queller (2011), Madgwick et al. (2018), Dhakshinamoorthy and Singh (2021)
	<i>Bacillus subtilis</i> ^a	Stalk-like aerial structure+ spores	Branda et al. (2001)
Nitrogen fixing	Filamentous cyanobacteria, e.g., <i>Anabaena cylindrica</i>	Sterile heterocyst+ vegetative cell	Adams (2000), Zhang et al. (2006), Rossetti et al. (2010), Herrero et al. (2016)
Beating flagella	<i>Volvox cateri</i>	Soma cell with flagella+ non-flagellated germ cells	Kirk (2005), Herron and Michod (2008)
	<i>Volvox</i> (others)	Soma cell with flagella+ non-flagellated germ cells	Shelton et al. (2012), Matt and Umen (2016)
Bacteriocin production	<i>Escherichia coli</i>	Colicin producing cells (3%) and others. Mechanism involves LexA repressor and SOS regulation. The producer is lysed (killed) during colicin release	Mulec et al. (2003), Cascales et al. (2007), Kamenšek et al. (2010), Mader et al. (2015), Mavridou et al. (2018)
	<i>Pseudomonas aeruginosa</i>	Some individuals heterogeneously synthesise and release pyocin through cell lysis (death)	Michel-Briand and Baysse (2002), Waite and Curtis (2009), Mei et al. (2021)
	<i>Xenorhabdus bovienii</i> and <i>Xenorhabdus koppenhoeferi</i>	Different bacteriocins are produced by different colonies and intraspecific inhibition is stronger than interspecific inhibition. The bacteriocins are similar to pyocin	Hawlana et al. (2012)
Triggering host inflammation response	<i>Salmonella enterica</i>	Some individuals heterogeneously express secretion system and sacrifice themselves to eliminate competitors	Ackermann et al. (2008), Sturm et al. (2011), Diard et al. (2013), Bumann and Cunrath (2017)
Virulence	<i>Cryptococcus gattii</i>	Some individuals adopt a tubular mitochondrion to facilitate others' growth	Voelz et al. (2014), Farrer et al. (2018)
Apoptosis	<i>Saccharomyces cerevisiae</i>	Some individuals undergo apoptosis after multicellular cluster formation.	Ratcliff et al. (2012)
<i>Group 2: Non-sterile helpers</i>			
Degrading extracellular substances/nutrients	<i>Pseudoalteromonas</i> sp. strain S91	Differential production in chitinolytic enzymes	Baty et al. (2000)
Antibiotic production	<i>Vibrionaceae</i> isolates	Genetic analyses show that within populations, broad-range antibiotics are produced by few genotypes, whereas all others are resistant, suggesting cooperation between conspecifics	Cordero et al. (2012)
	<i>Streptomyces coelicolor</i>	Bistable switch for antibiotic-producing phenotype	Mehra et al. (2008)
	<i>Streptomyces coelicolor</i>	Amplification and deletions to the chromosome causes differences in antibiotic production	Zhang et al. (2020)

(Continues)

TABLE 1 (Continued)

Specialisation	Species	Description	References
Biofilm formation	<i>Bacillus subtilis</i>	Matrix-producing cell type and others (bistable switch).	Branda et al. (2004), Branda et al. (2006), Dubnau and Losick (2006), Chai et al. (2008), Veening et al. (2008a), López and Kolter (2010), Marvasi et al. (2010), Marlow et al. (2014), Dragoš et al. (2018)
	<i>Bacillus subtilis</i>	Exoprotease-producing cell type and others	Veening et al. (2008a), Marlow et al. (2014)
	<i>Candida auris</i> (yeast pathogen)	Aggregative and non-aggregative phenotypes.	Brown et al. (2020)
Mobility chemical production	<i>Pseudomonas fluorescens</i>	One of the two strains produces wetting polymer.	Kim et al. (2016)

^aCell types include motility, surfactin production, matrix production, protease production, and sporulation (Vlamakis et al., 2013; van Gestel et al., 2015).

and all offspring disperse globally and compete to found groups in the next iteration of the group life cycle (global competition). The fecundity of each reproductive depends upon the level of cooperation in the group, which is determined by the proportion of helpers in the group.

We assumed that cooperation takes the form of producing a public good, which is shared with all members of this group. Bacteria and other microorganisms produce a large range of public goods, including iron scavenging siderophore molecules, and enzymes to digest proteins, aromatic compounds, or antibiotics (Diggle et al., 2007; Frost et al., 2018; Griffin et al., 2004; West et al., 2007). Public good models have been very useful for examining both cooperation and division of labor in microbes (Cooper & West, 2018; Frank, 2010; Lee et al., 2016; Sasaki & Uchida, 2013; West & Buckling, 2003). Other forms of cooperation such as beating flagella to keep a group of cells afloat can also be thought of as a form of public good cooperation (Herron & Michod, 2008; Michod, 2006). Another form of cooperation that involves division of labor is when cells sacrifice themselves to produce bacteriocin toxins that kill unrelated cells—this can be conceptualized as harming nonrelatives to help relatives, by reducing competition for resources, which is also a form of public good cooperation, directed at relatives (Gardner et al., 2004; Granato et al., 2019).

2.1.2 | Reproductive division of labor

We assumed that individuals in the last generation of the group life cycle develop into either pure reproductives or sterile helpers. Sterile helpers do not reproduce, but provide cooperative benefits that linearly increase the fecundity of pure reproductives in the group. Specifically, when a proportion P of individuals in the group are sterile helpers, the fecundity of a reproductive is proportional to: $1 - \epsilon + \epsilon P$, where $0 \leq \epsilon \leq 1$. Here, $1 - \epsilon$ represents the baseline fecundity, in the absence of any helpers, and ϵP represents the increase in fecundity gained from the cooperative behavior of sterile helpers. The parameter ϵ , which we call

the essentiality of cooperation, measures the degree to which reproductive fecundity is dependent on the amount of help in the group.

2.1.3 | The evolving target proportion of helpers (q)

Whether an individual becomes a sterile helper or a pure reproductive depends on a heritable trait and its mechanism for dividing labor. We assumed that there is an evolving trait, the target proportion of helpers, q , that specifies the proportion of helpers in the group that the individuals aim to achieve. This is akin to the optimal proportion of helpers for the lineage's founder. The target proportion of helpers, q , is a genotypic property of a focal individual, whereas the realized proportion of helpers in the group, P , is a phenotypic property of the whole group (depending upon the mechanism used to coordinate labor).

2.1.4 | Mechanisms for dividing labor

We examined two different mechanisms for dividing labor: fully random specialization and fully coordinated specialization. With random specialization, each individual becomes a helper with probability equal to its target proportion of helpers (Figure 1a; i.e., $P = q$ or $P \neq q$). In contrast, with coordinated specialization, individuals use within-lineage signaling to produce lineages with an exact proportion of q helpers (Figure 1b; i.e., $P = q$). We assumed that coordinators pay a relative cost of sending and receiving signals (θ).

2.1.5 | Mathematical analysis

We carried out a two-step invasion analysis. First, we calculated the evolutionary stable target proportion of helper for each mechanism, in uniform populations where every individual uses the same mechanism to divide labor (Section S1.2–S1.4). Second, we then examined

when a mutant strain with the alternative mechanism for dividing labor could invade the population.

2.1.6 | The relative fitness of random specializers

Let us assume that a focal founder employs fully random specialization with a target proportion of helpers, q , in a group that otherwise has a target proportion of helpers, Q . In this case, the expected fitness of the focal founder can be written as (Section S1.2):

$$w_{FR}(q, Q) = (1 - q)(1 - \epsilon + \epsilon q/l + \epsilon(l - 1)Q/l - \epsilon q/lm). \quad (1)$$

The first term in parentheses is the expected proportion of reproductives in the founder's lineage ($1 - q$). The second term in parentheses is the expected fecundity of each reproductive, which is determined by: the baseline fecundity, $1 - \epsilon$; the expected increase in fecundity from within-lineage help, $\epsilon q/l$; the expected increase in fecundity from across-lineage help, $\epsilon(l - 1)Q/l$; and the final term, $\epsilon q/lm$, is the expected fecundity cost due to random deviations from the target proportion of helpers. In particular, it does not matter whether the other founders use random or coordinated specialization because the expected levels of helping across the social group (i.e., Q) are the same for focal mutant lineage in either case.

We calculate the Evolutionarily Stable Strategy (ESS) for the target proportion of helpers (q_{FR}^*), which is the target proportion of helpers that cannot be invaded by any other strategy in a population of random specializers (Section S1.3 (Maynard Smith & Price, 1973)). This gives:

$$q_{FR}^* = \begin{cases} 0, & \text{if } \epsilon < lm/(l(m+1) - 1) \\ 1 - \frac{lm - \epsilon}{\epsilon(lm + m - 2)}, & \text{otherwise} \end{cases} \quad (2)$$

More essential cooperation (higher ϵ), smaller lineage sizes (smaller m), and fewer lineages (smaller l) lead to a higher level of cooperation in random groups (larger q_{FR}^*) (Section S1.3).

2.1.7 | The relative fitness of coordinated specializers

We now assume that the focal founder employs fully coordinated specialization to produce an exact proportion q of helpers within its lineage and that the other founders have an exact target proportion of helpers, Q . We assume that cells do not coordinate with cells from other lineages. We have the expected fitness as:

$$w_{FC}(q, Q) = (1 - \theta)(1 - q)(1 - \epsilon + \epsilon q/l + \epsilon(l - 1)Q/l). \quad (3)$$

The first term in parentheses captures the cost of coordination, the second term is the proportion of reproductives in the focal lineage, and the final term is the expected fecundity of each reproductive in the focal lineage (in the absence of coordination costs). This last term is equal to the expected fecundity of random specializers without the cost to random specialization (second last term of Equation (1); $\epsilon(l - 1)Q/l$). Once again, it does not matter in Equation

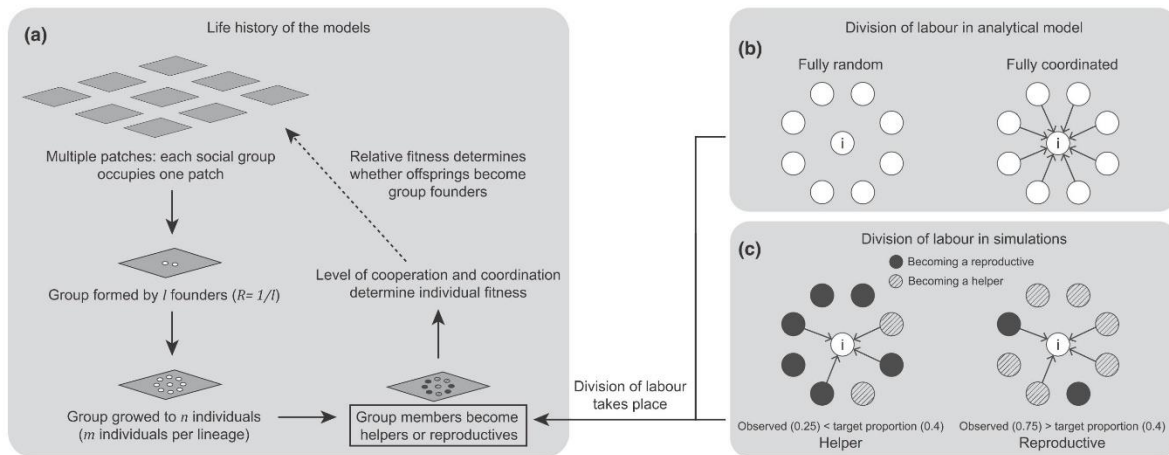


FIGURE 1 Life history assumptions. (a) We outline the life history assumptions common to both our analytical and simulation model. Parts b and c show the mechanisms by which division of labor occurs in our (b) analytical and (c) simulation models, respectively. In our analytical model (b), there are two mechanisms to divide labor: fully random specialization, where no communication is involved and each individual decides its phenotype probabilistically according to its target proportion of helper (a heritable trait, q), and fully coordinated specialization, where individuals assess the intended phenotype of all other members of the same lineage and adjust their developmental plan to match their target proportions of helpers. In our simulation model (c), the level of coordination is continuous, going from fully random to fully coordinated. We varied the level of coordination (s) by varying the proportion of the group that each individual signals and coordinates with. In the examples shown, we have assumed that the target proportion is 0.4, and so the focal individual would adopt a helper role in the left case and a reproductive role in the right case

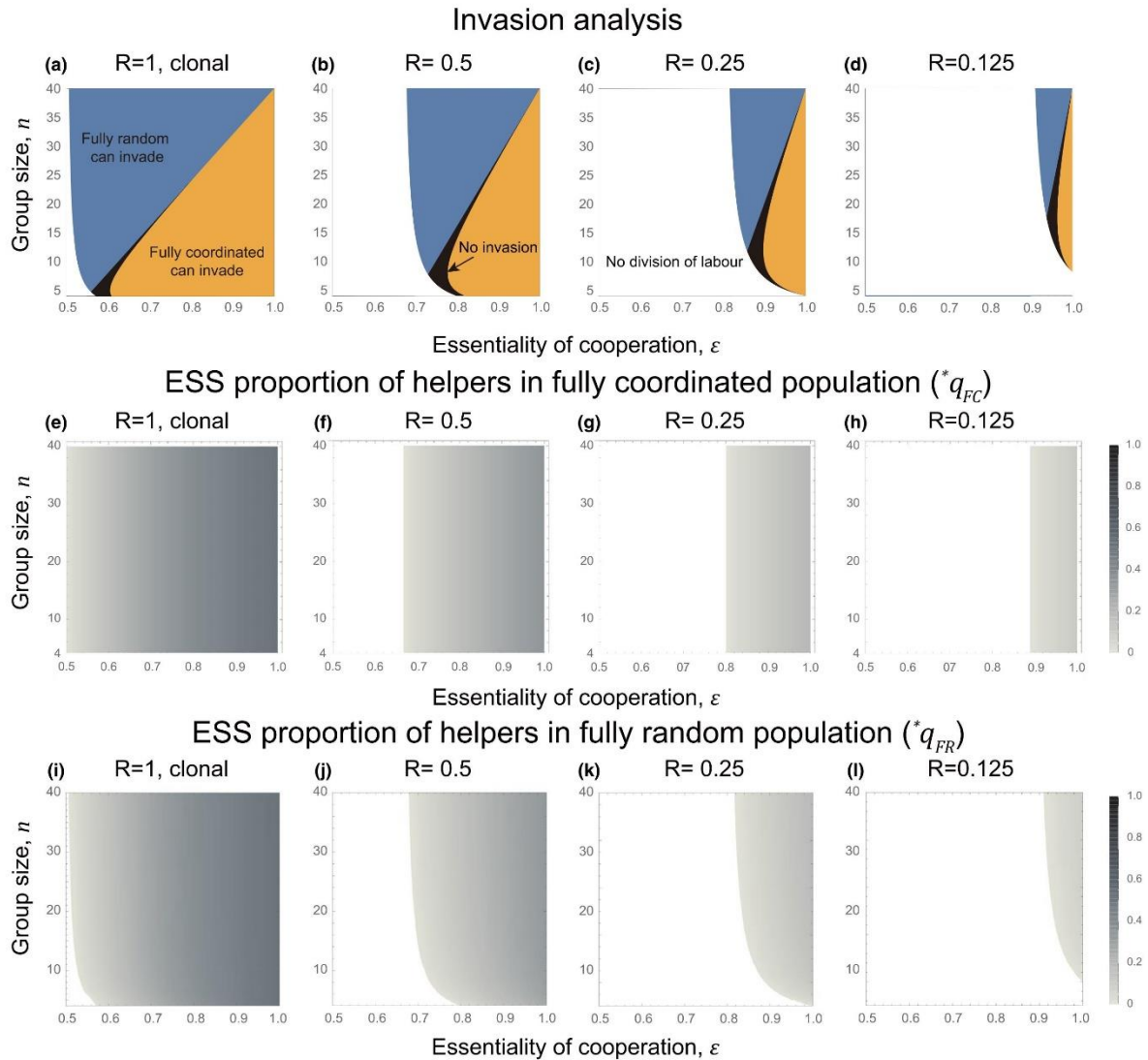


FIGURE 2 Invasion analysis and evolutionary stable strategy (ESS) in the analytical model. We performed invasion analyses to see if fully coordinated specialists can invade fully random specialists, and vice versa. We fixed the cost of coordination ($\theta = 0.025$) in all panels and varied the number of founders ($l = 1, 2, 4, 8$) to change the relatedness of populations. The different colors represent areas of parameter spaces where fully random specialist can invade fully coordinated population (blue), fully coordinated specialist can invade fully random population (orange), no invasion (black), and no division of labor (white). The x-axis is the essentiality of cooperation (ϵ) and the y-axis is the size of social group ($n = lm$). The different panels show: (a–d) the results of the invasion analysis; (e–h) the ESS proportion of helpers in populations with fully coordinated specialists (q_{FC}^*); (i–l) the ESS proportion of helpers in populations with fully random specialists (q_{FR}^*)

(3) whether the other (nonfocal) group founders employ coordination or random specialization when dividing labor (see also Section S1.2).

We can calculate the ESS target proportion of helpers in a population of coordinators, giving:

$$q_{FC}^* = \begin{cases} 0 & \text{if } \epsilon < l/(l+1) \\ 1 - \frac{l}{\epsilon(l+1)}, & \text{otherwise} \end{cases} \quad (4)$$

Again, we find that more essential cooperation (higher ϵ) and fewer lineages (lower l), favor a higher level of cooperation (larger q_{FC}^*) (Section S1.4). In contrast, because coordinated specialists produce a deterministic proportion of helpers, the ESS level of coordinated cooperation does not depend on the size of the lineage (m). The level of cooperation in a population of coordinators is greater than the level of cooperation in a population of random specialists ($q_C^* > q_R^*$, Section S1.5).

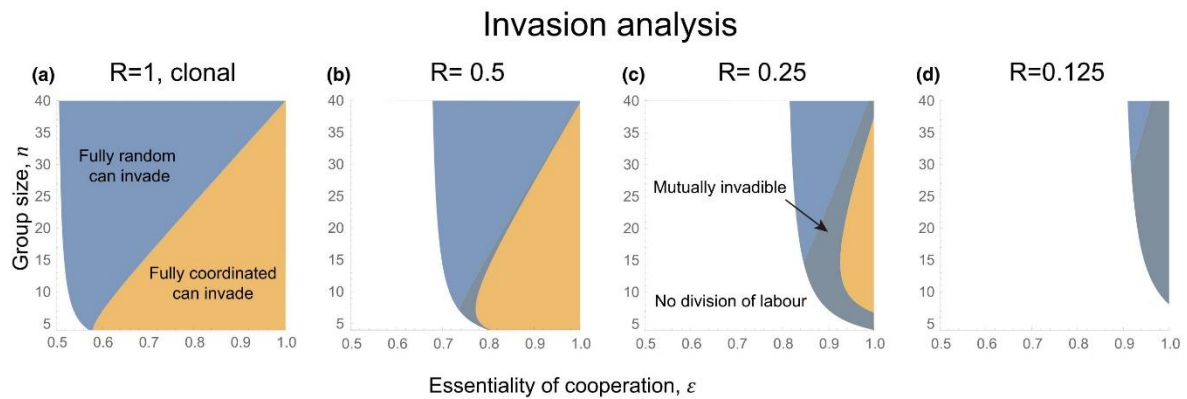


FIGURE 3 Invasion analysis of fully random or fully coordinated specialists when invaders can have different target proportion of helpers from the residents. We performed invasion analysis over various relatedness setting and see if fully coordinated specialists can invade fully random specialists, or vice versa. We fixed the cost of coordination ($\theta = 0.025$) in all panels and vary the number of founders ($l = 1, 2, 4, 8$) to change the relatedness of populations. Colored areas represent parameter spaces where fully random specialist can invade fully coordinated population (blue), fully coordinated specialist can invade fully random population (orange), mutually invadable (brown), and no division of labor (white). The x-axis is the essentiality of cooperation (ϵ) and the y-axis is the size of social group ($n = lm$). See Section S2 for more details of this analytical model

2.1.8 | Invasion analysis

We now determine the conditions in which each mechanism is either stable or invadable by the other mechanism. A focal mutant employing random specialization can invade a population of coordinated specialists if $w_{FR}(q_{FC}^*, q_{FC}^*) > w_{FC}(q_{FC}^*, q_{FC}^*)$, giving the condition:

$$\epsilon < \frac{\theta lm}{\theta lm(1 - q_{FC}^*) + q_{FC}^*} \quad (5)$$

Alternatively, a focal mutant employing coordinated specialization can invade a population of random specialists if $w_{FC}(q_{FR}^*, q_{FR}^*) > w_{FR}(q_{FR}^*, q_{FR}^*)$, giving the condition:

$$\epsilon > \frac{\theta lm}{\theta lm(1 - q_{FR}^*) + q_{FR}^*} \quad (6)$$

If the cost of coordination (θ), the number of lineages (l), and the final size of lineages (m) is sufficiently large ($\theta lm \geq 1$), then random specialization can always invade and is always stable to invasion. Otherwise (if $\theta lm < 1$), more essential cooperation (larger ϵ), less costly coordination (lower θ), fewer lineages (lower l), smaller lineage sizes (lower m), and a higher level of cooperation (larger q_c^* or q_R^*) favor the invasion of coordinated specialization.

In the above, we assumed that the level of cooperation of an invading mutant is equal to the ESS for the resident population, so that the only difference between competing mechanisms is the way that helpers are produced and not the relative target proportion of helpers. We find the same qualitative results when mutants employ the optimal target proportion of helpers for their mechanism to

divide labor (coordinated division favors a higher target proportion of helpers; modeling details are provided in Section S2). We also modeled the invasion under various costs of coordination and found coordinated specialization is more dominant when the cost is lower (Section S1.7).

2.2 | Analytical predictions

Our predictions are consistent with previous theory where groups were assumed to be clonal (Figure 2a, e, i; Cooper et al., 2020). When cooperation is more essential (higher ϵ), there is a larger opportunity cost from producing suboptimal proportions of helpers and so random specialization is disfavored. When the size of the lineage increases (higher m), the relative variance in the proportion of helpers produced by random specialization decreases, which leads to a smaller cost of stochasticity (Figure 2e,i; see also Section S1.6).

Moving from clonal groups ($l = 1$) to nonclonal groups ($l > 1$), we found that as relatedness decreased (higher l): (a) division of labor is less likely to be favored (Figure 2, left to right columns); and (b) a lower proportion of helpers is favored (Figure 2, left to right columns). Consistent with previous theory, these patterns reflect the smaller inclusive fitness benefit of altruism (sterile helping) toward individuals that are less likely to be kin (Section S1.3 and S1.4) (Cooper & West, 2018; Hamilton, 1964a, 1964b; Johnstone, 2000; Reeve et al., 1998; Reeve & Shen, 2006).

We found that groups with a lower relatedness (more lineages; higher l) favor the evolution of random specialization over coordinated specialization (Figure 2). When there are more lineages, the variance in the proportion of helpers produced by a focal lineage

has less of an impact on the total proportion of helpers across the group. This diminishes the expected cost of stochasticity to a focal founder employing random specialization. This result differs from previous theory, which had only considered the case of clonal groups ($R = 1$).

We also found that, as relatedness decreases, there is an increase in the size of the intermediate area where neither mechanism could invade the other (Figure 2); in our alternate analysis, there is an increase in the intermediate area where the two mechanisms could mutually invade the other (Figure 6; Section S2). By construction, our analytical models only allow for the evolution of fully coordinated or random specialization. We hypothesized that this expanding intermediate region corresponds to where partial coordination could be favored. To test this hypothesis, we next developed individual-based simulations in which intermediate mechanisms could evolve.

2.3 | Individual-based simulations

Our analytical model examined the extreme cases where division of labor was either completely random, or fully coordinated. In order to investigate the continuum between these two cases, and to determine whether coordination can "gradually" evolve, we developed an individual-based simulation.

2.3.1 | The evolving level of coordination (s)

We assumed that the extent to which individuals adjust their phenotype depending upon the phenotype of their group mates can vary continuously, as defined by their probability, s , of being "coordinated" with each group neighbor. If division of labor is at least partially coordinated ($s > 0$), an individual may interact with some group neighbors, via signals or cues, and can take account of these neighbors' intended phenotype when specializing. If $s = 0$, then an individual is not coordinated with any neighbors and we assume that the individual is a fully random specialist, with helper probability equal to its target proportion of helpers (q).

More specifically, the focal individual has probability s of establishing a one-way link with each group member and receives information about the neighbor's intended phenotype (arrows in Figure 1c). We modeled the signaling process in this way because in many systems the spatial arrangements of individuals remain relatively static when division of labor takes place (van Gestel et al., 2015; Keller & Surette, 2006; Yanni et al., 2020). Consequently, one way of thinking about the coordination parameter s is that it measures the relative proportion of the group that a focal individual is close enough to coordinate with.

We assumed the initial phenotype of all individuals is reproductive, as many organisms would start as newly divided cells but then specialize to become helpers later (Ackermann et al., 2008; Herrero

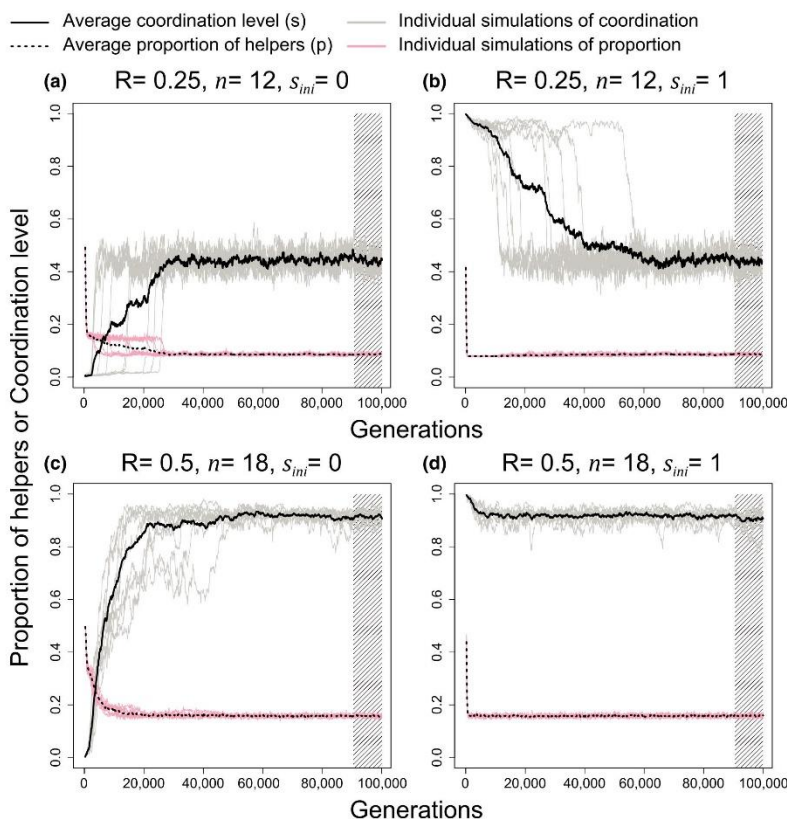


FIGURE 4 The coevolving dynamics of helper proportion and coordination level. Coordination is shown in gray and solid black line, while helper proportion is shown in pink and broken black lines. Each panel shows the dynamics of 10 repeated simulations and the essentiality of cooperation is set to 1.0. The panels show how some level of coordination can evolve from initially no coordination, and that there can be greater variance in the level of coordination, compared with the level of helping. Stripped areas are the generations used in plotting the heatmap (Figure 5). Note that group size (n) is equal to the number of lineages (l) times lineage size (m)

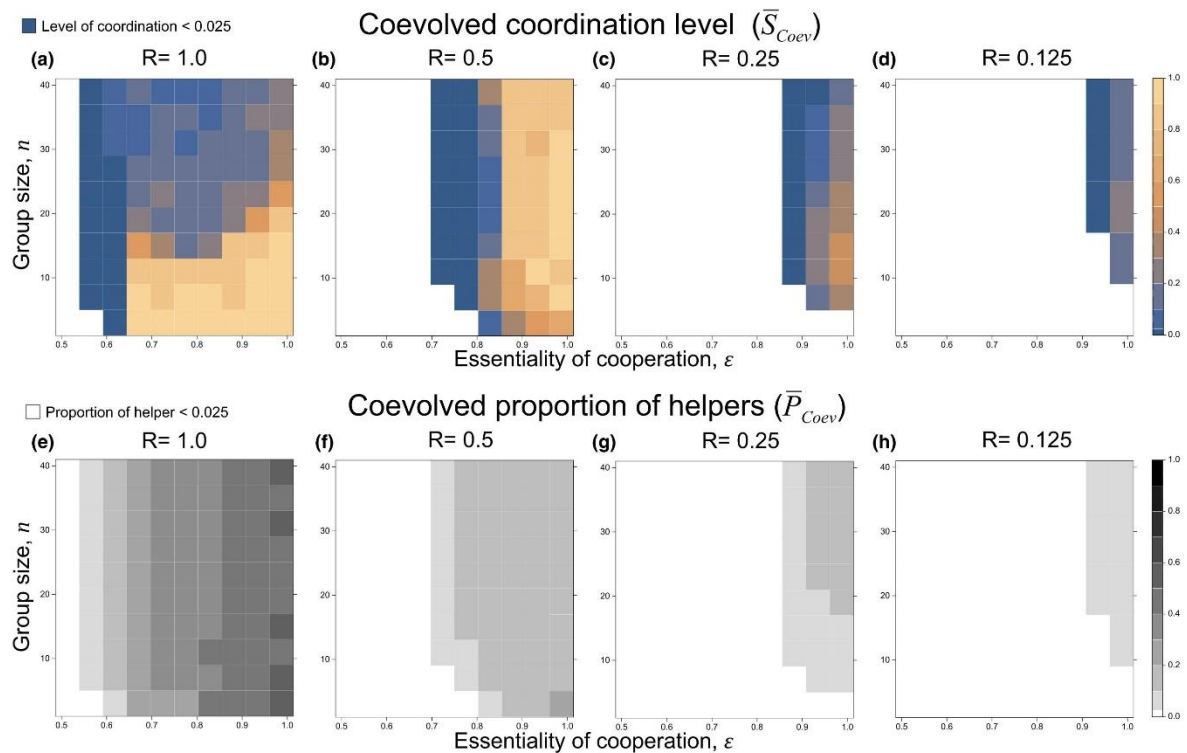


FIGURE 5 Coevolved level of coordination and proportion of helpers in the simulations. (a–d) The evolved coordination level plotted against the essentiality of cooperation (x-axis; ϵ) and group size (y-axis; $n = lm$). Each panel represents the results of different relatedness setting. The blueish colors represent the evolved mechanism of division of labor is closer to random specialization, whereas the orangish colors represent the evolved mechanism is closer to coordinated specialization. Each grid is an average of 10 repeated simulations where the average is taken from the last 10% of 10^5 generations. (e–h) The evolved proportion of helpers. Darker shades mean there are more helpers in the population. Coordination levels when proportion of helpers is below 0.025 is not plotted as we regard it as no division of labor

et al., 2016). After the coordination network is formed, the individuals within the group are randomly sampled, one individual at a time, to determine whether it changes its intended phenotype. The type-changing decision depends on comparing its target proportion of helpers with the observed proportion of “intended helpers” amongst all the cells that it interacts with. If the observed ratio is larger than target proportion, the sampled individual sets its developmental plan to become an “intended reproductive,” and vice versa (Figure 1c).

We designed the metabolic cost of coordination as a function of the level of coordination, $\text{cost}(s) = \theta(1 - e^{-5s})$, where θ , the cost coefficient, has the same value as in analytical model. This cost of coordination is nonlinear, with a decelerating slope (i.e., saturating with increasing s ; $\frac{d^2(\theta(1 - e^{-5s}))}{ds^2} < 0$) (Foster, 2004). The high initial cost can be thought of as the cost of building the coordination machinery (e.g., protein interaction networks), and then, increased coordination improves the efficiency of that machinery (Crespi, 2001; Wilkinson, 1988). In addition, the cost function has the same or very similar cost as the analytical model when $s = 0, 1$ to make the modeling results more comparable.

2.3.2 | Fitness calculation and the coevolving traits

We assumed that the fecundity of an individual, w , is given by:

$$w = (1 - \theta(1 - e^{-5s})) (1 - h) (1 - \epsilon + \epsilon P) \quad (7)$$

The first term in parentheses contains the cost of coordination; the second term specifies the individual's phenotype, where h is 1 for a helper and 0 for a reproductive. The third term captures the benefits of group cooperation, which is higher when there is a larger proportion of helpers. Note that the value of h for each individual is determined by both the target proportions of helpers and the level of coordination, and the two traits are coevolving in the simulations.

2.3.3 | Mutation of the coevolving traits

We assumed the traits can mutate between generations. For each trait of a new founder, the mutation rate is $p_{\text{mut}} = 0.01$. If a mutation

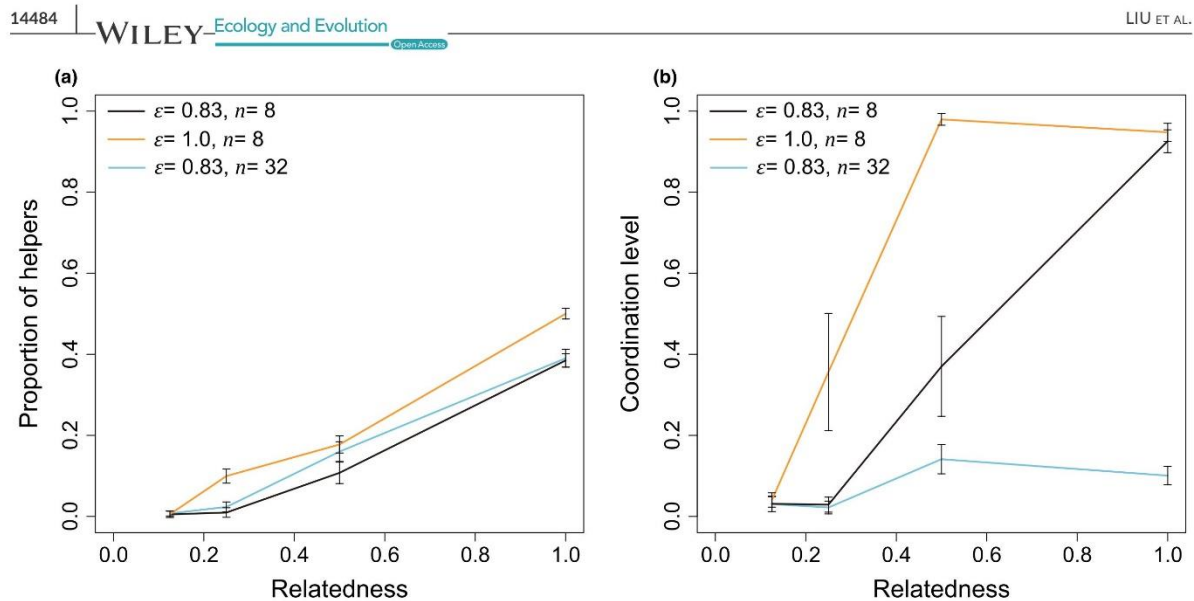


FIGURE 6 Effects of relatedness on: (a) proportion of helpers (p); and (b) level of coordination (s). Note the symbols in parameter settings represent essentiality (ε) and group size ($n = lm$). Each point is the average of the last 10% time-steps of the 10^5 -step simulations; error bar represents the standard error from 10 repetitions

occurs, we perturb the parental trait value by a normally distributed deviation with mean of 0 and standard deviation of 0.1, constrained at the boundaries [0,1].

2.3.4 | Analysis of the simulations

For each combination of parameters, we investigated two scenarios: (a) fully random specialization ($s = 0$), where only the target proportion of helpers (q) was allowed to evolve; (b) both the target proportion of helpers (q) and the level of coordination (s) were allowed to evolve. We used the case of fully random specialization ($s = 0$) as a "control" to examine the influence of coordination. In all cases, we repeated the simulation 10 times, ran 10^5 generations for each simulation, and let division of labor take place when the population size is around 10^4 individuals (i.e., $lm \ll 10^4/lm$) to ensure that the trait values converged to their evolutionary equilibria.

2.4 | Simulation Results

2.4.1 | Intermediate coordination coevolved from simulations

Examining dynamics over time, we found that intermediate coordination can evolve from either fully random specialization or fully coordinated specialization (Figure 4). The level of coordination favored can be intermediate between the extremes of full ($s = 1$) or no ($s = 0$) coordination. Comparing across different runs of the simulation, the

level of coordination ($\bar{s}_{\text{coev}}$) showed greater variation than the proportion of helpers ($\bar{p}_{\text{coev}}$) (Figure 4; gray lines are significantly more variable than pink lines; F test, $p < 10^{-5}$). Greater variation can be expected because the level of coordination has a smaller influence on fitness than the proportion of helpers, via its influence on the θ s term where θ is much smaller than 0.1 in Equation (7) (weaker stabilizing selection).

2.4.2 | Agreement with analytical models

Consistent with our analytical model, we found that as relatedness decreases: (a) division of labor is less likely to be favored (shaded area decreases as go across Figures 5e-h and 6a); (b) a lower proportion of helpers is favored (lighter shading as go across Figures 5e-h and 6a); (c) mechanism for dividing labor shifts from more coordinated to more random in general (Figures 5a-d and 6b). In clonal groups, we also found coordinated specialization is more favored when essentiality is high and group size is small (Figures 5a and 6b).

2.4.3 | Precision of coordination

As relatedness decreases, we found that less precise coordination is favored—the brightest shading goes from orange to brownish blue across Figure 5a-d. In other words, the maximal level of coordination is smaller when relatedness is low. This pattern may reflect that there are less helpers in low-relatedness population, reducing the relative advantage of more precision division. The pattern may also reflect random specialization being a cheating strategy that exploits

the costly coordination practiced by other lineages. The decreased maximal level of coordination along relatedness contrasts the analytical models as intermediate levels of coordination are not included in those models.

2.4.4 | Robustness of results

We confirmed the robustness of our conclusions with several additional simulations. Our simulation results were not changed when we varied the initial starting conditions (Section S3.3). We found the same qualitative pattern when the cost of coordination increased linearly or accelerating, rather than decelerating with the level of coordination (Section S3.5–S3.7). When analyzing purely random division, with no coordination, the results of our simulation were in close agreement with our analytical model (Section S3.1). If cells only coordinate within their own lineage, then lower levels of coordination are favored, because coordination is less able to reach the target proportion of helpers (Section S3.4). Quantitative differences between our analytical and simulation results appear to arise from our simulation allowing the proportion of helpers and the level of coordination to coevolve (Section S3.2).

3 | DISCUSSION

We found that as relatedness decreased (lower R), there was reduced selection for division of labor to be coordinated. We first tackled this issue analytically, examining the extremes of division of labor by fully coordinated and fully random specialization. In these models, we found that when relatedness was lower (lower R), random specialization was more likely to be favored (Figures 2 and 3). We then developed a simulation model that allowed us to examine intermediate levels of coordination. Our simulation showed that when relatedness was lower (lower R), that lower levels of coordination were favored to divide labor (Figures 5 and 6). These results differ from previous theory, which had only considered the case of clonal groups ($R = 1$; Cooper et al., 2020).

Why did a lower relatedness lead to reduced selection for coordinated division of labor? One factor is that when relatedness is lower, lower levels of helping are favored (Figures 2 and 5), and so helping has a smaller influence on fitness (Equations 2 and 4). Consequently, there is weaker selection to coordinate division of labor precisely. Another factor is that paying a personal cost to coordinate division of labor can be seen as a form of cooperation. A lower level of coordination, or random division of labor, which avoids the personal cost of coordination, can hence be seen as form of cheating. When relatedness is lower, there is reduced selection for cooperation, and increased selection for cheating.

Our results can help explain the distribution of mechanisms to divide labor across bacteria and other microorganisms. Many microbe species appear to use random specialization to produce division of labor, based upon "phenotypic noise" (Ackermann, 2015;

Dubnau & Losick, 2006; Lewis, 2010; Smits et al., 2006; Veening et al., 2008; West & Cooper, 2016). Further, many of these species are likely to interact in nonclonal populations ($R < 1$). For example, social bacteria *Myxococcus xanthus*, fungal pathogen *Cryptococcus gattii*, social amoeba *Dictyostelium discoideum*, and a range of species in the gut microbiome (Dragoš et al., 2018; Farrer et al., 2018; Fiegna & Velicer, 2005; Foster et al., 2002; Gilbert et al., 2007; Simonet & McNally, 2021; Voelz et al., 2014). We have shown that, in nonclonal populations, appreciable levels of coordination are only favored when cooperation is relatively essential (high c ; Figures 2 and 4). In contrast, the most striking examples of coordinated division of labor are in clonal populations, such as colonial green algae *Volvox carteri*, and filamentous cyanobacteria *Anabaena cylindrica* (Herrero et al., 2016; Kirk, 2001; Matt & Umen, 2016; Rossetti et al., 2010). Nonetheless, our conclusions here are speculative - as data on more species becomes available, it would be extremely useful to carry out a formal across species test of our predictions.

Our models also supported the predictions of previous theory. We found that as relatedness decreases: (a) division of labor is less likely to be favored (Figures 2 and 5); (b) a smaller proportion of helpers is favored (Figures 2 and 5). In addition, we found that coordinated division of labor is more likely to be favored when cooperation is more essential, or group size is smaller (Figures 2 and 5). These results agreed with previous theory examining either division of labor (Ackermann et al., 2008; Cooper & West, 2018; Michod & Roze, 1999; Cooper et al., 2020), or reproductive skew (Johnstone, 2000; Reeve et al., 1998; Reeve & Shen, 2006). Empirically, both experimental and comparative studies have found a lower proportion of helpers when relatedness is lower (Fisher et al., 2013; Langer et al., 2004; Madgwick et al., 2018).

Finally, there are at least two important avenues for future progress in this area. Empirically, data are required on both the molecular machinery for dividing labor in a wider range of species, and the relatedness structure of natural populations (Hall et al., 2020; Madgwick et al., 2018; Olm et al., 2021; Simonet & McNally, 2021; Speed & Balding, 2015). Data on these and other ecological parameters, such as group size or the relative importance (essentiality) of cooperation, would allow us to look for broad across species patterns. Theoretically, we have developed a deliberately simple model that could be applied widely. It would be very useful to develop more specific models, based on the mechanisms of particular species. As well as allowing specific coordination mechanisms to be modeled, this would allow other factors to be investigated: (a) division of labor being adjusted in response to population density (Bumann & Cunrath, 2017; Maldonado-Barragán & West, 2020; Mavridou et al., 2018); (b) strategies producing a "deceptive" coordination signal that could be selected for in low-relatedness populations; (c) incorporating spatial structure to model case-specific cooperative interactions; (d) phenotypes that can be changed in later life stages (Bergmiller & Ackermann, 2011; Strassmann & Queller, 2011); and (e) division of labor where all individuals are specialized to tasks not related to reproduction (Armbruster et al., 2019; van Gestel et al., 2015; Nikel et al., 2014).

ACKNOWLEDGMENTS

We thank Alan Grafen, Kevin Foster, and Jorge Peña for comments, suggestions, and/or discussion; Oxford-Taiwan and Oxford-Natural Motion graduate scholarships (ML), St John's College, Oxford (GAC), and the ERC (Advanced Grant 834164; SAW) for funding. The authors would like to acknowledge the use of the University of Oxford Advanced Research Computing (ARC; <http://dx.doi.org/10.5281/zenodo.22558>) facility in carrying out the simulations.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

Ming Liu: Conceptualization (equal); Formal analysis (equal); Investigation (lead); Methodology (supporting); Validation (equal); Visualization (lead); Writing-original draft (lead); Writing-review & editing (equal). **Stuart Andrew West:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Supervision (equal); Visualization (supporting); Writing-original draft (supporting); Writing-review & editing (equal). **Guy Alexander Cooper:** Conceptualization (equal); Formal analysis (equal); Investigation (supporting); Methodology (lead); Supervision (equal); Validation (equal); Visualization (supporting); Writing-original draft (supporting); Writing-review & editing (equal).

OPEN RESEARCH BADGES



This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://github.com/mingpapilio/Codes_DOL_Mechanisms_Nonclonal.

DATA AVAILABILITY STATEMENT

All results are generated using Mathematica and C. The codes and generated data used for this study are available at: https://github.com/mingpapilio/Codes_DOL_Mechanisms_Nonclonal.

ORCID

Ming Liu <https://orcid.org/0000-0002-5170-8688>

Stuart Andrew West <https://orcid.org/0000-0003-2152-3153>

Guy Alexander Cooper <https://orcid.org/0000-0002-1748-8183>

REFERENCES

- Ackermann, M. (2015). A functional perspective on phenotypic heterogeneity in microorganisms. *Nature Reviews Microbiology*, 13, 497–508. <https://doi.org/10.1038/nrmicro3491>
- Ackermann, M., Stecher, B., Freed, N. E., Songhet, P., Hardt, W.-D., & Doebeli, M. (2008). Self-destructive cooperation mediated by phenotypic noise. *Nature*, 454, 987–990. <https://doi.org/10.1038/nature07067>
- Adams, D. G. (2000). Heterocyst formation in cyanobacteria. *Current Opinion in Microbiology*, 3, 618–624. [https://doi.org/10.1016/S1369-5274\(00\)00150-8](https://doi.org/10.1016/S1369-5274(00)00150-8)
- Anderson, K. E., Linksvayer, T. A., & Smith, C. R. (2008). The causes and consequences of genetic caste determination in ants (Hymenoptera: Formicidae). *Myrmecological News*, 11, 119–132.
- Armbruster, C. R., Lee, C. K., Parker-Gilham, J., de Anda, J., Xia, A., Zhao, K., Murakami, K., Tseng, B. S., Hoffman, L. R., Jin, F., Harwood, C. S., Wong, G. C. L., & Parsek, M. R. (2019). Heterogeneity in surface sensing suggests a division of labor in *Pseudomonas aeruginosa* populations. *eLife*, 8, e45084. <https://doi.org/10.7554/eLife.45084>
- Baty, A. M., Eastburn, C. C., Diwu, Z., Techkarnjanaruk, S., Goodman, A. E., & Geesey, G. G. (2000). Differentiation of chitinase-active and non-chitinase-active subpopulations of a marine bacterium during chitin degradation. *Applied and Environmental Microbiology*, 66, 3566–3573. <https://doi.org/10.1128/AEM.66.8.3566-3573.2000>
- Bergmiller, T., & Ackermann, M. (2011). Pole age affects cell size and the timing of cell division in *Methylobacterium extorquens* AM1. *Journal of Bacteriology*, 193, 5216–5221. <https://doi.org/10.1128/JB.00329-11>
- Branda, S. S., Chu, F., Kearns, D. B., Losick, R., & Kolter, R. (2006). A major protein component of the *Bacillus subtilis* biofilm matrix. *Molecular Microbiology*, 59, 1229–1238. <https://doi.org/10.1111/j.1365-2958.2005.05020.x>
- Branda, S. S., González-Pastor, J. E., Ben-Yehuda, S., Losick, R., & Kolter, R. (2001). Fruiting body formation by *Bacillus subtilis*. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 11621–11626. <https://doi.org/10.1073/pnas.191384198>
- Branda, S. S., González-Pastor, J. E., Dervyn, E., Ehrlich, S. D., Losick, R., & Kolter, R. (2004). Genes involved in formation of structured multicellular communities by *Bacillus subtilis*. *Journal of Bacteriology*, 186, 3970–3979. <https://doi.org/10.1128/JB.186.12.3970-3979.2004>
- Brown, J. L., Delaney, C., Short, B., Butcher, M. C., McKloud, E., Williams, C., Kean, R., & Ramage, G. (2020). *Candida auris* phenotypic heterogeneity determines pathogenicity in vitro. *mSphere*, 5, e00371–e320. <https://doi.org/10.1128/mSphere.00371-20>
- Bruce, J. B., Cooper, G. A., Chabas, H., West, S. A., & Griffin, A. S. (2017). Cheating and resistance to cheating in natural populations of the bacterium *Pseudomonas fluorescens*. *Evolution*, 71, 2484–2495. <https://doi.org/10.1111/evo.13328>
- Bumann, D., & Cunrath, O. (2017). Heterogeneity of *Salmonella*-host interactions in infected host tissues. *Current Opinion in Microbiology*, 39, 57–63. <https://doi.org/10.1016/j.mib.2017.09.008>
- Butaitė, E., Baumgartner, M., Wyder, S., & Kümmerli, R. (2017). Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater *Pseudomonas* communities. *Nature Communications*, 8, 414. <https://doi.org/10.1038/s41467-017-00509-4>
- Cascales, E., Buchanan, S. K., Duché, D., Kleanthous, C., Lloubès, R., Postle, K., Riley, M., Slatin, S., & Cavard, D. (2007). Colicin biology. *Microbiology and Molecular Biology Reviews*, 71, 158–229. <https://doi.org/10.1128/MMBR.00036-06>
- Chai, Y., Chu, F., Kolter, R., & Losick, R. (2008). Bistability and biofilm formation in *Bacillus subtilis*. *Molecular Microbiology*, 67, 254–263. <https://doi.org/10.1111/j.1365-2958.2007.06040.x>
- Cooper, G. A., Liu, M., Peña, J., & West, S. A. (2020). The evolution of mechanisms to produce phenotypic heterogeneity in microorganisms. *bioRxiv*. <https://doi.org/10.1101/2020.02.25.964643>
- Cooper, G. A., & West, S. A. (2018). Division of labour and the evolution of extreme specialization. *Nature Ecology & Evolution*, 2, 1161–1167. <https://doi.org/10.1038/s41559-018-0564-9>
- Cordero, O. X., Wildschutte, H., Kirkup, B., Proehl, S., Ngo, L., Hussain, F., Le Roux, F., Mincer, T., & Polz, M. F. (2012). Ecological populations of bacteria act as socially cohesive units of antibiotic production and resistance. *Science*, 337, 1228–1231. <https://doi.org/10.1126/science.1219385>
- Crespi, B. J. (2001). The evolution of social behavior in microorganisms. *Trends in Ecology & Evolution*, 16, 178–183. [https://doi.org/10.1016/S0169-5347\(01\)02115-2](https://doi.org/10.1016/S0169-5347(01)02115-2)

- Dhakshinamoorthy, R., & Singh, S. P. (2021). Evolution of Reproductive division of labor – lessons learned from the Social Amoeba *Dictyostelium discoideum* During its multicellular development. *Frontiers in Cell and Developmental Biology*, 9. <http://dx.doi.org/10.3389/fcell.2021.599525>
- Diard, M., Garcia, V., Maier, L., Remus-Emsermann, M. N. P., Regoes, R. R., Ackermann, M., & Hardt, W.-D. (2013). Stabilization of cooperative virulence by the expression of an avirulent phenotype. *Nature*, 494, 353–356. <https://doi.org/10.1038/nature11913>
- Diggle, S. P., Griffin, A. S., Campbell, G. S., & West, S. A. (2007). Cooperation and conflict in quorum-sensing bacterial populations. *Nature*, 450, 411–414. <https://doi.org/10.1038/nature06279>
- Dragoš, A., Kieselwahr, H., Martin, M., Hsu, C.-Y., Hartmann, R., Wechsler, T., Eriksen, L., Brix, S., Drescher, K., Stanley-Wall, N., Kümmerli, R., & Kovács, Á. T. (2018). Division of labor during biofilm matrix production. *Current Biology*, 28, 1903–1913.e1905. <https://doi.org/10.1016/j.cub.2018.04.046>
- Dubnau, D., & Losick, R. (2006). Bistability in bacteria. *Molecular Microbiology*, 61, 564–572. <https://doi.org/10.1111/j.1365-2958.2006.05249.x>
- Farrer, R. A., Ford, C. B., Rhodes, J., Delorey, T., May, R. C., Fisher, M. C., Cloutman-Green, E., Balloux, F., & Cuomo, C. A. (2018). Transcriptional heterogeneity of *Cryptococcus gattii* VGII compared with non-VGII lineages underpins key pathogenicity pathways. *mSphere*, 3, e00445–e418. <https://doi.org/10.1128/mSphere.00445-18>
- Fiegna, F., & Velicer, G. J. (2005). Exploitative and hierarchical antagonism in a cooperative bacterium. *PLoS Biology*, 3, e370. <https://doi.org/10.1371/journal.pbio.0030370>
- Fisher, R. M., Cornwallis, C. K., & West, S. A. (2013). Group formation, relatedness, and the evolution of multicellularity. *Current Biology*, 23, 1120–1125. <https://doi.org/10.1016/j.cub.2013.05.004>
- Foster, K. R. (2004). Diminishing returns in social evolution: The not-so-tragic commons. *Journal of Evolutionary Biology*, 17, 1058–1072. <https://doi.org/10.1111/j.1420-9101.2004.00747.x>
- Foster, K. R., Fortunato, A., Strassmann, J. E., & Queller, D. C. (2002). The costs and benefits of being a chimera. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269, 2357–2362. <https://doi.org/10.1098/rspb.2002.2163>
- Frank, S. A. (2010). A general model of the public goods dilemma. *Journal of Evolutionary Biology*, 23, 1245–1250. <https://doi.org/10.1111/j.1420-9101.2010.01986.x>
- Frost, I., Smith, W. P. J., Mitri, S., Millan, A. S., Davit, Y., Osborne, J. M., Pitt-Francis, J. M., MacLean, R. C., & Foster, K. R. (2018). Cooperation, competition and antibiotic resistance in bacterial colonies. *The ISME Journal*, 12, 1582–1593. <https://doi.org/10.1038/s41396-018-0090-4>
- Gardner, A., West, S. A., & Buckling, A. (2004). Bacteriocins, spite and virulence. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271, 1529–1535. <https://doi.org/10.1098/rspb.2004.2756>
- Gilbert, O. M., Foster, K. R., Mehdiabadi, N. J., Strassmann, J. E., & Queller, D. C. (2007). High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 8913–8917. <https://doi.org/10.1073/pnas.0702723104>
- Granato, E. T., Meiller-Legrand, T. A., & Foster, K. R. (2019). The evolution and ecology of bacterial warfare. *Current Biology*, 29, R521–R537. <https://doi.org/10.1016/j.cub.2019.04.024>
- Griffin, A. S., West, S. A., & Buckling, A. (2004). Cooperation and competition in pathogenic bacteria. *Nature*, 430, 1024–1027. <https://doi.org/10.1038/nature02744>
- Hall, G. A., Speed, T. P., & Woodruff, C. J. (2020). Strain-level sample characterisation using long reads and MAPQ scores. *bioRxiv*, 2020.2010.2018.344739.
- Hamilton, W. D. (1964a). The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, 7, 1–16. [https://doi.org/10.1016/0022-5193\(64\)90038-4](https://doi.org/10.1016/0022-5193(64)90038-4)
- Hamilton, W. D. (1964b). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, 7, 17–52. [https://doi.org/10.1016/0022-5193\(64\)90039-6](https://doi.org/10.1016/0022-5193(64)90039-6)
- Hart, A. G., & Ratnieks, F. L. W. (2001). Task partitioning, division of labour and nest compartmentalisation collectively isolate hazardous waste in the leafcutting ant *Atta cephalotes*. *Behavioral Ecology and Sociobiology*, 49, 387–392. <https://doi.org/10.1007/s002650000312>
- Hawlena, H., Bashey, F., & Lively, C. M. (2012). Bacteriocin-mediated interactions within and between coexisting species. *Ecology and Evolution*, 2, 2521–2526. <https://doi.org/10.1002/ece3.354>
- Herrero, A., Stavans, J., & Flores, E. (2016). The multicellular nature of filamentous heterocyst-forming cyanobacteria. *FEMS Microbiology Reviews*, 40, 831–854. <https://doi.org/10.1093/femsre/fuw029>
- Herron, M. D., & Michod, R. E. (2008). Evolution of complexity in the volvocine algae: Transitions in individuality through Darwin's eye. *Evolution*, 62, 436–451. <https://doi.org/10.1111/j.1558-5646.2007.00304.x>
- Higgs, P. I., Hartzell, P. L., Holkenbrink, C., & Hoiczyk, E. (2014). *Myxococcus xanthus* vegetative and developmental cell heterogeneity. In Z. Yang & P. I. Higgs (Eds.), *Myxobacteria: Genomics, cellular and molecular biology* (pp. 51–77). Caister Academic Press. <https://doi.org/10.21775/9781910190913>
- Higgs, P. G., & Lehman, N. (2015). The RNA world: Molecular cooperation at the origins of life. *Nature Reviews Genetics*, 16, 7–17. <https://doi.org/10.1038/nrg3841>
- Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Harvard University Press.
- Hughes, W. O. H., & Boomsma, J. J. (2008). Genetic royal cheats in leaf-cutting ant societies. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 5150–5153. <https://doi.org/10.1073/pnas.0710262105>
- Ispolatov, I., Ackermann, M., & Doebe, M. (2012). Division of labour and the evolution of multicellularity. *Proceedings of the Royal Society B: Biological Sciences*, 279, 1768–1776. <https://doi.org/10.1098/rspb.2011.1999>
- Johnstone, R. A. (2000). Models of reproductive skew: A review and synthesis (Invited Article). *Ethology*, 106, 5–26. <https://doi.org/10.1046/j.1439-0310.2000.00529.x>
- Kaiser, D. (2004). Signaling in myxobacteria. *Annual Review of Microbiology*, 58, 75–98. <https://doi.org/10.1146/annurev.micro.58.030603.123620>
- Kamenšek, S., Podlesek, Z., Gillor, O., & Žgur-Bertok, D. (2010). Genes regulated by the *Escherichia coli* SOS repressor LexA exhibit heterogeneous expression. *BMC Microbiology*, 10, 283. <https://doi.org/10.1186/1471-2180-10-283>
- Keller, L., & Surette, M. G. (2006). Communication in bacteria: An ecological and evolutionary perspective. *Nature Reviews Microbiology*, 4, 249–258. <https://doi.org/10.1038/nrmicro1383>
- Kim, W., Levy, S. B., & Foster, K. R. (2016). Rapid radiation in bacteria leads to a division of labour. *Nature Communications*, 7, 10508. <https://doi.org/10.1038/ncomms10508>
- Kirk, D. L. (2001). Germ-soma differentiation in volvox. *Developmental Biology*, 238, 213–223. <https://doi.org/10.1006/dbio.2001.0402>
- Kirk, D. L. (2005). A twelve-step program for evolving multicellularity and a division of labor. *BioEssays*, 27, 299–310. <https://doi.org/10.1002/bies.20197>
- Kononova, A., Petters, T., & Søgaard-Andersen, L. (2010). Extracellular biology of *Myxococcus xanthus*. *FEMS Microbiology Reviews*, 34, 89–106. <https://doi.org/10.1111/j.1574-6976.2009.00194.x>
- Langer, P., Hogendoorn, K., & Keller, L. (2004). Tug-of-war over reproduction in a social bee. *Nature*, 428, 844–847. <https://doi.org/10.1038/nature02431>

- Lee, W., van Baalen, M., & Jansen, V. A. A. (2016). Siderophore production and the evolution of investment in a public good: An adaptive dynamics approach to kin selection. *Journal of Theoretical Biology*, 388, 61–71. <https://doi.org/10.1016/j.jtbi.2015.09.038>
- Levin, S. R., & West, S. A. (2017). The evolution of cooperation in simple molecular replicators. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20171967. <https://doi.org/10.1098/rspb.2017.1967>
- Lewis, K. (2010). Persister cells. *Annual Review of Microbiology*, 64, 357–372. <https://doi.org/10.1146/annurev.micro.112408.134306>
- Linksvayer, T. A. (2006). Direct, maternal, and subsocial genetic effects on individual and colony traits in an ant. *Evolution*, 60, 2552–2561. <https://doi.org/10.1111/j.0014-3820.2006.tb01889.x>
- López, D., & Kolter, R. (2010). Extracellular signals that define distinct and coexisting cell fates in *Bacillus subtilis*. *FEMS Microbiology Reviews*, 34, 134–149. <https://doi.org/10.1111/j.1574-6976.2009.00199.x>
- Mader, A., von Bronk, B., Ewald, B., Kesel, S., Schnetz, K., Frey, E., & Opitz, M. (2015). Amount of colicin release in *Escherichia coli* is regulated by lysis gene expression of the Colicin E2 operon. *PLoS One*, 10, e0119124. <https://doi.org/10.1371/journal.pone.0119124>
- Madgwick, P. G., Stewart, B., Belcher, L. J., Thompson, C. R. L., & Wolf, J. B. (2018). Strategic investment explains patterns of cooperation and cheating in a microbe. *Proceedings of the National Academy of Sciences of the United States of America*, 115, E4823–E4832. <https://doi.org/10.1073/pnas.1716087115>
- Maldonado-Barragán, A., & West, S. A. (2020). The cost and benefit of quorum sensing-controlled bacteriocin production in *Lactobacillus plantarum*. *Journal of Evolutionary Biology*, 33, 101–111. <https://doi.org/10.1111/jeb.13551>
- Marlow, V. L., Cianfanelli, F. R., Porter, M., Cairns, L. S., Dale, J. K., & Stanley-Wall, N. R. (2014). The prevalence and origin of exoprotease-producing cells in the *Bacillus subtilis* biofilm. *Microbiology*, 160, 56–66. <https://doi.org/10.1099/mic.0.072389-0>
- Marvasi, M., Visscher, P. T., & Casillas Martinez, L. (2010). Exopolymeric substances (EPS) from *Bacillus subtilis*: Polymers and genes encoding their synthesis. *FEMS Microbiology Letters*, 313, 1–9. <https://doi.org/10.1111/j.1574-6968.2010.02085.x>
- Matt, G., & Umen, J. (2016). Volvox: A simple algal model for embryogenesis, morphogenesis and cellular differentiation. *Developmental Biology*, 419, 99–113. <https://doi.org/10.1016/j.ydbio.2016.07.014>
- Mavridou, D. A. I., Gonzalez, D., Kim, W., West, S. A., & Foster, K. R. (2018). Bacteria use collective behavior to generate diverse combat strategies. *Current Biology*, 28, 345–355.e344. <https://doi.org/10.1016/j.cub.2017.12.030>
- Mehra, S., Charaniya, S., Takano, E., & Hu, W.-S. (2008). A bistable gene switch for antibiotic biosynthesis: The butyrolactone regulon in *Streptomyces coelicolor*. *PLoS One*, 3, e2724. <https://doi.org/10.1371/journal.pone.0002724>
- Mei, M., Thomas, J., Diggel, S. P., & Hogan, D. A. (2021). Heterogenous susceptibility to R-pyocins in populations of *Pseudomonas aeruginosa* sourced from cystic fibrosis lungs. *Mbio*, 12, e00458–e421. <https://doi.org/10.1128/mBio.00458-21>
- Michel-Briand, Y., & Bayse, C. (2002). The pyocins of *Pseudomonas aeruginosa*. *Biochimie*, 84, 499–510. [https://doi.org/10.1016/S0300-9084\(02\)01422-0](https://doi.org/10.1016/S0300-9084(02)01422-0)
- Michod, R. E. (2005). On the transfer of fitness from the cell to the multicellular organism. *Biology and Philosophy*, 20, 967–987. <https://doi.org/10.1007/s10539-005-9018-2>
- Michod, R. E. (2006). The group covariance effect and fitness trade-offs during evolutionary transitions in individuality. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 9113–9117. <https://doi.org/10.1073/pnas.0601080103>
- Michod, R. E., & Roze, D. (1999). Cooperation and conflict in the evolution of individuality. III. Transitions in the unit of fitness. In C. L. Nehaniv (Ed.), *Mathematical and computational biology: Computational morphogenesis, hierarchical complexity, and digital evolution*. (Lectures on mathematics in the life sciences, 26, pp. 47–92). American Mathematical Society.
- Mulec, J., Podlessek, Z., Mrak, P., Kopitar, A., Ihan, A., & Žgur-Bertok, D. (2003). A *cka-gfp* transcriptional fusion reveals that the Colicin K activity gene is induced in only 3 percent of the population. *Journal of Bacteriology*, 185, 654–659. <https://doi.org/10.1128/JB.185.2.654-659.2003>
- Nikel, P. I., Silva-Rocha, R., Benedetti, I., & de Lorenzo, V. (2014). The private life of environmental bacteria: Pollutant biodegradation at the single cell level. *Environmental Microbiology*, 16, 628–642. <https://doi.org/10.1111/1462-2920.12360>
- Olm, M. R., Crits-Christoph, A., Bouma-Gregson, K., Firek, B. A., Morowitz, M. J., & Banfield, J. F. (2021). InStrain profiles population microdiversity from metagenomic data and sensitively detects shared microbial strains. *Nature Biotechnology*, 39(6), 727–736. <https://doi.org/10.1038/s41587-020-00797-0>
- Oster, G. F., & Wilson, E. O. (1978). *Caste and ecology in the social insects*. Princeton University Press.
- Pepper, J. W. (2000). Relatedness in trait group models of social evolution. *Journal of Theoretical Biology*, 206, 355–368. <https://doi.org/10.1006/jtbi.2000.2132>
- Ratcliff, W. C., Denison, R. F., Borrello, M., & Travisano, M. (2012). Experimental evolution of multicellularity. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 1595–1600. <https://doi.org/10.1073/pnas.1115323109>
- Reeve, H. K., Emlen, S. T., & Keller, L. (1998). Reproductive sharing in animal societies: Reproductive incentives or incomplete control by dominant breeders? *Behavioral Ecology*, 9, 267–278. <https://doi.org/10.1093/beheco/9.3.267>
- Reeve, H. K., & Shen, S.-F. (2006). A missing model in reproductive skew theory: The bordered tug-of-war. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 8430–8434. <https://doi.org/10.1073/pnas.0603005103>
- Rossetti, V., Schirmeister, B. E., Bernasconi, M. V., & Bagheri, H. C. (2010). The evolutionary path to terminal differentiation and division of labor in cyanobacteria. *Journal of Theoretical Biology*, 262, 23–34. <https://doi.org/10.1016/j.jtbi.2009.09.009>
- Sasaki, T., & Uchida, S. (2013). The evolution of cooperation by social exclusion. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20122498. <https://doi.org/10.1098/rspb.2012.2498>
- Schiessl, K. T., Ross-Gillespie, A., Cornforth, D. M., Weigert, M., Bigosch, C., Brown, S. P., Ackermann, M., & Kümmerli, R. (2019). Individual-versus group-optimality in the production of secreted bacterial compounds. *Evolution*, 73, 675–688. <https://doi.org/10.1111/evo.13701>
- Schwander, T., Lo, N., Beekman, M., Oldroyd, B. P., & Keller, L. (2010). Nature versus nurture in social insect caste differentiation. *Trends in Ecology & Evolution*, 25, 275–282. <https://doi.org/10.1016/j.tree.2009.12.001>
- Shelton, D. E., Desnitskiy, A. G., & Michod, R. E. (2012). Distributions of reproductive and somatic cell numbers in diverse Volvox (Chlorophyta) species. *Evolutionary Ecology Research*, 14, 707–727.
- Simonet, C., & McNally, L. (2021). Kin selection explains the evolution of cooperation in the gut microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, 118, e2016046118. <https://doi.org/10.1073/pnas.2016046118>
- Simpson, C. (2012). The evolutionary history of division of labour. *Proceedings of the Royal Society B: Biological Sciences*, 279, 116–121. <https://doi.org/10.1098/rspb.2011.0766>
- Smith, C. R., Toth, A. L., Suarez, A. V., & Robinson, G. E. (2008). Genetic and genomic analyses of the division of labour in insect societies. *Nature Reviews Genetics*, 9, 735–748. <https://doi.org/10.1038/nrg2429>
- Smith, J. M. (1964). Group selection and kin selection. *Nature*, 201, 1145–1147. <https://doi.org/10.1038/2011145a0>

- Smith, J. M., & Price, G. R. (1973). The logic of animal conflict. *Nature*, 246, 15–18. <https://doi.org/10.1038/246015a0>
- Smits, W. K., Kuipers, O. P., & Veening, J.-W. (2006). Phenotypic variation in bacteria: The role of feedback regulation. *Nature Reviews Microbiology*, 4, 259–271. <https://doi.org/10.1038/nrmicro1381>
- Speed, D., & Balding, D. J. (2015). Relatedness in the post-genomic era: Is it still useful? *Nature Reviews Genetics*, 16, 33–44. <https://doi.org/10.1038/nrg3821>
- Strassmann, J. E., & Queller, D. C. (2011). Evolution of cooperation and control of cheating in a social microbe. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 10855–10862. <https://doi.org/10.1073/pnas.1102451108>
- Sturm, A., Heinemann, M., Arnoldini, M., Benecke, A., Ackermann, M., Benz, M., Dormann, J., & Hardt, W.-D. (2011). The cost of virulence: Retarded growth of *Salmonella typhimurium* cells expressing type III secretion system 1. *PLoS Pathogens*, 7, e1002143. <https://doi.org/10.1371/journal.ppat.1002143>
- Szathmáry, E., & Smith, J. M. (1995). The major evolutionary transitions. *Nature*, 374, 227–232. <https://doi.org/10.1038/374227a0>
- van Gestel, J., Vlamakis, H., & Kolter, R. (2015). Division of labor in biofilms: The ecology of cell differentiation. *Microbiology Spectrum*, 3(2). <http://dx.doi.org/10.1128/microbiolspec.mb-0002-2014>
- Veening, J.-W., Igoshin, O. A., Eijlander, R. T., Nijland, R., Hamoen, L. W., & Kuipers, O. P. (2008). Transient heterogeneity in extracellular protease production by *Bacillus subtilis*. *Molecular Systems Biology*, 4, 184. <https://doi.org/10.1038/msb.2008.18>
- Veening, J.-W., Smits, W. K., & Kuipers, O. P. (2008). Bistability, epigenetics, and bet-hedging in bacteria. *Annual Review of Microbiology*, 62, 193–210. <https://doi.org/10.1146/annurev.micro.62.081307.163002>
- Vlamakis, H., Chai, Y., Beauregard, P., Losick, R., & Kolter, R. (2013). Sticking together: Building a biofilm the *Bacillus subtilis* way. *Nature Reviews Microbiology*, 11, 157–168. <https://doi.org/10.1038/nrmicro.2960>
- Voelz, K., Johnston, S. A., Smith, L. M., Hall, R. A., Idnurm, A., & May, R. C. (2014). 'Division of labour' in response to host oxidative burst drives a fatal *Cryptococcus gattii* outbreak. *Nature Communications*, 5, 5194. <https://doi.org/10.1038/ncomms6194>
- Wahl, L. M. (2002). The division of labor: Genotypic versus phenotypic specialization. *The American Naturalist*, 160, 135–145. <https://doi.org/10.1086/340601>
- Waite, R. D., & Curtis, M. A. (2009). *Pseudomonas aeruginosa* PAO1 pyocin production affects population dynamics within mixed-culture biofilms. *Journal of Bacteriology*, 191, 1349–1354. <https://doi.org/10.1128/JB.01458-08>
- West, S. A., & Buckling, A. (2003). Cooperation, virulence and siderophore production in bacterial parasites. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270, 37–44. <https://doi.org/10.1098/rspb.2002.2209>
- West, S. A., & Cooper, G. A. (2016). Division of labour in microorganisms: An evolutionary perspective. *Nature Reviews Microbiology*, 14, 716–723. <https://doi.org/10.1038/nrmicro.2016.111>
- West, S. A., Diggle, S. P., Buckling, A., Gardner, A., & Griffin, A. S. (2007). The social lives of microbes. *Annual Review of Ecology, Evolution, and Systematics*, 38, 53–77. <https://doi.org/10.1146/annurev.ecolsys.38.091206.095740>
- West, S. A., Griffin, A. S., Gardner, A., & Diggle, S. P. (2006). Social evolution theory for microorganisms. *Nature Reviews Microbiology*, 4, 597–607. <https://doi.org/10.1038/nrmicro1461>
- Wilkinson, G. S. (1988). Reciprocal altruism in bats and other mammals. *Ethology and Sociobiology*, 9, 85–100. [https://doi.org/10.1016/0162-3095\(88\)90015-5](https://doi.org/10.1016/0162-3095(88)90015-5)
- Yanni, D., Jacobeen, S., Márquez-Zacarias, P., Weitz, J. S., Ratcliff, W. C., & Yunker, P. J. (2020). Topological constraints in early multicellularity favor reproductive division of labor. *eLife*, 9, e54348. <https://doi.org/10.7554/eLife.54348>
- Zhang, C.-C., Laurent, S., Sakr, S., Peng, L., & Bédou, S. (2006). Heterocyst differentiation and pattern formation in cyanobacteria: A chorus of signals. *Molecular Microbiology*, 59, 367–375. <https://doi.org/10.1111/j.1365-2958.2005.04979.x>
- Zhang, Z., Du, C., de Bary, F., Liem, M., Liakopoulos, A., van Wezel, G. P., Choi, Y. H., Claessen, D., & Rozen, D. E. (2020). Antibiotic production in *Streptomyces* is organized by a division of labor through terminal genomic differentiation. *Science Advances*, 6, eaay5781. <https://doi.org/10.1126/sciadv.aay5781>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Liu, M., West, S. A., & Cooper, G. A.

(2021). Relatedness and the evolution of mechanisms to divide labor in microorganisms. *Ecology and Evolution*, 11, 14475–14489. <https://doi.org/10.1002/ece3.8067>

Corrections

1. Equation 2, upper line: $\varepsilon < lm/((l + 1)m - 1)$
2. Equation 4, upper line: $\varepsilon < 1/(l + 1)$

3

Antagonistic effects of long-and short-term environmental variation on species coexistence

PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research



Cite this article: Liu M, Rubenstein DR, Cheong SA, Shen S-F. 2021 Antagonistic effects of long- and short-term environmental variation on species coexistence. *Proc. R. Soc. B* **288**: 20211491.
<https://doi.org/10.1098/rspb.2021.1491>

Received: 30 June 2021
Accepted: 13 August 2021

Subject Category:
Evolution

Subject Areas:
evolution, ecology, theoretical biology

Keywords:
fluctuating environment, temporal scale of variation, disturbance, thermal niche

Author for correspondence:
Sheng-Feng Shen
e-mail: shensf@sinica.edu.tw

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.5574339>.

THE ROYAL SOCIETY
PUBLISHING

Antagonistic effects of long- and short-term environmental variation on species coexistence

Ming Liu^{1,2}, Dustin R. Rubenstein^{3,4}, Siew Ann Cheong⁵ and Sheng-Feng Shen¹

¹Biodiversity Research Center, Academia Sinica, Taipei 11529, Taiwan

²Department of Zoology, University of Oxford, Oxford OX1 3SZ, UK

³Department of Ecology, Evolution and Environmental Biology, and ⁴Center for Integrative Animal Behavior, Columbia University, New York, NY 10027, USA

⁵Division of Physics and Applied Physics, School of Physical and Mathematical Sciences, Nanyang Technological University, 637371, Singapore

ML, 0000-0002-5170-8688; DRR, 0000-0002-4999-3723; SAC, 0000-0002-8589-6699; S-FS, 0000-0002-0631-6343

Assessing the impact of environmental fluctuations on species coexistence is critical for understanding biodiversity loss and the ecological impacts of climate change. Yet determining how properties like the intensity, frequency or duration of environmental fluctuations influence species coexistence remains challenging, presumably because previous studies have focused on indefinite coexistence. Here, we model the impact of environmental fluctuations at different temporal scales on species coexistence over a finite time period by employing the concepts of time-windowed averaging and performance curves to incorporate temporal niche differences within a stochastic Lotka–Volterra model. We discover that short- and long-term environmental variability has contrasting effects on transient species coexistence, such that short-term variation favours species coexistence, whereas long-term variation promotes competitive exclusion. This dichotomy occurs because small samples (e.g. environmental changes over long time periods) are more likely to show large deviations from the expected mean and are more difficult to predict than large samples (e.g. environmental changes over short time periods), as described in the central limit theorem. Consequently, we show that the complex set of relationships among environmental fluctuations and species coexistence found in previous studies can all be synthesized within a general framework by explicitly considering both long- and short-term environmental variation.

1. Introduction

Environmental fluctuations not only influence an organism's physiology and reproduction directly, but they can also impact an organism's fitness indirectly by driving species interactions [1–3]. Hutchinson [4] suggested that fluctuations in environmental conditions may allow for the coexistence of species competing for limiting resources. For example, one of the key arguments behind the intermediate disturbance hypothesis is that species can reach an equilibrium state and exclude other competing species under reduced environmental fluctuations, whereas increased fluctuations prevent species from reaching equilibrium and therefore prevent competing species from excluding others [4–8] (but see [9]). By contrast, May [10–12] argued that for competing species to coexist in a fluctuating environment, niche differences need to be greater because random environmental fluctuations may favour one species while occasionally excluding another. In a series of influential articles, Chesson *et al.* [13–16] developed the 'modern coexistence theory' of when and how environmental fluctuations mediate species coexistence through nonlinear averaging and storage effects. They found that species can coexist only when the fitness differences [17,18] between species are

smaller than their niche differences in a shared environment (i.e. differences in resource utilization in space and time) [17]. Accordingly, environmental fluctuations potentially promote species coexistence by minimizing average fitness differences (equalizing mechanism) [17] or by creating different temporal niches (stabilizing mechanism) [13,16].

The theory of species coexistence was mainly aimed at discussing indefinite coexistence [19–21] (i.e. species persist through infinite time), so fluctuating environments can be represented by the stationary distribution of environmental conditions because the environmental state at any given time recurs with a predictable long-run frequency [22]. Although the analysis of indefinite coexistence has helped us to better understand the mathematical nature of species coexistence, there are significant limitations in its practical application, including how species extinction can be affected by the time scale of environmental variation. For example, a species that specializes in arid environments could survive a certain period of dormancy in a wet year and reproduce again in the next dry year. However, if the wet conditions continue for a thousand years (as opposed to alternating dry and wet years over a short period of time), the probability of extinction of an arid specialist would be much higher [20,23]. Therefore, although the average distribution of the environment (the mean and variance of the environmental conditions) on a long time scale is the same for both the annual alternation of wet and dry years and the alternation of wet and dry years once every thousand years, the effects on the coexistence of species are obviously different. This situation is similar to the process behind the central limit theorem, which was proposed by de Moivre in 1733 and proved by Lyapunov in 1901 [24]. According to the central limit theorem, small samples (e.g. environmental variation over long time periods) are more likely to show large deviations from the expected mean than large samples (e.g. environmental variation over short time periods) and are less predictable. As many practical experiments have confirmed, species rarely (if ever) coexist indefinitely in the real world [25–27]. Therefore, considering the transient coexistence of species at ecologically meaningful time scales will be crucial for understanding real-world scenarios that might influence species coexistence, particularly in a period of increased global climate change where environmental fluctuations are increasing across much of the world.

Past research on the effects of temporal scales of environmental fluctuations on species coexistence has been based largely on the temporal autocorrelation of the environment. For example, red noise represents low-frequency environmental fluctuations, and thus the time scale of environmental fluctuations is longer. Schreiber [28] investigated the effect of temporal autocorrelation of the environment on species coexistence in relation to the link between environmental conditions and interspecific competition. He found that species can coexist in negatively autocorrelated environments through a different form of storage effect [28], a mechanism that emphasizes that populations are less sensitive to interspecific competition in unfavourable years and can therefore ‘store’ their population increments in favourable years [14,17]. On the other hand, Caswell & Cohen [29] found that the greater the temporal autocorrelation of environmental fluctuations, the more likely that species exclusion is promoted. However, these studies did not consider that the environment may have different magnitudes of variation at different time scales. Moreover, it is difficult to directly compare the distribution of the

environmental states at different time scales by using temporal autocorrelation to investigate environmental variation (e.g. the effect of the time scale of environmental variation on species coexistence under different mean environmental conditions). This limits our understanding of the effects of environmental variation on species coexistence at different time scales.

Here, we explore the patterns of species coexistence in fluctuating environments using a stochastic Lotka–Volterra competition model framework. We use windowed averaging to discuss the effects of temporal scales of environmental variation on the mean state of the transient environment. We also employ the newly developed standardized approach for characterizing temperature variation across temporal scales [30], adjusting the magnitude of environmental variation at different time scales to simulate environmental fluctuations in close proximity to real-world situations. We use thermal performance curves to explicitly describe temperature-dependent fitness [31,32]. Although we focus on temperature, our approach can be applied to other climatic measures like precipitation (though a different performance curve would be needed to represent it). In addition, we limit our study to transient coexistence [4,17,33] in order to explore environmental fluctuations at different time scales in nonequilibrium systems. Ultimately, our model provides a basic framework for understanding patterns of species coexistence in fluctuating and unpredictable environments, a topic that will have critical implications for studying and conserving biodiversity in an era of anthropogenic climate change.

2. Methods

(a) Population dynamics

We investigate the conditions promoting coexistence using a stochastic Lotka–Volterra competition model with two competing species regulated by the population density of its own and the opposing species. Although this design is similar to the classic form of this model [10,12], instead of integrating all fitness responses of environmental conditions, we leave them as continuous responses to the environment (i.e. temperature) and explicitly model environmental conditions through time. That is, the population dynamics follow

$$\frac{dN_i}{dt} = r_i N_i w_i(T) \left(1 - \frac{N_i}{K_i w_i(T)} - \frac{\alpha N_j}{K_i w_i(T)} \right) - d_i N_i (1 - w_i(T)), \quad (2.1)$$

where i, j identifies the species, T is temperature and N stands for population density. Similarly, K , r , α and d denote carrying capacities, intrinsic growth rates, levels of interspecific interaction and temperature-dependent mortality, respectively. We also assume that the populations will go extinct if the density is too low (i.e. $N_i = 0$ if $N_i < N_{\text{ext}} = 0.5$) in order to prevent the dynamics staying at extremely small positive values.

Although most parameters remain constant, the population growth rate changes with temperature and depends on the thermal performance function ($w_i(T)$), which is adapted from previously published estimates of thermal performance curves [34,35],

$$w_i(T) = \begin{cases} \exp\left(-\left(\frac{T - T_{\text{opt},i}}{2\sigma_i}\right)^2\right) + w_{\text{base},i} & T_{\text{opt},i} \geq T \\ 1 - \frac{\left(\frac{T - T_{\text{opt},i}}{T_{\text{opt},i} - T_{\text{max},i}}\right)^2}{w_{\text{base},i}} + w_{\text{base},i} & T_{\text{max},i} \geq T > T_{\text{opt},i} \\ w_{\text{base},i} & T > T_{\text{max},i} \end{cases} \quad (2.2)$$

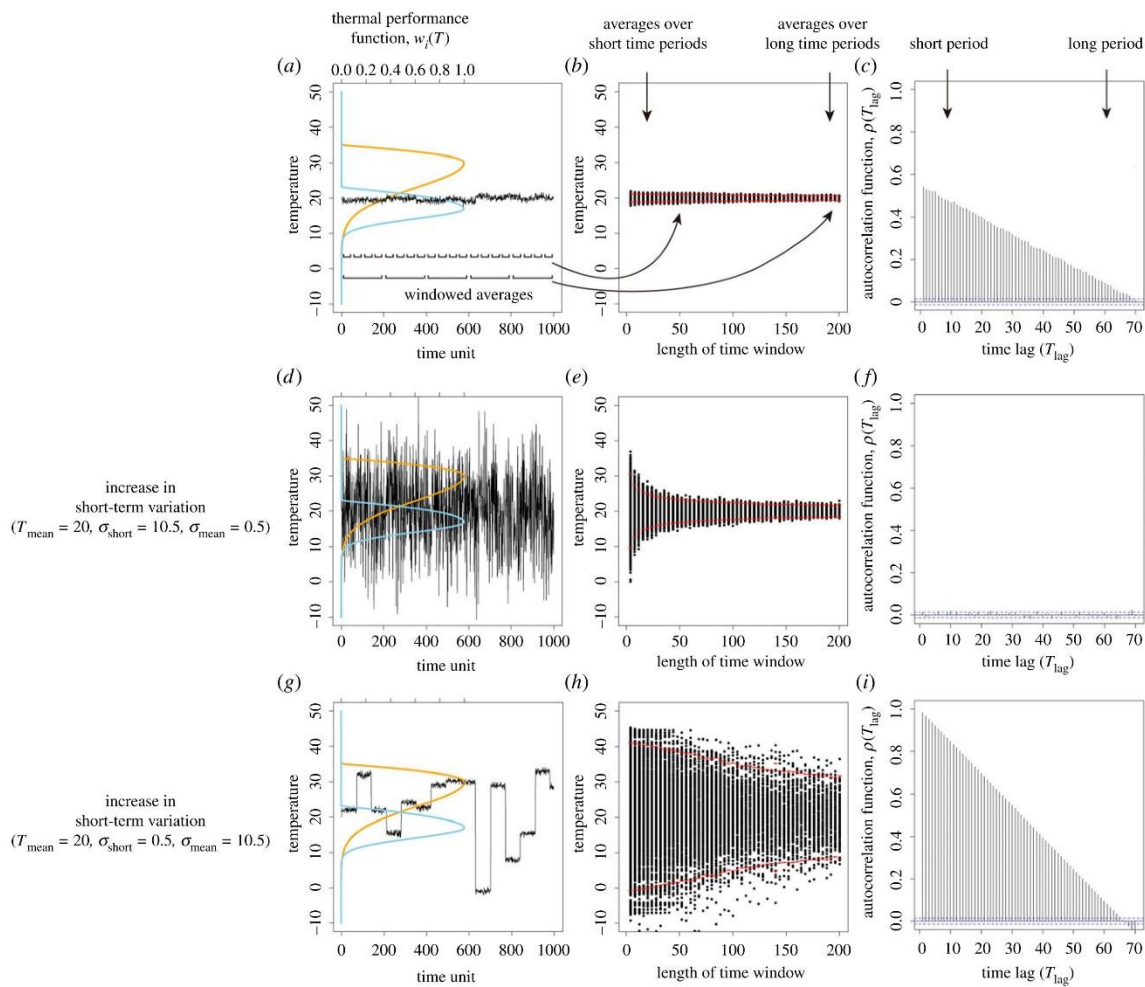


Figure 1. A demonstration of the species and environmental variation properties in our model. (a) Following the concept of biological performance curves, the difference between the two species can be depicted by the per capita growth rates across all environments (i.e. temperatures). We assume that there is a high temperature-adapted species (orange curve; species 1) and a low temperature-adapted species (blue curve; species 2) competing against one another (see also Methods). The solid black line describes the time series of temperature variation. (b) To better visualize the fluctuations at different temporal scales, we divide the time series into many fractions with equal durations (i.e. window lengths) and calculate the windowed averages of each fraction. This visualization allows us to visualize the rate of convergence of the windowed average, where the red dots are the average plus or minus two standard deviations of the windowed averages for different time windows. (c) Alternatively, the temperature time series can be analysed using the autocorrelation function, where autocorrelation coefficients are plotted along with time lag. The blue dashed lines indicate the confidence interval of the autocorrelation function; autocorrelation coefficients larger than the interval are statistically significant. (d–f) The time series, windowed averages and autocorrelation function of another temperature time series with an increased short-term variation. (g–i) The same analyses for the other temperature time series where long-term variation is increased. (Online version in colour.)

This function has a maximum value of 1 at T_{opt} . When T is below T_{opt} , thermal performance decreases exponentially with decreasing temperature, whereas when T is above T_{opt} , thermal performance decreases quadratically and eventually reaches the minimum value, w_{base} , at T_{max} ($w_{\text{base}} > 0$). We assume that there is a high temperature-adapted species (orange; species 1) and a low temperature-adapted species (blue; species 2) (figure 1a). Species 1 is a relative specialist at warm temperatures and species 2 is a relative specialist at cool temperatures ($T_{\text{opt},1} > T_{\text{opt},2}$). Although broadly similar in the shapes of their performance curves, we assume that species 1 performs well in a wider range of environments than species 2 ($\sigma_1 > \sigma_2$). From a biological perspective, our model can be applied to a variety of species for which thermal performance can be quantified; the performance component can be the survival of the species, its reproduction or other fitness components. Although in the literature the thermal performance curve is most often

applied to the performance of ectotherms at different body temperatures [36,37], the concept of the thermal performance curve has been extended to the performance of different fitness components of organisms at different environmental or body temperatures [34,38,39]. As a result, our model can be applied to both endotherms and ectotherms. For simplicity, we do not consider the possibility that the thermal performance curve may change over a relatively short time frame [30]. Instead, we assume that the thermal performance curve of a species is fixed.

(b) Temporal scales of environmental variation

We introduce stochasticity into the model by allowing environmental conditions (i.e. temperature) to change over time. We design two types of environmental variation with different temporal scales that differ in resampling frequency to capture this

idea. Specifically, temperatures are determined by the mean and variation of the short-term distribution,

$$T \sim \text{Norm}(T_{\text{smean}}, \sigma_{\text{short}}^2), \quad (2.3a)$$

where the mean of the short-term distribution and T_{smean} is sampled from a long-term distribution,

$$T_{\text{smean}} \sim \text{Norm}(T_{\text{mean}}, \sigma_{\text{long}}^2). \quad (2.3b)$$

Note that T is resampled in every time unit, T_{smean} is resampled in every $\delta = 70$ time units and population size is updated 200 times in each time unit. We ensure that changes in population size respond to changes in the environment in real time by updating the population size frequently. Although we did not directly consider the effect of species' generation length, we can account for this by adjusting the timing of environmental effects on population renewal to be less frequent, or by allowing the intrinsic growth rate to be smaller (meaning that populations grow or shrink more slowly). The result of doing this demonstrates that species with long generation times are relatively less sensitive to environmental changes in population size (electronic supplementary material, figure S5).

Under our settings, environmental fluctuations are controlled by three factors: (i) the mean temperature of the long-term distribution (T_{mean}), (ii) long-term variation (σ_{long}^2); and (iii) short-term variation (σ_{short}^2). Thus, we model a nonequilibrium system in the sense that environmental conditions vary stochastically and, therefore, the carrying capacities and growth rates of competing species can change dynamically, which prevents populations from reaching the equilibrium points of the Lotka–Volterra equations. Note that equilibrium can be defined more broadly; for example, an equilibrium state describes having some system converge and return to those points upon perturbation, not just the equilibrium points that we define here [25].

The characteristics of short- and long-term environmental variation can be visualized by using an autocorrelation function, a windowed average approach and a fast Fourier transformation. Temporal autocorrelation, used frequently in the literature, describes the probability that the same environmental conditions will remain constant over time [28,29,40]. In essence, when the autocorrelation is large, the chance that the environment remains constant over time is high, and vice versa (figure 1c,f,i). Further, we show that when the time scale of species coexistence is not indefinite and the environmental variation is stochastic, we can also use windowed averaging to resolve the difference between the long- and short-term environmental mean conditions due to the sampling bias as indicated by the central limit theorem (figure 1b,e and h, see also Introduction). Windowed averaging with temporal autocorrelation makes it easier to understand the transient coexistence of species over a finite period of time (e.g. hundreds, thousands or tens of thousands of years). Lastly, the time scale of environmental fluctuations can also be visualized through fast Fourier transformation (FFT) to decompose the time series of temperature into frequency and amplitude domains (electronic supplementary material, figure S1b,c) [30]. Since frequencies with the greatest amplitudes contribute more to the pattern of variation, if we change one type of variation at a time, we can see that larger long-term variation increases the amplitude of low frequencies more than that of higher frequencies (electronic supplementary material, figure S1i). Conversely, larger short-term variation increases the amplitudes of all frequencies uniformly (electronic supplementary material, figure S1f). Thus, increasing long-term variation makes the temperature time series more similar to red noise, while increasing short-term variation makes the time series closer to white noise.

Our temperature fluctuation settings capture the characteristics of stochastic fluctuations at different temporal scales. For instance, body temperature may vary over the course of a few

Table 1. List of model parameters.

name	values	description
r_1, r_2	0.5	scaling factor of intrinsic growth rate
K_1, K_2	10 000	scaling factor of carrying capacity
d_1, d_2	0.01	scaling factor of environmental-dependent mortality
α	0.4	level of interspecific competition
δ	70	relative duration of long-term variation to short-term variation
$T_{\text{opt},1}, T_{\text{opt},2}$	30, 17	optimum temperature of the two species
$T_{\text{max},1}, T_{\text{max},2}$	35, 23	upper boundary of fitness function of the two species
σ_1, σ_2	5, 2	width parameter of the fitness function for temperature below optimum
w_{base}	0.001	minimum value of fitness function, designed to avoid population deadlock at extreme temperatures
N_{ext}	0.5	the threshold at which a population goes extinct

minutes due to a difference in wind speed, whereas variation in weather (e.g. a tropical storm or heat wave) may last for weeks. Further, global events like El Niño may occur at considerably irregular intervals at the temporal scale of years. These examples illustrate how stochastic events can occur at both short- and long-term temporal scales. Importantly, long- and short-term variation should be viewed as two points along with a continuum of temporal environmental variation. Although we do not directly focus on the number of variation types or the relative temporal scale of updating the environmental conditions (i.e. δ), it is possible to extend our model to incorporate more complex and realistic patterns of environmental variation (see table 1 for a summary of all parameters).

3. Results

(a) Short- versus long-term environmental variation

We first explore how combinations of temperature variation at different time scales and different mean temperatures affect species coexistence under (i) continuous short-term variations with different long-term variation (figure 2a–d) and (ii) continuous long-term variations with different short-term variations (figure 2e–h). We found a uniform trend such that the proportion of replicate experiments with species coexistence increases with an increase in short-term variation, regardless of the magnitude of the long-term variation, when short-term variation is continuous along the x -axis and long-term variation is discrete across panels (figure 2a–d). Similarly, when that the range of continuous long-term variation is constant, the area of high coexistence also increases with increasing short-term variation (figure 2e–h). By contrast, we observe the opposite effect of long-term variation, which mainly promotes exclusion (figure 2a–d and e–h). To confirm the generality of this result, we also control for the sum of variances and only

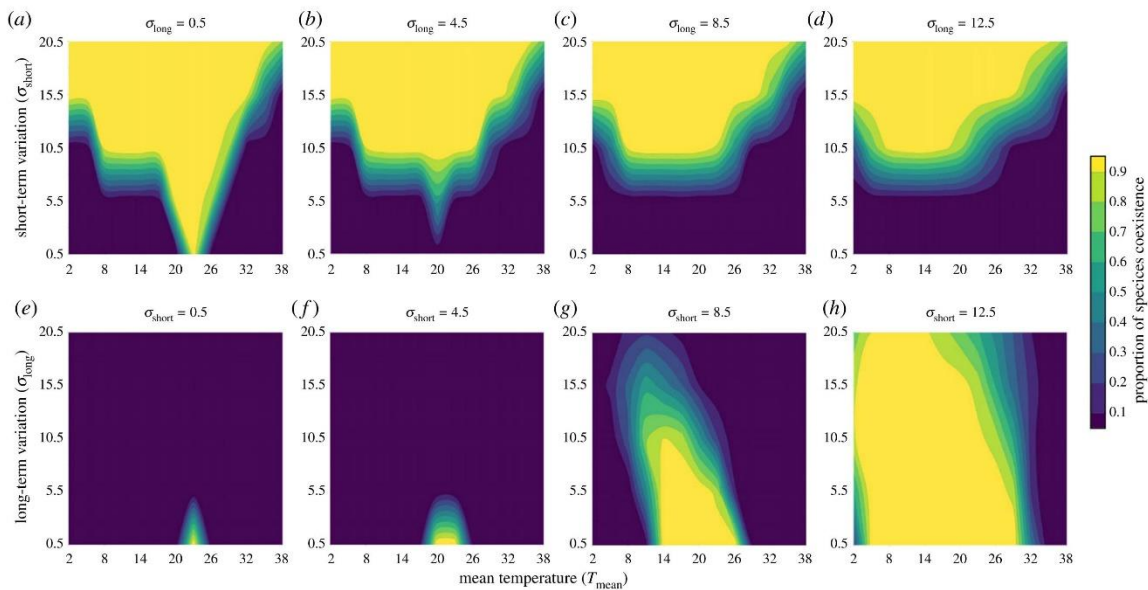


Figure 2. Short- and long-term temperature variations have contrasting impacts on species coexistence. (a–d) Proportion of simulations producing species coexistence dynamics, where brighter colours indicate greater proportions of coexisting species. Each panel has constant long-term variation (σ_{long} , labelled above each panel) but variable mean temperature (T_{mean} , x-axis) and short-term variation (σ_{short} , y-axis). Species coexistence occurs if both species sustain through 20 000 short-term variations and 286 long-term variations (4-million calculations of population change). A species becomes extinct if its density is too low ($N_i < N_{\text{ext}}$); each combination of mean and variability is repeated for 100 times. (e–h) The proportion of coexistence with the same definition, but each panel has a constant short-term variation (σ_{short} , labelled above each panel) and variable long-term variation (σ_{long} , y-axis). (Online version in colour.)

change the distribution of variances in the ratio of long- to short-term variation, finding qualitatively similar results (electronic supplementary material, figure S2). In addition, we also confirm that the environmental conditions examined here are such that both species can survive individually in the absence of competition. Therefore, our coexistence and exclusion results are largely driven by species competition (electronic supplementary material, figure S3). Together, these results suggest that greater short-term environmental variation promotes coexistence, whereas greater long-term environmental variation leads to a reduction in species coexistence.

(b) Mechanisms of long- and short-term environmental variation on species coexistence

Our results demonstrate that long-term environmental variation promotes competitive exclusion, while short-term environmental changes promote species coexistence (figures 2 and 3; electronic supplementary material, figure S3). Here, we show that when the environmental variation is large at long time scales and small at short time scales (figure 3a), the temporal autocorrelation of the temperature is larger at short time scales and decreases with longer time scales (figure 3b). Interestingly, the mean temperature varies considerably in this case across time periods, showing the windowed average property (figure 3c). Combining the short time period mean temperature with the above results of temporal autocorrelation, we find that the temperature is more likely to remain in a state for a long period of time while the variation is small at short time scales, thus promoting competitive exclusion (figure 3d). Conversely, when the environmental variation is large at short time scales and small at long time scales (figure 3q), the temperature has little temporal autocorrelation at either time scale (figure 3r). The mean temperature is more

variable in the short term because it changes many times in a short period of time, but it tends to converge as the time period becomes longer (figure 3s). Therefore, temperature changes a lot at short time scales but very little at long time scales, thus promoting species coexistence (figure 3t).

(c) Effects of environmental averaging on species coexistence

In the case of fixed environmental variation, the effects of the environmental mean on species coexistence are relatively easy to understand. If the mean temperature of the environment is close to the optimal performance temperature of species 1, then species 1 can easily outcompete species 2, resulting in the extinction of species 2 (figure 4a). If the average temperature is intermediate between the optimal temperatures of the two competing species, then the two competing species are likely to coexist (figure 4b). Finally, if the mean temperature is close to the optimal temperature of species 2, then species 1 is easily excluded by species 2 (figure 4c).

(d) Environmental variation can both promote and inhibit species coexistence

We have shown that while long-term environmental variation tends to hinder species coexistence and short-term variation tends to promote coexistence, the mean state of the environment also has a critical influence on the pattern of species coexistence. Therefore, by combining these three environmental characteristics, a wide variety of relationships between environmental variation and species coexistence can be generated. Here, we use the above framework to explain the relationship between species coexistence and environmental variation for four seemingly contradictory scenarios.

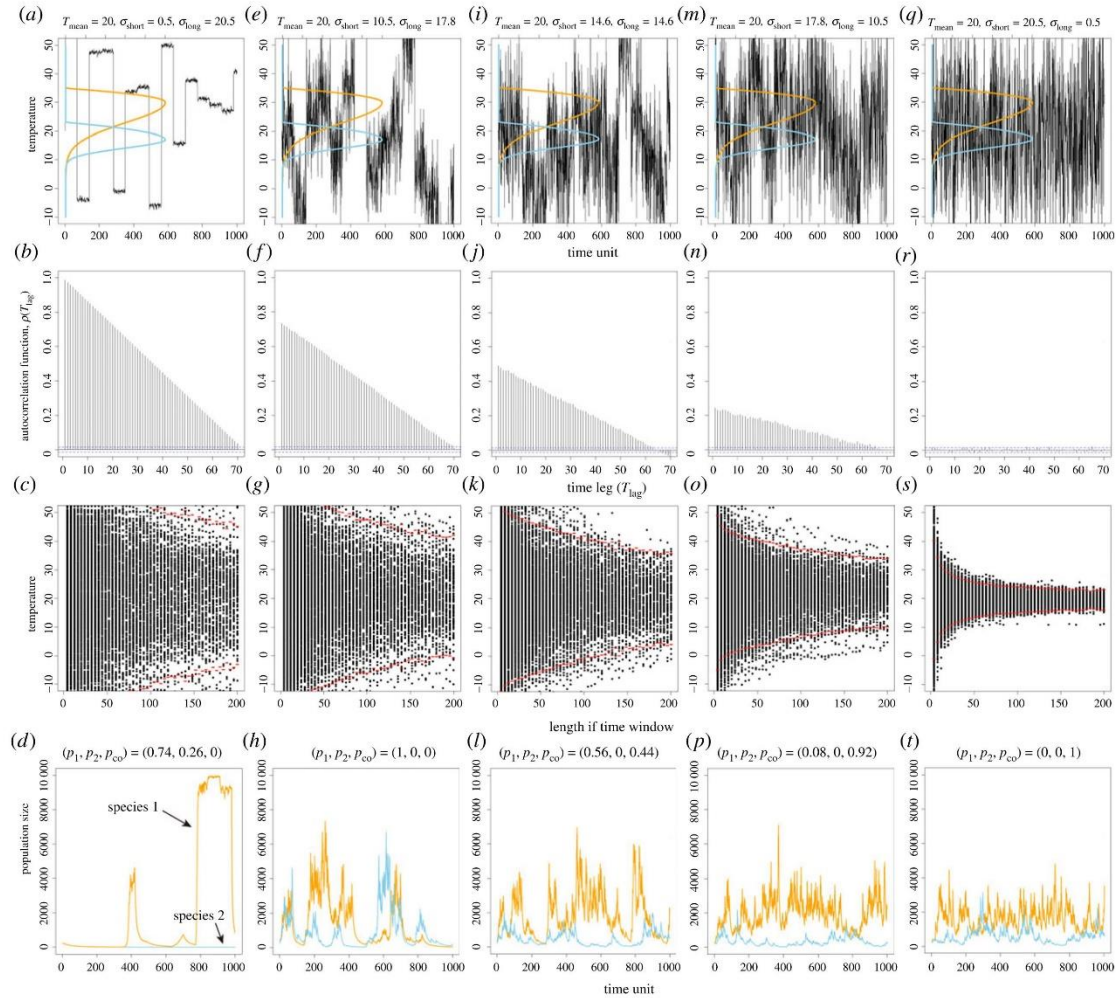


Figure 3. Under the same degree of environmental variability, time series with more short-term variation favour species coexistence. Here, we fix the sum of long- and short-term variation $(\sigma_{\text{long}} - 0.5)^2 + (\sigma_{\text{short}} - 0.5)^2 = 400$ and examine the fluctuations with different combinations of the two time scales of environmental variation. Specifically, we analyse the temperature time series and the probability of three population-dynamic outcomes: species 1 dominates (p_1), species 2 dominates (p_2) and species coexistence, (p_{co}). (a) The temperature time series when most of the variation occurs as long-term variation ($\sigma_{\text{long}} = 20.5$, $\sigma_{\text{short}} = 0.5$) and thermal performance curves of the two species. (b,c) Autocorrelation function and windowed averages of the temperature time series. (d) The population dynamics corresponding to (a) and the probabilities of coexistence or when a single species dominates. (e–t) The same analyses for another four variation settings where short-term variation increases, but long-term variation decreases. Note that we can only keep the total variance approximately constant. In other words, the difference in variances in the parameter space is marginal, but the variance is not actually constant. (Online version in colour.)

We found that the previous coexistence–exclusion–coexistence result occurs at a mean temperature (17 degrees) that lies in between the optimal temperatures of the two competing species and allows for coexistence in a stable environment (figures 3a and 4g). However, if the mean temperature increases (and becomes closer to the optimum of species 1), species coexistence only occurs when variation is large (figure 4f). Moreover, if the mean temperature increases further, even greater environmental variation will not generate species coexistence. In other words, when mean temperature deviates from the intermediate temperature that is non-optimal for both competing species and approaches the optimum of either species, the relationship between environmental variation and species coexistence may shift from coexistence–exclusion–coexistence (figure 4g–i) to one that promotes coexistence (figure 4d–f) to one that favours coexistence but has relatively weaker influence (figure 4a–c).

4. Discussion

We have shown that the mean and variability of environmental fluctuations can have complex yet predictable effects on patterns of species coexistence. Notably, environmental variation can either promote or hinder species coexistence depending on the temporal scale of variation. This is because short-term environmental variation, at least with our model settings, generally favours species coexistence, whereas long-term environmental variation promotes exclusion of competing species. Our model settings include competitive effects that are linear, competitive effects that are not directly affected by the environment and a range of temperature variation averages over a largely concave fitness surface. In addition, the mean environmental condition also plays a critical role in shaping the effects of environmental variation on species coexistence, depending on whether the mean condition approaches the optimal condition of one of the species or whether it occurs between

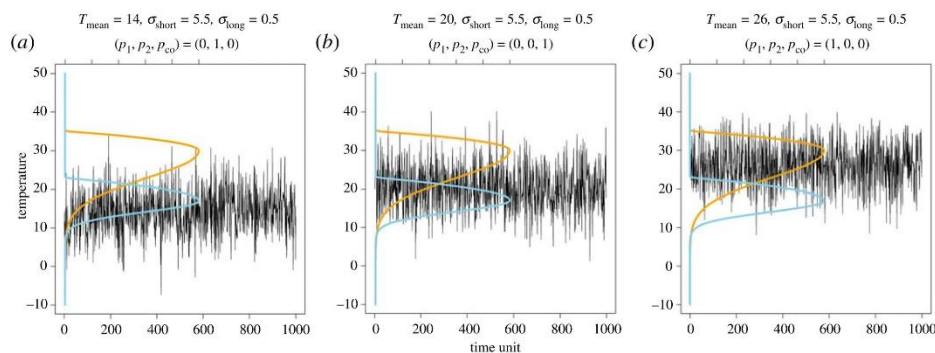


Figure 4. Mean temperature (T_{mean}) affects species coexistence even when long- and short-term variations remain unchanged. (a) When the mean is lower than the optimal temperature of species 2 (blue curve), species 2 excludes species 1. (b) However, when the mean is between the optima of the two species, species coexistence can occur. (c) Finally, when the mean is above the optimum of species 1, it excludes species 2. Temperature variability is the same in all three cases, where $\sigma_{\text{long}} = 0.5$, $\sigma_{\text{short}} = 5.5$. Additionally, p_1 , p_2 and p_{co} represent the probability of species 1 dominating, species 2 dominating and species coexistence, respectively. Note that the mean temperature is different from windowed averages (e.g. figure 3s), as the mean is the actual average of the temperature distribution used for generating time series. (Online version in colour.)

the optimal conditions of the competing species. In general, large short-term environmental variation promotes species coexistence. However, if the mean environmental conditions are intermediate between the optimal conditions for the two species, large long-term environmental variation will promote competitive exclusion in most of the parameter space. Moreover, when the mean environmental conditions are closer to the optimal conditions for one of the species, there is a small parameter space for large long-term environmental variation to promote species coexistence as a result of environmental variation that suits different species at different times. Thus, if environments fluctuate simultaneously on different temporal scales (e.g. daily, seasonal or annual patterns of temperature fluctuation), which commonly occurs in nature, diverse relationships between environmental variability and species coexistence are expected.

Many seemingly contradictory results of species coexistence and environmental fluctuations from previous studies can be viewed as special cases of a more general result described here (figure 5). For example, Hutchinson [4,5] discussed the impact of temporal scales of environmental variation on species coexistence in a nonequilibrium setting (i.e. species could go extinct by chance due to environmental fluctuations). He argued that both overly fast and overly slow fluctuations promote competitive exclusion between competing species because whichever species competes best on average will exclude the other. Thus, only environmental fluctuations occurring at an intermediate temporal scale will favour species coexistence. Interestingly, analyses of Hutchinson's theory, based on modern coexistence theory, generally conclude that Hutchinson's predictions about the time scales of environmental change and species coexistence are misleading [41]. This is because in the discussion of indefinite coexistence, the mean environmental condition rather than the time scale of environmental fluctuations affects the pattern of species coexistence. By contrast, our model partially agrees with Hutchinson's hypothesis such that intermediate- and long-term environmental fluctuations favour species coexistence and exclusion, respectively. Nevertheless, our model demonstrates that short-term fluctuations are also predicted to facilitate species coexistence rather than exclusion. The key difference between long- and short-term environmental fluctuations is that fast-changing

environments allow each competing species to experience their optimal and adverse environmental conditions prior to extinction, which prevents competitive exclusion, whereas slow-changing environments last longer and, thus, can favour one of the competing species in excluding the other. However, May *et al.* [10–12] proposed that niche differences need to be larger in fluctuating environments than in stable environments for species to coexist. Consequently, competitive exclusion is predicted to occur more easily in fluctuating environments than in stable environments. Yet our model shows that May and colleagues' prediction is a special case such that it is only valid for long-term environmental variation in a nonequilibrium, stochastic model setting.

Our model may also help resolve the long-standing debate over the intermediate disturbance hypothesis [6,7,42,43], which states that species richness of competing species will be 'maximized at intermediate frequencies and/or intensities of disturbance or environmental change' [42]. We show that there can be diverse patterns of species coexistence in relation to environmental variability. Therefore, with the right combination of long- and short-term environmental variation, intermediate disturbance *can* generate higher species richness relative to higher or lower disturbance scenarios (e.g. figure 5b, left arrow). However, it is also true that intermediate disturbance does not *always* lead to the highest species richness because species coexistence depends at least partially on the temporal scale of environmental variation. The main differences between our model and previous models are that (i) environments in our model fluctuate stochastically, and therefore species can go extinct by chance if they happen to experience unfavourable environments for an extended period of time [44–46], and (ii) we explicitly consider the temporal scale of environmental variation, while simultaneously considering the effect of the mean environmental condition (i.e. environmental mean, variance and their interaction are included in our model). Accordingly, we urge future studies testing the intermediate disturbance hypothesis to carefully distinguish between different properties of environmental disturbance (e.g. intensity and frequency) on the richness of competing species [30,47].

Since previous research on species coexistence has focused on indefinite coexistence, environmental fluctuations

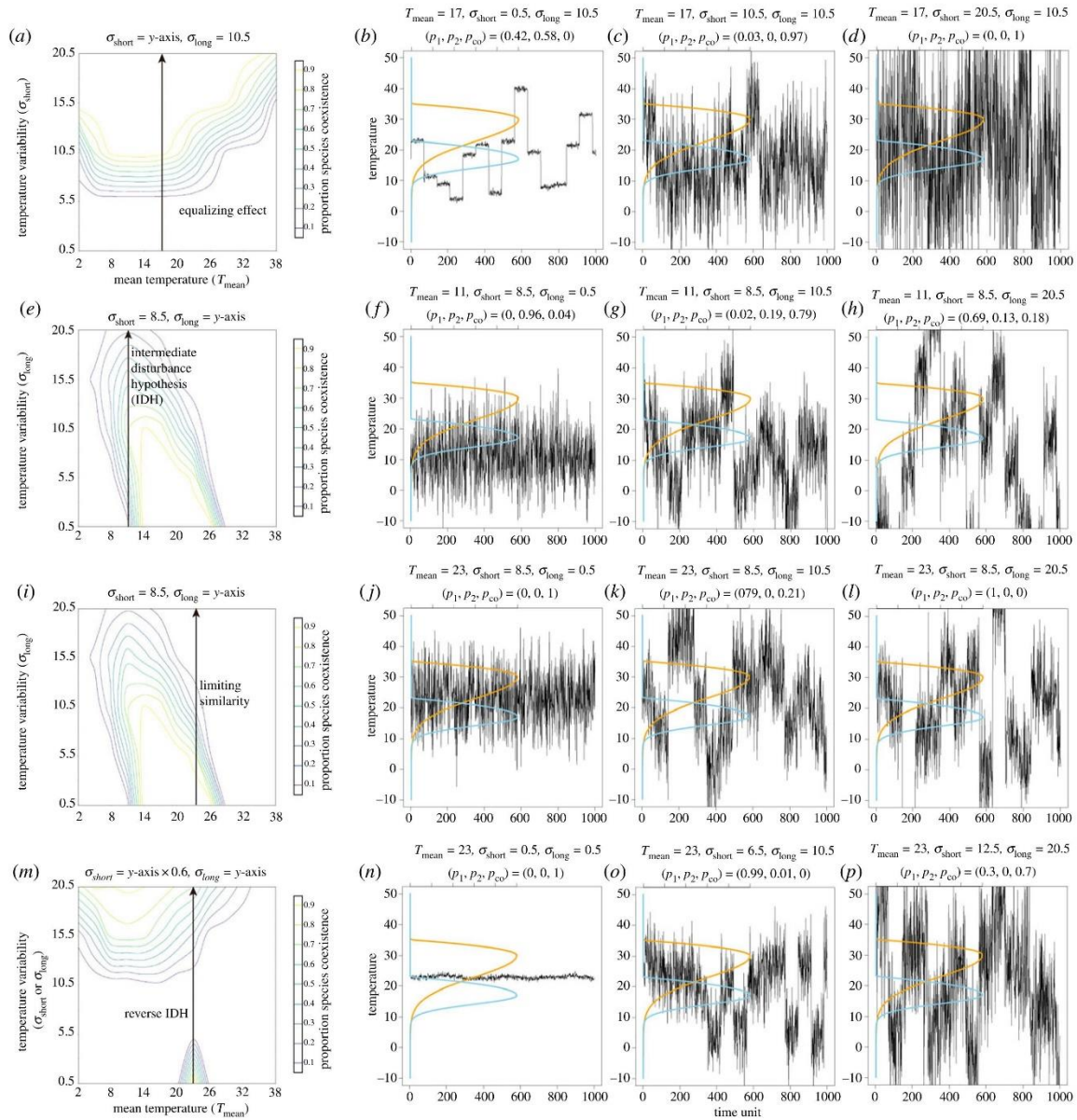


Figure 5. Different combinations of short-term temperature variation, long-term temperature variation and mean temperature can generate diverse patterns of species coexistence. (a–d) Coexistence can be promoted by greater temperature variability when the increase is in short-term variation (σ_{short}). (e–h) Coexistence may also occur when variability is intermediate when the increase is in long-term variation (σ_{long}) [4,6]. (i–l) Alternatively, coexistence can be hindered by temperature variability [10] when the increased variability is of long-term variation (σ_{long}) at a different mean temperature (T_{mean}). (m–p) Finally, it is also possible that coexistence is promoted when variability is very high or very low, when the increased variability contains both short- and long-term variation (both σ_{short} and σ_{long}). We provide examples of temperature time series for each pattern with the probability of species coexistence and single-species dominance above each panel. (Online version in colour.)

at different time scales have little effect on the relationship between the average environment and species coexistence. To achieve indefinite coexistence, species must be able to invade competing species with low population densities (termed mutual invisibility) [48,49]. Consequently, to achieve indefinite coexistence, there are usually two non-mutually exclusive mechanisms. The first is that species are less sensitive to the interspecific competition when population density is low or the environment is of poor quality [14,17,28]. The second is that the population growth rate increases when population density is low or the environment is of poor quality [16,17].

However, in our model, we deliberately do not assume that the coefficient of interspecific competition (α) is directly influenced by the environment. Yet the intensity of interspecific competition is still influenced by population size (αN), and therefore by environmental conditions. Thus, we do not assume that the population has a buffering effect in poor quality environments or at low population densities, so that we can explore more directly the effect of the environment on competitive exclusion and species coexistence (i.e. assuming that the interspecific competition coefficient becomes smaller when the population density is small or the environment is of poor

quality, the coexistence probability is bound to increase). In the terminology of modern coexistence theory, we do not assume a stabilizing effect; our transient coexistence is due to environmental changes that equalize the average performance of the two species (i.e. equalizing effect).

Previous discussions of indefinite coexistence have provided great insights into the mathematical investigation of species coexistence. Here, we echo past calls for discussion of non-equilibrium or transient coexistence [20,23], and the importance of understanding the spatial and temporal scales at which patterns occur, something that has long been emphasized in ecology [50]. We believe that exploring the temporal scales of species coexistence has critical implications for understanding real-world biodiversity loss. Because of the stochastic nature of the environment, the average state of the environment will be different for different time scales (e.g. decades, centuries and millennia) and thus will probably result in different patterns of species coexistence, as described in the central limit theorem [24].

In conclusion, we show that contrasting results from previously published studies linking environmental variation to species interactions [4,10,17] can be viewed as special cases of a more general framework that we develop here (figure 5). By explicitly taking different temporal scales of environmental variation into account [51,52], simultaneously

considering the mean environmental condition and modeling different types of stochastic environments, we develop a framework that can be used to explore rich patterns of species coexistence. This framework will be useful for developing testable predictions at a time when environmental fluctuations are increasing globally.

Data accessibility. All simulated data were generated using C. The codes and generated data used for this study are available at: https://github.com/mingpapilio/Codes_Coexistence_and_Variation.

Authors' contributions. M.L.: formal analysis, investigation, methodology, visualization, writing-original draft, writing-review and editing; D.R.R.: conceptualization, investigation, visualization, writing-review and editing; S.A.C.: formal analysis, investigation, methodology, validation, writing-review and editing; S.-F.S.: conceptualization, formal analysis, funding acquisition, investigation, supervision, visualization, writing-original draft, writing-review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

Funding. S.-F.S. was supported by Academia Sinica (Career Development Award and Investigator Award, grant no. AS-IA-106-L01) and Minister of Science and Technology of Taiwan (S.-F.S., grant nos. 100-2621-B-001-004-MY3 and 104-2311-B-001-028-MY3). D.R.R. was supported by the US National Science Foundation (grant nos. IOS-1439985 and IOS-1656098).

Acknowledgements. We thank Po-Ju Ke, Hong-Yan Shih and two anonymous referees for their helpful comments.

References

1. Tylianakis JM, Didham RK, Bascompte J, Wardle DA. 2008 Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* **11**, 1351–1363. (doi:10.1111/j.1461-0248.2008.01250.x)
2. Gilman SE, Urban MC, Tewksbury J, Gilchrist GW, Holt RD. 2010 A framework for community interactions under climate change. *Trends Ecol. Evol.* **25**, 325–331. (doi:10.1016/j.tree.2010.03.002)
3. Davis AJ, Jenkinson LS, Lawton JH, Shorrocks B, Wood S. 1998 Making mistakes when predicting shifts in species range in response to global warming. *Nature* **391**, 783–786. (doi:10.1038/35842)
4. Hutchinson GE. 1961 The paradox of the plankton. *Am. Nat.* **95**, 137–145. (doi:10.1086/282171)
5. Hutchinson GE. 1953 The concept of pattern in ecology. *Proc. Acad. Natl Sci. Philadelphia* **105**, 1–12.
6. Connell JH. 1978 Diversity in tropical rain forests and coral reefs. *Science* **199**, 1302–1310. (doi:10.1126/science.199.4335.1302)
7. Grime JP. 1973 Competitive exclusion in herbaceous vegetation. *Nature* **242**, 344–347. (doi:10.1038/242344a0)
8. Roxburgh SH, Shea K, Wilson JB. 2004 The intermediate disturbance hypothesis: patch dynamics and mechanisms of species coexistence. *Ecology* **85**, 359–371. (doi:10.1890/03-0266)
9. Chesson P, Huntly N. 1997 The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *Am. Nat.* **150**, 519–553. (doi:10.1086/286080)
10. May RM, MacArthur RH. 1972 Niche overlap as a function of environmental variability. *Proc. Natl Acad. Sci. USA* **69**, 1109–1113. (doi:10.1073/pnas.69.5.1109)
11. May RM. 1973 Stability in randomly fluctuating versus deterministic environments. *Am. Nat.* **107**, 621–650.
12. May RM. 1974 On the theory of niche overlap. *Theor. Popul. Biol.* **5**, 297–332. (doi:10.1016/0040-5809(74)90055-0)
13. Chesson PL, Warner RR. 1981 Environmental variability promotes coexistence in lottery competitive systems. *Am. Nat.* **117**, 923–943. (doi:10.1086/283778)
14. Chesson PL. 1983 *Coexistence of competitors in a stochastic environment: the storage effect*, pp. 188–198. Berlin, Germany: Springer.
15. Chesson PL. 1988 *Interactions between environment and competition: how fluctuations mediate coexistence and competitive exclusion*, pp. 51–71. Berlin, Germany: Springer.
16. Chesson P. 1994 Multispecies competition in variable environments. *Theor. Popul. Biol.* **45**, 227–276. (doi:10.1006/tpbi.1994.1013)
17. Chesson P. 2000 Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* **31**, 343–366. (doi:10.1146/annurev.ecolsys.31.1.343)
18. Chesson P. 2018 Updates on mechanisms of maintenance of species diversity. *J. Ecol.* **106**, 1773–1794. (doi:10.1111/1365-2745.13035)
19. Armstrong RA, McGehee R. 1976 Coexistence of species competing for shared resources. *Theor. Popul. Biol.* **9**, 317–328. (doi:10.1016/0040-5809(76)90051-4)
20. Hubbell SP. 2001 *The unified neutral theory of biodiversity and biogeography*. Princeton, NJ: Princeton University Press.
21. Ellner SP, Snyder RE, Adler PB, Hooker G. 2019 An expanded modern coexistence theory for empirical applications. *Ecol. Lett.* **22**, 3–18. (doi:10.1111/ele.13159)
22. Chesson P. 2017 AEDT: a new concept for ecological dynamics in the ever-changing world. *PLoS Biol.* **15**, e2002634. (doi:10.1371/journal.pbio.2002634)
23. Cooper G. 2001 Must there be a balance of nature? *Biol. Phil.* **16**, 481–506. (doi:10.1023/A:1011935220219)
24. Tijms H. 2007 *Understanding probability: chance rules in everyday life*, 2nd edn. Cambridge, UK: Cambridge University Press.
25. Rohde K. 2005 *Nonequilibrium ecology*. Cambridge, UK: Cambridge University Press.
26. Donohue I et al. 2016 Navigating the complexity of ecological stability. *Ecol. Lett.* **19**, 1172–1185. (doi:10.1111/ele.12648)
27. Shimadzu H, Dornelas M, Henderson PA, Magurran AE. 2013 Diversity is maintained by seasonal variation in species abundance. *BMC Biol.* **11**, 98. (doi:10.1186/1741-7007-11-98)
28. Schreiber SJ. 2021 Positively and negatively autocorrelated environmental fluctuations have opposing effects on species coexistence. *Am. Nat.* **197**, 405–414. (doi:10.1086/713066)
29. Caswell H, Cohen JE. 1995 Red, white and blue: environmental variance spectra and coexistence in metapopulations. *J. Theor. Biol.* **176**, 301–316. (doi:10.1006/jtbi.1995.0200)

30. Dillon ME, Woods HA, Wang G, Fey SB, Vasseur DA, Telemeo RS, Marshall K, Pincebourde S. 2016 Life in the frequency domain: the biological impacts of changes in climate variability at multiple time scales. *Integr. Comp. Biol.* **56**, 14–30. (doi:10.1093/icb/icw024)
31. Huey RB, Kingsolver JG. 1989 Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* **4**, 131–135. (doi:10.1016/0169-5347(89)90211-5)
32. Angilletta Jr MJ, Angilletta MJ. 2009 *Thermal adaptation: a theoretical and empirical synthesis*. Oxford, UK: Oxford University Press.
33. Loreau M. 2010 The maintenance and functional consequences of species diversity. In *From populations to ecosystems*, pp. 19–55, Princeton, NJ: Princeton University Press.
34. Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008 Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl Acad. Sci. USA* **105**, 6668–6672. (doi:10.1073/pnas.0709472105)
35. Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CD, McCann KS, Savage V, Tunney TD, O'Connor MI. 2014 Increased temperature variation poses a greater risk to species than climate warming. *Proc. R. Soc. B* **281**, 20132612. (doi:10.1098/rspb.2013.2612)
36. Huey RB, Stevenson RD. 2015 Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* **19**, 357–366. (doi:10.1093/icb/19.1.357)
37. Sinclair BJ *et al.* 2016 Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* **19**, 1372–1385. (doi:10.1111/ele.12686)
38. Tsai HY, Rubenstein DR, Chen BF, Liu M, Chan SF, Chen DP, Sun SJ, Yuan TN, Shen SF. 2020 Antagonistic effects of intraspecific cooperation and interspecific competition on thermal performance. *eLife* **9**, e57022. (doi:10.7554/eLife.57022)
39. Levesque DL, Marshall KE. 2021 Do endotherms have thermal performance curves? *J. Exp. Biol.* **224**, jeb141309. (doi:10.1242/jeb.141309)
40. Schreiber SJ, Benaim M, Atchadé KAS. 2011 Persistence in fluctuating environments. *J. Math. Biol.* **62**, 655–683. (doi:10.1007/s00285-010-0349-5)
41. Li L, Chesson P. 2016 The effects of dynamical rates on species coexistence in a variable environment: the paradox of the plankton revisited. *Am. Nat.* **188**, E46–E58. (doi:10.1086/687111)
42. Fox JW. 2013 The intermediate disturbance hypothesis should be abandoned. *Trends Ecol. Evol.* **28**, 86–92. (doi:10.1016/j.tree.2012.08.014)
43. Huston MA. 2014 Disturbance, productivity, and species diversity: empiricism vs. logic in ecological theory. *Ecology* **95**, 2382–2396. (doi:10.1890/13-1397.1)
44. Adler PB, Ellner SP, Levine JM. 2010 Coexistence of perennial plants: an embarrassment of niches. *Ecol. Lett.* **13**, 1019–1029. (doi:10.1111/j.1461-0248.2010.01496.x)
45. Gravel D, Guichard F, Hochberg ME. 2011 Species coexistence in a variable world. *Ecol. Lett.* **14**, 828–839. (doi:10.1111/j.1461-0248.2011.01643.x)
46. Adler PB, Drake JM. 2008 Environmental variation, stochastic extinction, and competitive coexistence. *Am. Nat.* **172**, E186–E195. (doi:10.1086/591678)
47. Vázquez DP, Gianoli E, Morris WF, Bozinovic F. 2017 Ecological and evolutionary impacts of changing climatic variability. *Biol. Rev. (Cambridge)* **92**, 22–42. (doi:10.1111/brv.12216)
48. Grainger TN, Levine JM, Gilbert B. 2019 The invasion criterion: a common currency for ecological research. *Trends Ecol. Evol.* **34**, 925–935. (doi:10.1016/j.tree.2019.05.007)
49. Siepielski AM, McPeck MA. 2010 On the evidence for species coexistence: a critique of the coexistence program. *Ecology* **91**, 3153–3164. (doi:10.1890/10-0154.1)
50. Levin SA. 1992 The problem of pattern and scale in ecology: the Robert H. MacArthur award lecture. *Ecology* **73**, 1943–1967. (doi:10.2307/1941447)
51. Shibasaki S, Mobilia M, Mitri S. 2021 Exclusion of the fittest predicts microbial community diversity in fluctuating environments. *bioRxiv*. (doi:10.1101/2020.07.22.216010)
52. Liu M, Rubenstein DR, Liu W-C, Shen S-F. 2019 A continuum of biological adaptations to environmental fluctuation. *Proc. R. Soc. B* **286**, 20191623. (doi:10.1098/rspb.2019.1623)

4

The evolution of manipulative cheating

The evolution of manipulative cheating

Ming Liu^{1*}, Stuart Andrew West¹, Geoff Wild²

¹Department of Biology, University of Oxford, Oxford, United Kingdom; ²Department of Mathematics, The University of Western Ontario, London, Canada

Abstract A social cheat is typically assumed to be an individual that does not perform a cooperative behaviour, or performs less of it, but can still exploit the cooperative behaviour of others. However, empirical data suggests that cheating can be more subtle, involving evolutionary arms races over the ability to both exploit and resist exploitation. These complications have not been captured by evolutionary theory, which lags behind empirical studies in this area. We bridge this gap with a mixture of game-theoretical models and individual-based simulations, examining what conditions favour more elaborate patterns of cheating. We found that as well as adjusting their own behaviour, individuals can be selected to manipulate the behaviour of others, which we term 'manipulative cheating'. Further, we found that manipulative cheating can lead to dynamic oscillations (arms races), between selfishness, manipulation, and suppression of manipulation. Our results can help explain both variation in the level of cheating, and genetic variation in the extent to which individuals can be exploited by cheats.

Editor's evaluation

This theoretical paper examines the conditions under which manipulative cheating can evolve. Manipulative cheating is a form of coercion where individuals can benefit by actively manipulating others to help them at a cost to their own fitness. The paper seamlessly integrates a multilevel selective framework and inclusive fitness, with a rigorous analysis of the joint dynamics of selfishness, manipulation, and suppression of manipulation. The results are novel and important, as they help us to better understand the evolution of cooperation and the spectrum of social cheating, potentially opening up new directions for theoretical and empirical work.

*For correspondence:
ming.liu@biology.ox.ac.uk

Competing interest: The authors declare that no competing interests exist.

Funding: See page 12

Preprinted: 29 May 2022

Received: 27 May 2022

Accepted: 03 October 2022

Published: 04 October 2022

Reviewing Editor: Sara Mitri, University of Lausanne, Switzerland

© Copyright Liu et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Introduction

Many social interactions involve a trade-off between investing in behaviours or traits that increase the productivity of the group, versus those that increase an individual's share of group productivity (*Frank, 1998; Bourke, 2011; Queller and Strassmann, 2018*; Figure 1a). A female social insect larva could become a queen or a worker (*Hamilton, 1972*). A slime mould cell could become a stalk or a spore within a fruiting body (*Strassmann et al., 2000*). A bacterial cell could invest costly resources into producing some 'public good' that benefits the local group of cells, such as iron scavenging siderophore molecules (*Griffin et al., 2004*).

A standard assumption is that individuals can behave more selfishly by investing less in cooperative behaviours that benefit the group, and by taking a larger share of the available resources (*Ghoul et al., 2014*). For example, a slime mould lineage could 'cheat' by having a larger proportion of its cells develop into spore cells, or a bacterial cell could cheat by producing fewer siderophores (*Strassmann et al., 2000; Jiricny et al., 2010*). When cheats can invade, theory predicts that this will lead to either uniform cooperation at a lower level or the coexistence of cheats and cooperators (*Queller, 1984; Frank, 1994; Frank, 1998; West and Buckling, 2003; MacLean and Gudelj, 2006; Ross-Gillespie et al., 2007; Gore et al., 2009; Frank, 2010c; Frank, 2010a; Frank, 2010b; Patel et al.,*

2019). Coexistence is only expected when some factor leads to the fitness of cheats being higher when they are less common (frequency-dependent selection).

In contrast, the empirical data suggests that there can be more complex forms of cheating and resistance to cheating within social groups. For example, in the slime *Dictyostelium discoideum*: (i) lineages appear to be able to evolve resistance to cheating, where they prevent other lineages from obtaining a higher proportion of spore cells (Buttery et al., 2009; Khare et al., 2009; Levin et al., 2015); (ii) genomic data suggests an ongoing evolutionary arms race, and not just selection towards an optimal level of cooperation (Ostrowski et al., 2015; Noh et al., 2018; Ostrowski, 2019). In some ant species, genetic variation appears to be maintained in the extent to which different patriline contribute to the production of workers in a colony, a phenomenon termed ‘royal cheating’ (Hughes et al., 2003; Linksvayer, 2006; Anderson et al., 2008; Hughes and Boomsma, 2008; Mitchell et al., 2012; Stürup et al., 2014). In *Pseudomonas* bacteria, strains appear to vary in both the extent to which they can cheat by exploiting the siderophores produced by other strains and the extent to which they can be cheated by other strains (Kümmerli et al., 2009; Bruce et al., 2017; Butaitė et al., 2017). In all these cases, cheating appears to involve doing more than just cooperating at a lower level. Other examples of more complex forms of cheating can be found in bacteria, viruses, and replicator RNA (Pollak et al., 2016; Furubayashi et al., 2020; Bruce et al., 2021; Mizuuchi et al., 2022).

Our aim is to explain how these more complex forms of cheating can be maintained in a population. As mentioned above, most evolutionary models assume that an individual can control their own investment into selfish and cooperative behaviours – put simply, an individual cheats by investing less in cooperation (Queller, 1984; MacLean and Gudelj, 2006; Ross-Gillespie et al., 2007; Gore et al., 2009; Frank, 2010c; Patel et al., 2019). We investigate another possibility, that individuals can manipulate the behaviour of others. For example, an ant patriline may increase the likelihood that its own larvae are raised as queens, while reducing the likelihood that the larvae of other patrilines are reared. Or a bacteria cell lineage could increase the public goods produced by another lineage. Such ‘manipulative cheating’ could involve a variety of mechanisms, ranging from coercive communication to more direct influencing.

We develop a series of models to examine whether manipulative cheating can be favoured, and if it will lead to variation in the extent to which individuals can exploit others. In addition, we explore how once manipulation has evolved, there could be selection to evolve resistance or suppression of that cheating. We use a mixture of game theory and simulation, to examine scenarios where resistance can evolve relatively simply, or where there are multiple mechanisms of manipulative cheating that each require a different form of resistance. Our model assumes an asexual haploid population, but we also examine the consequences of recombination. We find that natural selection can maintain a variety of strategies at equilibrium, as well as the possibility for ongoing oscillating dynamics, such as evolutionary arms races. While our focus is on ‘manipulation’, our more general aim is to investigate scenarios where cheating is more than just ‘cooperate less’.

Materials and methods

Model overview

Our aim is to investigate the general feasibility of manipulation, in a way that could be applicable across a range of species and does not rely on the specifics of any single species. Consequently, we have chosen a deliberately simple approach to maintain tractability. We use a combination of modelling approaches to examine the evolutionary stability and dynamics of manipulative cheating.

First, we use an equilibrium game theoretical approach to examine under what conditions manipulative cheating could be favoured. Our model is based on the classic tragedy of the commons model (Frank, 1994; Frank, 1996; Frank, 1998; Dionisio and Gordo, 2006; Frank, 2010a; Frank, 2010b), where individuals can invest in their own selfish reproduction, or the productivity of the group (Figure 1a; Appendix 1—1.2). We extend this model by assuming that: (i) individuals can invest effort into manipulating the behaviour of others to make them cooperate at a higher rate (behave less selfishly; Figure 1b); and (ii) individuals can also invest effort into suppressing (blocking) the extent to which they are manipulated by others (Figure 1c). We then test the robustness of our game-theoretical results by comparing them with individual-based simulations.

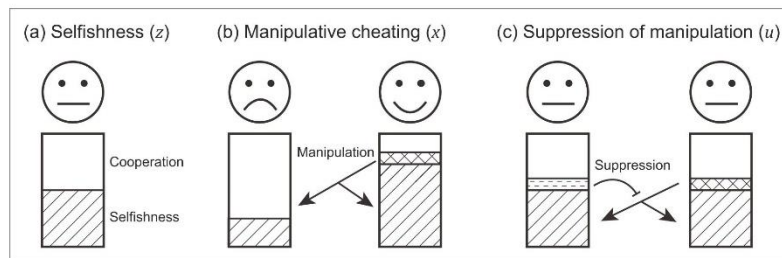


Figure 1. Schematic view of the three evolving traits in our model. Each individual can put effort into three actions: (a) invest resources into reproduction (z_i ; represented by stripe texture) or group productivity (average cooperation; $1 - \bar{z}$); (b) manipulate others (x_i ; represented by the grid texture), to make them invest more into group productivity; and (c) suppress (block) the manipulation from others (u_i ; represented by horizontal dashed texture).

Second, we examine the consequence of manipulative cheating when multiple different mechanisms of manipulation can evolve, which each requires a different mechanism to suppress. We consider cases where traits are introduced at the same time point or different time points (mutation) and focus on the temporal trait dynamics. As multiple mechanisms could generate complications between traits which cannot be easily presented by equilibrium analysis, we focus on dynamic results from individual-based simulations.

Our general aim is to investigate scenarios where cheating can be more than just ‘cooperate less’. We do this by developing models focused on the special case of manipulation and its suppression. Other possibilities would include developing coevolutionary genetic models to look at genotype-specific cheating-resistance, analogous to gene-for-gene or matching alleles models of host-parasite coevolution, or to model a different form of coercion (Sasaki, 2000; Agrawal and Lively, 2002).

Model design

We assume a haploid and asexual population inhabiting an infinite number of identical habitat patches, where each patch supports a single social group and lets social interactions take place. We also assume the fitness of each individual is proportional to the productivity of the social group multiplied by the individual’s share of group productivity, and all offspring compete globally for patches in the next generation. Within each social group, each individual divides its effort into cooperation with group members, which aids group productivity or selfishly reproducing (gaining a greater share of the group productivity). We consider three coevolving traits:

Selfishness (z): We assume that when individuals put more effort into reproduction (z), they: (i) obtain a larger share of the group productivity; (ii) put less effort into cooperation, which reduces group productivity. Specifically, if a focal individual i invests z_i into reproduction, and the group average of all individuals in the group is $\bar{z}$ ($0 < z < 1$). In other words, the parameter z measures the level of ‘selfishness’ (Figure 1a; Frank, 1994; Frank, 1996; Frank, 1998; Dionisio and Gordo, 2006; Frank, 2010a; Frank, 2010b).

Manipulative cheating (x): We assume that a focal individual can invest x in manipulation, which causes other individuals to invest fewer resources into their own reproduction, and instead invest those resources into the reproduction of the focal individual (manipulator; $0 < x < 1$; Figure 1b). This manipulation requires effort and so reduces the resources that the manipulator contributes to group productivity (by $\bar{x}$). Manipulation x therefore takes reproduction from others, whereas selfishness z shifts an individual’s allocation between cooperation and reproduction. Consequently, manipulation is redistributing the share of all individual’s reproduction while z is changing the effort spent in reproduction. For simplicity, we have assumed there is no ‘loss’ in reproductive effort during the process of manipulation, but we found similar results when manipulation also leads to a personal reproduction cost (Appendix 1–3).

Suppression of manipulative cheating (u): We add the possibility for individuals to suppress (block) manipulation ($0 < u < 1$; Figure 1c). When suppression exists, a manipulator can only take a proportion

$1 - u^b$ of reproduction, where b is a shape parameter (see below). Like the other two traits, suppression takes effort and reduces the effort put into group productivity (by $\bar{u}$).

Combining the effects of all three traits lead to:

$$w_i = \frac{z_i + (1 - u_i^b) x_i^a z_{-i} - (1 - u_i^b) x_{-i}^a z_i}{\bar{z}} (1 - \bar{z} - \bar{x} - \bar{u}) \quad (1)$$

where z_i is the selfishness of the focal individual, $(1 - u_i^b) x_i^a z_{-i}$ is the gain from manipulating others, $(1 - u_i^b) x_{-i}^a z_i$ is the loss from being manipulated, and $1 - \bar{z} - \bar{x} - \bar{u}$ is the group productivity. Because the sum of gain and loss across all individuals in the group is zero, the average effort in reproduction is just $\bar{z}$. Thus, the first term is the relative share of group productivity of the focal individual (i.e. $\frac{z_i + (1 - u_i^b) x_i^a z_{-i} - (1 - u_i^b) x_{-i}^a z_i}{\bar{z}}$). In other words, our manipulative cheating trait involves an individual actor

causing a recipient (other group members) to behave less selfishly (more cooperatively), in a way that is beneficial to the actor, but costly to the recipient. This contrasts with previous theory which examined the consequences of changing the benefit and cost of social behaviour (Frank, 1995), or when relatives are manipulated to perform a social behaviour that provides an indirect benefit to the actor (González-Forero and Gavrillets, 2013; Appendix 1—1.3). We provide a more detailed derivation of equation 1 in Appendix 1—1.1. In the special case of no manipulation or suppression, equation 1 simplifies to the classic tragedy of the commons model (Frank, 1994; Frank, 1996; Frank, 1998; West et al., 2002; Dionisio and Gordo, 2006; Frank, 2010a; Frank, 2010b).

We allow the effects of manipulation and suppression to be nonlinear by the shape parameters, a and b . Nonlinearities can arise for a number of reasons, such as mechanisms of molecular diffusion, or interference between the behaviour of different individuals (Müller et al., 2006; Wiley, 2013; Rogers et al., 2017). The parameter a determines whether the benefit from increased investment into manipulation is decelerating ($a < 1$), linear ($a = 1$) or accelerating ($a > 1$). Similarly, the shape parameter b determines the shape of the benefit of increased investment into suppression. We have focused on the decelerating forms because this will often be the case for biological traits (Appendix 1—5.1).

We use two approaches to analyse the model with different assumptions about recombination, or the degree of linkage, between traits. Our first analysis uses the neighbour modulated fitness of Taylor and Frank, 1996, where one trait is modified at a time to find the convergent stable strategy (Model Analysis and Appendix 1—1.1–1.2). The analysis assumes all three traits evolve independently; this is equivalent to a model that assumes a sexual diploid with additive interactions between alleles at a given locus and without recombination (Taylor, 1996; Day and Taylor, 1998; Kisdi and Geritz, 1999; Geritz and Kisdi, 2000). The second approach is through individual-based simulations, where recombination process can be explicitly modelled through how traits are inherited between generations (Appendix 1—7). The main simulation results also assume the absence of recombination. Nevertheless, we relax the assumption and include recombination by allowing haploid asexual individuals the chances to swap traits in Appendix 1—4.4 and 4.8.

We found it useful to think about manipulation, suppression, and equation 1 with a lottery-ticket metaphor. Imagine reproduction as the buying of a lottery ticket – producing an offspring, that has some chance of ‘winning’ (reproducing) in the next generation. Individuals can either invest resources into buying the lottery tickets produced by the group (z ; selfish), or invest resources into helping the group produce more lottery tickets ($1 - z$; cooperation). Each group produces its own lottery tickets and more tickets means a higher chance for the focal group to win. Being more selfish (higher z) means obtaining a larger fraction of the lottery tickets produced by the group, but also putting fewer resources into producing tickets (and so the group procures fewer tickets). In contrast, manipulation (higher x) allows individuals to steal lottery tickets from others within their group. Suppression, u , would involve blocking lottery tickets from being stolen. We consider biological examples of manipulation and suppression in the discussion section.

Results

Model analysis

We seek the evolutionarily stable strategy (ESS), which is the individual strategy that cannot be invaded by any other rare strategy. Using the neighbour-modulated fitness approach of **Taylor and Frank, 1996**, we find:

$$\begin{cases} \Delta W_z = \left(\bar{R}_{-i} \frac{-\left(\frac{n-1}{n} - x^a\right)(1-u^b)}{z} + \frac{\frac{n-1}{n} - x^a(1-u^b)}{z} \right) (1-z-x-u) - \bar{R} \\ \Delta W_x = \left(\bar{R}_{-i} (-ax^{a-1}) (1-u^b) + ax^{a-1} (1-u^b) \right) (1-z-x-u) - \bar{R} \\ \Delta W_u = \left(\bar{R}_{-i} (-x^a) bu^{b-1} + x^a bu^{b-1} \right) (1-z-x-u) - \bar{R} \end{cases} \quad (2)$$

where traits are analysed one at a time, where ΔW_z means the change in fitness due to a slight increase in selfishness in the focal individual, and analogous for ΔW_x and ΔW_u . In addition, $\bar{R}_{-i}$ denotes the relatedness of focal individual to all other group members, $\bar{R}$ indicate the whole-group relatedness (**Pepper, 2000**; Appendix 1—1.2). The two relatedness terms can be linked—for example, if the group size is n , then $\bar{R} = 1/n + ((n-1)/n) \bar{R}_{-i}$. For simplicity, we assume all traits only operate within the group so that $\bar{R}_{-i}$ does not differ between traits. More general form allowing for cases when different traits are at different social scales is considered in the derivation in Appendix 1—1. We also assume each trait evolves independently and there is no allele-level interaction between each trait. Our analyses identify the candidate ESS or convergently stable strategy (**Eshel, 1983**; **Christiansen, 1991**), which can be used to make comparative statics predictions (**Frank, 1998**).

Each of the three equations in **Equation 2** represents the change to the inclusive fitness of an individual that occurs when the individual increases the level at which it expresses the focal trait (**Hamilton, 1964a**; **Hamilton, 1964b**). Because all three expressions share similar structure, we can simplify them into

$$\Delta W_{\text{trait}} = (\bar{R}_{-i} (-A) + A) (1-z-x-u) - \bar{R}, \quad (3)$$

where A is different for each trait and subscript ‘trait’ means the type of trait we are analysing, which could be z , x , or u . The first term on the right, $-A$, represents the relatedness-weighted reduction in neighbours’ share of group productivity due to an individual’s selfishness or manipulation (an inclusive-fitness cost; e.g. $-\left(\frac{n-1}{n} - x^a\right)(1-u^b)/z$ for selfishness). The second term, A , represents the increase in personal share of group productivity made by a selfish or manipulative individual (an inclusive-fitness benefit; e.g. $\frac{\frac{n-1}{n} - x^a(1-u^b)}{z}$ for selfishness). The third term, group productivity, represents a relatedness-weighted reduction in fitness of the average group member, self-included, owing to selfishness, manipulation, or suppression (i.e. $1-z-x-u$). The cost in group productivity is spread equally among all individuals in the group, including the actor. The last term, $\bar{R}$, represents the indirect cost of reducing the share of group productivity obtained by relatives.

Equation 2 could not be solved analytically, and so we found solutions with an iterative numerical method (**Figure 2**; Appendix 1—6.2). We tested the robustness of our solutions with an individual-based simulation, which includes mutation and drift. Our simulations and numerical predictions showed close agreement (**Figure 2**; Appendix 1—7).

We found that the ESS selfishness, manipulation, and suppression are all predicted to show negative relationships with relatedness (**Figure 2**). At a lower relatedness, individuals are predicted to: (i) selfishly keep a higher fraction of their resources for themselves (as predicted by the classic tragedy of the commons model); (ii) invest more heavily in manipulating others to help them (as also predicted in a model without suppression; Appendix 1—2); (iii) invest more heavily in suppressing manipulation perpetrated by others (when manipulation is at higher levels). These predictions qualitatively hold for a broad range of parameter space, with linear or non-linear returns on increased investment in manipulation and suppression, and show that manipulative cheating as well as its suppression can be favoured by natural selection.

We predicted that the level of selfishness was greater than the level of manipulation, which was greater than the level of suppression ($z > x > u$). To a large extent this reflects that benefit of

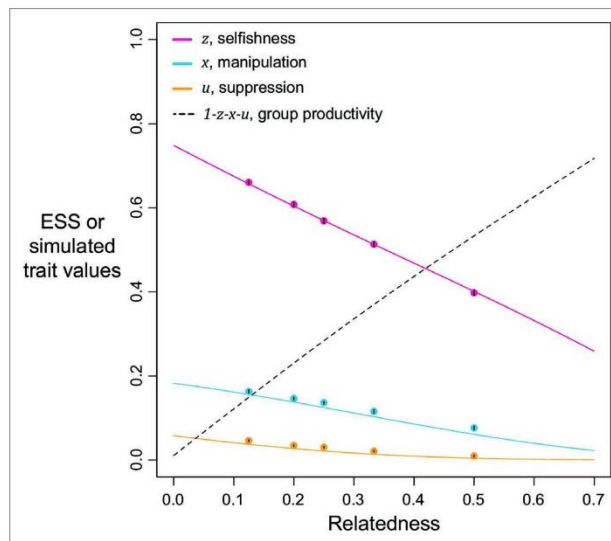


Figure 2. Selfishness, manipulation, and suppression of manipulation. The panels show the ESS predictions and simulation results for our three-trait model. The solid lines show the ESS predictions, and the dots show the results of our individual-based simulations ($a = b = 0.5$). The black segments within each dot show the standard deviation of trait values of the last 10% duration of 10 repeated simulations (Appendix 1–7).

manipulation depends upon the level of selfishness, and the benefit of suppression depends upon the level of manipulation. However, the quantitative predictions depend on details such as the specific values of the shape parameters (see below), and these relations need not always hold. Our simulations, which involved mutations, led to relatively static predictions when there is only one mechanism to manipulate and suppress, in agreement with our analytical results, supporting the results as ESSs (dots versus lines in **Figure 2**).

The quantitative ESS predictions depend upon the shape parameters (**Figure 3**). When the shape parameter of manipulation, a , is more decelerating, selfishness becomes lower, suppression becomes higher, and manipulation becomes higher until suppression evolved (**Figure 3a–c**). Secondly, when the shape parameter of suppression, b , is more decelerating, selfishness becomes higher, manipulation becomes lower, and suppression becomes higher until there is no manipulation to suppress (**Figure 3g–i**). Thirdly, when the two shape parameters covary together, the impact on ESS trait values is a combination of the two forementioned effects (**Figure 3d–f**). Both manipulation and suppression can be favoured across a wide range of parameter values.

Multiple mechanisms for manipulative cheating and suppressing

In our above model, we have focused on evolutionary equilibrium, assuming there is only a single mechanism to manipulate others. In nature, manipulation could take many forms, and each requires a different form of suppression. This could lead to complicated dynamics, such as a genetic arms race, where different forms of manipulation arise and are then suppressed over time. Because the rise and fall of trait values are potentially transient, we move away from game-theoretical analysis and focus on non-equilibrium scenarios through individual-based simulations. We present two different cases here: one with multiple mechanisms where all are introduced at the same time, and the other with multiple mechanisms where new mechanisms are introduced to the population at different time points (via mutation).

Assuming there are k mechanisms to manipulate and suppress, fitness is given by

$$w_i = \left(z_i + (1 - u_{1,-i}^b) z_{-i} x_{1,i}^a - (1 - u_{1,i}^b) z_i x_{1,-i}^a + (1 - u_{2,-i}^b) z_{-i} x_{2,i}^a \right. \\ \left. - (1 - u_{2,i}^b) z_i x_{2,-i}^a + \dots + (1 - u_{k,-i}^b) z_{-i} x_{k,i}^a - (1 - u_{k,i}^b) z_i x_{k,-i}^a \right) \bar{z}^{-1}, \quad (4) \\ (1 - \bar{z} - \bar{x}_1 - \bar{u}_1 - \bar{x}_2 - \bar{u}_2 - \dots - \bar{x}_k - \bar{u}_k)$$

where $x_1, x_2, \dots, x_n$ denote the n mechanisms of manipulation and $u_1, u_2, \dots, u_n$ represent suppression. Additional terms of gain and loss from manipulation and being manipulated are added to the first parenthesis, and group productivity can potentially be decreased by all the $2k + 1$ traits.

We first examine this potentially non-equilibrium scenario by assuming all mechanisms are present from the beginning and consider the simplest case of two mechanisms ($k = 2$). By simulating various combinations of shape parameters and relatedness, we found that levels of selfishness and manipulation do not always tend to a single equilibrium. Instead, they can oscillate in a periodic style (Figure 4a). Although the oscillation in suppression is less profound, it also fluctuates in a smaller amplitude in response to the changes in levels of manipulation. We used harmonic regression to test for periodic oscillations, as opposed to random noise (Figure 4b and Appendix 1—4.2).

We found oscillations occur when relatedness is relatively low ($R < 1/6$) and when shape parameters are intermediate decelerating ($0.6 < a, b < 0.85$). In these intermediate settings, the optimal levels of manipulation and selfishness are similar, which could lead to a similar pay-off between investing in

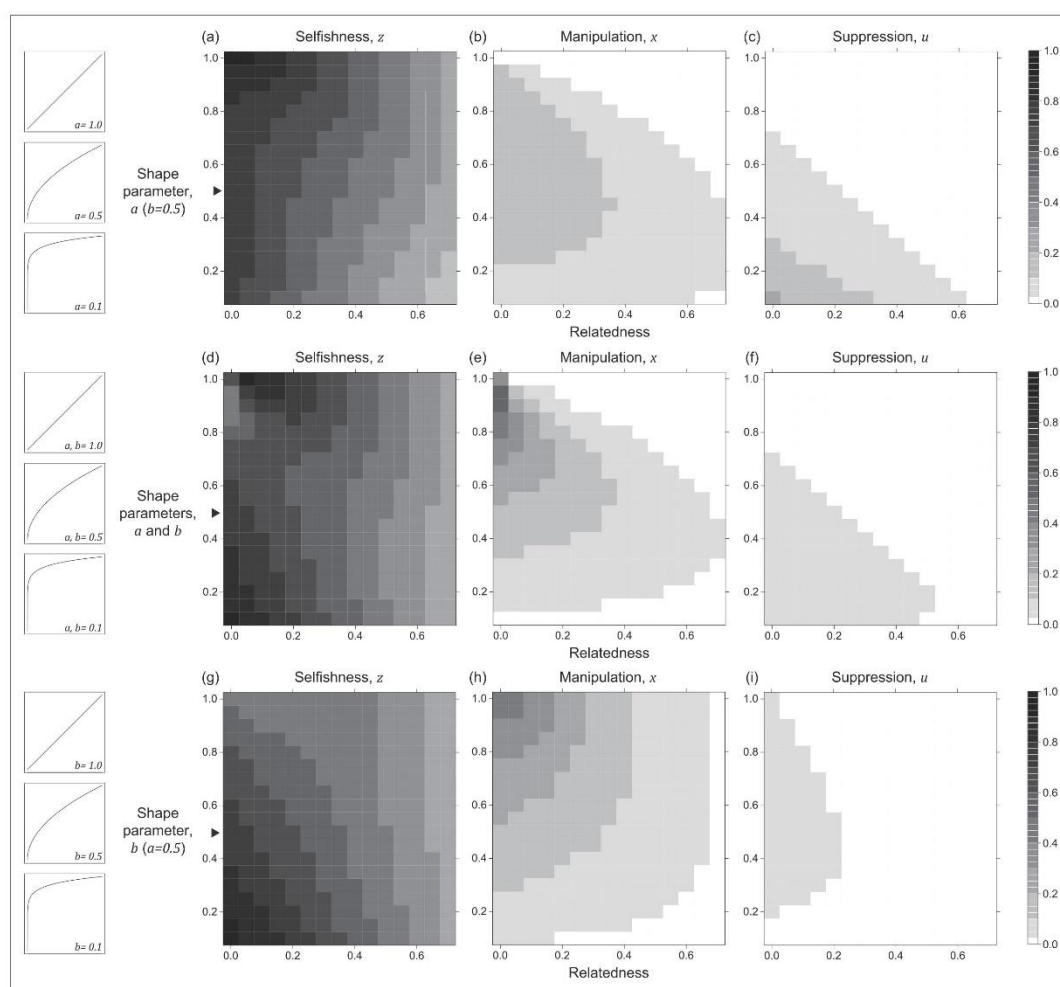


Figure 3. Nonlinear returns of manipulative cheating and its suppression. Panels (a), (b), and (c) show the predicted ESS level of selfishness (z^*), manipulation (x^*), and suppression (u^*) respectively, for different values of relatedness (R , x-axis). From top to bottom, each row of panels presents varying different parameters on y-axis: the shape parameter for manipulative cheating (a-c; a), both parameters (d-f; a and b), and the shape parameter for suppression (g-i; b). The black triangle indicates the horizontal row where the parameter settings are identical to each other and to Figure 2. We have focused on decelerating function for shape parameters because it resembles the nature of communication (Appendix 1—6.1).

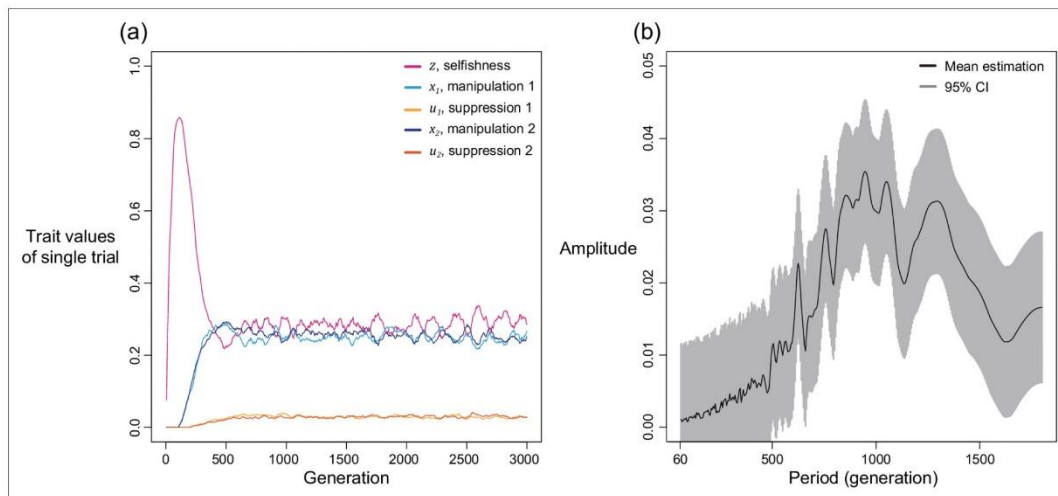


Figure 4. Selfishness and manipulative cheating traits can oscillate when there are two mechanisms for manipulation and suppression. **(a)** An example of oscillating dynamics when shape parameters are intermediate, and relatedness is relatively low ($R = 1/8$; $a = b = 0.7$). Broader analysis and more details are provided in Appendix 1—4. **(b)** The estimated amplitude of the time series of selfishness by harmonic regression. Harmonic regression is an analysis of time series to show the significance of periodic oscillation, where low period means frequent recurrent pattern, and high period means recurrence after a long span. Here, 10 repeated simulations were used and only the last 75% of the 3000 generations was analysed, after selfishness is roughly stabilised. The analysis shows maximal amplitude is found at period of 815 generations with p-value of 1.8035×10^{-5} . Mean estimation is shown in black curve whereas the 95% confidence interval is illustrated as the grey area (F-test; $\mu \pm 2\sigma$).

them. Thus, any small perturbation in trait values could disturb the dynamics and generate oscillation. In support of the hypothesis that oscillation is a consequence of interactions between selfishness and manipulation, we found that oscillation can be prevented when selfishness cannot mutate (Appendix 1—4.3) or when there is no linkage between traits (Appendix 1—4.4).

We also found that oscillations can occur when there are more than two mechanisms to manipulate and suppress (Appendix 1—4.5), becomes slower when mutation rate is smaller (Appendix 1—4.6), and less likely when there is a personal reproduction cost to manipulation and suppression (Appendix 1—4.7). The dynamics between cheats and cooperators can involve frequency dependent selection (Ross-Gillespie et al., 2007; Gore et al., 2009; Patel et al., 2019). In this model, we were examining evolution in a continuous trait space and so we were unable to test for frequency-dependent selection in the way that can be done with interactions between cheats and cooperator (i.e. 2 types, as opposed to continuous; Allison, 2005; Yurtsev et al., 2013).

We also found that recombination between traits could eliminate the oscillations (Appendix 1—4.4 and 4.8). We added recombination to our simulation in two ways. The first way is independently inheriting each trait when creating a new group founder for the next generation (Appendix 1—4.4). The second way is through allocating all founders in a founder pool and randomly grouped them into pairs. Each pair then exchange one random trait value with a recombination rate (Appendix 1—4.8). As a result, the first way is more discrete while the second way is more continuous. Because the group productivity term makes individuals with sum of all trait values exceeding 1 have no fitness, a positively deviated trait value cannot be protected by other traits simultaneously become negatively deviated under the presence of recombination. This finding coincides with previous literature that recombination prevents evolutionary branching into distinct genotypes, because it removes the linkage between strategies (linkage disequilibrium; Abrams et al., 1993; Dieckmann and Doebeli, 1999). Consequently, oscillations are more likely to be seen in asexual species.

We now consider a slightly different scenario (Figure 5). In the above analysis, we assumed that all manipulation and suppression mechanisms were available at the start of the simulation. An alternative possibility is that different mechanisms can arise over time, by mutation. When a manipulation trait arises, it could be selected for. Furthermore, once a form of manipulation becomes sufficiently common then a mutation that allows suppression of that manipulation could be selected for. These

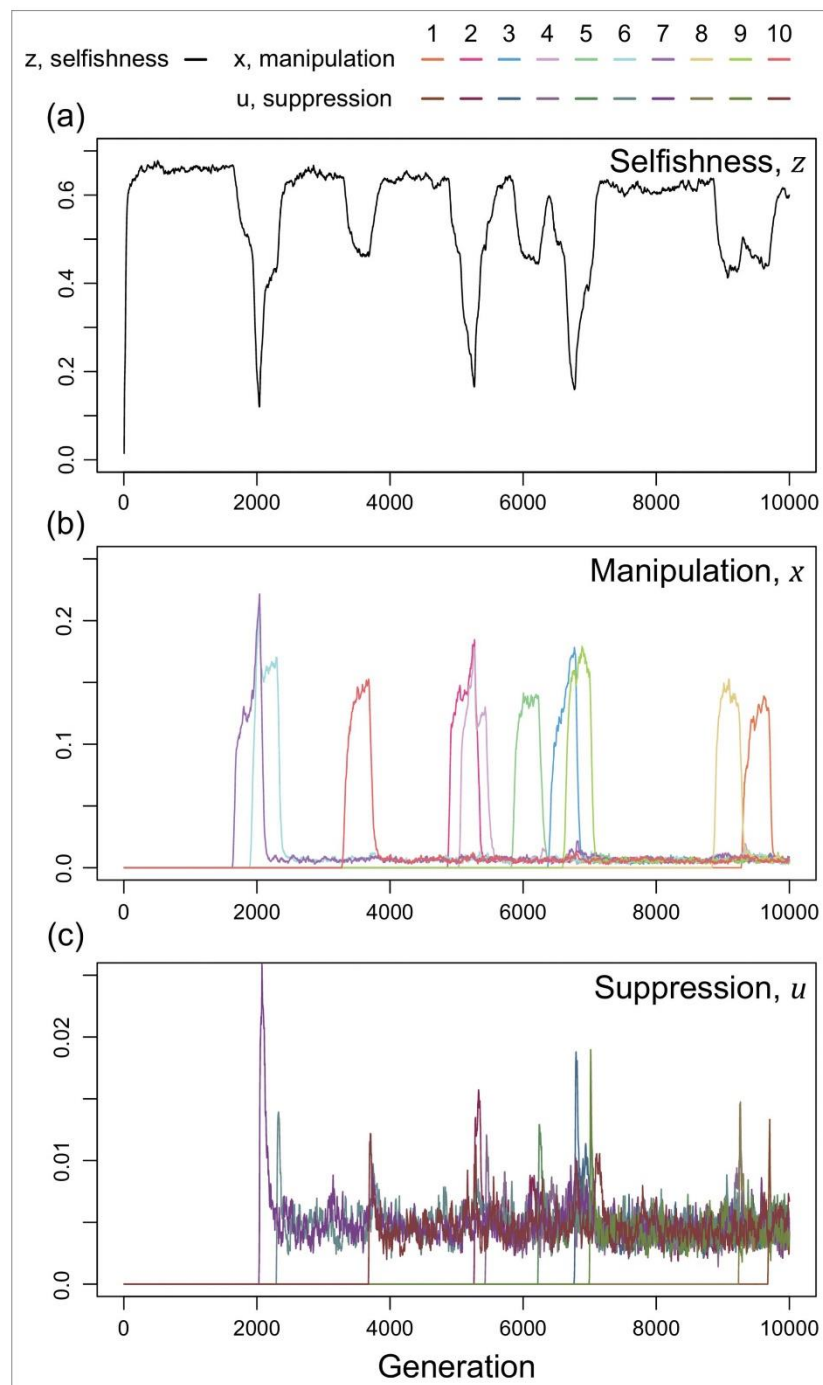


Figure 5. Time series of trait dynamics of the simulations when new mechanisms for manipulation and suppression are introduced at different time points. Selfishness is shown in the upper panel, the ten manipulative traits are shown in the middle panel, and the ten suppressive traits are shown in the bottom panel. The y-axis is scaled differently across the panels, to match the levels of variation. Each manipulative and suppressive trait are

Figure 5 continued on next page

Figure 5 continued

mutated to non-zero values in arbitrary time points for better visualisation. The shape parameters are set to $a = 0.5$ for manipulation and $b = 0.01$ for suppression, and the relatedness is $R = 1/3$. All data are from a single representative simulation.

different forms of manipulation could involve very different traits, or new forms of an old trait, that would require a different suppression mechanism. This scenario could potentially lead to an evolutionary arms race where new forms of manipulation evolve and are then suppressed. We modelled this scenario by introducing mutations for new manipulative traits at random time points, and then mutations for the suppression of manipulation 400 generations afterwards for illustrative purpose.

We found that this scenario also leads to a form of arms race (Figure 5). When a new form of manipulation arises, it initially spreads. This spread of manipulation favours lower levels of selfishness, because the manipulation reduces the benefit of selfishly investing in reproduction. In addition, once a manipulation is common, any mutation that allows suppression of that manipulation will be favoured, and hence increase in frequency. As suppression spreads, this selects for reduced investment in manipulation (which would just be blocked), and hence also increased selfish investment in reproduction (which won't be manipulated anymore) (Figure 5). Overall, this leads to a pattern of recurring dynamics whenever a new manipulative trait arises, and where individuals can show variable levels of selfishness, manipulation and suppression.

For illustrative purposes, our example in Figure 5 shows the gradual evolution of 10 different manipulation mechanisms and focuses on the parameter space where suppression can almost completely block manipulation in a very effective way ($a = 0.5$, $b = 0.01$) (Scott and West, 2019). We examine other scenarios in Appendix 1—5 (Appendix 1—figure 14 & Appendix 1—figure 15). The repeated invasion of different forms of manipulation, and their suppression, can be seen as a form of trait substitution sequence (Champagnat, 2006; Méléard and Tran, 2009). Recombination does not remove this arms race because it results from different traits spreading in turn, rather than oscillations of linked traits.

Altogether, our results from the multiple mechanisms of manipulation suggest that: (i) multiple types of manipulation can arise and / or be maintained in a population; (ii) the manipulation traits and their suppression can sometimes show oscillating dynamics, where the levels of selfishness, manipulation, and/ or suppression oscillate. The oscillation originates either from interactions between coevolving traits (endogenous; Figure 4) or from the introduction of new mechanisms (exogenous; Figure 5). These results provide possible explanations for cases where there appears to be genetic variation for the extent or form of cheating and/or cheating suppression, such as the variance between ant patrilines (Hughes and Boomsma, 2008), slime moulds strains (clones; Buttery et al., 2009; Khare et al., 2009; Levin et al., 2015), or different bacteria strains (Kümmerli et al., 2009; Bruce et al., 2017; Butaitė et al., 2017).

Discussion

We found that: (1) manipulative cheating can be favoured across a wide variety of conditions; (2) manipulation is more likely to be favoured when relatedness is relatively low; (3) suppression of manipulation can also be favoured. In addition, when we allowed for multiple mechanisms (types) of manipulation, we found that non-equilibrium dynamics could occur, such as oscillations. Oscillations could be generated in either (i) a coevolutionary interaction between selfishness and manipulation; or (ii) a genetic arms race between different forms of manipulative cheating and their specific suppressors. These non-equilibrium dynamics could help explain the genetic diversity that has been observed for both cheating and the response to cheating across the biological world (Hughes et al., 2003; Linksvayer, 2006; Anderson et al., 2008; Hughes and Boomsma, 2008; Buttery et al., 2009; Khare et al., 2009; Kümmerli et al., 2009; Mitchell et al., 2012; Stürup et al., 2014; Levin et al., 2015; Ostrowski et al., 2015; Pollak et al., 2016; Bruce et al., 2017; Butaitė et al., 2017; Noh et al., 2018; Ostrowski, 2019; Furubayashi et al., 2020; Bruce et al., 2021; Mizuuchi et al., 2022).

Our general aim was to investigate scenarios where cheating is characterised by more than just 'cooperate less'. We did this by developing a theoretical model where individuals could manipulate others, to make them cooperate more. There is a range of other possibilities for more complex forms

of cheating, such as obtaining a disproportionate share of some public good, where we suggest that a similar logic would apply (i.e. the evolution of 'more efficient' cheating). A key distinction here is that we are not modelling some evolutionarily stable signalling system which benefits the signaller and receiver (*Maynard Smith and Harper, 2003*). We have instead modelled coercion, which provides no benefit to the recipient of that coercion. Consequently, the response would be to evolve to suppress or ignore that coercion, rather than evolve to some evolutionarily stable mutually beneficial response. It would be useful to examine the consequences of different genetic systems, especially those with asymmetric inheritance such as haplodiploidy. Another useful extension would be to examine caste determination mechanism in social insect, and whether predictions depended upon whether castes are determined by environmental or genetic cues (*Schwander et al., 2010*).

Manipulative cheating could take many different forms in different species. In bacteria and other microorganisms, individuals could produce coercive molecules that make other cells invest more into the production of public goods, and invest less into reproduction. In species that form fruiting bodies, manipulation could cause the cells of other lineages to be more likely to become stalk cells (*Buttery et al., 2009; Khare et al., 2009; Levin et al., 2015*). In organisms such as social insects, the method of manipulation would depend upon how caste is determined (*Schwander et al., 2010*). In species where workers control the caste of offspring, manipulation could cause workers from other lineages (different queens or patriline) to be less likely to rear their own offspring as reproductive, and be more likely to rear the offspring of the manipulating lineage as reproductive (*Hamilton, 1972*). In species where larvae control their own caste, manipulation could decrease the likelihood that other individuals develop as reproductive (*Wenseleers and Ratnieks, 2004*). Alternatively, manipulation could occur through more direct approaches, such as selectively killing the eggs from other lineages. These mechanisms all share the same concept that the 'more manipulative' lineage gains a greater share of group productivity.

Similarly, suppression could involve a variety of different mechanisms, from evolving to ignore coercion, to blocking behaviours that steal resources or kill individuals, to making fewer public goods. Experiments on slime moulds have found that strains can evolve to become more resistant to cheats (*Levin et al., 2015*). Bacteria appear to be able to produce more specific and less exploitable public goods (*Kümmerli et al., 2009; Bruce et al., 2017; Butaitė et al., 2017*). Different lineages of cooperative RNA replicators can survive better against different cheating replicators (*Mizuuchi et al., 2022*). These cases share the same concept that cooperators can somewhat defend against or suppress the cheats. Other possible mechanisms are discussed elsewhere, for bacteria and viruses.

Previous theoretical work has focused on conflict suppression and hence how the tragedy of the commons could be avoided (*Frank, 1995; West et al., 2002; Frank, 2003; Wenseleers et al., 2003; Wenseleers et al., 2004; Ratnieks et al., 2006*). In these previous papers, selfishness could be selected against, by mechanisms such as policing, sanctions, and social reputation, which alter the costs and benefits of behaving selfishly (*Frank, 1995; Milinski et al., 2002; Dionisio and Gordo, 2006; Dionisio and Gordo, 2007; El Mouden et al., 2010*). In contrast, we have examined a different route to increased selfish behaviour – the manipulation of others via manipulative cheating. Furthermore, we have shown that if there are multiple manipulation traits, then genotypic diversity can be maintained for selfishness, manipulative cheating, and/or its suppression. This diversity result links to previous work on quorum sensing in bacteria, which has shown how cycles of 'cheating' and 'cheating immunity' can arise (*Eldar, 2011; Pollak et al., 2016*). Several theoretical models have examined the coevolution of offense and defence traits in ecological interactions (*Press and Dyson, 2012; Hilbe et al., 2013*) and in sexual conflict (*Gavrilets, 2000; Gavrilets et al., 2001; Rowe et al., 2005*). These models examined specific ecological scenarios, such as memory of previous interactions or runaway processes. Other recent models have examined a different manipulation question- how individuals can be favoured to manipulate relatives when this leads to an indirect fitness benefit to the manipulator, and the manipulated individual is not selected to resist (*González-Forero and Gavrilets, 2013; González-Forero, 2014; González-Forero, 2015; González-Forero and Peña, 2021*). Manipulation can also be favoured in mutualisms if it leads to better terms of trading (*Wyatt et al., 2016*). More generally, manipulation and counteradaptations to prevent elimination can arise in many forms of communication (*Dawkins et al., 1979; Maynard Smith and Harper, 2003*).

To conclude, we have shown how cheating can take more complex forms than just lower levels of cooperation. We have shown that individuals can also be selected to manipulate the behaviour

of others, for their own selfish gain. Furthermore, this can lead to evolutionary arms races between attempts to manipulate and to suppress that manipulation. A major task for the future is to determine the extent to which more complex forms of cheating occur in the natural world (Pollak *et al.*, 2016; Meir *et al.*, 2020; Leeks *et al.*, 2021). In many cases, this would require elucidation of the underlying mechanisms, which could be completely different across different species. The potential for manipulation and its suppression could arise at all levels of life, from RNA replicators to complex animal societies.

Acknowledgements

We thank Thomas W Scott, Guy A Cooper and the rest of West group for the input since the early development stages of this project; for discussions with Matishalin Patel, Xiang-Yi Li Richter, and Daisuke Kyogoku, who provided us with valuable literature. We are grateful for the three anonymous reviewers for their helpful feedback.

Additional information

Funding

Funder	Grant reference number	Author
European Research Council	Horizon 2020 Advanced Grant 834164	Stuart Andrew West Ming Liu
Ministry of Education	Oxford-Taiwan Graduate Scholarships	Ming Liu

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Author contributions

Ming Liu, Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review and editing; Stuart Andrew West, Conceptualization, Supervision, Funding acquisition, Investigation, Writing – original draft, Project administration, Writing – review and editing; Geoff Wild, Conceptualization, Formal analysis, Validation, Investigation, Writing – original draft, Writing – review and editing

Author ORCIDs

Ming Liu  <http://orcid.org/0000-0002-5170-8688>
 Stuart Andrew West  <http://orcid.org/0000-0003-2152-3153>
 Geoff Wild  <http://orcid.org/0000-0001-7821-7304>

Decision letter and Author response

Decision letter <https://doi.org/10.7554/eLife.80611.sa1>
 Author response <https://doi.org/10.7554/eLife.80611.sa2>

Additional files

Supplementary files

- MDAR checklist

Data availability

All results are generated using C and Python. The codes and data used for this study are available at: https://github.com/mingpapilio/Codes_Manipulative_Cheat, (copy archived at [swh:1:rev:e526dcaff01d51c334b90977cf17793c2c255e67](https://www.swh.io/rev/e526dcaff01d51c334b90977cf17793c2c255e67)).

References

Abrams PA, Matsuda H, Harada Y. 1993. Evolutionarily unstable fitness maxima and stable fitness minima of continuous traits. *Evolutionary Ecology* 7:465–487. DOI: <https://doi.org/10.1007/BF01237642>

- Agrawal A, Lively CM. 2002. Infection genetics: gene-for-gene versus matching-alleles models and all points in between. *Evolutionary Ecology Research* 4:91–107.
- Allison SD. 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecology Letters* 8:626–635. DOI: <https://doi.org/10.1111/j.1461-0248.2005.00756.x>
- Anderson KE, Linksvayer TA, Smith CR. 2008. The causes and consequences of genetic caste determination in ants (hymenoptera: formicidae). *Myrmecological News* 11:119–132.
- Bonner JT. 1982. Evolutionary strategies and developmental constraints in the cellular slime molds. *The American Naturalist* 119:530–552. DOI: <https://doi.org/10.1086/283930>
- Bourke AFG. 2011. Principles of Social Evolution. Oxford University Press. DOI: <https://doi.org/10.1093/acprof:oso/9780199231157.001.0001>
- Bruce JB, Cooper GA, Chabas H, West SA, Griffin AS. 2017. Cheating and resistance to cheating in natural populations of the bacterium *Pseudomonas fluorescens*. *Evolution; International Journal of Organic Evolution* 71:2484–2495. DOI: <https://doi.org/10.1111/evo.13328>, PMID: 28833073
- Bruce JB, Lion S, Buckling A, Westra ER, Gandon S. 2021. Regulation of prophage induction and lysogenization by phage communication systems. *Current Biology* 31:5046–5051. DOI: <https://doi.org/10.1016/j.cub.2021.08.073>, PMID: 34562385
- Butaitė E, Baumgartner M, Wyder S, Kümmerli R. 2017. Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater *Pseudomonas* communities. *Nature Communications* 8:414. DOI: <https://doi.org/10.1038/s41467-017-00509-4>, PMID: 28871205
- Buttery NJ, Rozen DE, Wolf JB, Thompson CRL. 2009. Quantification of social behavior in *D. discoideum* reveals complex fixed and facultative strategies. *Current Biology* 19:1373–1377. DOI: <https://doi.org/10.1016/j.cub.2009.06.058>, PMID: 19631539
- Champagnat N. 2006. A microscopic interpretation for adaptive dynamics trait substitution sequence models. *Stochastic Processes and Their Applications* 116:1127–1160. DOI: <https://doi.org/10.1016/j.spa.2006.01.004>
- Christiansen FB. 1991. On conditions for evolutionary stability for a continuously varying character. *The American Naturalist* 138:37–50. DOI: <https://doi.org/10.1086/285203>
- Dawkins R, Krebs JR, Maynard Smith J, Holliday R. 1979. Arms races between and within species. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 205:489–511. DOI: <https://doi.org/10.1098/rspb.1979.0081>, PMID: 42057
- Day T, Taylor PD. 1998. Unifying genetic and game theoretic models of kin selection for continuous traits. *Journal of Theoretical Biology* 194:391–407. DOI: <https://doi.org/10.1006/jtbi.1998.0762>, PMID: 9778445
- Dieckmann U, Doebeli M. 1999. On the origin of species by sympatric speciation. *Nature* 400:354–357. DOI: <https://doi.org/10.1038/22521>, PMID: 10432112
- Dionisio F, Gordo I. 2006. The tragedy of the commons, the public goods dilemma, and the meaning of rivalry and excludability in evolutionary biology. *Evolutionary Ecology Research* 8:321–332.
- Dionisio F, Gordo I. 2007. Controlling excludability in the evolution of cooperation. *Evolutionary Ecology Research* 9:365–373.
- Eldar A. 2011. Social conflict drives the evolutionary divergence of quorum sensing. *PNAS* 108:13635–13640. DOI: <https://doi.org/10.1073/pnas.1102923108>, PMID: 21807995
- El Mouden C, West SA, Gardner A. 2010. The enforcement of cooperation by policing. *Evolution; International Journal of Organic Evolution* 64:2139–2152. DOI: <https://doi.org/10.1111/j.1558-5646.2010.00963.x>, PMID: 20148957
- Eshel I. 1983. Evolutionary and continuous stability. *Journal of Theoretical Biology* 103:99–111. DOI: [https://doi.org/10.1016/0022-5193\(83\)90201-1](https://doi.org/10.1016/0022-5193(83)90201-1)
- Frank SA. 1994. Kin selection and virulence in the evolution of protocells and parasites. *Proceedings. Biological Sciences* 258:153–161. DOI: <https://doi.org/10.1098/rspb.1994.0156>, PMID: 7838853
- Frank SA. 1995. Mutual policing and repression of competition in the evolution of cooperative groups. *Nature* 377:520–522. DOI: <https://doi.org/10.1038/377520a0>, PMID: 7566147
- Frank SA. 1996. Models of parasite virulence. *The Quarterly Review of Biology* 71:37–78. DOI: <https://doi.org/10.1086/419267>, PMID: 8919665
- Frank SA. 1998. Foundations of Social Evolution. Princeton University Press. DOI: <https://doi.org/10.1515/9780691206820>
- Frank SA. 2003. Perspective: repression of competition and the evolution of cooperation. *Evolution; International Journal of Organic Evolution* 57:693–705. DOI: <https://doi.org/10.1111/j.0014-3820.2003.tb00283.x>, PMID: 12778541
- Frank SA. 2010a. A general model of the public goods dilemma. *Journal of Evolutionary Biology* 23:1245–1250. DOI: <https://doi.org/10.1111/j.1420-9101.2010.01986.x>, PMID: 20345809
- Frank SA. 2010b. Demography and the tragedy of the commons. *Journal of Evolutionary Biology* 23:32–39. DOI: <https://doi.org/10.1111/j.1420-9101.2009.01893.x>, PMID: 19912449
- Frank SA. 2010c. Microbial secretor-cheater dynamics. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 365:2515–2522. DOI: <https://doi.org/10.1098/rstb.2010.0003>, PMID: 20643741
- Furubayashi T, Ueda K, Bansho Y, Motooka D, Nakamura S, Mizuuchi R, Ichihashi N. 2020. Emergence and diversification of a host-parasite RNA ecosystem through Darwinian evolution. *eLife* 9:e56038. DOI: <https://doi.org/10.7554/eLife.56038>, PMID: 32690137
- Gardner A, West SA. 2004. Cooperation and punishment, especially in humans. *The American Naturalist* 164:753–764. DOI: <https://doi.org/10.1086/425623>, PMID: 29641920

- Gardner A, West SA, Barton NH. 2007. The relation between multilocus population genetics and social evolution theory. *The American Naturalist* **169**:207–226. DOI: <https://doi.org/10.1086/510602>, PMID: 17211805
- Gavrilets S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403**:886–889. DOI: <https://doi.org/10.1038/35002564>, PMID: 10706284
- Gavrilets S, Arnqvist G, Friberg U. 2001. The evolution of female mate choice by sexual conflict. *Proceedings. Biological Sciences* **268**:531–539. DOI: <https://doi.org/10.1098/rspb.2000.1382>, PMID: 11296866
- Geritz SA, Kisdi E. 2000. Adaptive dynamics in diploid, sexual populations and the evolution of reproductive isolation. *Proceedings. Biological Sciences* **267**:1671–1678. DOI: <https://doi.org/10.1098/rspb.2000.1194>, PMID: 11467431
- Ghoul M, Griffin AS, West SA. 2014. Toward an evolutionary definition of cheating. *Evolution; International Journal of Organic Evolution* **68**:318–331. DOI: <https://doi.org/10.1111/evo.12266>, PMID: 24131102
- Gill J, Johnson P. 2002. *Research Methods for Managers*. Sage.
- González-Forero M, Gavrilets S. 2013. Evolution of manipulated behavior. *The American Naturalist* **182**:439–451. DOI: <https://doi.org/10.1086/671932>, PMID: 24021397
- González-Forero M. 2014. An evolutionary resolution of manipulation conflict. *Evolution; International Journal of Organic Evolution* **68**:2038–2051. DOI: <https://doi.org/10.1111/evo.12420>, PMID: 24725252
- González-Forero M. 2015. Stable eusociality via maternal manipulation when resistance is costless. *Journal of Evolutionary Biology* **28**:2208–2223. DOI: <https://doi.org/10.1111/jeb.12744>, PMID: 26341103
- González-Forero M, Peña J. 2021. Eusociality through conflict dissolution. *Proceedings. Biological Sciences* **288**:20210386. DOI: <https://doi.org/10.1098/rspb.2021.0386>, PMID: 33878926
- Gore J, Youk H, van Oudenaarden A. 2009. Snowdrift game dynamics and facultative cheating in yeast. *Nature* **459**:253–256. DOI: <https://doi.org/10.1038/nature07921>, PMID: 19349960
- Griffin AS, West SA, Buckling A. 2004. Cooperation and competition in pathogenic bacteria. *Nature* **430**:1024–1027. DOI: <https://doi.org/10.1038/nature02744>, PMID: 15329720
- Hamilton WD. 1964a. The genetical evolution of social behaviour. I. *Journal of Theoretical Biology* **7**:1–16. DOI: [https://doi.org/10.1016/0022-5193\(64\)90038-4](https://doi.org/10.1016/0022-5193(64)90038-4), PMID: 5875341
- Hamilton WD. 1964b. The genetical evolution of social behaviour. II. *Journal of Theoretical Biology* **7**:17–52. DOI: [https://doi.org/10.1016/0022-5193\(64\)90039-6](https://doi.org/10.1016/0022-5193(64)90039-6), PMID: 5875340
- Hamilton WD. 1972. Altruism and related phenomena, mainly in social insects. *Annual Review of Ecology and Systematics* **3**:193–232. DOI: <https://doi.org/10.1146/annurev.es.03.110172.001205>
- Hilbe C, Nowak MA, Traulsen A. 2013. Adaptive dynamics of extortion and compliance. *PLOS ONE* **8**:e77886. DOI: <https://doi.org/10.1371/journal.pone.0077886>, PMID: 24223739
- Hudson RE, Aukema JE, Rispe C, Roze D. 2002. Altruism, cheating, and anticheater adaptations in cellular slime molds. *The American Naturalist* **160**:31–43. DOI: <https://doi.org/10.1086/340613>, PMID: 18707497
- Hughes WOH, Sumner S, Van Borm S, Boomsma JJ. 2003. Worker caste polymorphism has a genetic basis in acromyrmex leaf-cutting ants. *PNAS* **100**:9394–9397. DOI: <https://doi.org/10.1073/pnas.1633701100>, PMID: 12878720
- Hughes WOH, Boomsma JJ. 2008. Genetic royal cheats in leaf-cutting ant societies. *PNAS* **105**:5150–5153. DOI: <https://doi.org/10.1073/pnas.0710262105>, PMID: 18339809
- Jiricny N, Diggle SP, West SA, Evans BA, Ballantyne G, Ross-Gillespie A, Griffin AS. 2010. Fitness correlates with the extent of cheating in a bacterium. *Journal of Evolutionary Biology* **23**:738–747. DOI: <https://doi.org/10.1111/j.1420-9101.2010.01939.x>, PMID: 20210835
- Khare A, Santorelli LA, Strassmann JE, Queller DC, Kuspa A, Shaulsky G. 2009. Cheater-resistance is not futile. *Nature* **461**:980–982. DOI: <https://doi.org/10.1038/nature08472>, PMID: 19794414
- Kisdi É, Geritz SAH. 1999. Adaptive dynamics in allele space: evolution of genetic polymorphism by small mutations in a heterogeneous environment. *Evolution; International Journal of Organic Evolution* **53**:993–1008. DOI: <https://doi.org/10.1111/j.1558-5646.1999.tb04515.x>, PMID: 28565508
- Kümmerli R, Griffin AS, West SA, Buckling A, Harrison F. 2009. Viscous medium promotes cooperation in the pathogenic bacterium *Pseudomonas aeruginosa*. *Proceedings. Biological Sciences* **276**:3531–3538. DOI: <https://doi.org/10.1098/rspb.2009.0861>, PMID: 19605393
- Leeks A, West SA, Ghoul M. 2021. The evolution of cheating in viruses. *Nature Communications* **12**:6928. DOI: <https://doi.org/10.1038/s41467-021-27293-6>, PMID: 34836945
- Lehmann L, Rousset F, Roze D, Keller L. 2007. Strong reciprocity or strong ferocity? a population genetic view of the evolution of altruistic punishment. *The American Naturalist* **170**:21–36. DOI: <https://doi.org/10.1086/518568>, PMID: 17853989
- Levin SR, Brock DA, Queller DC, Strassmann JE. 2015. Concurrent coevolution of intra-organismal cheaters and resistors. *Journal of Evolutionary Biology* **28**:756–765. DOI: <https://doi.org/10.1111/jeb.12618>, PMID: 25772340
- Linksvayer TA. 2006. Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant. *Evolution; International Journal of Organic Evolution* **60**:2552–2561. DOI: <https://doi.org/10.1554/06-011.1>, PMID: 17263116
- Liu M, West SA, Cooper GA. 2021. Relatedness and the evolution of mechanisms to divide labor in microorganisms. *Ecology and Evolution* **11**:14475–14489. DOI: <https://doi.org/10.1002/ece3.8067>, PMID: 34765120
- MacLean RC, Gudelj I. 2006. Resource competition and social conflict in experimental populations of yeast. *Nature* **441**:498–501. DOI: <https://doi.org/10.1038/nature04624>, PMID: 16724064

- Maynard Smith J. 1964. Group selection and kin selection. *Nature* 201:1145–1147. DOI: <https://doi.org/10.1038/2011145a0>
- Maynard Smith J, Harper D. 2003. *Animal Signals*. Oxford University Press. DOI: <https://doi.org/10.1016/j.cub.2013.07.070>
- Meir M, Harel N, Miller D, Gelbart M, Eldar A, Gophna U, Stern A. 2020. Competition between social cheater viruses is driven by mechanistically different cheating strategies. *Science Advances* 6:eabb7990. DOI: <https://doi.org/10.1126/sciadv.abb7990>, PMID: 32937370
- Méléard S, Tran VC. 2009. Trait substitution sequence process and canonical equation for age-structured populations. *Journal of Mathematical Biology* 58:881–921. DOI: <https://doi.org/10.1007/s00285-008-0202-2>, PMID: 18668245
- Milinski M, Semmann D, Krambeck HJ. 2002. Reputation helps solve the “tragedy of the commons.” *Nature* 415:424–426. DOI: <https://doi.org/10.1038/415424a>, PMID: 11807552
- Mitchell RE, Frost CL, Hughes WOH. 2012. Size and asymmetry: are there costs to winning the royalty race? *Journal of Evolutionary Biology* 25:522–531. DOI: <https://doi.org/10.1111/j.1420-9101.2011.02444.x>, PMID: 22239486
- Mizuuchi R, Furubayashi T, Ichihashi N. 2022. Evolutionary transition from a single RNA replicator to a multiple replicator network. *Nature Communications* 13:1460. DOI: <https://doi.org/10.1038/s41467-022-29113-x>, PMID: 35304447
- Müller J, Kuttler C, Hense BA, Rothballer M, Hartmann A. 2006. Cell-Cell communication by quorum sensing and dimension-reduction. *Journal of Mathematical Biology* 53:672–702. DOI: <https://doi.org/10.1007/s00285-006-0024-z>, PMID: 16897015
- Noh S, Geist KS, Tian X, Strassmann JE, Queller DC. 2018. Genetic signatures of microbial altruism and cheating in social amoebas in the wild. *PNAS* 115:3096–3101. DOI: <https://doi.org/10.1073/pnas.1720324115>, PMID: 29507206
- Ostrowski EA, Shen Y, Tian X, Suggang R, Jiang H, Qu J, Katoh-Kurasawa M, Brock DA, Dinh C, Lara-Garduno F, Lee SL, Kovar CL, Dinh HH, Korchina V, Jackson L, Patil S, Han Y, Chaboub L, Shaulsky G, Muzny DM, et al. 2015. Genomic signatures of cooperation and conflict in the social amoeba. *Current Biology* 25:1661–1665. DOI: <https://doi.org/10.1016/j.cub.2015.04.059>, PMID: 26051890
- Ostrowski E.A. 2019. Enforcing cooperation in the social amoebae. *Current Biology* 29:R474–R484. DOI: <https://doi.org/10.1016/j.cub.2019.04.022>, PMID: 31163162
- Patel M, Raymond B, Bonsall MB, West SA. 2019. Crystal toxins and the volunteer's dilemma in bacteria. *Journal of Evolutionary Biology* 32:310–319. DOI: <https://doi.org/10.1111/jeb.13415>, PMID: 30672052
- Pepper JW. 2000. Relatedness in trait group models of social evolution. *Journal of Theoretical Biology* 206:355–368. DOI: <https://doi.org/10.1006/jtbi.2000.2132>, PMID: 10988021
- Pollak S, Omer-Bendori S, Even-Tov E, Lipsman V, Bareia T, Ben-Zion I, Eldar A. 2016. Facultative cheating supports the coexistence of diverse quorum-sensing alleles. *PNAS* 113:2152–2157. DOI: <https://doi.org/10.1073/pnas.1520615113>, PMID: 26787913
- Press WH, Dyson FJ. 2012. Iterated prisoner's dilemma contains strategies that dominate any evolutionary opponent. *PNAS* 109:10409–10413. DOI: <https://doi.org/10.1073/pnas.1206569109>, PMID: 22615375
- Queller DC. 1984. Kin selection and frequency dependence: a game theoretic approach. *Biological Journal of the Linnean Society* 23:133–143. DOI: <https://doi.org/10.1111/j.1095-8312.1984.tb00133.x>
- Queller DC, Strassmann JE. 2018. Evolutionary conflict. *Annual Review of Ecology, Evolution, and Systematics* 49:73–93. DOI: <https://doi.org/10.1146/annurev-ecolsys-110617-062527>
- Ratnieks FLW, Foster KR, Wenseleers T. 2006. Conflict resolution in insect societies. *Annual Review of Entomology* 51:581–608. DOI: <https://doi.org/10.1146/annurev.ento.51.110104.151003>, PMID: 16332224
- Rogers DW, McConnell E, Miller EL, Greig D. 2017. Diminishing returns on intragenic repeat number expansion in the production of signaling peptides. *Molecular Biology and Evolution* 34:3176–3185. DOI: <https://doi.org/10.1093/molbev/msx243>, PMID: 28961820
- Ross-Gillespie A, Gardner A, West SA, Griffin AS. 2007. Frequency dependence and cooperation: theory and a test with bacteria. *The American Naturalist* 170:331–342. DOI: <https://doi.org/10.1086/519860>, PMID: 17879185
- Rowe L, Cameron E, Day T. 2005. Escalation, retreat, and female indifference as alternative outcomes of sexually antagonistic coevolution. *The American Naturalist* 165 Suppl 5:S5–S18. DOI: <https://doi.org/10.1086/429395>, PMID: 15795862
- Sasaki A. 2000. Host-Parasite coevolution in a multilocus gene-for-gene system. *Proceedings. Biological Sciences* 267:2183–2188. DOI: <https://doi.org/10.1098/rspb.2000.1267>, PMID: 11413631
- Schwander T, Lo N, Beekman M, Oldroyd BP, Keller L. 2010. Nature versus nurture in social insect caste differentiation. *Trends in Ecology & Evolution* 25:275–282. DOI: <https://doi.org/10.1016/j.tree.2009.12.001>, PMID: 20106547
- Scott TW, West SA. 2019. Adaptation is maintained by the Parliament of genes. *Nature Communications* 10:5163. DOI: <https://doi.org/10.1038/s41467-019-13169-3>, PMID: 31727886
- Strassmann JE, Zhu Y, Queller DC. 2000. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* 408:965–967. DOI: <https://doi.org/10.1038/35050087>, PMID: 11140681
- Stürup M, Nash DR, Hughes WOH, Boomsma JJ. 2014. Sperm mixing in the polyandrous leaf-cutting ant *Acromyrmex echinator*. *Ecology and Evolution* 4:3571–3582. DOI: <https://doi.org/10.1002/ece3.1176>, PMID: 25478149

- Taylor PD.** 1996. Inclusive fitness arguments in genetic models of behaviour. *Journal of Mathematical Biology* **34**:654–674. DOI: <https://doi.org/10.1007/BF02409753>, PMID: 8691088
- Taylor PD, Frank SA.** 1996. How to make a kin selection model. *Journal of Theoretical Biology* **180**:27–37. DOI: <https://doi.org/10.1006/jtbi.1996.0075>, PMID: 8763356
- Tibbetts EA, Liu M, Laub EC, Shen SF.** 2020. Complex signals alter recognition accuracy and conspecific acceptance thresholds. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **375**:20190482. DOI: <https://doi.org/10.1098/rstb.2019.0482>, PMID: 32420854
- Wenseleers T, Ratnieks FLW, Billen J.** 2003. Caste fate conflict in swarm-founding social Hymenoptera: an inclusive fitness analysis. *Journal of Evolutionary Biology* **16**:647–658. DOI: <https://doi.org/10.1046/j.1420-9101.2003.00574.x>, PMID: 14632228
- Wenseleers T, Helanterä H, Hart A, Ratnieks FLW.** 2004. Worker reproduction and policing in insect societies: an ESS analysis. *Journal of Evolutionary Biology* **17**:1035–1047. DOI: <https://doi.org/10.1111/j.1420-9101.2004.00751.x>, PMID: 15312076
- Wenseleers T, Ratnieks FLW.** 2004. Tragedy of the commons in melipona bees. *Proceedings. Biological Sciences* **271** Suppl 5:S310–S312. DOI: <https://doi.org/10.1098/rsbl.2003.0159>, PMID: 15504003
- West SA, Kiers ET, Simms EL, Denison RF.** 2002. Sanctions and mutualism stability: why do rhizobia fix nitrogen? *Proceedings. Biological Sciences* **269**:685–694. DOI: <https://doi.org/10.1098/rspb.2001.1878>, PMID: 11934359
- West SA, Buckling A.** 2003. Cooperation, virulence and siderophore production in bacterial parasites. *Proceedings. Biological Sciences* **270**:37–44. DOI: <https://doi.org/10.1098/rspb.2002.2209>, PMID: 12590769
- Wild G.** 2011. Inclusive fitness from multitype branching processes. *Bulletin of Mathematical Biology* **73**:1028–1051. DOI: <https://doi.org/10.1007/s11538-010-9551-2>, PMID: 20532644
- Wiley RH.** 2013. Signal detection, noise, and the evolution of communication. Brumm H (Ed). *Animal Communication and Noise*. Berlin Heidelberg Berlin, Heidelberg: Springer. p. 7–30. DOI: https://doi.org/10.1007/978-3-642-41494-7_2
- Wyatt GAK, Kiers ET, Gardner A, West SA.** 2016. Restricting mutualistic partners to enforce trade reliance. *Nature Communications* **7**:10322. DOI: <https://doi.org/10.1038/ncomms10322>
- Yurtsev EA, Chao HX, Datta MS, Artemova T, Gore J.** 2013. Bacterial cheating drives the population dynamics of cooperative antibiotic resistance plasmids. *Molecular Systems Biology* **9**:683. DOI: <https://doi.org/10.1038/msb.2013.39>, PMID: 23917989

Corrections

1. The second paragraph of model design: the revised second sentence is “Specifically, we considered a focal individual i invests z_i effort into reproduction, and the group average of all individuals in the group is $\bar{z}$ ($0 < z < 1$).”
2. Two lines above equation 1: missed a close parenthesis at the end.
3. The second paragraph on page 8 of 38: misspelled suppress.

5

Equilibria and oscillations in cheat–cooperator dynamics

OXFORD

Evolution Letters, 2023, XX(XX), 1–12

<https://doi.org/10.1093/evlett/grad032>

Letter

Equilibria and oscillations in cheat-cooperator dynamics

Ming Liu¹, Geoff Wild², Stuart A. West¹¹Department of Biology, University of Oxford, Oxford, United Kingdom²Department of Mathematics, The University of Western Ontario, London, ON, CanadaCorresponding author: Department of Biology, University of Oxford, 11a Mansfield Road, Oxford, Oxfordshire, OX1 3SZ, United Kingdom. Email: ming.liu@biology.ox.ac.uk

Abstract

Cooperative societies can be threatened by cheats, who invest less in cooperation and exploit the contributions of others. The impact of cheats depends on the extent to which they are maintained in the population. However, different empirical studies, across organisms ranging from RNA replicators to bacteria, have shown diverse cheat-cooperator dynamics. These vary from approaching a stable equilibrium to dynamic cyclical oscillations. The reason for this variation remains unclear. Here, we develop a theoretical model to identify the factors that determine whether dynamics should tend toward stable equilibria or cyclical oscillations. Our analyses show that (1) a combination of both periodic population bottlenecks and density-dependent selection on cheating is required to produce cyclical oscillations and (2) the extent of frequency-dependent selection for cheating can influence the amplitude of these oscillations but does not lead to oscillations alone. Furthermore, we show that stochastic group formation (demographic stochasticity) can generate different forms of oscillation, over a longer time scale, across growth cycles. Our results provide experimentally testable hypotheses for the processes underlying cheat-cooperator dynamics.

Keywords: serial passage, density dependence, frequency dependence, cyclic dynamics, population bottleneck, stochastic group formation

Lay Summary

Cooperators can be exploited by cheats. Cheats are individuals who gain the benefits of cooperation without contributing their fair share. Cheats can be found across the tree of life, including viruses, bacteria, slime moulds, and social insects. The outcomes of the interactions between cheats and cooperators can vary widely, from relatively stable proportions of cheats to cyclical oscillations, where the proportion of cheats goes up and down. We developed a theoretical model to explain the dynamics between cheats and cooperators. We discovered that the emergence of cyclical oscillations relies on the simultaneous occurrence of two key mechanisms: periodic population bottlenecks, characterized by recurrent reductions in population size, and density-dependent selection acting on cheating behavior, where the fitness of cheats increases with the abundance of cooperators. We then explored the impact of frequency-dependent selection, where the fitness of cheats increases with the proportion of cooperator within the population. We found that while frequency-dependent selection can influence the amplitude of oscillations, it is insufficient to generate oscillations in the proportion of cheats. Moreover, our examination of stochastic group formation, which is introducing random variations in group composition, revealed another form of oscillation and added an intriguing layer of complexity to cheat-cooperator dynamics. Our results provide hypotheses that could be tested in either experimental or natural populations.

Introduction

All populations of cooperative organisms are at risk of being invaded by cheats, defined as individuals who avoid the costs of cooperation but benefit from the cooperation of others (Ghoul et al., 2014; Trivers, 1971). Cheats have been observed at many levels of biology, from genes to viruses, and bacteria to animals (Ghoul & Mitri, 2016; He et al., 2019; Hughes & Boomsma, 2008; Jaenike, 2001; Strassmann & Queller, 2011; Tapia et al., 2019). In some cases, cheats are individuals who do not produce a public good, such as replicase enzyme in viruses or iron-scavenging molecules in bacteria (Griffin et al., 2004; Leeks et al., 2021). In other cases, cheating can take more active or devious forms, such as when a cuckoo or other brood parasite tricks individuals of another species into rearing their eggs (Davies, 2015). In the extreme, if cheats spread to fixation, then cooperation can be lost (Andersen et al., 2015). Consequently, the influence of cheats will depend on their

evolutionary dynamics. Will cheating be transient, spread to an equilibrium level, go to fixation, or should we even expect cycles of cheating (Fig. 1A–C)?

Very different cheat dynamics have been observed across empirical studies. In many laboratory experiments with bacteria the dynamics approach an equilibrium, where cheats are either lost, go to fixation, or maintained at a relatively stable proportion (i.e., frequency; Figure 1D and E) (Diggle et al., 2007; Frost et al., 2018; Griffin et al., 2004; Pollitt et al., 2014; Rumbaugh et al., 2012; Schluter et al., 2016). In contrast, laboratory experiments with RNA replicators and data from natural populations of bacteria have shown cheats cyclically oscillating in proportion (Furubayashi et al., 2020; Kümmerli et al., 2015; Mizuuchi et al., 2022; Raymond et al., 2012). Cyclical oscillations in the proportion of cheats are also observed when culturing viruses (Frensing et al., 2013; Tapia et al., 2019). Indeed, these cycles are so common

Received April 25, 2023; revisions received June 29, 2023; accepted July 10, 2023

© The Author(s) 2023. Published by Oxford University Press on behalf of The Society for the Study of Evolution (SSE) and European Society for Evolutionary Biology (ESEN).

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

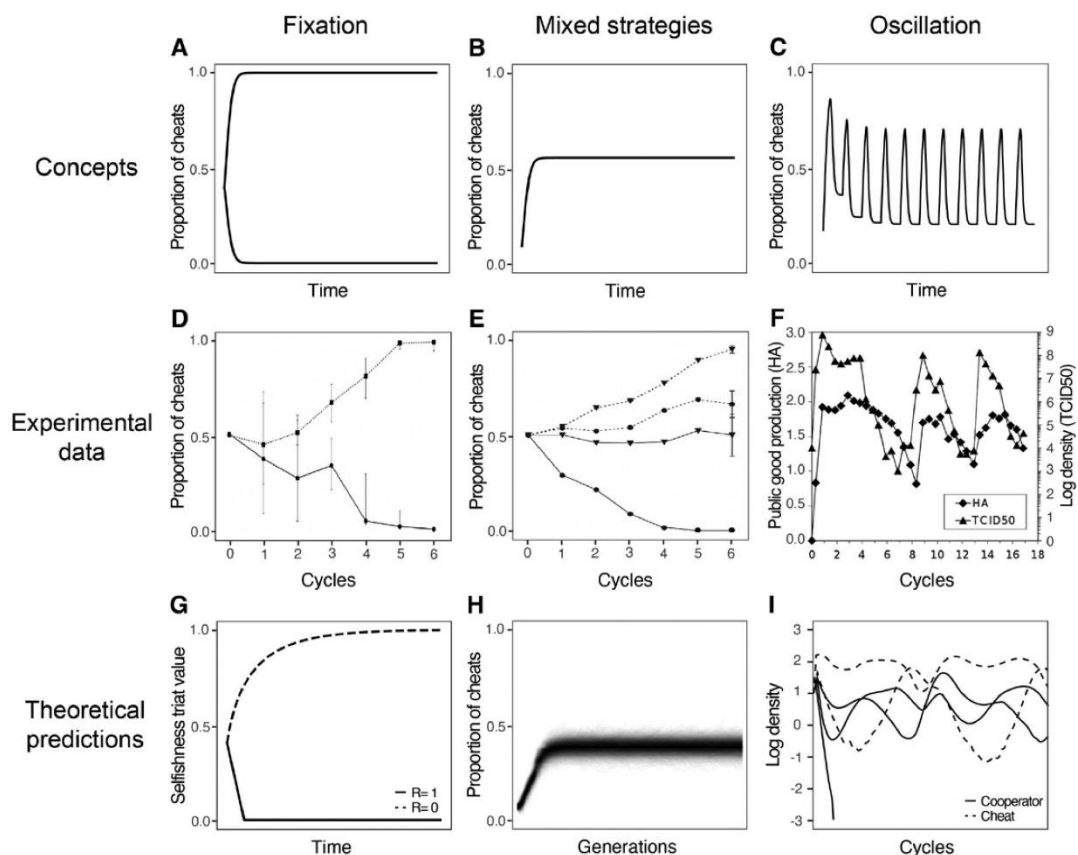


Figure 1. Different types of cheat-cooperator dynamics. (A–C) Conceptual illustrations of different cheat-cooperator dynamics: (A) either cheats or cooperators go to fixation; (B) cooperators and cheats are maintained at an equilibrium proportion in a population (coexistence); (C) oscillations through time. (D–F) Empirical examples of different cheat-cooperator dynamics: (D) proportion of cheats that do not quorum sense in the bacteria *P. aeruginosa* (Rumbaugh et al., 2012); (E) proportion of cheats that does not produce siderophores in the bacteria *P. aeruginosa* (Griffin et al., 2004); (F) production of public goods by influenza virus and its total population density (Frensing et al., 2013). (G–I) Previous theoretical examples of different cheat-cooperator dynamics: (G) Taylor and Frank (1996); (H) Killingback et al. (2010); (I) Mizuuchi et al. (2022).

across different virus species that they have been given a name—the von Magnus effect (Kirkwood & Bangham, 1994; Tapia et al., 2019; von Magnus, 1951; Zwart et al., 2013) (Figure 1F). It is not clear why the dynamics of cheats varies so much across these empirical studies. Why do cheats show stable dynamics in some studies, but oscillate in proportion in others?

The dynamics of cheating is also important for assessing the validity of some intervention strategies for treating parasites. Synthetic viral cheats, termed “therapeutic interfering particles,” are being developed to interfere with and suppress viral infections (Dimmock & Easton, 2014; Leeks et al., 2021; Metzger et al., 2011). Similar strategies have been suggested for bacteria, using cheats to introduce either less virulent strains or medically beneficial alleles such as antibiotic susceptibility into infections (Brown et al., 2009). It has also been shown both theoretically and experimentally that it can be harder for parasites to evolve resistance against intervention strategies that disrupt cooperation, than against treatments such as antibiotic treatments that kill individuals (André & Godelle, 2005; Dieltjens et al., 2020). Disrupting cooperation turns all individuals into cheats, making it hard for a “resistance” mutation that switches cooperation back on to spread (Mellbye & Schuster, 2011). In all of these cases, the

effectiveness of the intervention will depend upon the cheat-cooperator dynamics. For example, if the proportion of cheats can oscillate in ways that allow the cheats to go extinct, then the intervention will be less useful (Rezzoagli et al., 2019).

Theoretical models have made contrasting predictions for the evolutionary dynamics of cheating. Hamilton found that the fitness of cheats did not depend on their proportion in the population, and so cheats would either be disfavored or go to fixation (Figure 1G) (Hamilton, 1964). More recent studies have found that the fitness of cheats can be greater when they are less common (frequency dependence) or when populations are more dense (density dependence) (Gore et al., 2009; MacLean & Gudelj, 2006; Madgwick et al., 2018; Patel et al., 2019; Queller, 1984; Ross-Gillespie et al., 2007; 2009; Scholz & Greenberg, 2015; van Gestel et al., 2014). These processes can result in both cooperators and cheats being maintained at some equilibrium proportion in a population (coexistence; Figure 1H). Other models have predicted nonequilibrium dynamics, in which the frequencies of cheats and cooperators oscillate over time (Figure 1I) (Mizuuchi et al., 2022; Sanchez & Gore, 2013; Weitz et al., 2016). Because many of these models were tailored to specific experimental systems and contained multiple different features, it is not clear why they give rise

to different predictions. Why do we get stable equilibrium with some models, versus oscillations in cheat proportion in others?

We investigate theoretically how different life-history features can interact to produce different forms of cheat-cooperator dynamics. We use a combination of numerical and simulation approaches to examine the influence of four factors that could shape the dynamics of cheating: frequency dependence, density dependence, periodic population bottlenecks, and stochastic group formation. These factors have been suggested to be important in previous theories but never examined together. Our main aims are to (1) understand what maintains oscillations in the proportion of cheats and cooperators, as opposed to a tendency toward equilibrium; (2) use the results of our model to explain variation across previous theoretical and empirical studies; and (3) provide experimental designs for testing the empirical influence of different factors on cheat-cooperator dynamics.

Methods and results

We developed a cheat-cooperator model that allows for several factors that could potentially alter the form of dynamics. We investigated how the proportion of cheat is influenced by frequency and density dependence on cooperators, periodic population bottlenecks, and stochastic group formation. Frequency dependence is when cheats' growth rate depends on the proportion of cooperators within population; density dependence is when it depends on the number of cooperators. By changing the parameters of our model, we could include or remove these different factors, and therefore test their different influences as well as their interactions.

The model

We derived our model from competitive Lotka-Volterra equations where a system of differential equations described the change in population density over infinitesimally small increments of time:

$$\begin{cases} \frac{dN_{co}}{dt} = rN_{co} \left(1 - \frac{N_{co} + \alpha N_{ch}}{K}\right) \\ \frac{dN_{ch}}{dt} = h r N_{ch} \left(1 - \frac{\alpha N_{co} + N_{ch}}{K}\right) \end{cases} \quad (1)$$

where N_{co} and N_{ch} are the density of cooperator and cheats, r is the intrinsic growth rate, α is the relative strength of between-strain population regulation to within-strain regulation, and K is the carrying capacity of the system (Lotka, 1925; Volterra, 1927). Equation 1 is comparable to a population with a limited supply of nutrients that stops growing once the nutrient has been depleted (i.e., the population reaches its carrying capacity). We simplified the interactions between populations so that cooperators and cheats have the same regulation on each other (i.e., $\alpha = 1$). The growth rate of cheats is the same, except for the benefit coefficient of cheating (h). The benefit coefficient rescales the growth rate of cheats: If $h < 1$, cheats can never grow as fast as cooperators; if $h \geq 1$, cheats can grow equally well or better than cooperators.

We then extended equation 1 to examine the consequences of when population density and the frequency of cheats influence the relative fitness of cheats. We assumed:

$$\begin{cases} \frac{dN_{co}}{dt} = rN_{co} \left(1 - \frac{N_{co} + \alpha N_{ch}}{K}\right) \\ \frac{dN_{ch}}{dt} = \left(1 - a + \frac{a}{1 + e^{-s_d(N_{co} - t_d)}}\right) \left(1 - b + \frac{b}{1 + e^{-s_f(N_{co}/(N_{co} + N_{ch}) - t_f)}}\right) h r N_{ch} \left(1 - \frac{\alpha N_{co} + N_{ch}}{K}\right) \end{cases} \quad (2)$$

where two additional terms are multiplied to the rate of changes in the cheat density: the density dependence term in blue texts and the frequency dependence term in orange text. The

cheat density dependence term $\left(1 - a + \frac{a}{1 + e^{-s_d(N_{co} - t_d)}}\right)$ describes how the relative fitness of cheats increases at higher population densities because they are better able to exploit cooperators. For example, when bacteria are at higher densities, cheats will on average be closer to cooperators and hence better able to exploit cooperative behaviors, such as the production of public goods molecules (Kümmerli et al., 2009; Ross-Gillespie et al., 2009). The importance of cheat density dependence is weighted by the parameter a , where $a = 0$ means that there is no density dependence. The shape parameter (s_d) controls the steepness of the density dependence function by changing the function from a binary response (higher s_d) to a gradual transition (lower s_d). The threshold parameter (t_d) controls the location of the density dependence function, where a high threshold means the transition in cheats' growth rate occurs at a higher density of cooperators. This way in which the relative fitness of cheats can vary with density (cheat density dependence) is different and needs to be distinguished from how population growth decreases as the population density approaches the carrying capacity (population density regulation, $1 - \frac{N_{co} + N_{ch}}{K}$). In Supplementary Section 3.1 and Supplementary Figure S3, we provide a graphical explanation of these model parameters.

The cheat frequency dependence term $\left(1 - b + \frac{b}{1 + e^{-s_f(N_{co}/(N_{co} + N_{ch}) - t_f)}}\right)$ describes how the relative fitness of cheats increases when they are at lower frequencies (proportion) in the population because they are better able to exploit cheats. For example, when bacteria or yeast cheats are a lower fraction in the population then they are more likely to be interacting with cooperators (Ross-Gillespie et al., 2007; Gore et al., 2009). The importance of cheat frequency dependence is weighted by the parameter b , where $b = 0$ means that there is no frequency dependence. The shape (s_f) and threshold (t_f) parameters determine the steepness and location of the frequency dependence function.

The focus of our result will be on the effects of weightings of density and frequency dependence (i.e., a and b) because the difference in growth rate between cheats and cooperators is the product of (1) the density dependence term; (2) the frequency dependence term; and (3) the benefit coefficient of cheating (h). As the density and frequency of cooperators can vary through time, the relative benefit of cheating can also have temporal fluctuation. All parameter values are summarized in Supplementary Table S1 and discussed further in Supplementary Information.

Scenario 1: Frequency and density dependence

We started by examining an unstructured and well-mixed (panmictic) population, without population bottlenecks or stochastic group formation. Biologically speaking, scenario 1 is analogous to chemostats in experimental evolution, where abiotic factors are kept constant through time (Gresham & Dunham, 2014; Novick & Szilard, 1950).

Result 1: Density and frequency dependence are insufficient for dynamic oscillations

By independently varying the levels of density ($0 \leq a \leq 1$) and frequency ($0 \leq b \leq 1$) dependence, we found that neither density nor frequency dependence led to dynamic oscillations. Here we are referring to density dependence in terms of how density influences the relative fitness of cheats (equation 2), and not how the overall population density influences the rate of growth towards the carrying capacity (equation 1). In all cases, we found that our

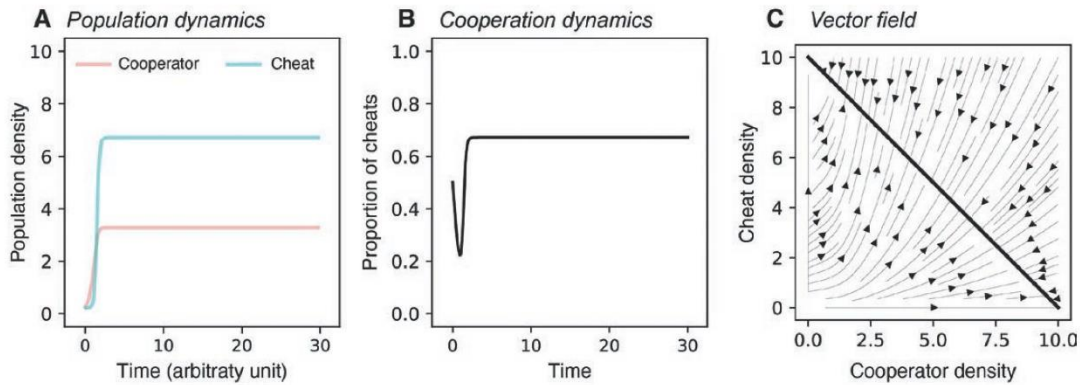


Figure 2. Dynamics of the model in a simple set up. (A) Density of cooperator and cheat through time (i.e., population dynamics; N_{co} and N_{ch}). (B) Proportion of cheats through time (i.e., cooperation dynamics; $N_{ch}/(N_{co} + N_{ch})$). (C) Vector field plot showing the model would have static dynamics once population densities reach carrying capacity (shown in thick black line) ($a = 1$, $b = 0$; other parameter values are listed in Supplementary Table S1).

model approached an equilibrium, with the coexistence of cooperators and cheats at a certain ratio (Figure 2A and B; analytical analysis in Supplementary Section 2.2).

This model reaches equilibria because the population density regulation term, $1 - \frac{N_{co} + N_{ch}}{K}$, leads to the populations of both cooperators and cheats remaining fixed once the carrying capacity is reached ($N_{co} + N_{ch} = K$). We can also examine the dynamics via a vector plot, which shows the direction of changes in population densities from any state (Otto & Day, 2007). In other words, the arrows in a vector plot would indicate how the population dynamic should change in the two-dimension parameter space of the densities of cheats and cooperators. Our vector plot confirms our predictions, by showing that all dynamics converge to the equilibrium point (line) where $N_{co} + N_{ch} = 10$ (Figure 2C; thick black line). The level of frequency dependence (b) can alter the form of dynamics from approach to an equilibrium (Supplementary Section 2.1). These results are consistent with a number of previous theoretical studies have shown how density or frequency dependence can lead to coexistence between cheats and cooperators at some equilibrium frequency (Gore et al., 2009; MacLean & Gudelj, 2006; Patel et al., 2019; Queller, 1984; Ross-Gillespie et al., 2007; 2009).

Scenario 2: Periodic population bottlenecks

We then relaxed the assumption that the population is free from disturbance by assuming it goes through periodic bottlenecks. We dilute the population densities of both cheats and cooperators by a dilution ratio, D , once every T_{grow} time units. This leads to the population dynamics following the algorithm of

$$\begin{cases} \text{if } t < T_{grow} : \text{equation 1, } t = t + dt \\ \text{if } t = T_{grow} : N_{ch} = N_{ch}/D, N_{co} = N_{co}/D, t = 0 \end{cases} \quad (3)$$

where T_{grow} is the duration of each growth period. Biologically speaking, expression 3 is analogous to serial passages, or growth cycles and host-host transmission, in experimental evolution (Gresham & Dunham, 2014; Lenski et al., 1991). Some theoretical studies have discussed the optimal dilution ratio for experimental evolution, but density or frequency dependence was not included (Wahl & Gerrish, 2001; Wahl et al., 2002). Another study has looked into cooperation dynamics under different

bottleneck settings, but without temporal dynamics (Brockhurst et al., 2007).

Result 2: The combination of periodic population bottlenecks and density dependence generates cheat-cooperator oscillations

We found that the combination of periodic population bottlenecks and cheat density dependence could lead to dynamic cycles, where the proportion of cheats and cooperators oscillates over time, rather than approaching an equilibrium (e.g., $a = 1$, $b = 0$, $D = 10$; Figure 3A and B). This combination leads to oscillations because the population bottleneck moves populations from high density, where cheats are increasing in proportion, to low density, where cooperators increase in proportion. It is this change in whether cooperators or cheats are preferentially favored that keeps the oscillations going. After a bottleneck, cooperators are favored and so increase in proportion. Then as the population grows, the relative fitness of cheats increases, until eventually cheats have a higher fitness and then increase in proportion (Supplementary Section 3.5).

In contrast, the combination of population bottlenecks and frequency dependence did not lead to dynamic oscillations (e.g., $a = 0$, $b = 1$; Figure 3C and D). This combination does not lead to oscillations because the population bottleneck did not change whether cheats or cooperators were favored. If cheats are initially introduced at a low proportion, and have a fitness advantage, they will then increase in proportion, causing their relative fitness to decrease. Eventually, the proportion is reached at which the fitness of cheats and cooperators is equal, and so an equilibrium is reached. Population bottlenecks do not change this qualitative pattern because they change density not frequency. Consequently, bottlenecks do not lead to a scenario where cooperators are at an advantage, which would require a change in the proportion of cheats, rather than just the population density.

Additionally, the extent of frequency dependence (b) can interact with the extent of density dependence (a) to determine the amplitude of cyclic oscillations (Figure 3E). The oscillation amplitude is the maximum difference in proportion of cheats once the dynamics have stabilized (the bar next to Figure 3B). The amplitude of oscillation increases with increasing cheat density dependence (colors become brighter as one moves

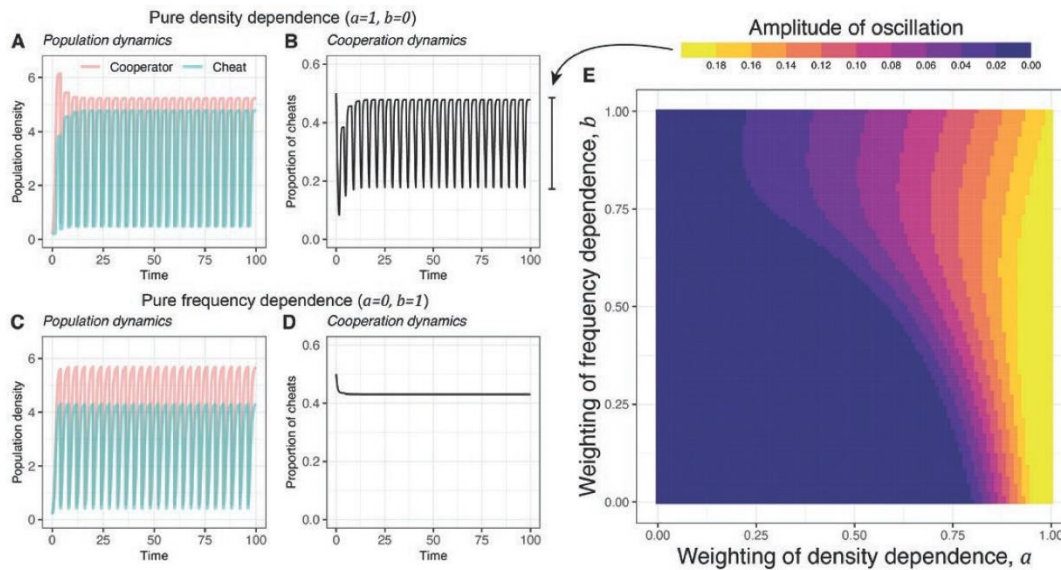


Figure 3. Density dependence ($a > 0$) is crucial for oscillation in frequency dynamics. (A, B) Density and frequency dynamics when density dependence of cheats on cooperators is at the strongest level and frequency dependence is absent ($a = 1, b = 0$). (C, D) Dynamics when density dependence is absent and frequency dependence is strongest ($a = 0, b = 1$). (E) Broader exploration of amplitude in oscillation, which is defined as the maximal difference in proportion of cheats, across all combinations of density and frequency dependence weighting.

from left to right). Frequency dependence has mixed effects on amplitude of oscillation, depending on the weighting of density dependence—increasing oscillation when density dependence is low, but reducing oscillations when density weighting is high ($a \approx 1$). This complex pattern happens because frequency dependence can change the differences in relative fitness of cheats, and if the differences become larger, the amplitude of oscillation can grow.

We examined the effects of all model parameters on the amplitude of oscillation in supplementary information. In Supplementary Sections 3.4 and 3.5, we showed that increasing the benefit of cheating ($h, h > 1$) decreases the range where oscillation happens because cheats can spread even at lower densities. There is no oscillation in cheat proportion when $h = 1$ because cheats can never out-grow cooperators. In Supplementary Section 3.6, we showed that decreasing the dilution ratio (D) reduces the amplitude of oscillation. Supplementary Section 3.7 suggested sufficient growth time (T_{grow}) is needed to generate oscillating dynamics. In Supplementary Section 3.8, we changed the values of shape parameters and found steeper density dependence functions (larger s_d) increase the amplitude of oscillation, while steeper frequency dependence functions (larger s_f) reduce the optimal frequency weighting (b) where oscillation is favored. In Supplementary Section 3.9, a higher threshold of density dependence function (larger t_d) reduces the range of density weighting (a) where cheat proportion oscillates; a higher threshold of frequency dependence function (larger t_f) also reduces the optimal frequency weighting (b) for oscillation. Lastly, we showed our setting of ODE solver and intrinsic growth rate is robust against periodic population bottlenecks (Supplementary Sections 3.2 and 3.3).

Scenario 3: Stochastic group formation

Finally, we relaxed the assumption of unstructured and panmictic populations. We examined the stochastic consequences of

small populations or incomplete population mixing processes (Constable et al., 2016; Hilbe et al., 2018; Mizuuchi et al., 2022). We could not examine the role of stochasticity with our nonlinear deterministic population dynamical model (equation 2), and so we instead developed an individual-based simulation. We modeled a population growing at the unit of subpopulations, where each subpopulation is partially mixed before going through periodic population bottleneck, following Mizuuchi et al. (2022). However, we simplified their system to only two possible strains (one cooperator strain and one cheat strain) and replaced the ordinary differential equations with equation 2 to model population growth of cooperators and cheats with density and/or frequency-dependent benefits.

Our simulation divides the population into M subpopulations (social groups), where the interactions between cheats and cooperators take place. The life cycle then involves two phases. First, a growth phase in the subpopulations. Second, a phase of subpopulation mixing, before samples (bottlenecks) are taken to initiate the next round of subpopulation growth. Population mixing is only partial between subpopulations, with each new subpopulation being formed from a sample of just two subpopulations, rather than all subpopulations. This design helps slow down the spread of cheats or cooperators. We repeated the random sampling of two subpopulations and creation of two new subpopulations for $F \times M$ times to start the next growth cycle, where F is the mixing coefficient (i.e., larger F means more mixing process and closer to global mixing). Following the experimental protocols and algorithms by Mizuuchi et al. (2022), we converted the population densities to integers when they were sampled, summed, and after diluted. This process increases stochasticity in finite populations and has been found to help generate oscillating dynamics (Mizuuchi et al., 2022). A full list of parameters is listed in Supplementary Table S1 and the simulation process is illustrated in Supplementary Section 4.1.

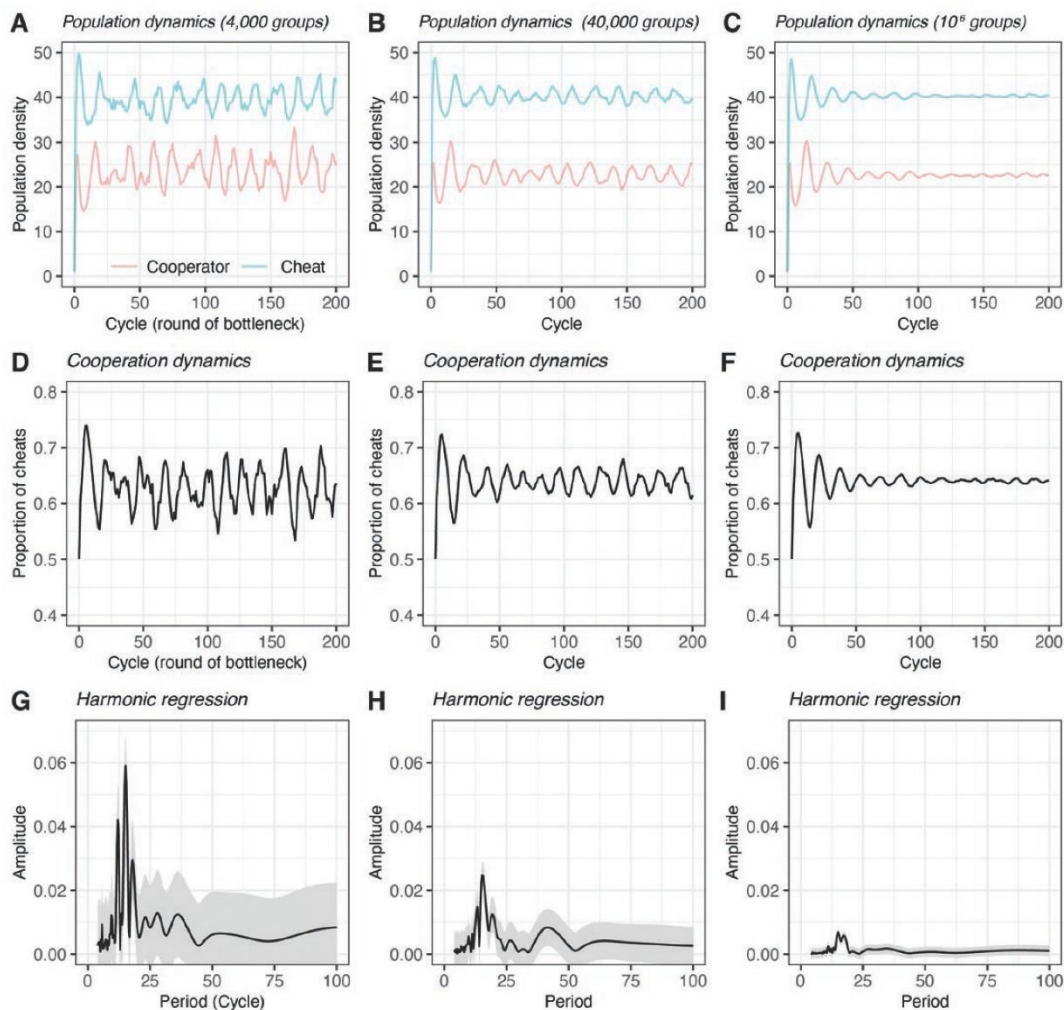


Figure 4. Stochasticity creates an additional layer of oscillation across growth cycles. (A–C) Population dynamics at various degree of stochasticities, where increasing number of subpopulations results in an increased global population size, thus decreasing stochasticity (because the carrying capacity of each subpopulation is the same). (D–F) Cooperation dynamics of the density time series in (A–C). (G–I) Harmonic regression analyzed the time series of cheat frequencies from cycle 51 to cycle 200 and showed the estimated amplitude at each period with confidence intervals. Periodicity of cheat frequencies increases if certain periods have much higher amplitude than other periods (e.g., G and H compared to I). The results presented here are density-dependence-only model, where $a = 1$ and $b = 0$.

Our simulation allows us to investigate whether stochasticity influences cheat-cooperator dynamics by varying the number of subpopulations (M), the carrying capacity of each subpopulation (K), and the mixing coefficient (F). These three parameters have experimental analogues and can change stochasticity in different ways. Increasing the number of subpopulations (M) increases the size of the entire population, thus increases the sample size of random sampling and initializing new subpopulations, and decreases stochasticity. Increasing carrying capacity (K) increases the number of individuals available to be distributed into new subpopulations, thus decreases the stochasticity in each process. Increasing the mixing coefficient (F) can reduce stochasticity by making each subpopulation more similar to each other, but increased mixing also disrupts the population structure and facilitates the spread of cheats or cooperators to other groups.

Result 3: Stochasticity can also generate cheat-cooperator oscillations

We found that stochasticity, combined with density dependence and population bottlenecks, can lead to oscillations in the proportion of cheats across cyclic population bottlenecks (e.g., $a = 1$, $b = 0$; Figure 4A and D). This oscillation is different from result 2, where the oscillation has identical dynamics in each growth cycle; instead, the oscillation here occurs across (rather than within) bottlenecks and is somewhat noisier (i.e., Figure 4 quantifies time at the unit of growth cycle, where each growth cycle lasts T_{grow}). We quantified the cyclic nature of these oscillations with harmonic regression (Halberg et al., 1967; Young et al., 1999), which examines the extent to which sine waves can explain the observed time series data. The results showed a peak of amplitudes at 10–20 growth cycles (Figure 4G), meaning that

cheat proportion roughly repeats the same oscillating dynamic every 10–20 growth cycles under the focal parameter setting. In addition, we investigated the role of stochasticity by examining how these oscillating dynamics disappear as stochasticity is reduced. The cyclic oscillation becomes smaller with intermediate number of subpopulations (Figure 4B and E) and quickly attenuated in large number of subpopulations (Figure 4C and F). Harmonic regression further showed that the size of peak was smaller when there were more subpopulations (Figure 4G–I). This pattern occurs because increasing sample size results in a less stochastic distribution of cheat proportion across all subpopulations, as suggested by central limit theorem. Similarly, by increasing the number of individuals in each subpopulation, we found increasing carrying capacity (K) also produces smaller oscillation in Supplementary Section 4.2.

In order to understand the cause of oscillating dynamics in cheat proportion in scenario 3, we continued our analysis to a broader scale of density and frequency dependence. We analyzed three properties: the amplitude of oscillation in cheat proportion, where larger values mean the cooperation dynamics are more variable or periodic (Figure 5A); cheat's average relative fitness, where values greater than 1 indicate cheats are fitter than cooperators (Supplementary Section 4.3 and Supplementary Figure S17); and the coefficient of variation, where larger values suggest relative fitness is more variable between subpopulations (Figure 5B). These factors are potentially important because they inform us about the prevalence of oscillations, and the fitness landscape across different scenarios. We found strong density dependence and weak-to-moderate frequency dependence can generate oscillating dynamics ($a > 0.8$, $b < 0.7$). Under these combinations of density and frequency dependence, cheats have relative fitness between 1 and 1.5, which means cheats can slightly outcompete cooperators on average. Furthermore, these

combinations produce the highest coefficient of variation across the entire parameter space. This finding coincides with previous work on synthetic microbial system, where large variation was generated through strong population bottlenecks (Chuang et al., 2009). In short, we found that oscillating dynamics in cheat proportion is associated with small fitness advantage of cheats over cooperators, and high variation of fitness between subpopulations.

Why does stochasticity lead to oscillations? We found that oscillations occurred when there is greater variations in the fitness of cheats, such that cheats have greater fitness than cooperators in some patches but lower in others (Figure 5B). We hypothesized that this fitness variation can lead to cycles, and not just noise, because limited mixing between groups delays the rates where a group can reach an equilibrium proportion of cheats. We tested this hypothesis by changing the degree of mixing between groups. In support of our hypothesis, we found that greater mixing produces a less periodic dynamic—as the mixing coefficient (F) increases, the peak signal in harmonic regression becomes smaller, while the nonpeak signals become larger (Supplementary Section 4.4 and Supplementary Figure S18, but see Supplementary Section 4.5 for a special exception).

Discussion

Our model predicted three different forms of cheat-cooperator dynamics: approach to a stable proportion of cheats (Figure 2); cyclic oscillations of the proportion cheats within growth cycles (Figure 3); and cyclic oscillations of the proportion cheats across growth cycles (Figure 4). A combination of both periodic population bottlenecks (growth cycles or serial passages) and density dependence was required to produce cyclic oscillations which repeat each growth cycles (Figure 3E). The stochasticity

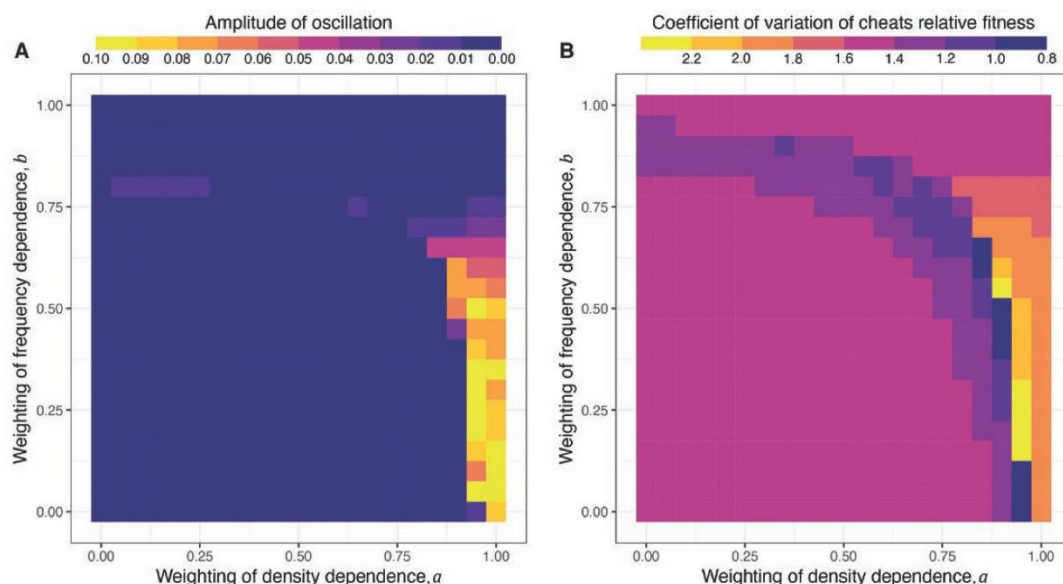


Figure 5. The impacts of density and frequency dependence on oscillations across growth cycles and fitness landscape in scenario 3. (A) Amplitude of oscillation is calculated from the maximal signal of cheat proportion after discrete Fourier transformation. Brighter colors represent greater oscillation. (B) Coefficient of variation of relative fitness of cheats is calculated from the relative fitness of all subpopulations, using standard deviation divided by average, for each growth cycle. In all panels, each grid is the average of 10 repeated simulations. All simulations last 200 growth cycles and data was collected from cycle 51 to cycle 200. The number of subpopulations is set to 4,000.

Table 1. The list of published theory papers.

Paper	Focus	Oscillate?	Experiment?	Equivalent
Eco-evolutionary models				
Sanchez and Gore (2013)	Cooperation dynamics	Yes (within cycle)	Yes	Density dependence + growth cycle
Yurtsev et al. (2013)	Cooperation dynamics under antibiotic stress	Yes (within cycle)	Yes	Density dependence + growth cycle
Vetsigian (2017)	Coexistence of strains with different toxin production and resistance	Yes	No	Density dependence + growth cycle + stochasticity
Mizuuchi et al. (2022)	Cooperation dynamics	Yes	Yes	Density dependence + growth cycle + stochasticity
Replicator dynamics				
Brown and Taddei (2007)	Cooperation dynamics with durable public goods	Yes	No	N/A
Weitz et al. (2016)	Cooperation dynamics with game–environment feedback	Yes	No	N/A

introduced by small populations or small number of subpopulations was required to produce oscillations in the proportion of cheats across growth cycles (Figure 4 and Supplementary Section S16).

Our model can explain the variation in predictions between previous theoretical models (Table 1). First, comparing with our scenario 1, models that assumed frequency or density dependence, but without periodic population bottlenecks, predicted evolution toward an equilibrium proportion of cheats (Brown et al., 2009; Patel et al., 2019; Ross-Gillespie et al., 2007, 2009). Second, comparing with our scenario 2, models that assumed both periodic population growth, and cheat density dependence, predicted oscillations within growth cycles (Sanchez & Gore, 2013; Yurtsev et al., 2013). Third, comparing with our scenario 3, models that allowed for stochasticity, as well as periodic growth cycles and cheat density dependence, led to oscillations across growth cycles (Mizuuchi et al., 2022; Vetsigian, 2017). Brown and Taddei (2007) found a different mechanism of oscillation through durable public goods which remains exploitable for several generations. Because the dynamics of public goods is slower than cheat proportion, there is a delay in the consequence of current players' strategies, which results in cyclic dynamics. Similarly, Weitz et al. (2016) predict oscillations for another different reason. In their model, cheats initially have an advantage, but degrade the environment, till a switch point is reached at which point cooperators have an advantage. As cooperators become more common, the environment improves, until the switch at which cheats are again favored. This leads to oscillations because a switch point is assumed, which swaps which strategy has an advantage—as opposed to the more gradual functions in our scenario 1, which allowed an equilibrium to be reached.

Our model can help explain the variation in cheat-cooperator dynamics that have been observed across previous empirical studies. First, oscillations have been observed in a long-term experimental evolution study of synthesized cooperative and cheat (defective) RNA replicators (Furubayashi et al., 2020; Mizuuchi et al., 2022). This system appears to fit the assumptions of our scenario 3, where we would predict oscillating dynamics across periodic growth cycles. In their experiment, a periodic population bottleneck was imposed, both types of replicators can reproduce during coinfection, and the limiting factor for reproduction is the population size of host bacterial cells. Second, oscillations between cooperators and cheats (defective interfering particles) are common when culturing viruses (Frensing et al., 2013; Grabau & Holland, 1982; Huang, 1973; Palma & Huang,

1974; Roux et al., 1991; Tapia et al., 2019; von Magnus, 1951). This culturing of viruses involves growth cycles with cheat density dependence, and so is analogous to our scenario 2. Several theoretical models have examined these dynamics within predator–prey framework (Bangham & Kirkwood, 1990; Frank, 2000; Heldt et al., 2013; Kirkwood & Bangham, 1994; Shirogane et al., 2021). Predator–prey equations approximate defective interfering particles because there can be a very high benefit to cheating (high h), such that cheats can effectively eliminate (eat) cooperators when they coinfect a cell (Leeks et al., 2021). These models provide another mechanism for oscillating dynamics under a more specific biological setting. Third, oscillations of cheat proportion have also been observed in natural populations of the bacteria *Bacillus thuringiensis*, where the life cycle is consistent with our scenario 2: There is appreciable density dependence and winter imposes a periodic population bottleneck each year (Raymond et al., 2012).

There are also several experimental evolution studies in bacteria and viruses, where cyclical oscillations were not observed (Diggle et al., 2007; Frost et al., 2018; Griffin et al., 2004; Meir et al., 2020; Özkaya et al., 2018; Pollitt et al., 2014; Rumbaugh et al., 2012; Turner & Chao, 1999). The dynamics by which stable equilibria were reached in those cases suggest that cheat density dependence was not strong enough to change which strategy had the higher fitness, after the periodic bottleneck. One possible explanation for this is that the strength of density dependence can be lower in the well-mixed liquid populations that are routinely used in such experiments (Kümmerli et al., 2009; Ross-Gillespie et al., 2009). This and other possibilities could be tested experimentally (see below). Even with periodic population bottlenecks and cheat density dependence, our model only predicts cyclic oscillations under certain parameter conditions—not at all times.

Our model suggests numerous experimental designs that could be used to test how different factors influence cheat-cooperator dynamics. Considering oscillations within growth cycles, the degree of population bottleneck or strength of density dependence could be reduced, to test whether this eliminates cycles (Figure 6A–E). For example, by diluting populations less each growth cycle, or by adding less nutrients, to restrict growth to low population densities. Another possibility is to parametrize the strength of density dependence, to test whether it is low or lower in studies where cyclic oscillations have not been observed. An even more extreme manipulation would be to compare populations in chemostats, to populations with periodic growth

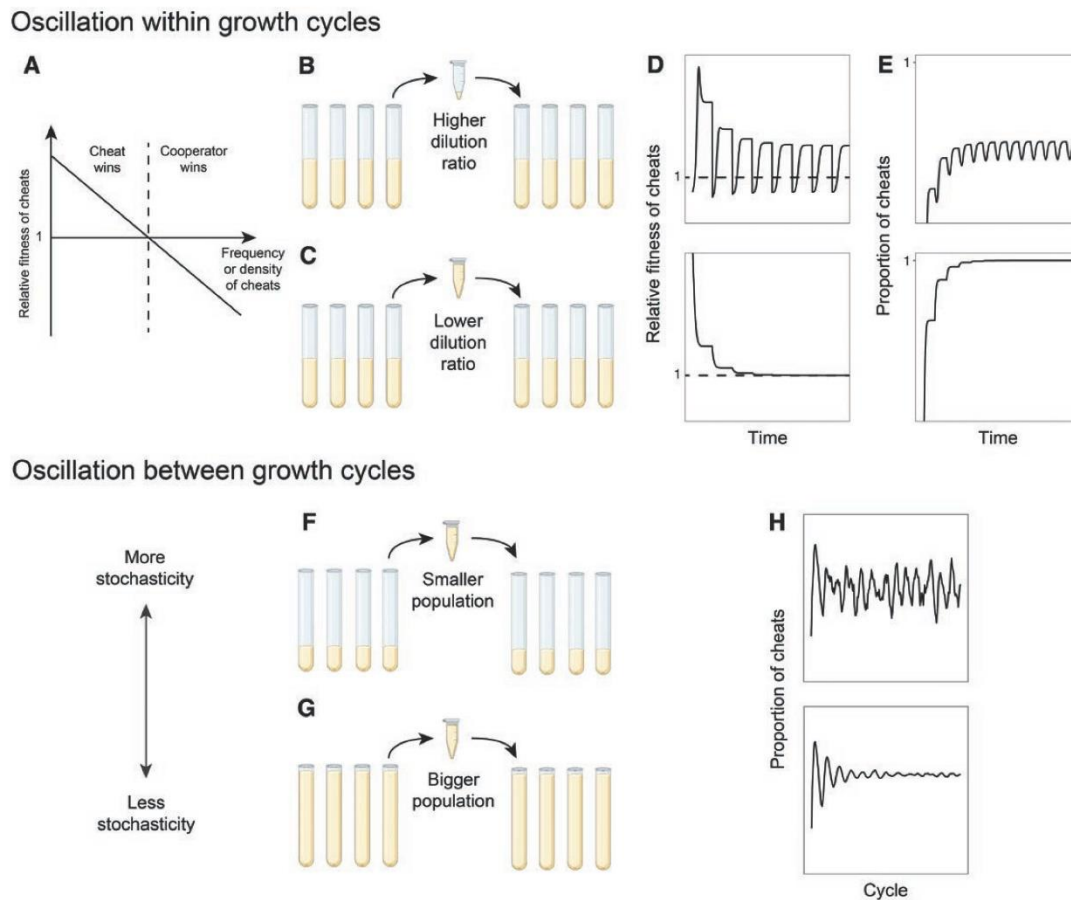


Figure 6. Possible experimental designs for testing the role of different factors in leading to oscillations. (A–E) Changing dilution ratio may create chances for relative fitness of cheats to cross horizontal line of equal fitness (e.g., dashed line in D), and let the direction of selection alternate within each growth cycle. The direction of selection would only change when the relative fitness of cheats crosses unity (dashed line), so that selection sometimes favors cooperators and other times favor cheats. (F–H) Increasing population size could result in a decrease in stochasticity and stop oscillation. Created with BioRender.com.

cycles. As for oscillation across periodic growth cycles, the degree of stochasticity could be reduced to examine whether it eliminates oscillations (Figure 6F–H). For instance, by increasing the total volume of medium, or by providing more nutrients, to let the population grow to a larger size. Our results predict oscillation would diminish when stochasticity is lower. Nevertheless, our plan would require enough data points to allow time series tools (e.g., harmonic regression, discrete Fourier transformation, and equivalent autoregressive tools) to distinguish periodic oscillation from pure noise. Our model could also be applied to data from macro-organisms, which experience growth cycles through seasonality.

The theoretical framework developed in this article is flexible and can be easily extended to consider other biological scenarios. For instance, spatial structure can lead to the coexistence of cheats and cooperator (Nowak & May, 1992)—this could be incorporated by assuming explicit spatial arrangements between each patch or subpopulation (with social groups located next to one another). Spatial structure could help generate oscillations because individuals cannot disperse to distant patches, and so some cooperators will be less likely to encounter and hence be exploited by cheats. This is analogous

to the stochastic scenario that we investigated in our individual-based simulation (Figures 4 and 5 and Supplementary Figure 18). Spatial structure provides an even more extreme case of low mixing. Another possibility is temporal variation in environmental quality, which can be implemented by varying carrying capacity through time. One benefit of using the current framework is that competitive Lotka–Volterra equations relax the assumptions of constant population size and constant degree of generation overlap. Relaxing these two assumptions has been found to support the selection of specialist against generalist (Gilchrist, 1995; Liu et al., 2019) and the coexistence between competing strains (Huang et al., 2015; Liu et al., 2021). Nevertheless, competitive Lotka–Volterra equations always predict coexistence of strategies when there is no external disturbance, such as noise, growth cycles, or temporal change (scenario 1). Future work could examine scenarios where this is not the case.

Our model could also be modified to examine other types of interaction (Jones et al., 2009; Lion, 2018). We examined a relatively simple cheat–cooperator system. One possible extension is to consider symbiotic or pathogenic bacteria, where being a cheat or cooperator has a large influence on the interaction with

their host and could therefore even alter the host dynamics. This could be examined with a three-level host-cooperator-cheat model. Another possibility is multipartite viruses, where the genome is split into different segments, which can be transmitted separately, and where the different segments can be complementary or mutually dependent (Leeks et al., 2021, 2023; Lowen, 2018). The stability of this interaction could be compromised by oscillations in frequency, and so it would be extremely useful to examine what conditions prevent oscillations. Still, another possibility is to consider the coevolution of multiple social traits, such as rock-paper-scissors dynamics (Inglis et al., 2016; Kerr et al., 2002). In all these cases, it would be useful to examine the effects of population bottlenecks in structured or unstructured population.

Supplementary material

Supplementary material is available online at *Evolution Letters*.

Data availability

All codes and data of this study are available at: https://github.com/mingpapi/Codes_Oscillation.

Author contribution

M.L., G.W., and S.A.W. conceptualized the project together. G.W. did the analytical solutions; M.L. made the numerical and simulation investigation. M.L. and S.A.W. wrote the first draft; M.L., G.W., and S.A.W. reviewed and edited the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgments

We thank Matishalin Patel, Asher Leeks, Thomas Scott, Laurence Belcher, George Shillcock, and the rest of the social evolution group for their helpful feedback. S.W. and M.L. were supported by European Research Council (Horizon 2020 Advanced Grant 834164), M.L. was supported by Oxford-Taiwan Graduate Scholarship, and G.W. was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada (RGPIN-2019-06626).

References

- Andersen, S. B., Marvig, R. L., Molin, S., Krogh Johansen, H., & Griffin, A. S. (2015). Long-term social dynamics drive loss of function in pathogenic bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, **112**(34), 10756–10761. <https://doi.org/10.1073/pnas.1508324112>
- André, J. -B., & Godelle, B. (2005). Multicellular organization in bacteria as a target for drug therapy. *Ecology Letters*, **8**, 800–810.
- Bangham, C. M., & Kirkwood, T. B. L. (1990). Defective interfering particles: Effects in modulating virus growth and persistence. *Virology*, **179**, 821–826.
- Brockhurst, M. A., Buckling, A., & Gardner, A. (2007). Cooperation peaks at intermediate disturbance. *Current Biology*, **17**(9), 761–765. <https://doi.org/10.1016/j.cub.2007.02.057>
- Brown, S. P., & Taddei, F. (2007). The durability of public goods changes the dynamics and nature of social dilemmas. *PLoS One*, **2**(7), e593. <https://doi.org/10.1371/journal.pone.0000593>
- Brown, S. P., West, S. A., Diggle, S. P., & Griffin, A. S. (2009). Social evolution in micro-organisms and a Trojan horse approach to medical intervention strategies. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**(1533), 3157–3168. <https://doi.org/10.1098/rstb.2009.0055>
- Chuang, J. S., Rivoire, O., & Leibler, S. (2009). Simpson's paradox in a synthetic microbial system. *Science*, **323**(5911), 272–275. <https://doi.org/10.1126/science.1166739>
- Constable, G. W. A., Rogers, T., McKane, A. J., & Tarnita, C. E. (2016). Demographic noise can reverse the direction of deterministic selection. *Proceedings of the National Academy of Sciences of the United States of America*, **113**(32), E4745–E4754. <https://doi.org/10.1073/pnas.1603693113>
- Davies, N. (2015). *Cuckoo: Cheating by nature*. Bloomsbury Publishing.
- Dieltjens, L., Appermans, K., Lissens, M., Lories, B., Kim, W., Van der Eycken, E. V., Foster, K. R., & Steenackers, H. P. (2020). Inhibiting bacterial cooperation is an evolutionarily robust anti-bio-film strategy. *Nature Communications*, **11**(1), 107. <https://doi.org/10.1038/s41467-019-13660-x>
- Diggle, S. P., Griffin, A. S., Campbell, G. S., & West, S. A. (2007). Cooperation and conflict in quorum-sensing bacterial populations. *Nature*, **450**(7168), 411–414. <https://doi.org/10.1038/nature06279>
- Dimmock, N. J., & Easton, A. J. (2014). Defective interfering influenza virus RNAs: Time to reevaluate their clinical potential as broad-spectrum antivirals? *Journal of Virology*, **88**(10), 5217–5227. <https://doi.org/10.1128/JVI.03193-13>
- Frank, S. A. (2000). Within-host spatial dynamics of viruses and defective interfering particles. *Journal of Theoretical Biology*, **206**(2), 279–290. <https://doi.org/10.1006/jtbi.2000.2120>
- Freising, T., Heldt, F. S., Pflugmacher, A., Behrendt, I., Jordan, I., Flockerzi, D., Genzel, Y., & Reichl, U. (2013). Continuous influenza virus production in cell culture shows a periodic accumulation of defective interfering particles. *PLoS One*, **8**(9), e72288. <https://doi.org/10.1371/journal.pone.0072288>
- Frost, I., Smith, W. P. J., Mitri, S., Millan, A. S., Davit, Y., Osborne, J. M., Pitt-Francis, J. M., MacLean, R. C., & Foster, K. R. (2018). Cooperation, competition and antibiotic resistance in bacterial colonies. *The ISME Journal*, **12**(6), 1582–1593. <https://doi.org/10.1038/s41396-018-0090-4>
- Furubayashi, T., Ueda, K., Bansho, Y., Motooka, D., Nakamura, S., Mizuuchi, R., & Ichihashi, N. (2020). Emergence and diversification of a host-parasite RNA ecosystem through Darwinian evolution. *eLife*, **9**, e56038. <https://doi.org/10.7554/eLife.56038>
- Ghoul, M., Griffin, A. S., & West, S. A. (2014). Toward an evolutionary definition of cheating. *Evolution*, **68**(2), 318–331. <https://doi.org/10.1111/evo.12266>
- Ghoul, M., & Mitri, S. (2016). The ecology and evolution of microbial competition. *Trends in Microbiology*, **24**(10), 833–845. <https://doi.org/10.1016/j.tim.2016.06.011>
- Gilchrist, G. W. (1995). Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *American Naturalist*, **146**(2), 252–270. <https://doi.org/10.1086/285797>
- Gore, J., Youk, H., & van Oudenaarden, A. (2009). Snowdrift game dynamics and facultative cheating in yeast. *Nature*, **459**(7244), 253–256. <https://doi.org/10.1038/nature07921>
- Grabau, E. A., & Holland, J. J. (1982). Analysis of viral and defective-interfering nucleocapsids in acute and persistent infection by rhabdoviruses. *Journal of General Virology*, **60**(Pt. 1), 87–97. <https://doi.org/10.1099/0022-1317-60-1-87>
- Gresham, D., & Dunham, M. J. (2014). The enduring utility of continuous culturing in experimental evolution. *Genomics*, **104**(6 Pt A), 399–405. <https://doi.org/10.1016/j.ygeno.2014.09.015>

- Griffin, A. S., West, S. A., & Buckling, A. (2004). Cooperation and competition in pathogenic bacteria. *Nature*, **430**(7003), 1024–1027. <https://doi.org/10.1038/nature02744>
- Halberg, F., Tong, Y. L., & Johnson, E. A. (1967). Circadian system phase—An aspect of temporal morphology; procedures and illustrative examples. In H. von Mayersbach (Ed.), *The Cellular Aspects of Biorhythms: Symposium on Rhythmic Research* Sponsored by the VIIIth International Congress of Anatomy Wiesbaden, 8–14 August 1965 (pp. 20–48). Springer.
- Hamilton, W. D. (1964). The genetical evolution of social behaviour. *Journal of Theoretical Biology*, **7**(1), 1–16. [https://doi.org/10.1016/0022-5193\(64\)90038-4](https://doi.org/10.1016/0022-5193(64)90038-4); 17–52. [https://doi.org/10.1016/0022-5193\(64\)90039-6](https://doi.org/10.1016/0022-5193(64)90039-6).
- He, L., Wang, Q., Gu, Z., Liao, Q., Palukaitis, P., & Du, Z. (2019). A conserved RNA structure is essential for a satellite RNA-mediated inhibition of helper virus accumulation. *Nucleic Acids Research*, **47**(15), 8255–8271. <https://doi.org/10.1093/nar/gkz564>
- Heldt, F. S., Frensing, T., Pflugmacher, A., Gröpler, R., Peschel, B., & Reichl, U. (2013). Multiscale modeling of influenza A virus infection supports the development of direct-acting antivirals. *PLoS Computational Biology*, **9**(11), e1003372. <https://doi.org/10.1371/journal.pcbi.1003372>
- Hilbe, C., Šimsa, S., Chatterjee, K., & Nowak, M. A. (2018). Evolution of cooperation in stochastic games. *Nature*, **559**, 246–249.
- Huang, A. S. (1973). Defective interfering viruses. *Annual Review of Microbiology*, **27**, 101–117. <https://doi.org/10.1146/annurev.mi.27.100173.000533>
- Huang, W., Hauert, C., & Traulsen, A. (2015). Stochastic game dynamics under demographic fluctuations. *Proceedings of the National Academy of Sciences of the United States of America*, **112**(29), 9064–9069. <https://doi.org/10.1073/pnas.1418745112>
- Hughes, W. O. H., & Boomsma, J. J. (2008). Genetic royal cheats in leaf-cutting ant societies. *Proceedings of the National Academy of Sciences of the United States of America*, **105**(13), 5150–5153. <https://doi.org/10.1073/pnas.0710262105>
- Inglis, R. F., Biernaskie, J. M., Gardner, A., & Kümmerli, R. (2016). Presence of a loner strain maintains cooperation and diversity in well-mixed bacterial communities. *Proceedings of the Royal Society, Series B: Biological Sciences*, **283**(1822), 20152682. <https://doi.org/10.1098/rspb.2015.2682>
- Jaenike, J. (2001). Sex chromosome meiotic drive. *Annual Review of Ecology and Systematics*, **32**(1), 25–49. <https://doi.org/10.1146/annurev.ecolsys.32.081501.113958>
- Jones, E. I., Ferrière, R., & Bronstein, J. L. (2009). Eco-evolutionary dynamics of mutualists and exploiters. *American Naturalist*, **174**(6), 780–794. <https://doi.org/10.1086/647971>
- Kerr, B., Riley, M. A., Feldman, M. W., & Bohannan, B. J. M. (2002). Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature*, **418**(6894), 171–174. <https://doi.org/10.1038/nature00823>
- Killingback, T., Doebeli, M., & Hauert, C. (2010). Diversity of cooperation in the tragedy of the commons. *Biological Theory*, **5**(1), 3–6. https://doi.org/10.1162/biot_a_00019
- Kirkwood, T. B., & Bangham, C. R. (1994). Cycles, chaos, and evolution in virus cultures: A model of defective interfering particles. *Proceedings of the National Academy of Sciences of the United States of America*, **91**(18), 8685–8689. <https://doi.org/10.1073/pnas.91.18.8685>
- Kümmerli, R., Griffin, A. S., West, S. A., Buckling, A., & Harrison, F. (2009). Viscous medium promotes cooperation in the pathogenic bacterium *Pseudomonas aeruginosa*. *Proceedings of the Royal Society, Series B: Biological Sciences*, **276**(1672), 3531–3538. <https://doi.org/10.1098/rspb.2009.0861>
- Kümmerli, R., Santorelli, L. A., Granato, E. T., Dumas, Z., Dobay, A., Griffin, A. S., & West, S. A. (2015). Co-evolutionary dynamics between public good producers and cheats in the bacterium *Pseudomonas aeruginosa*. *Journal of Evolutionary Biology*, **28**(12), 2264–2274. <https://doi.org/10.1111/jeb.12751>
- Leeks, A., West, S. A., & Ghoul, M. (2021). The evolution of cheating in viruses. *Nature Communications*, **12**(1), 6928. <https://doi.org/10.1038/s41467-021-27293-6>
- Leeks, A., Young, P. G., Turner, P. E., Wild, G., & West, S. A. (2023). Cheating leads to the evolution of multipartite viruses. *PLoS Biology*, **21**(4), e3002092. <https://doi.org/10.1371/journal.pbio.3002092>
- Lenski, R. E., Rose, M. R., Simpson, S. C., & Tadler, S. C. (1991). Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *American Naturalist*, **138**, 1315–1341.
- Lion, S. (2018). Theoretical approaches in evolutionary ecology: Environmental feedback as a unifying perspective. *American Naturalist*, **191**(1), 21–44. <https://doi.org/10.1086/694865>
- Liu, M., Rubenstein, D. R., Cheong, S. A., & Shen, S.-F. (2021). Antagonistic effects of long- and short-term environmental variation on species coexistence. *Proceedings of the Royal Society, Series B: Biological Sciences*, **288**(1958), 20211491. <https://doi.org/10.1098/rspb.2021.1491>
- Liu, M., Rubenstein, D. R., Liu, W.-C., & Shen, S.-F. (2019). A continuum of biological adaptations to environmental fluctuation. *Proceedings of the Royal Society, Series B: Biological Sciences*, **286**(1912), 20191623. <https://doi.org/10.1098/rspb.2019.1623>
- Lotka, A. J. (1925). *Elements of physical biology*. Williams & Wilkins.
- Lowen, A. C. (2018). It's in the mix: Reassortment of segmented viral genomes. *PLoS Pathogens*, **14**(9), e1007200. <https://doi.org/10.1371/journal.ppat.1007200>
- MacLean, R. C., & Gudelj, I. (2006). Resource competition and social conflict in experimental populations of yeast. *Nature*, **441**(7092), 498–501. <https://doi.org/10.1038/nature04624>
- Madgwick, P. G., Stewart, B., Belcher, L. J., Thompson, C. R. L., & Wolf, J. B. (2018). Strategic investment explains patterns of cooperation and cheating in a microbe. *Proceedings of the National Academy of Sciences of the United States of America*, **115**(21), E4823–E4832. <https://doi.org/10.1073/pnas.1716087115>
- Meir, M., Harel, N., Miller, D., Gelbart, M., Eldar, A., Gophna, U., & Stern, A. (2020). Competition between social cheater viruses is driven by mechanistically different cheating strategies. *Science Advances*, **6**(34), eabb7990. <https://doi.org/10.1126/sciadv.abb7990>
- Mellbye, B., & Schuster, M. (2011). The sociomicrobiology of antiviral drug resistance: A proof of concept. *mBio*, **2**(5), 1. <https://doi.org/10.1128/mbio.00131-00111>
- Metzger, V. T., Lloyd-Smith, J. O., & Weinberger, L. S. (2011). Autonomous targeting of infectious superspreaders using engineered transmissible therapies. *PLoS Computational Biology*, **7**(3), e1002015. <https://doi.org/10.1371/journal.pcbi.1002015>
- Mizuuchi, R., Furubayashi, T., & Ichihashi, N. (2022). Evolutionary transition from a single RNA replicator to a multiple replicator network. *Nature Communications*, **13**(1), 1460. <https://doi.org/10.1038/s41467-022-29113-x>
- Novick, A., & Szilard, L. (1950). Description of the chemostat. *Science*, **112**(2920), 715–716. <https://doi.org/10.1126/science.112.2920.715>
- Nowak, M. A., & May, R. M. (1992). Evolutionary games and spatial chaos. *Nature*, **359**(6398), 826–829. <https://doi.org/10.1038/359826a0>

- Otto, S. P., & Day, T. (2007). *A biologist's guide to mathematical modeling in ecology and evolution*. Princeton University Press.
- Özkaya, O., Balbontin, R., Gordo, I., & Xavier, K. B. (2018). Cheating on cheaters stabilizes cooperation in *Pseudomonas aeruginosa*. *Current Biology*, **28**(13), 2070–2080.e6. <https://doi.org/10.1016/j.cub.2018.04.093>
- Palma, E. L., & Huang, A. (1974). Cyclic production of vesicular stomatitis virus caused by defective interfering particles. *The Journal of Infectious Diseases*, **129**(4), 402–410. <https://doi.org/10.1093/infdis/129.4.402>
- Patel, M., Raymond, B., Bonsall, M. B., & West, S. A. (2019). Crystal toxins and the volunteer's dilemma in bacteria. *Journal of Evolutionary Biology*, **32**(4), 310–319. <https://doi.org/10.1111/jeb.13415>
- Pollitt, E. J. G., West, S. A., Crusz, S. A., Burton-Chellew, M. N., & Diggle, S. P. (2014). Cooperation, quorum sensing, and evolution of virulence in *Staphylococcus aureus*. *Infection and Immunity*, **82**(3), 1045–1051. <https://doi.org/10.1128/IAI.01216-13>
- Queller, D. C. (1984). Kin selection and frequency dependence: A game theoretic approach. *Biological Journal of the Linnean Society*, **23**(2–3), 133–143. <https://doi.org/10.1111/j.1095-8312.1984.tb00133.x>
- Raymond, B., West, S. A., Griffin, A. S., & Bonsall, M. B. (2012). The dynamics of cooperative bacterial virulence in the field. *Science*, **337**(6090), 85–88. <https://doi.org/10.1126/science.1218196>
- Rezzoagli, C., Granato, E. T., & Kümmerli, R. (2019). In-vivo microscopy reveals the impact of *Pseudomonas aeruginosa* social interactions on host colonization. *The ISME Journal*, **13**(10), 2403–2414. <https://doi.org/10.1038/s41396-019-0442-8>
- Ross-Gillespie, A., Gardner, A., Buckling, A., West, S. A., & Griffin, A. S. (2009). Density dependence and cooperation: Theory and a test with bacteria. *Evolution*, **63**, 2315–2325.
- Ross-Gillespie, A., Gardner, A., West, S. A., & Griffin, A. S. (2007). Frequency dependence and cooperation: Theory and a test with bacteria. *American Naturalist*, **170**, 331–342.
- Roux, L., Simon, A. E., & Holland, J. J. (1991). Effects of defective interfering viruses on virus replication and pathogenesis in vitro and in vivo. In K. Maramorosch, F. A. Murphy, & A. J. Shatkin (Eds.), *Advances in virus research* (pp. 181–211). Academic Press.
- Rumbaugh, K. P., Trivedi, U., Watters, C., Burton-Chellew, M. N., Diggle, S. P., & West, S. A. (2012). Kin selection, quorum sensing and virulence in pathogenic bacteria. *Proceedings of the Royal Society, Series B: Biological Sciences*, **279**(1742), 3584–3588. <https://doi.org/10.1098/rspb.2012.0843>
- Sanchez, A., & Gore, J. (2013). Feedback between population and evolutionary dynamics determines the fate of social microbial populations. *PLoS Biology*, **11**(4), e1001547. <https://doi.org/10.1371/journal.pbio.1001547>
- Schluter, J., Schoech, A. P., Foster, K. R., & Mitri, S. (2016). The evolution of quorum sensing as a mechanism to infer kinship. *PLoS Computational Biology*, **12**(4), e1004848. <https://doi.org/10.1371/journal.pcbi.1004848>
- Scholz, R. L., & Greenberg, E. P. (2015). Sociality in *Escherichia coli*: Enterochelin is a private good at low cell density and can be shared at high cell density. *Journal of Bacteriology*, **197**(13), 2122–2128. <https://doi.org/10.1128/JB.02596-14>
- Shirogane, Y., Rousseau, E., Voznica, J., Xiao, Y., Su, W., Catching, A., Whitfield, Z. J., Rouzine, I. M., Bianco, S., & Andino, R. (2021). Experimental and mathematical insights on the interactions between poliovirus and a defective interfering genome. *PLoS Pathogens*, **17**(9), e1009277. <https://doi.org/10.1371/journal.ppat.1009277>
- Strassmann, J. E., & Queller, D. C. (2011). Evolution of cooperation and control of cheating in a social microbe. *Proceedings of the National Academy of Sciences of the United States of America*, **108**(Suppl 2), 10855–10862. <https://doi.org/10.1073/pnas.1102451108>
- Tapia, F., Wohlfarth, D., Sandig, V., Jordan, I., Genzel, Y., & Reichl, U. (2019). Continuous influenza virus production in a tubular bioreactor system provides stable titers and avoids the “von Magnus effect.”. *PLoS One*, **14**(11), e0224317. <https://doi.org/10.1371/journal.pone.0224317>
- Taylor, P. D., & Frank, S. D. (1996). How to make a kin selection model. *Journal of Theoretical Biology*, **180**(1), 27–37. <https://doi.org/10.1006/jtbi.1996.0075>
- Trivers, R. L. (1971). The evolution of reciprocal altruism. *The Quarterly Review of Biology*, **46**(1), 35–57. <https://doi.org/10.1086/406755>
- Turner, P. E., & Chao, L. (1999). Prisoner's dilemma in an RNA virus. *Nature*, **398**(6726), 441–443. <https://doi.org/10.1038/18913>
- van Gestel, J., Weissing, F. J., Kuipers, O. P., & Kovács, A. T. (2014). Density of founder cells affects spatial pattern formation and cooperation in *Bacillus subtilis* biofilms. *The ISME Journal*, **8**(10), 2069–2079. <https://doi.org/10.1038/ismej.2014.52>
- Vetsigian, K. (2017). Diverse modes of eco-evolutionary dynamics in communities of antibiotic-producing microorganisms. *Nature Ecology and Evolution*, **1**(7), 0189.
- Volterra, V. (1927). *Variazioni e Fluttuazioni del Numero d'individui in Specie Animali Conviventi*. Società anonima tipografica “Leonardo da Vinci.”
- von Magnus, P. (1951). Propagation of the PR8 strain of influenza virus in chick embryos. *Acta Pathologica Microbiologica Scandinavica*, **28**, 278–293.
- Wahl, L. M., & Gerrish, P. J. (2001). The probability that beneficial mutations are lost in populations with periodic bottlenecks. *Evolution*, **55**(12), 2606–2610. <https://doi.org/10.1111/j.0014-3820.2001.tb00772.x>
- Wahl, L. M., Gerrish, P. J., & Saika-Voivod, I. (2002). Evaluating the impact of population bottlenecks in experimental evolution. *Genetics*, **162**(2), 961–971. <https://doi.org/10.1093/genetics/162.2.961>
- Weitz, J. S., Eksin, C., Paarporn, K., Brown, S. P., & Ratcliff, W. C. (2016). An oscillating tragedy of the commons in replicator dynamics with game-environment feedback. *Proceedings of the National Academy of Sciences of the United States of America*, **113**(47), E7518–E7525. <https://doi.org/10.1073/pnas.1604096113>
- Young, P. C., Pedregal, D. J., & Tych, W. (1999). Dynamic harmonic regression. *Journal of Forecasting*, **18**(6), 369–394. [https://doi.org/10.1002/\(sici\)1099-131x\(199911\)18:6<369::aid-for748>3.0.co;2-k](https://doi.org/10.1002/(sici)1099-131x(199911)18:6<369::aid-for748>3.0.co;2-k)
- Yurtsev, E. A., Chao, H. X., Datta, M. S., Artemova, T., & Gore, J. (2013). Bacterial cheating drives the population dynamics of cooperative antibiotic resistance plasmids. *Molecular Systems Biology*, **9**, 683. <https://doi.org/10.1038/msb.2013.39>
- Zwart, M. P., Pijlman, G. P., Sardanyés, J., Duarte, J., Januário, C., & Elena, S. F. (2013). Complex dynamics of defective interfering baculoviruses during serial passage in insect cells. *Journal of Biological Physics*, **39**(2), 327–342. <https://doi.org/10.1007/s10867-013-9317-9>

Corrections

1. Final line of the left column of the third page of the paper: remove “s” from “blue texts”.
2. Page 3 of the paper, the paragraph on frequency dependence: “... describes how the relative fitness of cheats increases when they **are** at lower frequencies (proportion) in the population because they are better able to exploit **cooperators**.”

6

What can climatic dataset tell
us about evolution: an analysis
on environmental predictabilities
and correlations

What can climatic dataset tell us about evolution: an analysis on environmental predictabilities and correlations

Ming Liu¹, Charlie K. Cornwallis², Stuart A. West¹

¹Department of Biology, University of Oxford

²Department of Biology, Lund University

Abstract

Understanding how the environment shapes the course of evolution is one of the key issues in evolutionary biology. Biologists have used climatic variables, such as temperature and precipitation, from global meteorological datasets to analyse the correlation and causation between traits and environments. These variables are commonly quantified in terms of average, standard deviation (or variance) and predictability. However, the meaning and calculation of predictability has varied in the past literature. Furthermore, these quantities can be correlated with each other, obscuring their relative influences. We systematically compared different measures of predictability and analysed the potential correlation problem using a recently published dataset on cooperative breeding in birds. First, we found that different potential measures of predictability could give completely opposite patterns. Second, we found strong correlations between the average, standard deviation, and predictability of temperature, but weaker correlations between the precipitation quantities. Third, we found that adding predictability to the phylogenetic regression analyses resulted in worse models. Taken together, these results suggest that care needs to be taken when using or interpreting different aspects of the same climatic variable.

Keywords: harsh environment, climatic time series, global climatic dataset, macroecology, climate change

1. Introduction

Variation in abiotic environmental conditions has been argued to be important for evolutionary biology, macroecology, and conservation biology. Environmental conditions such as temperature and rainfall can play crucial roles in determining the degree of biological activity. Biologists collect climatic quantities from meteorological datasets and use these quantities to represent environments on a global scale. Evolutionary biologists have used across species comparative studies to examine if certain traits tend to be found in certain environments. For example, cooperation and dormancy are more likely to be found in harsher environments (Cornwallis *et al.* 2017; Shen *et al.* 2017). From a macroecology and biogeographic perspective, ecologists examine the drivers of spatial variation in species richness, such as trophic interactions and environmental properties, and how these properties correlate with climatic quantities (Palmer 1994; Evans *et al.* 2005). From a conservation biology perspective, monitoring environmental variation is crucial to understand population decline, or recovery, especially under the ongoing climate change (Wiens & Graham 2005; Wiens *et al.* 2010).

Meteorological datasets are used to measure environmental variation. Usually two variables are focused on— temperature and rainfall— which can be quantified in multiple ways. The simplest quantification is to measure the average and standard deviation (SD). The average, or the arithmetic mean, tells us about the overall level of quantity (i.e., hot or cold; dry or wet). The SD captures how much rainfall or temperature fluctuate over time, sometimes also measured by variance. For example, it has been argued that environments with a high average temperature and/or highly variable rainfall are relatively harsh environments (Cornwallis *et al.* 2017; Firman *et al.* 2020; Martin *et al.* 2020). These two quantities on temperature and rainfall have been widely

used in biological studies (Lawing & Polly 2011; Diamant *et al.* 2021).

Another possible aspect of environmental variation is 'predictability'. Environmental predictability has been measured in multiple ways. Predictability has sometimes been used interchangeably with variability (SD), where a low predictability means highly variable (Jetz & Rubenstein 2011). Another example is to link unpredictability to a life history outcome, such as higher nest failures (Caro *et al.* 2016). More usually though, predictability refers to the sequence of measurements and ask if past datapoints are correlated to future datapoints (Colwell 1974; Koenig & Knops 2000; Norden *et al.* 2007). In other words, if there is a strong correlation, such as highly repeatable seasons, then the environmental variables such as rainfall and temperature would show high predictability. Predictability from this perspective is a measure of periodicity, and there is a number of methods that can be applied.

There remain at least three open questions for attempts to examine the ecological and environmental consequences of predictability. First, to what extent do results depend upon how predictability is measured. Some methods measure relatively, scaled to the mean, while others measure it absolutely. Does predictability need to be scaled in this way, to measure its biological importance, or does this confound predictability with variability? Second, predictability can be highly correlated with the average and SD (variability) of environmental variables. Consequently, the biological significance of predictability, relative to these other measures, is unclear. To what extent is it useful to include predictability in studies, examining the consequences of environmental variability? Third, a consideration of environmental predictability implies predictability in the context of the organisms being studied. How well do meteorological measurements correspond to the ability of organisms to predict their environment?

83

84 We addressed the first two questions with three aims: (1) We used a commonly
85 used meteorological dataset to compare three different methodologies for measuring
86 the predictability of temperature and rainfall. These methods have potentially different
87 biological meanings, and we examined how these predictabilities distribute on the globe.
88 (2) We used a previously published dataset on cooperative breeding in birds (Cornwallis
89 *et al.* 2017) to test the extent to which different measures provide novel and useful
90 information. We examined the correlation between the average, SD and predictability
91 of both temperature and precipitation. (3) We examined the extent to which adding
92 predictability can improve a model that already includes the average and SD, for both
93 temperature and rainfall, using the same dataset for cooperative breeding trait in birds.
94 We then addressed the third question on how meteorologically measured predictability
95 corresponds to predictability from the perspective of living organisms in discussion.

96

97 **2. How to measure predictability?**

98 We compared three different methods for quantifying predictability: Colwell's
99 predictability with fixed binning, Colwell's predictability with dynamic binning, and
100 normalised spectral entropy. The first two methods are developed by Robert Colwell
101 (Colwell 1974). This method puts observations into data categories and uses Shannon
102 information statistic, also known as information entropy, to calculate uncertainty across
103 time and data categories to obtain Colwell's predictability. Colwell's method is the
104 mainstream method over the last decade (Fisher *et al.* 2013; Cornwallis *et al.* 2017;
105 Fristoe *et al.* 2017; Griesser *et al.* 2017; Ringen *et al.* 2019; Firman *et al.* 2020; Martin
106 *et al.* 2020; Diamant *et al.* 2021). The third method is imported from spatial ecology
107 and movement ecology, that some recent papers have suggested using spectral entropy
108 to calculate the periodicity of time series data (Zaccarelli *et al.* 2013; Riotte-Lambert

& Matthiopoulos 2020). The reason we chose these three methods is because of popularity (for Colwell's measurements), and because of potential methodological benefit (for normalised spectral entropy).

Because all three methods apply the concept of information entropy to measure the degree of uncertainty, we will first explain the concept of such entropy. We will then describe the three methods in more details, and use two simulated time series to illustrate the similarities and differences between the three methods.

2.1 The concept of entropy in information theory

Entropy is one of the fundamental concepts in thermodynamics and it measures the degree of disorder, or uncertainty. The most commonly used formulation was developed by Claude E. Shannon (Shannon 1948),

$$H(X) = \sum_{i=1}^s -p_i \ln(p_i) / \ln(s) \quad (1)$$

where X is the data, i is the index in observation categories, s is the number of categories, and p is the proportion of observation belong to the focal category. $H(X)$ is called normalised information entropy in information theory, and normalisation is added to facilitate comparison between different data. $H(X)$ is maximised when all p have equal values, and is minimised when one p is 1 and all others are 0. In other words, the bigger H indicates greater uncertainty of predicting which group a random data point is in.

While all three methods use the same formulation of $H(X)$, the key difference between them is how categories are defined. Colwell's predictability, both fixed binning

and dynamic binning, uses discrete numeric grouping for observations to create categories, while normalised spectral entropy uses frequencies of waves to define categories.

2.2 Method 1: Colwell's predictability with fixed binning

Colwell's predictability focuses on the yearly cycles by processing data from the same time of the year into the same column (X) of a table, and counting how many occurrences for each numeric data category (Y) are on the rows. We call this table the processed occurrence table. For example, if we have monthly temperature data for 5 years, then the data processing step is to make an occurrence table with 12 columns, where each column is a month, and the sum of each column is 5. Then, Colwell's predictability is defined as

$$P_{Colwell} = 1 - \frac{H(XY) - H(X)}{\ln(s)}, \quad (2)$$

where $H(X)$ is the uncertainty over time, $H(XY)$ is the uncertainty over all data points, and s is the number of data categories. Because the number of datapoints for each month is constant (i.e., 5), $H(X)$ is also a constant. Thus, Colwell's predictability emphasises on how spread out is the datapoints across data categories, or the rows, at each month. If there is one row containing 5 occurrences, and all other rows having 0 occurrence, then Colwell's predictability would be maximised because $H(XY)$ is minimised (i.e., $H(XY) = (-\ln(1) - 4 * 0 * \ln(0)) / \ln(5) = 0$). On the contrary, if there are five rows containing 1 occurrence, which is the most possible spread-out distribution for 5 observations, then Colwell's predictability would be minimised because $H(XY)$ is maximised (i.e., $H(XY) = -5 * 1/5 * \ln(1/5) / \ln(5) = 1$).

The final detail here is how rows are defined in the processed occurrence table. That is, how should a data category, Y , be defined? For instance, one could define it as 1°C , or any arbitrary value, because they are interested in certain thermal resolution of temperature time series data. Then, for any given time series, we could maintain the same resolution of the data because the bin width of each data category does not change with the range of the input data. We call this binning method as fixed binning method.

We use two example time series to elaborate further details of this fixed binning method (Fig. 1a). Time series 1 was generated from a sine wave of yearly cycle with the addition of some white noise. The sine wave fluctuates between 1 and -1, while the white noise ranges between -0.25 and 0.25. Each datapoint represents a monthly average temperature for a five-year period. For simplicity, we did not shift this temperature time series to more common values (e.g., 15°C) and shifting the data would not change the results. Time series 2 is time series 1 multiplied by 5 times and the only difference between them is the scale of variation. Then, we make the processed occurrence table for both time series, with 1°C width for each data category on the rows, ranging from -6 to $+6^{\circ}\text{C}$. As we can see in Fig. 1b, the processed occurrence table for time series 1 is less spread out and concentrate on a few rows, whereas the processed occurrence table for time series 2 is much more spread out on the rows. Consequently, using Colwell's predictability with fixed binning method, we find time series 1 is more predictable, with a predictability of 0.924, than time series 2, with a predictability of 0.803.

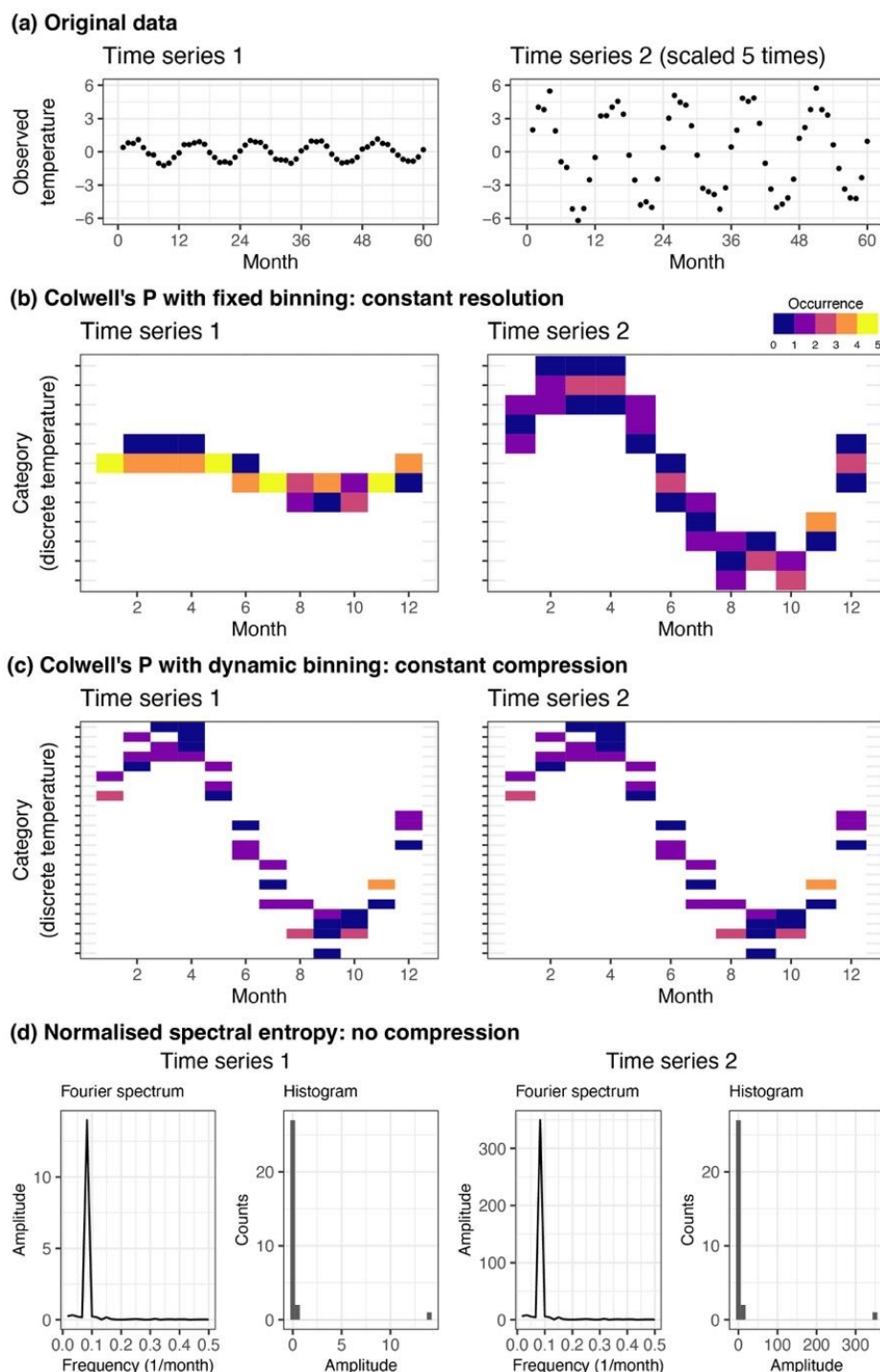


Figure 1. Three different types of predictabilities on two example time series. (a) The raw observations from the two time series data. Time series 1 is generated through a sine wave plus random numbers bounded between -0.25 and 0.25. Time series 2 is time series 1 multiplied by a factor of 5. (b-d) The processed data, or intermediate step, used in calculating three types of predictability. We set the bin width to 1 for fixed binning, and set 25 categories for dynamic binning. Brighter colour indicates there is a higher occurrence.

190

191 *2.3 Method2: Colwell's predictability with dynamic binning*

192 An alternative approach for creating data categories could be keeping the number of
 193 valid rows constant between input data, and let the bin width varies. That is, the width
 194 of a data category changes with the difference of maximal and minimal observation in
 195 a time series. An example is shown in Fig. 1c, where both time series are processed into
 196 tables with 25 rows. We call this method 'dynamic binning' because the definition of a
 197 single bin (i.e., data category) is dynamic and depend to the scale of input data. Because
 198 data are compressed at the same level, we have equal predictabilities for the two
 199 example time series: 0.691.

200

201 *2.4 Method 3: normalised spectral entropy*

202 Another method is normalised spectral entropy, which has been used for quantifying
 203 uncertainty and predictability in spatial and movement ecology. The method does not
 204 calculate entropy from a processed occurrence table, but from a Fourier spectrum
 205 produced by discrete Fourier transformation. Discrete Fourier transformation is one of
 206 the standard methods in time series analysis, and has been extensively used in signal
 207 processing, solving partial differential equations, and many other applications
 208 (Shumway *et al.* 2000). It uses a discrete set of sine waves to describe the input data
 209 set: if a dataset is very noisy, such as a white noise, then the Fourier spectrum would
 210 have roughly equal amplitudes at each frequency; if a data is very periodic, then the
 211 Fourier spectrum would have some peaks in a few frequencies and almost zero
 212 amplitudes in all other frequencies.

213

214 Normalised spectral entropy analyse the distribution of amplitudes, using each
 215 frequency as an amplitude entry (histogram in Fig. 1d). Specifically,

$$P_{SpcEntropy} = 1 - H(X) = 1 - \frac{\sum_i^s -p_i \ln(p_i)}{\ln(s)}, \quad (3)$$

where X is the Fourier spectrum, p_i is the amplitude of focal frequency divided by the sum of amplitudes, and s is the number of frequencies in the spectrum (equal to half the length of the time series; Zaccarelli *et al.* (2013)). If the amplitudes are all about the same with no peaks, then p_i would also be at similar levels and result in a low predictability. In contrast, if there is one single peak at a frequency, then a single p_i would be much greater than all others and result in a high predictability. As a result, spectral entropy is quantifying the periodicity of the input data. Going back to our two example time series, because the amplitude scales up evenly across all frequency (at a factor of squared scaling that time series 2 has 25 times larger amplitudes than time series 1), the normalised spectral entropy are identical between both time series: 0.796.

2.5 Comparison and biological interpretation of predictabilities

The three methods capture predictability with very different biological insights. While method 2 and 3 use different ways to capture periodicity, method 1 quantifies periodicity scaled by the variance of the input time series. This is summarised by the previous method text and Fig. 1, that the predictability from method 2& 3 does not vary between time series 1& 2, however, predictability from method 1 is higher for the less variable time series 1 and lower for the more variable time series 2. We will discuss the properties of the three methods in more detail in the following paragraphs.

Colwell's predictability with fixed binning keeps the same data resolution across all locations (Method 1). In other words, one degree difference in Celsius means the same thing irrespective of whether the temperature shows low (e.g., tropics) or high (e.g.,

temperate) variation. However, it also means the degree of compression, which results from binning observations into data categories, goes up when the variance of a data is smaller and consequently a higher predictability. For example, we might have less compressed data when the range of the data is larger, and more compressed data when the range is smaller (time series 1 and 2 in Fig. 1b). This measure is therefore not just periodicity, it combines information from periodicity and variability (SD).

In contrast, Colwell's predictability with dynamic binning keeps the degree of compression constant (Method 2). The benefit of this approach is that it provides a cleaner measure of periodicity, that is independent of the average or variability (SD). The cost of this approach is the calculation unit is not consistent across the entire globe – a difference in one degree Celsius will count for more when the SD of the input time series is lower.

Normalised spectral entropy focuses on the uncertainty among frequencies, which is even more independent of the variance of the data (Method 3). One potential benefit is spectral entropy looks into multiple possibilities of autocorrelations, while Colwell's calculation only focuses on the correlation between years (12 months lag). This difference means multiple-year events, such as El Nino and La Nina, which have important biological implications, are treated as independent samples in Colwell's calculation, while spectral entropy can potentially capture the correlation across multiple years. Another potential benefit is spectral entropy does not compress the data and almost no information is lost during the transformation process (except the phase of each sine wave). Ideally speaking, we make better inference for the time series when more information is available. Nevertheless, the downside of spectral entropy is the same as method 2, that the calculation unit is not constant, and a one-degree Celsius

difference matters more when the time series is less variable. The examples and all method source codes are available in SI section 5.

3. Using the measures of predictability

3.1 How does predictability distribute across the world?

3.1.1 Data collection

We first examined the environmental predictability patterns generated by these three methods on the global scale. We collected climatic data from the climate research unit (CRUTS v4.06; Harris *et al.* (2020)), by taking monthly measurements of temperature and precipitation (rainfall). We collected the data from January 1901 to December 2020, at a spatial resolution of $0.5^{\circ} \times 0.5^{\circ}$ longitude-latitude grids (~ 55.5 km north-to-south, east-to-west varies with latitude). For each grid, we process the climatic data into average, SD, and the three predictabilities over the 1440 months.

3.1.2 The global pattern

The three methods for measuring temperature predictability generated different global patterns (Fig. 2a-c). Colwell's predictability with fixed binning (Method 1) is highest around equator, but the other two methods (2 & 3) find that predictability is lowest at those regions (Fig. 2a vs. Fig.2b&c). Colwell's predictability with dynamic binning and normalised spectral entropy are measuring periodicity (Methods 2 & 3). This is greatest in areas which show more consistent seasonality, which is in more temperate areas, which move between warmer summers and colder winters. On the opposite end, because of low seasonality, any temporal patterns would have greater impacts on the predictability calculation for method 2 & 3, and lead to lower predictabilities in tropical places. In contrast, Colwell's predictability with fixed binning takes account for both periodicity and variability, resulting in higher predictability in less seasonal places –

because they show less variations (Method 1).

The three methods for quantifying precipitation predictability also showed different global patterns (Fig. 2d-f). All methods suggest rainfall is highly predictable in the Amazon rainforest, Arabian desert, and some regions of Himalayans, but they disagree with each other on Africa, Northern America, and Eurasia. This inconsistency is likely because of Colwell’s predictability uses log-2-based binning in both method 1 & 2, whereas method 3 does not have a similar transformation process before Fourier transformation. We also made the global maps for averages and SDs of temperature and precipitation in SI section 2.2.

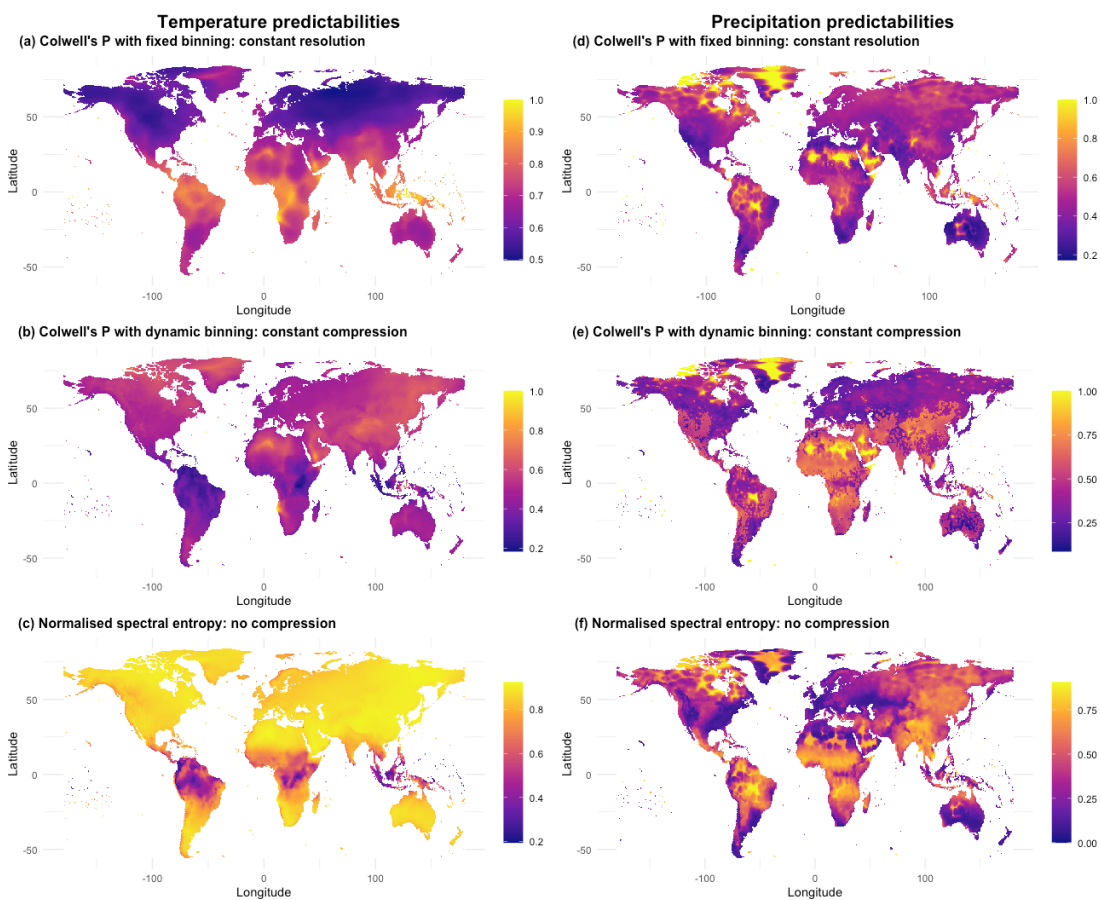


Figure 2. Predictabilities on a global scale. (a-c) The three predictabilities for temperature. Methodological details for binning in Colwell’s predictability are described in Fig. 1. (d-f) The three predictabilities for precipitation. Following previous

literature and Colwell's original paper, the bins for precipitation data when calculating Colwell's predictability is on log2 scale. Normalised spectral entropy uses the same calculation method in panel c& f. For temperature, the different methods show higher predictabilities in either low seasonality and tropical places (method 1, fixed binning; a), or high seasonality and temperate places (method 2 & 3; b & c). For precipitation, the different methods disagree with each other on where predictability is high or low (d-f).

3.1.3 Conclusion 1: Different predictabilities are quantifying different properties

As summarised in 3.1.2, we found the three predictabilities have contrastingly different patterns for temperature data, and inconsistent patterns for precipitation data. These results suggest they are probably quantifying different properties of climatic time series data.

3.2 How much does different climatic metrics correlate with each other?

3.2.1 Data collection: a reanalysis

We reused a global bird data set published in 2017 to test the usefulness of different measures of environmental variables (Cornwallis *et al.* 2017). The data contains 286 bird species, classified as whether they are a cooperative breeder. For each species, we used the species distribution data provided in the dataset to calculate the average meteorological quantities across space. For simplicity, we assume species abundance is uniform across space and the average is taken with equal weighting for each spatial grid. We then analyse the correlation between each climatic quantity by the unit of species. We first examined the correlation between predictabilities and average or SD, then we also checked how much does the average and SD correlate within temperature or precipitation. Some quantities have been transformed before checking the correlation and the transformation was made to improve the normality. SI section 4 contains the Q-

Q plots for original data and various common transformations, and section 2.3 has the scatter plot for the predictabilities of the entire dataset (N= 67420).

3.2.2 Correlation between averages and standard deviations

We started by analysing the correlation between averages and SDs to make the results more complete. Interestingly, we found very strong correlation between each SD and average (Fig. 3). In temperature, this correlation is likely because of seasonality, that hotter places are more tropical and less seasonal (Fig. 3a). In precipitation, the positive correlation is potentially because rainfall is a type of counting data, and there is more space to vary when the average is higher (Fig. 3b). Moreover, we also found correlations in other combinations of climatic variables and quantities (e.g., average temperature and SD of precipitation) in SI section 3.1. Together, these results highlight the potential problem of including both averages and SDs into regression models, as they are not independent to each other.

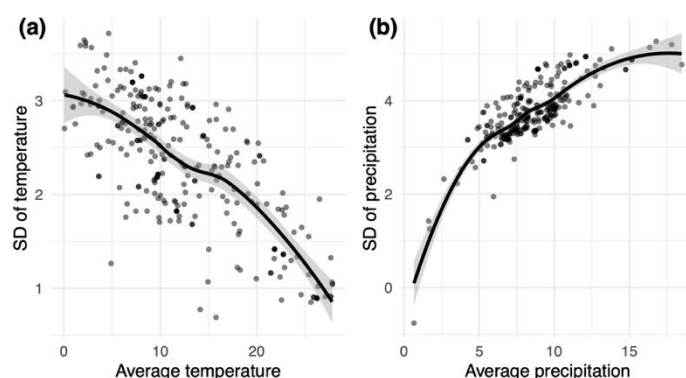


Figure 3. The correlation between averages and standard deviations. (a) Temperature data. (b) Precipitation data. The solid curve and shaded area is calculated from locally estimated scatterplot smoothing (LOESS). In order to improve normality, the average precipitation and standard deviation of temperature are square root transformed, while the standard deviation of precipitation is natural log transformed.

3.2.3 *Correlation between predictabilities and other quantities*

We then extend the correlation analysis to predictabilities against averages and SDs. We found very strong correlations between all temperature predictabilities and the average or the SD (Fig. 4a-f). In highly seasonal spaces (low average and high SD of temperature), Colwell's predictability with fixed binning (method 1) has low predictability values while the other two methods have high predictability values compared to less seasonal spaces. These results suggest predictabilities have overlapping properties with average and SD, and they are not independent with average and SD of temperature.

We found much lower correlations between precipitation predictabilities and the average or the SD (Fig. 4g-l). Although they all follow a decrease-increase pattern, the size of confidence interval expands dramatically when moving away from the centre of the x-axes (shaded area in each panel). The lower level of correlations is likely because there is no equivalent strong factor as seasonality to precipitation as in temperature. The driver of precipitation can be more local, predominantly determined by ocean currents, atmospheric circulation, and terrain.

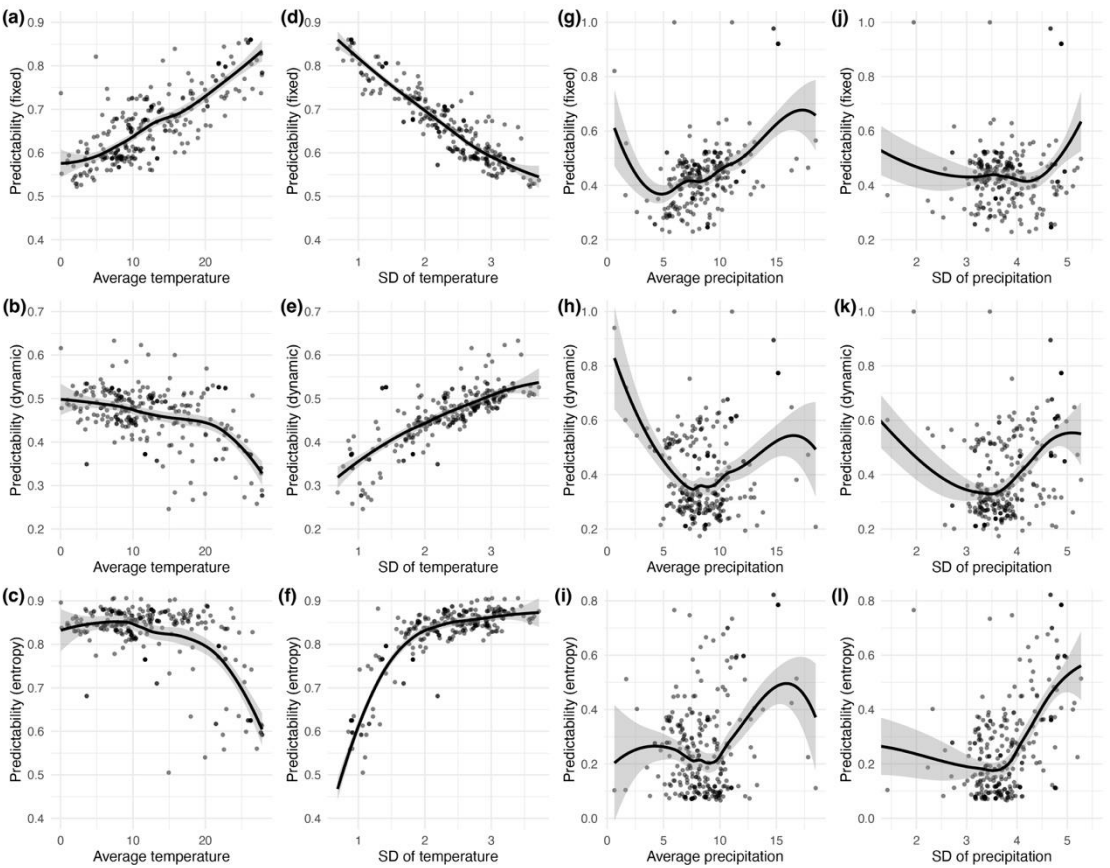


Figure 4. Correlation analysis of predictabilities and other climatic quantities. (a-c) Against average temperature. (d-f) Against standard deviation of temperature. (g-i) Against average precipitation. (j-l) Against standard deviation of precipitation. Same transformations are made in this figure as in Fig. 3. In each panel, the solid curve and shaded area is calculated from locally estimated scatterplot smoothing (LOESS).

3.2.4 Conclusion2: climatic metrics are highly correlated

Through correlation analyses, we found that, not just predictabilities are correlated with averages and SDs, but the latter two quantities also correlate with each other. The only exceptions with low correlations are precipitation predictabilities against other precipitations quantities, and the averages and SDs of temperature against average precipitation.

3.3 How much does including predictability and SDs add to statistical regression models?

3.3.1 Statistical methods and interpretations

Despite the strong correlation between climatic quantities, we could still add predictabilities and SDs to regression models and ask how much they improve the models. We used phylogenetic least square regression models (PGLS) to answer this question, because this method allows us to test model selection, and calculate the percentage of variance explained by each predictor in the model (i.e., partial R^2). We constructed three categories of models: (1) averages only, (2) averages+ SDs, and (3) averages+ SDs+ predictabilities. We then calculated the Akaike information criteria (AIC) and overall R^2 to compare model fitting. We also performed variance inflation factor (VIF) testing from the equivalent GLM models to examine the extent of multicollinearity. We carried out the analyses under R v4.3.0, phylolm package v2.6.2, rr2 package v1.1.0, and car package v3.1.2.

Before going into the analyses, here is a brief explanation on how to read each bit of results. First, if a model has lower AIC than another, then the model is suggested to be a better model. AIC balances between the number of predictors in the model, and the increased interpretability of the model. There are some criticisms against AIC (Anderson & Burnham 2002), but we use it here for simplicity and generality. Second, if a model has higher overall R^2 than another, then the model can explain more variance in the input data. We used likelihood R^2 because it is the simplest available tool for logistic PGLS regression models (Ives 2019). Third, VIF gives us an indication on how much the standard error in the estimate coefficient is inflated (Miles 2014). If an VIF is 4, it means the standard error is 2 times (square root of 4) larger than if that predictor had no correlation with other predictors. Describing the degree of collinearity

is subjective, and here we term moderately collinearity as when the VIF ranges between 2 and 4, and high collinearity as when the VIF is larger than 4.

3.3.2 Analysis

By comparing the PGLS models, we found adding predictability made worse models (Table 1). Specifically, all models with predictabilities (model C1, C2, and C3) have a higher AIC and a lower R^2 , compared to the model without predictabilities (model B). These results support that previous correlation analyses (Fig. 4) and suggest against including predictabilities into the models. In SI section 3.2-3.4, we performed a more rigorous nested model selection process, and similarly found that including different predictabilities results in different optimal models. In addition, by comparing the models with and without SDs (model A& B), we found adding SDs only slightly improve the R^2 by 3%. This small improvement aligns with the correlation analysis on averages and SDs (Fig. 3) and implies limited effects of adding variability measurements.

Table 1. Summary of model fitting for all models on cooperative breeding trait in the bird dataset from Cornwallis et al. (2017).

	Model A: averages only (2 predictors)	Model B: averages and SDs (4 predictors)	Model C1: averages, SDs, and Colwell'P (fixed; 6 predictors)	Model C2: averages, SDs, and Colwell'P (dynamic; 6 predictors)	Model C3: averages, SDs, and spectral entropy (6 predictors)
AIC	192.321	190.065	200.722	204.988	202.598
R2	0.348	0.378	0.346	0.325	0.337

In terms of multicollinearity, we found only the model with averages (model A) has low collinearity, whereas all other models have moderate to strong collinearity (Table 2). In particular, the model with averages and SDs has moderate levels of

collinearity in all predictors. Furthermore, four out of six predictors have strong collinearities in the model with Colwell's predictability using fixed binning (model C1), and one out of six predictors has a strong collinearity in the other two models with alternative predictability measures (model C2 & C3). The high level of collinearity suggests these models are potentially not good models because the assumptions in regression have been violated.

Table 2. Variance inflation factor of each model in table 1.

	Average temperature	Average precipitation	SD of temperature	SD of precipitation	Temperature predictability	Precipitation predictability
Model A	1.221	1.221	NA	NA	NA	NA
Model B	2.441	3.211	2.980	3.271	NA	NA
Model C1	2.691	7.113	5.917	6.014	6.453	2.321
Model C2	2.969	3.251	4.754	3.399	2.439	1.489
Model C3	3.070	3.367	5.338	3.715	3.202	1.789

3.3.3 Conclusion 3: adding predictabilities makes poorer models; adding SDs makes small improvements

From the two tables above, we can summarise that adding predictabilities does not improve the model, but instead makes it worse in our analyses. The models with any of the three ways of quantifying predictabilities, compared to the one with averages and SDs, is worse in AIC, overall R^2 , and multicollinearity. In addition, we found that adding SDs only slightly improve the model, at the cost of increased multicollinearity from low to moderate.

4. Discussion

4.1 Summary of results

We found that different potential measures of predictability can give very different results. For temperature data, Colwell's predictability with dynamic binning and

normalised spectral entropy (method 2& 3) measure periodicity, which is higher in temperate places because of high seasonality. In contrast, Colwell's predictability with fixed binning (method 1) measures a mixture of quantities and has higher values in tropical places, where temperature is less variable. Precipitation predictability shows mixed patterns between the three quantification methods. The reason for the lack of similarity between methods 2 & 3 for precipitation could be the additional log2 transformation in the binning processes for both methods 1 & 2, which was not done for the temperature data (these procedures are adopted from previous literature).

We found that the average, SD, and predictability of temperature were all highly correlated. All of these correlations are related to seasonality, as hotter places are typically closer to equator, have smaller temperature variability (SD), higher predictability with method 1, and lower predictabilities with method 2 & 3. On the other hand, we found the average and SD of precipitation are strongly correlated, but much lower correlations between precipitation predictabilities and other precipitation quantities.

Finally, we examined the extent to which the SD and predictability of each variable (temperature and precipitation) were able to help explain the distribution of cooperative breeding in birds. We found that adding the predictability of temperature and precipitation led to a worse model, as measured by both a higher AIC and lower R^2 . We found that adding the SD of temperature and precipitation led to a better model, but that effect was small, increasing R^2 from only 0.35 to 0.38. These analyses suggest that predictability does not add any extra information, and that adding SD provides only a small benefit, compared to model with just the average of temperature and precipitation. This result reflects the high correlation between the average, SD and

predictability for both temperature and rainfall (Fig. 3 & 4).

4.2 Different methodologies of predictability quantify different things

As different methods give different results, the best measure to use could depend upon the aims of the study. Colwell's predictability with fixed binning (method 1) suggests that more tropical climates are more predictable, because they show smaller variation. Colwell's predictability with dynamic binning and normalised spectral entropy (method 2 & 3) suggest that more temperate climates are more predictable because they show stronger seasonality (larger variation, but seasonally predictable). Consequently, what matters is whether an empirical study is investigating periodicity or variability, A potential complication here is that because it is partially a measure of variability, Colwell's predictability with fixed binning will correlate with the SD of that environmental variable, meaning that any information it supplies will be at least partially redundant.

4.3 Terminology of meteorological quantities

Our results also emphasise that confusion can be generated by terminology, because different measures of predictability are not measuring the same thing. Colwell's predictability with dynamic binning and normalised spectral entropy are measuring periodicity – over yearly or yearly and longer cycles respectively (Fig. 1c & d). Consequently, it might be clearer to just refer to them as 'periodicity'. On the contrary, Colwell's predictability with fixed binning is measuring periodicity rescaled by variability (Fig. 1b), thus could be called as 'rescaled periodicity'. Nevertheless, because Colwell's predictability with fixed binning is, by design, correlated with SD or other measures of variability, limiting the usefulness of adding this to a model that already includes another measure of variability.

513

514 *4.4 Explanatory power of meteorological quantities*

515 Our results on the correlations between meteorological quantities raise the issue of how
516 much explanatory power these quantities add. These high correlations coincide with a
517 recent study on a similar global meteorological database (WorldClim) and found there
518 are only 5 independent variables out of the 19 quantities (Dinnage 2023). Together, both
519 results suggest future studies to take extra caution when using similar datasets, because
520 high correlations might (1) violate the assumptions in regressive models, and (2) make
521 a model selection process invalid because of multicollinearity.

522

523 These results lead to an immediate question, what are the available options to
524 remedy the high correlations between these meteorological quantities? One option that
525 has been used in previous literature is the principal component analysis (PCA) (Botero
526 *et al.* 2014; Fristoe *et al.* 2017; Griesser *et al.* 2017; Firman *et al.* 2020). Because each
527 principal component (PC) axis is a linear combination of several climatic quantities,
528 the problem of correlation can be minimised though combining the correlated ones on
529 the same axis. However, the downside of this method is biological interpretation of
530 these PC axes can sometimes be challenging, and PCA does not provide information of
531 which quantity is the main contributor of explaining the variance in data. A possible
532 solution for these problems in PCA is to make a series of single-predictor regression
533 models for each PC axes, and find the one that can explain more variance than others.
534 Then we could say the axis is closest to that meteorological quantity. Alternatively, we
535 can also discard PCA and use the method presented in this paper. That is, check
536 correlations before making regression models, and always test the usefulness of adding
537 extra predictors to the models. Both approaches would minimise the chance of violating
538 the assumptions in the statistical analyses.

539

540 *4.5 Predictability for real organisms*

541 Finally, from an organism's perspective, predictability can imply different things when
542 organisms have different life histories. In some cases, climate is always predictable as
543 the life history decisions can be made on condition of the recent weather events. For
544 instance, the termination of dormant stage in insect larvae and freshwater invertebrate
545 eggs are closely linked to the increasing temperature or a recent raining event (Forrest
546 & Thomson 2011; Stenert *et al.* 2017). In other cases, climate is always unpredictable
547 because the life history decisions are not conditional of recent weather events. For
548 example, upon global warming, the great tit population in Netherland makes more
549 variable egg laying date when spring temperature is warmer (Deutsch *et al.* 2008). This
550 difference is mainly because, through cognition processes or conditional developmental
551 response, organisms respond to the same changes in environmental conditions in many
552 different ways. Therefore, there seems to be no single answer on how organisms
553 forecast future environmental conditions, or on whether a time series is more
554 'predictable' across all organisms than another time series.

555

556

References

1. Anderson, D.A. & Burnham K.P. (2002). Avoiding Pitfalls When Using Information-Theoretic Methods. *The Journal of Wildlife Management*, 66, 912-918.
2. Botero, C.A., Dor, R., McCain, C.M. & Safran, R.J. (2014). Environmental harshness is positively correlated with intraspecific divergence in mammals and birds. *Molecular Ecology*, 23, 259-268.
3. Capilla-Lasheras, P., Harrison, X., Wood, E.M., Wilson, A.J. & Young, A.J. (2021). Altruistic bet-hedging and the evolution of cooperation in a Kalahari bird. *Science Advances*, 7, eabe8980.
4. Caro, S.M., Griffin, A.S., Hinde, C.A. & West, S.A. (2016). Unpredictable environments lead to the evolution of parental neglect in birds. *Nature Communications*, 7, 10985.
5. Colwell, R.K. (1974). Predictability, constancy, and contingency of periodic phenomena. *Ecology*, 55, 1148-1153.
6. Cornwallis, C.K., Botero, C.A., Rubenstein, D.R., Downing, P.A., West, S.A. & Griffin, A.S. (2017). Cooperation facilitates the colonization of harsh environments. *Nature Ecology and Evolution*, 1, 0057.
7. Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. *et al.* (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, 105, 6668-6672.
8. Diamant, E.S., Falk, J.J. & Rubenstein, D.R. (2021). Male-like female morphs in hummingbirds: the evolution of a widespread sex-limited plumage polymorphism. *Proceedings of the Royal Society, Series B: Biological Sciences*, 288, 20203004.
9. Dinnage, R. (2023). How many variables does Wordclim have, really? Generative A.I. unravels the intrinsic dimension of bioclimatic variables. *bioRxiv*, 2023.2006.2012.544623.
10. Evans, K.L., Warren, P.H. & Gaston, K.J. (2005). Species–energy relationships at the macroecological scale: a review of the mechanisms. *Biological Reviews*, 80, 1-25.
11. Firman, R.C., Rubenstein, D.R., Moran, J.M., Rowe, K.C. & Buzatto, B.A. (2020). Extreme and variable climatic conditions drive the evolution of sociality in Australian rodents. *Current Biology*, 30, 691-697. e693.
12. Fisher, D.O., Dickman, C.R., Jones, M.E. & Blomberg, S.P. (2013). Sperm competition drives the evolution of suicidal reproduction in mammals. *Proceedings of the National Academy of Sciences*, 110, 17910-17914.

13. Forrest, J.R.K. & Thomson, J.D. (2011). An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecological Monographs*, 81, 469-491.
14. Fristoe, T.S., Iwaniuk, A.N. & Botero, C.A. (2017). Big brains stabilize populations and facilitate colonization of variable habitats in birds. *Nature Ecology and Evolution*, 1, 1706-1715.
15. Griesser, M., Drobniak, S.M., Nakagawa, S. & Botero, C.A. (2017). Family living sets the stage for cooperative breeding and ecological resilience in birds. *PLoS Biology*, 15, e2000483.
16. Harris, I., Osborn, T.J., Jones, P. & Lister, D. (2020). Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. *Scientific data*, 7, 1-18.
17. Ives, A.R. (2019). R2s for correlated data: phylogenetic models, LMMs, and GLMMs. *Systematic Biology*, 68, 234-251.
18. Jetz, W. & Rubenstein, D.R. (2011). Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Current Biology*, 21, 72-78.
19. Kennedy, P., Higginson, A.D., Radford, A.N. & Sumner, S. (2018). Altruism in a volatile world. *Nature*, 555, 359-362.
20. Koenig, W.D. & Knops, J.M.H. (2000). Patterns of annual seed production by northern hemisphere trees: a global perspective. *The American Naturalist*, 155, 59-69.
21. Lawing, A.M. & Polly, P.D. (2011). Pleistocene climate, phylogeny, and climate envelope models: an integrative approach to better understand species' response to climate change. *PLoS One*, 6, e28554.
22. Martin, J., Ringen, E., Duda, P. & Jaeggi, A. (2020). Harsh environments promote alloparental care across human societies. *Proceedings of the Royal Society, Series B: Biological Sciences*, 287, 20200758.
23. Miles, J. (2014). Tolerance and variance inflation factor. In: *Wiley StatsRef: Statistics Reference Online*.
24. Norden, N., Chave, J., Belbenoit, P., Caubère, A., Châtelet, P., Forget, P.-M. *et al.* (2007). Mast fruiting is a frequent strategy in woody species of eastern south America. *PLoS One*, 2, e1079.
25. Palmer, M.W. (1994). Variation in species richness: towards a unification of hypotheses. *Folia Geobotanica et Phytotaxonomica*, 29, 511-530.
26. Ringen, E.J., Duda, P. & Jaeggi, A.V. (2019). The evolution of daily food sharing: A Bayesian phylogenetic analysis. *Evolution and Human Behavior*, 40, 375-384.

27. Riotte-Lambert, L. & Matthiopoulos, J. (2020). Environmental predictability as a cause and consequence of animal movement. *Trends in Ecology and Evolution*, 35, 163-174.
28. Shannon, C.E. (1948). A mathematical theory of communication. *Bell System Technical Journal*, 27, 379-423.
29. Shen, S.-F., Emlen, S.T., Koenig, W.D. & Rubenstein, D.R. (2017). The ecology of cooperative breeding behaviour. *Ecology Letters*, 20, 708-720.
30. Shumway, R.H., Stoffer, D.S. & Stoffer, D.S. (2000). *Time series analysis and its applications*. Springer.
31. Stenert, C., Wüsth, R., Pires, M.M., Freiry, R.F., Nielsen, D. & Maltchik, L. (2017). Composition of cladoceran dormant stages in intermittent ponds with different hydroperiod lengths. *Ecological Research*, 32, 921-930.
32. Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V. *et al.* (2010). Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters*, 13, 1310-1324.
33. Wiens, J.J. & Graham, C.H. (2005). Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics*, 36, 519-539.
34. Zaccarelli, N., Li, B.-L., Petrosillo, I. & Zurlini, G. (2013). Order and disorder in ecological time-series: introducing normalized spectral entropy. *Ecological Indicators*, 28, 22-30.

What can climatic dataset tell us about evolution: an analysis on environmental predictabilities and correlations

Supplementary Information

Ming Liu, Charlie K. Cornwallis, and Stuart A. West

October 18, 2023

Contents

1 Overview	2
2 General information and global analysis	2
2.1 Table of abbreviations	2
2.2 Global map of averages and standard deviations	3
2.3 Global correlation analysis for predictabilities	4
3 Additional statistical analysis for the bird dataset	5
3.1 Correlation between other possible combinations of meteorological quantities	5
3.2 Summary of all models in main text	5
3.2.1 Model with averages only (Model A)	6
3.2.2 Model with averages and SDs (Model B)	6
3.2.3 Model with averages, SDs, and predictability with fixed binning (Model C1)	7
3.2.4 Model with averages, SDs, and predictability with dynamic binning (Model C2)	7
3.2.5 Model with averages, SDs, and predictability with spectral entropy (Model C3)	8
3.3 Nested model selection from model B (no predictabilities)	8
3.3.1 Model selection	9
3.3.2 Collinearity analysis	9
3.3.3 Summary of the best model	9
3.3.4 Partial R2	10
3.4 Nested model selection from model C1 (Colwell's predictability with fixed binning)	10
3.4.1 Model selection	10
3.4.2 Multicollinearity analysis	11
3.4.3 Summary of the best model	11
3.4.4 Partial R2	11
3.5 Nested model selection from model C2 (Colwell's predictability with dynamic binning)	11
3.5.1 Model selection	12
3.5.2 Multicollinearity analysis	12
3.5.3 Summary of the best model	12
3.5.4 Partial R2	13
3.6 Nested model selection from model C3 (normalised spectral entropy)	13
3.6.1 Model selection	13
3.6.2 Multicollinearity analysis	14
3.6.3 Summary of the best model	14
3.6.4 Partial R2	14
4 Normality check of the bird dataset	15

4.1	Q-Q plots for the meteorological quantities	15
4.2	Alternative transformations	16
4.2.1	SD of Temperature	16
4.2.2	Average precipitation	16
4.2.3	SD of precipitation	17
5	The source codes for Figure 1	17

1 Overview

This document is the supplementary information for the manuscript **What can climatic dataset tell us about evolution: an analysis on environmental predictabilities and correlations**. There are four sections: (1) additional analysis for the global climate (meteorological) dataset and a general abbreviation table, (2) detailed model summaries of the ones presented in the main text and nested model selection with or without predictabilities, (3) normality checks and transformations for each transformed meteorological quantity, and (4) the source code of Figure 1. The first section serves to help reading the rest of the document and to present some additional results to strengthen the main results in Fig. 2-4. The second section also has additional results to support the main results (i.e., table 1& 2). But more importantly, we present an alternative way to examine the effects of including predictabilities in to regression models in this section: how does different predictabilities change the consequence of nested model selection? The third section is a simple sanity check to show our data has reasonable level of normality and the transformations for some quantities improved their normality.

Some sections of the document contain a lot of details, such as model summary of the best regression models, however, there is a summary paragraph at the beginning of these lengthy sections to recapture the important bits of these analyses.

2 General information and global analysis

2.1 Table of abbreviations

Table S1: The abbreviations used in axes titles and column names of the data. All data are plotted after transformation, as listed in this table, unless otherwise specified. See section 4.2 for alternative common transformation options.

Name	Description	Transformation
Temperature		
<i>TMPavg</i>	Average	none
<i>TMPsd</i>	Standard deviation (SD)	sqrt
<i>TMPprd_Bot</i>	Colwell's predictability using fixed binning	none
<i>TMPprd_Dym</i>	Colwell's predictabilty with dynamic binning	none
<i>TMPprd_Ent</i>	Periodicity with normalised spectral entropy	none
Precipitation (rainfall)		
<i>PREavg</i>	Average	sqrt
<i>PREsd</i>	SD	log (ln)
<i>PREprd_Bot</i>	Colwell's predictability using fixed binning	none
<i>PREprd_Dym</i>	Colwell's predictabilty with dynamic binning	none
<i>PREprd_Ent</i>	Normalised spectral entropy	none

2.2 Global map of averages and standard deviations

To make the global maps complete, we plot the average and standard deviation (SD) of both temperature and precipitation in Fig. S1. The map correspond well with Fig. 4, that places with higher average temperatures have lower SDs (Fig. S1a& b), and places with higher average precipitations have higher SDs (Fig. S1c& d).

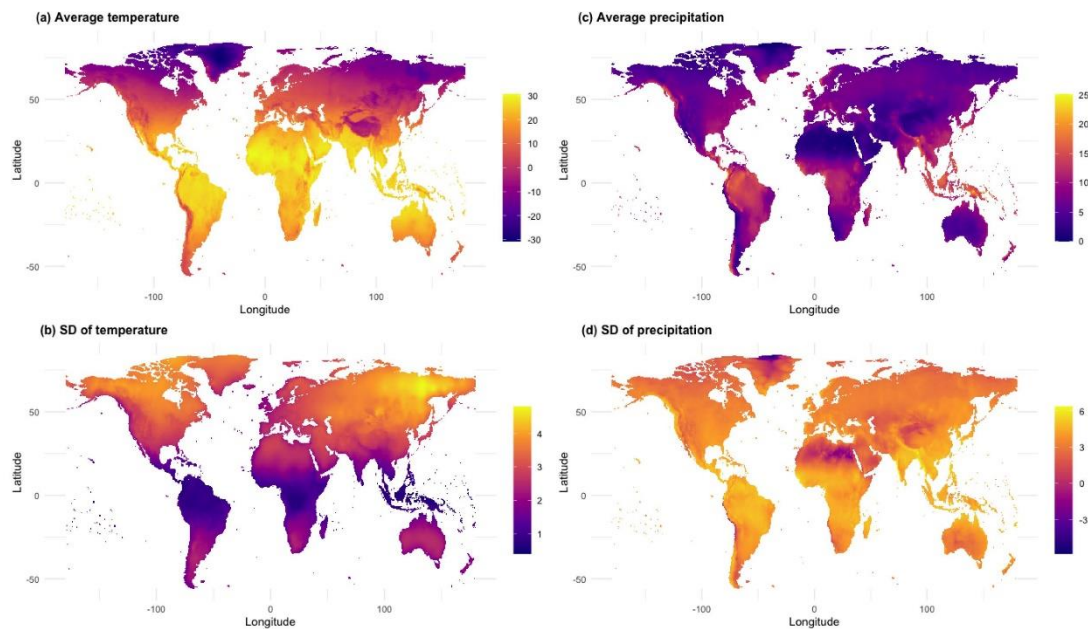


Figure S1: Global map of averages and standard deviations of temperature and precipitation.

2.3 Global correlation analysis for predictabilities

Fig. S2 present the scatter plots of predictabilities against average or SD of climatic variables. Because of the very large sample size, which makes all regressions significant, we did not plot regression lines in this figure. Similar to Fig. 3 in the main text, we found strong correlations in temperature predictabilities (Fig. S2a-f), and much less correlation in precipitation predictabilities (Fig. S2g-l). In addition, the directions of correlation in temperature predictabilities match with the ones in Fig. 3: fixed binning (method 1) positively correlated with average temperature but negatively correlated with SD of temperature; the other two methods negatively correlated with average temperature but positively correlated with SD of temperature. These results suggest the pattern presented in Fig. 3 is not a special case of the global climate data.

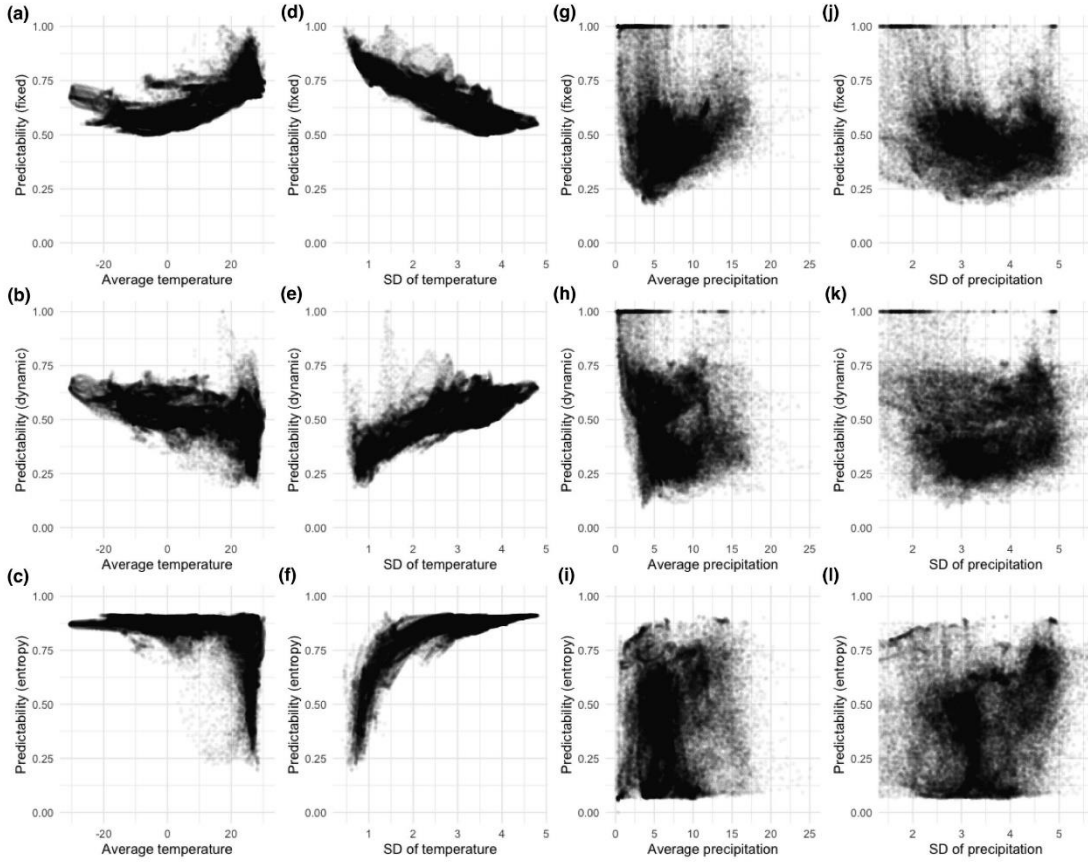


Figure S2: Correlation of predictabilities in the full global dataset.

3 Additional statistical analysis for the bird dataset

3.1 Correlation between other possible combinations of meteorological quantities

In addition to the correlations between average and SD within the same climatic variable (i.e., temperature or precipitation), as presented in Fig. 4, we also examined the potential correlations of other possible combinations (Fig. S3). We found no strong correlation between average temperature and average precipitation (Fig. S3a), or between average precipitation and SD of temperature (Fig. S3c). In contrast, there is some correlation between average temperature and SD of precipitation (Fig. S3b), and between SDs of temperature and precipitation (Fig. S3d). These correlations, together with the ones in Fig. 4, causes the increased variance inflation factor (VIF) in table 2 of the main text between model A and model B.

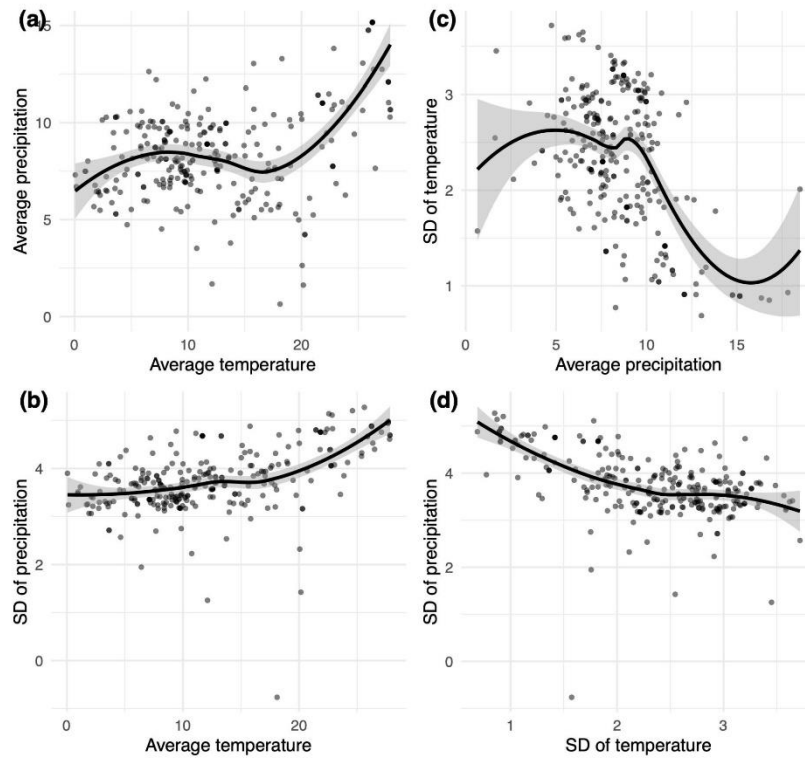


Figure S3: Additional correlation analyses for Fig. 4. Same and Fig. 4 in the main text, the curve and shaded area in each panel is calculated from loess regression.

3.2 Summary of all models in main text

We found the following meteorological quantities statistically significant ($p < 0.05$):

Averages only

- Model A: TMPavg and PREavg

Averages+ SDs

- Model B: PREavg

Averages+ SDs+ predictabilities

- Model C1 (fixed): TMPprd and PREprd
- Model C2 (dynamic): PREavg and PREsd
- Model C3 (entropy): PREavg and PREsd

The similarity between model C2 and C3 and their difference with model C1 correspond to the contrasting patterns in temperature predictabilities and their sensitivity/insensitivity to SDs (Fig. 1 and Fig. 2a-c). Please see the rest of this section for more details of model summaries.

3.2.1 Model with averages only (Model A)

```
##
## Call:
## phyloglm(formula = COOP2 ~ TMPavg + sqrt(PREavg), data = bird_data2,
##   phy = bird_tree, method = "logistic_MPLE", btol = 30)
##           AIC      logLik Pen.logLik
##      192.32      -92.16      -85.62
##
## Method: logistic_MPLE
## Mean tip height: 120.1095
## Parameter estimate(s):
## alpha: 0.02600371
##
## Coefficients:
##              Estimate      StdErr z.value  p.value
## (Intercept) -3.642832   1.057613 -3.4444 0.0005723 ***
## TMPavg       0.150122   0.048256  3.1110 0.0018647 **
## sqrt(PREavg) -0.138511   0.083979 -1.6494 0.0990755 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Note: Wald-type p-values for coefficients, conditional on alpha=0.02600371
```

3.2.2 Model with averages and SDs (Model B)

```
##
## Call:
## phyloglm(formula = COOP2 ~ TMPavg + sqrt(PREavg) + sqrt(TMPsd) +
##   log(PREsd), data = bird_data2, phy = bird_tree, method = "logistic_MPLE",
##   btol = 30)
##           AIC      logLik Pen.logLik
##      190.06      -89.03      -81.00
##
## Method: logistic_MPLE
## Mean tip height: 120.1095
## Parameter estimate(s):
## alpha: 0.02608535
##
## Coefficients:
##              Estimate      StdErr z.value p.value
## (Intercept) -1.226282   2.364129 -0.5187 0.60397
## TMPavg       0.089134   0.052101  1.7108 0.08712 .
## sqrt(PREavg) -0.297262   0.139053 -2.1378 0.03254 *
## sqrt(TMPsd)  -0.843894   0.583805 -1.4455 0.14832
## log(PREsd)   0.475233   0.470721  1.0096 0.31269
## ---
```



```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Note: Wald-type p-values for coefficients, conditional on alpha=0.02608535
```

3.2.3 Model with averages, SDs, and predictability with fixed binning (Model C1)

```
##
## Call:
## phyloglm(formula = COOP2 ~ TMPavg + sqrt(PREavg) + sqrt(TMPsd) +
##   log(PREsd) + TMPprd_Bot + PREprd_Bot, data = bird_data2,
##   phy = bird_tree, method = "logistic_MPLE", btol = 30)
##      AIC      logLik Pen.logLik
## 200.72    -92.36    -85.50
##
## Method: logistic_MPLE
## Mean tip height: 120.1095
## Parameter estimate(s):
## alpha: 0.008496072
##
## Coefficients:
##              Estimate      StdErr z.value  p.value
## (Intercept) -8.7484547  4.6613393 -1.8768 0.060544 .
## TMPavg       0.0170060  0.0349354  0.4868 0.626411
## sqrt(PREavg) 0.0052694  0.1399349  0.0377 0.969962
## sqrt(TMPsd)  0.3554659  0.5239228  0.6785 0.497474
## log(PREsd)   -0.1123348  0.4864411 -0.2309 0.817368
## TMPprd_Bot   13.9966568  5.3779584  2.6026 0.009252 **
## PREprd_Bot   -6.8286565  2.3274112 -2.9340 0.003346 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Note: Wald-type p-values for coefficients, conditional on alpha=0.008496072
```

3.2.4 Model with averages, SDs, and predictability with dynamic binning (Model C2)

```
##
## Call:
## phyloglm(formula = COOP2 ~ TMPavg + sqrt(PREavg) + sqrt(TMPsd) +
##   log(PREsd) + TMPprd_Dym + PREprd_Dym, data = bird_data2,
##   phy = bird_tree, method = "logistic_MPLE", btol = 30)
##      AIC      logLik Pen.logLik
## 204.99    -94.49    -85.52
##
## Method: logistic_MPLE
## Mean tip height: 120.1095
## Parameter estimate(s):
## alpha: 0.009442826
##
## Coefficients:
##              Estimate      StdErr z.value  p.value
## (Intercept) -0.744764  1.688926 -0.4410 0.6592352
## TMPavg       0.037525  0.029523  1.2710 0.2037177
## sqrt(PREavg) -0.325264  0.095280 -3.4138 0.0006407 ***
## sqrt(TMPsd)  -0.570604  0.389269 -1.4658 0.1426927
## log(PREsd)   0.956442  0.369130  2.5911 0.0095677 **
```

```
## TMPprd_Dym    -0.117390  2.762736 -0.0425 0.9661076
## PREprd_Dym    -0.794792  0.893403 -0.8896 0.3736683
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Note: Wald-type p-values for coefficients, conditional on alpha=0.009442826
```

3.2.5 Model with averages, SDs, and predictability with spectral entropy (Model C3)

```
##
## Call:
## phyloglm(formula = COOP2 ~ TMPavg + sqrt(PREavg) + sqrt(TMPsd) +
##          log(PREsd) + TMPprd_Ent + PREprd_Ent, data = bird_data2,
##          phy = bird_tree, method = "logistic_MPLE", btol = 30)
##          AIC      logLik Pen.logLik
##          202.6      -93.3      -83.9
##
## Method: logistic_MPLE
## Mean tip height: 120.1095
## Parameter estimate(s):
## alpha: 0.009710718
##
## Coefficients:
##              Estimate      StdErr z.value  p.value
## (Intercept)   0.118440   1.900791  0.0623 0.9503154
## TMPavg        0.043408   0.028645  1.5154 0.1296797
## sqrt(PREavg) -0.328544   0.096879 -3.3913 0.0006957 ***
## sqrt(TMPsd)  -0.165929   0.377204 -0.4399 0.6600147
## log(PREsd)    1.027901   0.381804  2.6922 0.0070978 **
## TMPprd_Ent   -2.742913   2.273806 -1.2063 0.2276983
## PREprd_Ent   -0.846097   0.817656 -1.0348 0.3007703
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Note: Wald-type p-values for coefficients, conditional on alpha=0.009710718
```

3.3 Nested model selection from model B (no predictabilities)

From this section to the end of section 3, we repeated nested model selection with different starting models. Nested model selection is the process where the starting model is the most complex model with all of the potentially important factors (i.e., predictors), then try to minimize the number of factors by removing them one by one. Our selection criteria is AIC, that is, if all of the reduced models have a higher AIC than the more complex model, then the model selection process ends. We apply the same process for model B, C1, C2, and C3, where one model does not contain predictability and the rest three have predictabilities calculated from one of the three method in Fig. 1. All models are logistic phylogenetic generalized least square (PGLS) regression models, but the multicollinearity test (VIF test) is done with the equivalent GLM model because there is no available tools to test multicollinearity for PGLS models.

The best model selected from model B is model B itself, with four predictors: **TMPavg**, **PREavg**, **TMPsd**, **PREsd**. The model has moderate degrees of collinearity (all VIFs < 3.3). There is only one significant predictor: average precipitation, with a partial R^2 of 4.5 %. (Note that partial R^2 from likelihood method is an estimation and values might not be additive.)

3.3.1 Model selection

Table S2: Model selection process from model B (no predictability).

Type	TMPavg	PREavg	TMPsd	PREsd	AIC
Full model					
<i>PGLS</i>	v	v	v	v	190.065
Drop 1 predictor					
<i>PGLS</i>		v	v	v	203.013
<i>PGLS</i>	v		v	v	194.224
<i>PGLS</i>	v	v		v	201.601
<i>PGLS</i>	v	v	v		204.638

Because all models with one fewer predictor have larger AIC, the best model is the full model.

3.3.2 Collinearity analysis

```
##      TMPavg sqrt(PREavg)  sqrt(TMPsd)  log(PREsd)
##      2.441206      3.210549      2.980364      3.271430
```

3.3.3 Summary of the best model

```
##
## Call:
## phyloglm(formula = COOP2 ~ TMPavg + sqrt(PREavg) + sqrt(TMPsd) +
##      log(PREsd), data = bird_data2, phy = bird_tree, method = "logistic_MPLE",
##      btol = 30)
##      AIC      logLik Pen.logLik
##      190.06      -89.03      -81.00
##
## Method: logistic_MPLE
## Mean tip height: 120.1095
## Parameter estimate(s):
## alpha: 0.02608535
##
## Coefficients:
##      Estimate StdErr z.value p.value
## (Intercept) -1.226282  2.364129 -0.5187 0.60397
## TMPavg      0.089134  0.052101  1.7108 0.08712 .
## sqrt(PREavg) -0.297262  0.139053 -2.1378 0.03254 *
## sqrt(TMPsd)  -0.843894  0.583805 -1.4455 0.14832
## log(PREsd)   0.475233  0.470721  1.0096 0.31269
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Note: Wald-type p-values for coefficients, conditional on alpha=0.02608535
```

3.3.4 Partial R²

Table S3: Partial R² of the fittest model.

Variables	R ²
<i>Overall</i>	0.378
<i>All fixed effects</i>	0.181
<i>Phylogenetic effects</i>	0.222
<i>TMPavg</i>	0.104
<i>PREavg</i>	0.045
<i>TMPsd</i>	0.095
<i>PREsd</i>	0.114

3.4 Nested model selection from model C1 (Colwell's predictability with fixed binning)

The best model selected from model C1 is contains three predictors: **TMPsd**, **TMPprd**, **PREprd**. The model has high degrees of collinearity (two VIFs out of three are larger than 4.8). There is only one significant predictor: precipitation predictability, with a partial R^2 of 10.6 %.

3.4.1 Model selection

Table S4: Model selection process (Colwell's predictability with fixed binning).

Type	TMPavg	PREavg	TMPsd	PREsd	TMPprd	PREprd	AIC
Full model							
<i>PGLS</i>	v	v	v	v	v	v	200.722
Drop 1 predictor							
<i>PGLS</i>		v	v	v	v	v	180.879
<i>PGLS</i>	v		v	v	v	v	182.422
<i>PGLS</i>	v	v		v	v	v	198.596
<i>PGLS</i>	v	v	v		v	v	180.835
<i>PGLS</i>	v	v	v	v		v	200.78
<i>PGLS</i>	v	v	v	v	v		199.462
Drop 2 predictors							
<i>PGLS</i>		v	v		v	v	178.63
<i>PGLS</i>	v		v		v	v	198.855
<i>PGLS</i>	v	v			v	v	182.345
<i>PGLS</i>	v	v	v			v	198.207
<i>PGLS</i>	v	v	v	v	v		206.847
Drop 3 predictors							
<i>PGLS</i>			v		v	v	176.614
<i>PGLS</i>		v			v	v	177.974
<i>PGLS</i>		v	v			v	188.922
<i>PGLS</i>		v	v		v		187.304
Drop 4 predictors							
<i>PGLS</i>					v	v	177.155
<i>PGLS</i>			v			v	184.002
<i>PGLS</i>			v		v		189.199

The best model is with (1) TMPsd, (2) TMPprd, and (3) PREprd.

3.4.2 Multicollinearity analysis

```
## sqrt(TMPsd)  TMPprd_Bot  PREprd_Bot
##    4.829679    4.916658    1.074507
```

3.4.3 Summary of the best model

```
##
## Call:
## phyloglm(formula = COOP2 ~ sqrt(TMPsd) + TMPprd_Bot + PREprd_Bot,
##   data = bird_data2, phy = bird_tree, method = "logistic_MPLE",
##   btol = 30)
##           AIC      logLik Pen.logLik
##    176.61    -83.31    -84.96
##
## Method: logistic_MPLE
## Mean tip height: 120.1095
## Parameter estimate(s):
## alpha: 0.02311312
##
## Coefficients:
##              Estimate      StdErr z.value p.value
## (Intercept) -11.268102   7.730172 -1.4577 0.14493
## sqrt(TMPsd)  0.099156   0.892179  0.1111 0.91151
## TMPprd_Bot   16.089311   8.861510  1.8156 0.06943 .
## PREprd_Bot   -7.267435   3.059078 -2.3757 0.01752 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Note: Wald-type p-values for coefficients, conditional on alpha=0.02311312
```

3.4.4 Partial R2

Table S5: Partial R2 of the fittest model.

Variables	R2
<i>Overall</i>	0.43
<i>All fixed effects</i>	0.251
<i>Phylogenetic effects</i>	0.238
<i>TMPsd</i>	0.02
<i>TMPprd</i>	0.07
<i>PREprd</i>	0.106

3.5 Nested model selection from model C2 (Colwell's predictability with dynamic binning)

The best model selected from model C2 is contains four predictors: **TMPavg**, **PREavg**, **PREsd**, **TMPprd**. The model has moderate degrees of collinearity (all VIFs < 3.4). There are two significant predictors: (1) average precipitation. with a partial R^2 of 9.1 %, and (2) average temperature, with a partial R^2 of 4.3 %.

3.5.1 Model selection

Table S6: Model selection process (Colwell's predictability with fixed binning).

Type	TMPavg	PREavg	TMPsd	PREsd	TMPprd	PREprd	AIC
Full model							
<i>PGLS</i>	v	v	v	v	v	v	204.988
Drop 1 predictor							
<i>PGLS</i>		v	v	v	v	v	201.601
<i>PGLS</i>	v		v	v	v	v	199.354
<i>PGLS</i>	v	v		v	v	v	199.333
<i>PGLS</i>	v	v	v		v	v	210.106
<i>PGLS</i>	v	v	v	v		v	201.052
<i>PGLS</i>	v	v	v	v	v		202.663
Drop 2 predictors							
<i>PGLS</i>		v		v	v	v	197.812
<i>PGLS</i>	v			v	v	v	197.545
<i>PGLS</i>	v	v			v	v	204.448
<i>PGLS</i>	v	v		v		v	194.682
<i>PGLS</i>	v	v		v	v		193.659
Drop 3 predictors							
<i>PGLS</i>		v		v	v		197.66
<i>PGLS</i>	v			v	v		204.743
<i>PGLS</i>	v	v			v		201.414
<i>PGLS</i>	v	v		v			201.601

The best model is with (1) TMPavg, (2) PREavg, (3) PREsd, and (4) TMPprd.

3.5.2 Multicollinearity analysis

```
##      TMPavg sqrt(PREavg)   log(PREsd)   TMPprd_Dym
##      1.485916      3.083736      3.356830      1.569149
```

3.5.3 Summary of the best model

```
##
## Call:
## phyloglm(formula = COOP2 ~ TMPavg + sqrt(PREavg) + log(PREsd) +
##      TMPprd_Dym, data = bird_data2, phy = bird_tree, method = "logistic_MPLE",
##      btol = 30)
##      AIC      logLik Pen.logLik
##      193.66     -90.83     -83.97
##
## Method: logistic_MPLE
## Mean tip height: 120.1095
## Parameter estimate(s):
## alpha: 0.02944318
##
## Coefficients:
##              Estimate      StdErr z.value p.value
## (Intercept) -2.008919   2.173520 -0.9243 0.355346
## TMPavg       0.098448   0.035727  2.7556 0.005859 **
```

```
## sqrt(PREavg) -0.272405 0.120346 -2.2635 0.023604 *
## log(PREsd) 0.612199 0.455745 1.3433 0.179177
## TMPprd_Dym -3.154738 3.060667 -1.0307 0.302665
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Note: Wald-type p-values for coefficients, conditional on alpha=0.02944318
```

3.5.4 Partial R2

Table S7: Partial R2 of the fittest model.

Variables	R2
<i>Overall</i>	0.361
<i>All fixed effects</i>	0.159
<i>Phylogenetic effects</i>	0.209
<i>TMPavg</i>	0.043
<i>PREavg</i>	0.091
<i>PREsd</i>	0.069
<i>TMPprd</i>	0.07

3.6 Nested model selection from model C3 (normalised spectral entropy)

The best model selected from model C3 is contains five predictors: **PREavg**, **TMPsd**, **PREsd**, **TMPprd**, **PREprd**. The model has moderate degrees of collinearity (all VIFs < 3.4). There are two significant predictors: (1) average precipitation. with a partial R^2 of 10.9 %, and (2) SD of temperature, with a partial R^2 of 3.7 %.

3.6.1 Model selection

Table S8: Model selection process (normalised spectral entropy).

Type	TMPavg	PREavg	TMPsd	PREsd	TMPprd	PREprd	AIC
Full model							
<i>PGLS</i>	v	v	v	v	v	v	202.598
Drop 1 predictor							
<i>PGLS</i>		v	v	v	v	v	192.697
<i>PGLS</i>	v		v	v	v	v	214.982
<i>PGLS</i>	v	v		v	v	v	200.568
<i>PGLS</i>	v	v	v		v	v	207.618
<i>PGLS</i>	v	v	v	v		v	202.462
<i>PGLS</i>	v	v	v	v	v		200.015
Drop 2 predictors							
<i>PGLS</i>			v	v	v	v	206.565
<i>PGLS</i>		v		v	v	v	195.799
<i>PGLS</i>		v	v		v	v	197.099
<i>PGLS</i>		v	v	v		v	199.222
<i>PGLS</i>		v	v	v	v		204.44

The best model is with (1) PREavg, (2) TMPsd, (3) PREsd, (4) TMPprd, and (5) PREprd.

3.6.2 Multicollinearity analysis

```
## sqrt(PREavg) sqrt(TMPsd) log(PREsd) TMPprd_Ent PREprd_Ent
## 3.065424 2.693238 3.366935 2.589245 1.508869
```

3.6.3 Summary of the best model

```
##
## Call:
## phyloglm(formula = COOP2 ~ sqrt(PREavg) + sqrt(TMPsd) + log(PREsd) +
## TMPprd_Ent + PREprd_Ent, data = bird_data2, phy = bird_tree,
## method = "logistic_MPLE", btol = 30)
## AIC logLik Pen.logLik
## 192.70 -89.35 -85.53
##
## Method: logistic_MPLE
## Mean tip height: 120.1095
## Parameter estimate(s):
## alpha: 0.02810589
##
## Coefficients:
## Estimate StdErr z.value p.value
## (Intercept) 0.91867 2.64415 0.3474 0.728263
## sqrt(PREavg) -0.42178 0.14299 -2.9497 0.003181 **
## sqrt(TMPsd) -1.34659 0.59572 -2.2604 0.023794 *
## log(PREsd) 1.04315 0.54902 1.9000 0.057431 .
## TMPprd_Ent -1.07190 2.69993 -0.3970 0.691361
## PREprd_Ent 0.42086 1.23316 0.3413 0.732887
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Note: Wald-type p-values for coefficients, conditional on alpha=0.02810589
```

3.6.4 Partial R2

Table S9: Partial R2 of the fittest model.

Variables	R2
<i>Overall</i>	0.375
<i>All fixed effects</i>	0.178
<i>Phylogenetic effects</i>	0.234
<i>PREavg</i>	0.109
<i>TMPsd</i>	0.037
<i>PREsd</i>	0.046
<i>TMPprd</i>	0.061
<i>PREprd</i>	0.096

4 Normality check of the bird dataset

4.1 Q-Q plots for the meteorological quantities

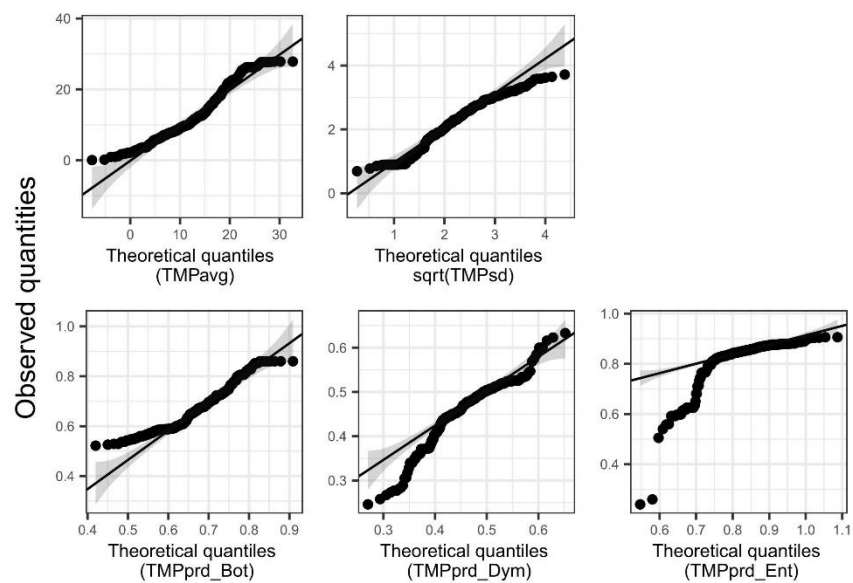


Figure S4: Q-Q plots for temperature quantities.

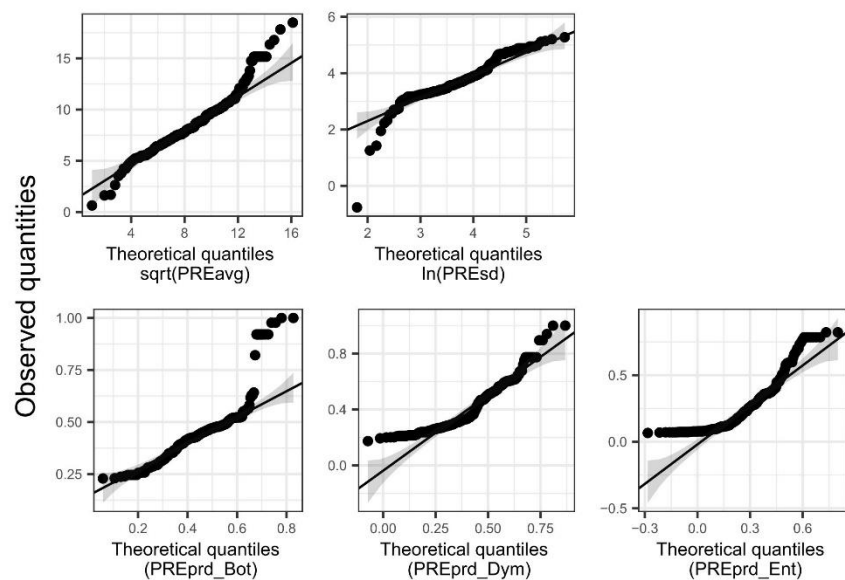


Figure S5: Q-Q plots for precipitation quantities.

4.2 Alternative transformations

4.2.1 SD of Temperature

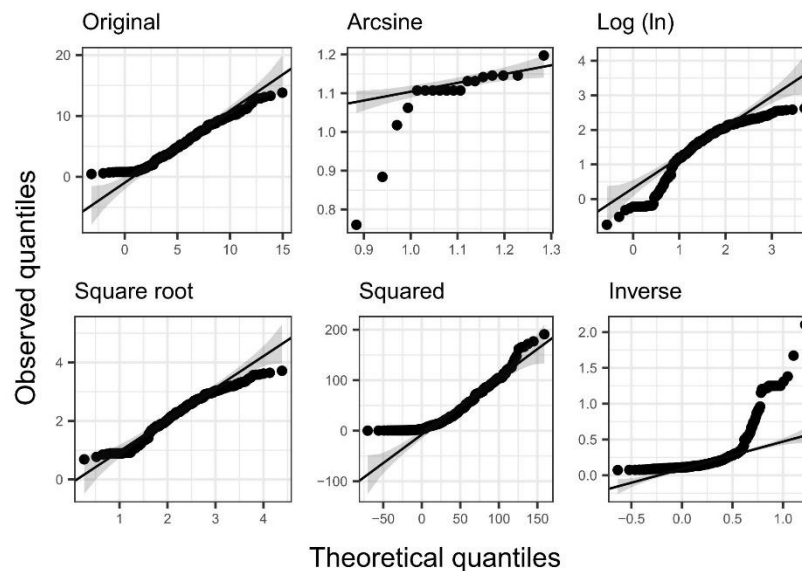


Figure S6: Alternative transformations for the SD of temperature

4.2.2 Average precipitation

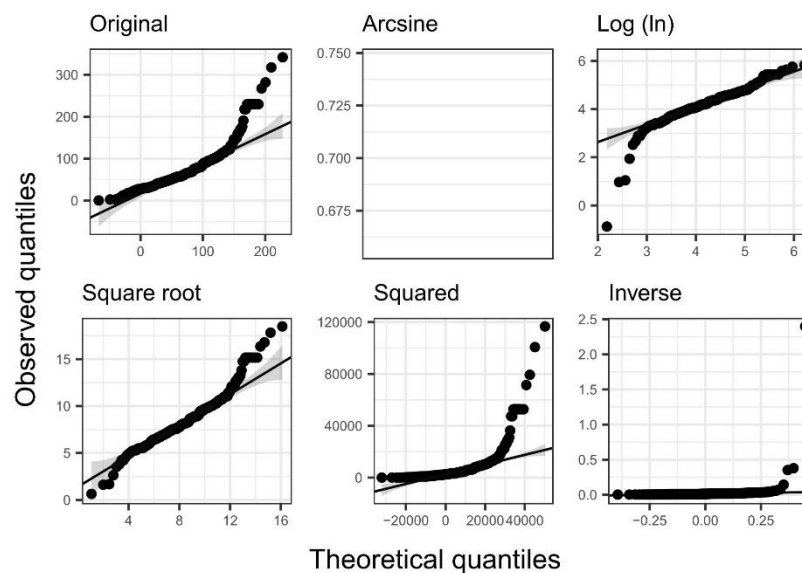


Figure S7: Alternative transformations for average precipitation

4.2.3 SD of precipitation

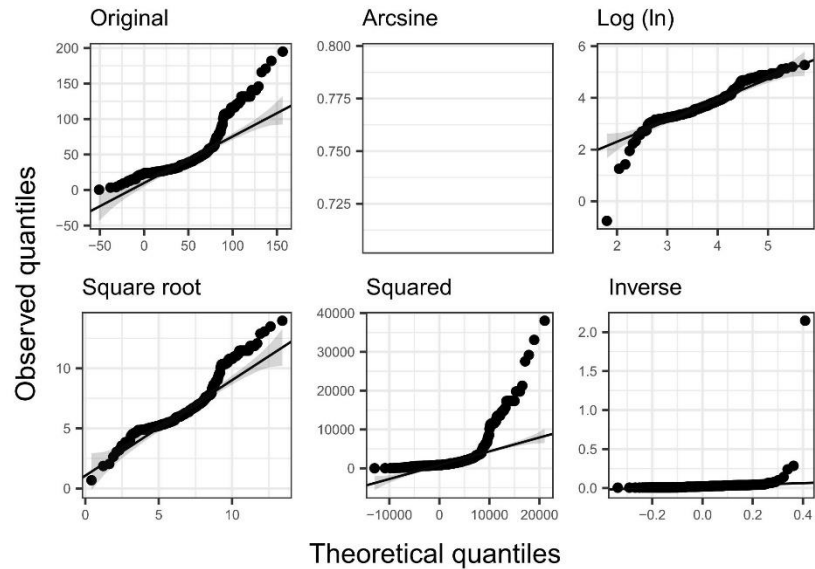


Figure S8: Alternative transformations for the SD of precipitation

5 The source codes for Figure 1

```
# Loading packages
library(data.table)
library(dplyr)
library(ggplot2)
library(ggpubr)
theme_set(theme_bw())

#####
# 1. Making the time series ###
#####

# Make an index
xx<- seq(1, 60, length.out= 60)
# Make a time series of white noise
wt.noise<- runif(60, min= -0.25, max= 0.25)
# Make the examples
# Time series 1
ts1<- sin(xx*2*pi/12)+ wt.noise
# Time series 2
ts2<- 5*sin(xx*2*pi/12)+ 5*wt.noise
# Plot the data (the first row in Fig. 1)
ts1.plot<- ggplot(data= data.frame(x= xx, y=ts1), aes(x=x,y=y))+
  geom_point(size= 0.75)+
  ggtitle("Time series 1")+
  scale_x_continuous(breaks=seq(0, 60, 12))+
  coord_cartesian(ylim = c(-6, 6), xlim = c(0, 60))+
```

```

xlab("Month")+ ylab(expression(atop("Observed",paste("temperature")))))

ts2.plot<- ggplot(data= data.frame(x= xx, y=ts2), aes(x=x,y=y))+
  geom_point(size= 0.75)+
  ggtitle("Time series 2")+
  scale_x_continuous(breaks=seq(0, 60, 12))+
  coord_cartesian(ylim = c(-6, 6), xlim = c(0, 60))+
  xlab("Month")+ ylab("")
#####
# 2. Colwell's P with fixed binning analysis ###
#####
# For ts1 ###
input.ts<- ts1
# Specify the break points
breaks<- seq(-6, 6, length.out= 13)
# Convert the time series into processed occurrence table
focal.ts<- data.frame(obs= input.ts)
ts.leng<- length(focal.ts[,1])
focal.ts$month<- rep(1:12, times=ts.leng/12) ## Assuming starting from January ##
focal.ts$class<- cut(focal.ts$obs, breaks, right = FALSE, include.lowest = TRUE)
focal.table<- with(focal.ts, table(class, month))
## Calculation (modified from R::hydrostats package)
X <- apply(focal.table, 2, sum, na.rm = T)
Y <- apply(focal.table, 1, sum, na.rm = T)
Z <- sum(focal.table, na.rm = TRUE)
HX <- -1 * sum((X/Z) * log(X/Z, base= 2), na.rm = TRUE)
HY <- -1 * sum((Y/Z) * log(Y/Z, base= 2), na.rm = TRUE)
HXY <- -1 * sum((focal.table/Z) * log(focal.table/Z, base= 2), na.rm = TRUE)
P <- round(1 - (HXY - HX)/log(length(breaks), base= 2), 3)
# Plot ts1 ###
plot.table<- melt(focal.table)
plot.table[plot.table==0]<- NA
ts1.Fixed<- ggplot(plot.table, aes(month, class, fill= value))+
  geom_tile()+
  ggtitle("Time series 1")+
  scale_fill_viridis_b(limits = c(0, 5), n.breaks=5,
    option = "plasma", name= "",na.value= "white")+
  xlab("Month")+ ylab(expression(atop("Category",paste("(discrete temperature)"))))+
  scale_x_continuous(breaks=seq(2, 12, 2))+
  theme(axis.text.y=element_blank(), #remove y axis labels
    legend.position = "none")
# For ts2 ###
input.ts<- ts2
# Specify the break points
breaks<- seq(-6, 6, length.out= 13)
# Convert the time series into processed occurrence table
focal.ts<- data.frame(obs= input.ts)
ts.leng<- length(focal.ts[,1])
focal.ts$month<- rep(1:12, times=ts.leng/12) ## Assuming starting from January ##
focal.ts$class<- cut(focal.ts$obs, breaks, right = FALSE, include.lowest = TRUE)
focal.table<- with(focal.ts, table(class, month))
## Calculation (modified from R::hydrostats package)
X <- apply(focal.table, 2, sum, na.rm = T)

```

```

Y <- apply(focal.table, 1, sum, na.rm = T)
Z <- sum(focal.table, na.rm = TRUE)
HX <- -1 * sum((X/Z) * log(X/Z, base= 2), na.rm = TRUE)
HY <- -1 * sum((Y/Z) * log(Y/Z, base= 2), na.rm = TRUE)
HXY <- -1 * sum((focal.table/Z) * log(focal.table/Z, base= 2), na.rm = TRUE)
P <- round(1 - (HXY - HX)/log(length(breaks), base= 2), 3)
# Plot ts2 ####
plot.table<- melt(focal.table)
plot.table[plot.table==0]<- NA
ts2.Fixed<- ggplot(plot.table, aes(month, class, fill= value))+
  geom_tile()+
  ggtitle("Time series 2")+
  scale_fill_viridis_b(limits = c(0, 5), n.breaks=5,
    option = "plasma", name= "",na.value= "white")+
  xlab("Month")+ ylab("")+
  scale_x_continuous(breaks=seq(2, 12, 2))+
  theme(axis.text.y=element_blank(), #remove y axis labels
    legend.position = "none")
#####
# 3. Colwell's P with dynamic binning ####
#####
# For ts1 ####
# Specify the break points
breaks= 24
# Convert the time series into processed occurrence table
input.ts<- ts1
focal.ts<- data.frame(obs= input.ts)
ts.leng<- length(focal.ts[,1])
focal.ts$month<- rep(1:12, times=ts.leng/12) ## Assuming starting from January ##
focal.ts$class<- cut(focal.ts$obs, breaks, right = FALSE, include.lowest = TRUE)
focal.table<- with(focal.ts, table(class, month))
## Calculation (copied from hydrostats package)
X <- apply(focal.table, 2, sum, na.rm = T)
Y <- apply(focal.table, 1, sum, na.rm = T)
Z <- sum(focal.table, na.rm = TRUE)
HX <- -1 * sum((X/Z) * log(X/Z, base= 2), na.rm = TRUE)
HY <- -1 * sum((Y/Z) * log(Y/Z, base= 2), na.rm = TRUE)
HXY <- -1 * sum((focal.table/Z) * log(focal.table/Z, base= 2), na.rm = TRUE)
P <- round(1 - (HXY - HX)/log(breaks, base= 2), 3)
# Plot ts1 ####
plot.table<- melt(focal.table)
plot.table[plot.table==0]<- NA
ts1.dynamic<- ggplot(plot.table, aes(month, class, fill= value))+
  geom_tile()+
  ggtitle("Time series 1")+
  scale_fill_viridis_b(limits = c(0, 5), n.breaks=5,
    option = "plasma", name= "",na.value= "white")+
  xlab("Month")+ ylab(expression(atop("Category",paste("(discrete temperature)"))))+
  scale_x_continuous(breaks=seq(2, 12, 2))+
  theme(axis.text.y=element_blank(), #remove y axis labels
    legend.position = "none")
# For ts2 ####
# Convert the time series into processed occurrence table

```



```

input.ts<- ts2
focal.ts<- data.frame(obs= input.ts)
ts.leng<- length(focal.ts[,1])
focal.ts$month<- rep(1:12, times=ts.leng/12) ## Assuming starting from January ##
focal.ts$class<- cut(focal.ts$obs, breaks, right = FALSE, include.lowest = TRUE)
focal.table<- with(focal.ts, table(class, month))
## Calculation (copied from hydrostats package)
X <- apply(focal.table, 2, sum, na.rm = T)
Y <- apply(focal.table, 1, sum, na.rm = T)
Z <- sum(focal.table, na.rm = TRUE)
HX <- -1 * sum((X/Z) * log(X/Z, base= 2), na.rm = TRUE)
HY <- -1 * sum((Y/Z) * log(Y/Z, base= 2), na.rm = TRUE)
HXY <- -1 * sum((focal.table/Z) * log(focal.table/Z, base= 2), na.rm = TRUE)
P <- round(1 - (HXY - HX)/log(breaks, base= 2), 3)
# Plot ts2 #####
plot.table<- melt(focal.table)
plot.table[plot.table==0]<- NA
ts2.dynamic<- ggplot(plot.table, aes(month, class, fill= value))+
  geom_tile()+
  ggtitle("Time series 2")+
  scale_fill_viridis_b(limits = c(0, 5), n.breaks=5,
    option = "plasma", name= "",na.value= "white")+
  xlab("Month")+ ylab("")+
  scale_x_continuous(breaks=seq(2, 12, 2))+
  theme(axis.text.y=element_blank(), #remove y axis labels
    legend.position = "none")
#####
# 4. Normalised spectral entropy ###
#####
# Convert the time series into frequency domain by using fast Fourier transformation (FFT)
ts1.spcm<- spectrum(ts1, plot=F)
ts2.spcm<- spectrum(ts2, plot=F)
ts1.spcm.df<- data.frame(freq=ts1.spcm$freq, spec=ts1.spcm$spec)
ts2.spcm.df<- data.frame(freq=ts2.spcm$freq, spec=ts2.spcm$spec)
# Make each panels
# Fourier spectrum
ts1.spcm.plot<- ggplot(ts1.spcm.df, aes(x=freq, y=spec))+
  ggtitle("Fourier spectrum")+
  xlab("Frequency (1/month)") + ylab("Amplitude")+
  geom_line()+
  theme(plot.title = element_text(size=9),
    axis.title.x=element_text(size=9), axis.title.y=element_text(size=9))
# Histogram of amplitudes
ts1.hist<- ggplot(ts1.spcm.df,aes(x=spec))+
  ggtitle("Histogram")+
  xlab("Amplitude") + ylab("Counts")+
  geom_histogram()+
  theme(plot.title = element_text(size=9),
    axis.title.x=element_text(size=9), axis.title.y=element_text(size=9))
# Fourier spectrum
ts2.spcm.plot<- ggplot(ts2.spcm.df, aes(x=freq, y=spec))+
  ggtitle("Fourier spectrum")+
  xlab("Frequency (1/month)") + ylab("Amplitude")+

```

```

geom_line()+
  theme(plot.title = element_text(size=9),
        axis.title.x=element_text(size=9), axis.title.y=element_text(size=9))
# Histogram of amplitudes
ts2.hist<- ggplot(ts2.spcm.df,aes(x=spec))+
  ggtitle("Histogram")+
  xlab("Amplitude")+ ylab("Counts")+
  geom_histogram()+
  theme(plot.title = element_text(size=9),
        axis.title.x=element_text(size=9), axis.title.y=element_text(size=9))
#####
# 5. Combine figure panels into a figure ###
#####
raw_plots<- ggarrange(ts1.plot, ts2.plot, ncol=2)
raw_plots<- annotate_figure(raw_plots, top= text_grob("(a) Original data",
                                                    hjust=2.63, face="bold"))

#
Fixed<- ggarrange(ts1.Fixed,ts2.Fixed, ncol=2)
Fixed<- annotate_figure(Fixed,
  top= text_grob("(b) Colwell's P with fixed binning: constant resolution",
                hjust=0.79, face="bold"))

#
Dynamic<- ggarrange(ts1.dynamic,ts2.dynamic, ncol=2)
Dynamic<- annotate_figure(Dynamic,
  top= text_grob("(c) Colwell's P with dynamic binning: constant compression",
                hjust=0.71, face="bold"))

#
ts1.ent<- ggarrange(ts1.spcm.plot,ts1.hist, ncol=2)
ts1.ent<- annotate_figure(ts1.ent, top= text_grob("Time series 1"))
ts2.ent<- ggarrange(ts2.spcm.plot,ts2.hist, ncol=2)
ts2.ent<- annotate_figure(ts2.ent, top= text_grob("Time series 2"))
Ent<- ggarrange(ts1.ent, ts2.ent, ncol=2)
Ent<- annotate_figure(Ent,
  top= text_grob("(d) Normalised spectral entropy: no compression",
                hjust= 0.86, face= "bold"))

# Merge all panels
ggarrange(raw_plots, Fixed, Dynamic, Ent, nrow=4, heights=c(0.75,1,1,1))

```

7

Discussion

1. Overview

Each of the proceeding chapters has their own discussion. Consequently, in this last chapter, I briefly summarise the key findings and discuss the potential extensions.

2. Relatedness and the evolution of mechanisms to divide labour in microorganisms (Chapter 2)

In Chapter 2, I examined the optimal level of coordination to divide labour, in response to genetic relatedness and group size. I found natural selection can favour intermediate levels of coordination, and that the level of coordination decreases as individuals are less related to each other. Empirical validation for the findings here would require understanding the molecular mechanisms of coordination before one could quantify the level of coordination for each social group. The closest systems are slime moulds and *Myxococcus* bacteria, where multiple cells form a fruiting body together - some cells become reproductive spores while others become sterile supporting structures (Kaiser 2004; Konovalova *et al.* 2010; Strassmann & Queller 2011; Dhakshinamoorthy & Singh 2021). These fruiting bodies can be clonal or nonclonal, and suggest the importance of understanding the effects of nonclonality (Foster *et al.* 2002; Fiegna & Velicer 2005). Nevertheless, currently there is not enough knowledge to quantify the degree of coordination and we could test if groups are less coordinated when less related once this challenge is overcome.

There are several possible extensions for this project: nonclonal division of labour with spatial structure networks, and non-reproductive division of labour in clonal or nonclonal groups. First, spatial structure has been suggested to facilitate task specialisation. In our previous paper (Appendix 6), we found when some individuals are more connected than other individuals, then they would be selected to become

reproductives and others would be selected to become helpers. This is because the better-connected cells gain a greater share of public goods. However, the previous paper did not look beyond clonality. Nonclonality opens two questions: (1) Can spatial structure still help division of labour, even if the proportion of helpers drops as relatedness goes down? (2) When there are options for forming spatial structure and performing coordination, which mechanism would be selected, and does it change when groups are less related? Answering these questions could help us explain why helper proportion is a bimodal distribution between species in the empirical data (Cooper *et al.* 2023), and why spatial structure is not found in all species with reproductive division of labour.

The second extension is to non-reproductive division of labour, where individuals are specialised to different tasks like metabolising specific nutrients. This scenario is different from reproductive division of labour because no individual reduces its reproductive potential. On the empirical side, several species have been found to divide non-reproductive labour, such as secreting surfactant or matrix protein, or performing a two-step metabolism to digest nutrient (Laue *et al.* 2006; Lawrence *et al.* 2007; Nikel *et al.* 2014; van Gestel *et al.* 2015; Armbruster *et al.* 2019). Little is known, on the theoretical side, about when non-reproductive division of labour is favoured over a generalist strategy (Wahl 2002). As a result, there is a need for theoretical development. In addition, it is unclear whether coordination is also an important mechanism in non-reproductive division of labour. Thus, a series of theories could be built for non-reproductive division of labour.

3. Antagonistic effects of long- and short-term environmental variation on species coexistence (Chapter 3)

In chapter 3, I found different time scales of environmental fluctuations have opposite effects on strain coexistence: short-term variation helps the maintenance of diversity while long-term variation facilitates competitive exclusion. Our theoretical framework also unified previously seeming contradicting hypotheses on diversity and environmental variability, as all possible patterns can be generated when more than one time scale of environmental fluctuation is considered in the model. On the empirical side, there is some evidence to support our predictions in macroecology studies (Chan *et al.* 2016; Cohen *et al.* 2020; Kefford *et al.* 2022), however, experiments would be needed to directly test the predictions. The easiest system to set up is probably bacterial communities because they grow very fast, and their growing environment can be easily manipulated.

Our framework can be applied to many different aspects of biology, such as cooperation dynamic under ‘transient’ time scale, and the effects of environmental fluctuations on predator-prey systems. Transient time scale is the opposite of indefinite time scale, on which classic analytical models typically make predictions (MacArthur 1972; Chesson 2000; Siepielski & McPeck 2010). The key difference here, is classic models usually assume a large number for the environmental conditions the strain (or species) experience, so the dynamics can be analysed through the true distribution of the environmental fluctuations. However, this assumption is violated when some variations are on a relative long-term in the ecological time scale because the window averages of environmental conditions converge on a much slower pace (Fig. 1 of Chapter 3). As a result, the cooperation dynamics can be very different from those that the Price equation suggests (Price 1970; Gardner 2020; dos Santos *et al.* 2023). For

instance, under the presence of long-term environmental fluctuations, cooperators could have a greater chance to get rid of cheats. Similarly, we can move beyond competition framework and explore similar types of effects under predator-prey interactions. For instance, it is possible that long-term environmental fluctuations can interfere with the classic predator-prey cycles and remove the atto-fox issue for Lotka-Volterra predator prey equations, thus making more realistic predictions (Anderson *et al.* 1981; Mollison 1991; Fowler 2021).

4. The evolution of manipulative cheating (Chapter 4)

In Chapter 4, I found arms-race-like dynamics in the degree of cheating can be found when manipulative cheating and its suppression is included to the classic tragedy of the commons model. These findings can help explain the empirical findings of genetic differences in the level of cooperation in ants, bacteria, and viruses. Although the direct proof for manipulative cheat needs molecular evidence, similar to Chapter 2, there are some immediate extensions for the model to better fit the actual biology. For example, we can extend the model from a haploid to haplodiploid to fit the genetic system of social insects (Hölldobler & Wilson 1990; de la Filia *et al.* 2015). The consequences of including an extra copy of gene might depend on the dominance relationship, and it is possible to generate more complex dynamics with this extra layer of biological detail (Crozier 2008; Davies & Gardner 2018).

5. Equilibria and oscillations in cheat-cooperator dynamics (Chapter 5)

In Chapter 5, I found periodic population bottlenecks and density dependence of cheat's growth rate on cooperator's abundance are important for creating oscillating dynamics in the proportion of cheats. Allowing for stochastic group formation also allows another level of oscillation, between growth cycles. Empirically speaking, by far there is only

evidence for the oscillation between growth cycles (i.e., the ones that require stochastic group formation), but population dynamic monitoring with very high time resolution is feasible for experiments with automated instruments. Validation on our first model prediction can be done if experiments with such high time resolution could be carried out.

Besides experimental validation, there are also many potential theoretical extensions for answering important biological questions. For instance, the cheat-cooperation relationship could have more than two strains involved and form a rock-paper-scissors relationship (Inglis *et al.* 2016; Lowen 2018). It is unknown whether similar oscillating dynamics in strain ratios can also be created with growth cycles and density dependence. Another extension is including resource dynamics where both cheats and cooperators exploit the resource to grow. This resource would represent host density in viral dynamics and cheats would be the viral particles with incomplete genome but replicate faster. Investigating when cheats could be removed in the system has important clinical application for phage therapy, which is a proposed methods to use phage to treat antimicrobial resistance bacteria (Horiuchi 1983; Hatfull *et al.* 2022). Lastly, we can also incorporate temporal and spatial fluctuations to the model and examine if these fluctuations help produce oscillating dynamics.

6. What can climatic dataset tell us about evolution: an analysis on environmental predictabilities and correlations (Chapter 6)

In chapter 6, I found predictability, a quantity used in comparative studies to explain diversity on a global scale, can have contrasting patterns depend on which calculation method is used. I further showed many meteorological quantities are highly correlated, suggesting that biologists should take extra caution when working with these quantities.

This project was a systematic diagnosis of a meteorological dataset, but could be extended in two ways. First, we could further examine other datasets, like ERA5 and WorldClim (Fick & Hijmans 2017; Hersbach *et al.* 2020). Some datasets have a much higher time resolution (hourly in ERA5, while others are monthly) or spatial resolution (1km in WorldClim, while others are 0.25° or 0.5°), plus different observation data or algorithm is used in each dataset, making it is unclear about the outcome for a similar analysis in different datasets. Second, we could apply the recent generative model framework to the dataset we analysed, the CRUTS (Harris *et al.* 2020; Dinnage 2023). In that study, a generative model found the 19 variables in WorldClim dataset only contains 5 independent axes (Dinnage 2023). It is not clear about the generality of the result, nor whether we can use the generated 5 axes for formal statistical analyses in comparative studies. Answering these questions could potentially change how people have been using meteorological data in understanding evolution.

7. Conclusion

This thesis highlights the importance of eco-evolutionary feedbacks, where ecological and evolutionary processes interact with each other (Lion 2018; Govaert *et al.* 2019). This type of feedback has received an increasing attention as there is growing number of empirical evidence showing ecological and evolutionary processes can operate at a similar time scale (Post & Palkovacs 2009; Hiltunen *et al.* 2014; Hendry 2016). In particular, my works have pointed out the crucial role of temporal scaling in these feedbacks in two ways: (1) the relative strength of random noise to selection forces; and (2) the relative speed of extinction process to environmental fluctuations.

The importance of relative strengths of noises and selective forces has been recognised in theoretical biology. For instance, when selection and mutation are both

present in a population, it may reach a balance between the two forces. In the context of eco-evolutionary feedbacks, the noise may not be created by mutation, but by other processes like demographic stochasticity, linkage of coevolving traits, and small number effects (e.g., that the ratio of division 1 can only be 0, 0.5, or 1 in a group of 2 individuals). These processes have been investigated in Chapters 2, 4, 5. One might think these mechanisms would only slow down the selection outcome, but they actually can change the trait dynamics from static to oscillations (Chapter 4), or even reverse the selection outcome (Constable *et al.* 2016; Hilbe *et al.* 2018). Because biological populations are rarely close to infinity, considering these effects could greatly improve our ability to make better implications and predictions in nature.

The relative speed of extinction process to environmental fluctuations, on the other hand, is rather underexplored. This factor is important because if the pace of extinction (e.g., through competitive exclusion) is very fast, then only a small number of environmental conditions would be experienced during the extinction process. Because of the small number, there can be a great degree of stochasticity in the experienced environmental condition. This point has been elucidated in Chapters 3& 5, where the time scale of fluctuations or the duration of growth cycles matter to the outcomes of selection. In addition, the global meteorological dataset used in Chapter 6 is highly related because it contains time series over a relatively long period of time (e.g., > 100 years for bacteria) and can be used to verify the hypotheses and logic in these arguments. Since there is a continuing advance in the spatial and temporal resolution of empirical data, future research on this temporal stochasticity and the effects of time scales could be promising.

References

1. Anderson, R.M., Jackson, H.C., May, R.M. & Smith, A.M. (1981). Population dynamics of fox rabies in Europe. *Nature*, 289, 765-771.
2. Armbruster, C.R., Lee, C.K., Parker-Gilham, J., de Anda, J., Xia, A., Zhao, K. *et al.* (2019). Heterogeneity in surface sensing suggests a division of labor in *Pseudomonas aeruginosa* populations. *eLife*, 8, e45084.
3. Chan, W.-P., Chen, I.-C., Colwell, R.K., Liu, W.-C., Huang, C.-y. & Shen, S.-F. (2016). Seasonal and daily climate variation have opposite effects on species elevational range size. *Science*, 351, 1437-1439.
4. Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 343-366.
5. Cohen, J.M., Fink, D. & Zuckerberg, B. (2020). Avian responses to extreme weather across functional traits and temporal scales. *Global Change Biology*, 26, 4240-4250.
6. Constable, G.W.A., Rogers, T., McKane, A.J. & Tarnita, C.E. (2016). Demographic noise can reverse the direction of deterministic selection. *Proceedings of the National Academy of Sciences*, 113, E4745-E4754.
7. Cooper, G.A., Liu, M., Zhang, Z., Fisher, R.M. & West, S.A. (2023). The evolution of predominantly sterile social groups. *under review in Evolution Letters*.
8. Crozier, R.H. (2008). Advanced eusociality, kin selection and male haploidy. *Australian Journal of Entomology*, 47, 2-8.
9. Davies, N.G. & Gardner, A. (2018). Monogamy promotes altruistic sterility in insect societies. *Royal Society Open Science*, 5, 172190.
10. de la Filia, A.G., Bain, S.A. & Ross, L. (2015). Haplodiploidy and the reproductive ecology of Arthropods. *Current Opinion in Insect Science*, 9, 36-43.
11. Dhakshinamoorthy, R. & Singh, S.P. (2021). Evolution of reproductive division of labor – lessons learned from the social amoeba *Dictyostelium discoideum* during its multicellular development. *Frontiers in Cell and Developmental Biology*, 9.
12. Dinnage, R. (2023). How many variables does Wordclim have, really? Generative A.I. unravels the intrinsic dimension of bioclimatic variables. *bioRxiv*, 2023.2006.2012.544623.
13. dos Santos, M., Downing, P.A., Griffin, Ashleigh S., Cornwallis, Charlie K. & West, Stuart A. (2023). Altruism and natural selection in a variable environment. *in resubmission to Nature*.
14. Fick, S.E. & Hijmans, R.J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37, 4302-4315.

15. Fiegna, F. & Velicer, G.J. (2005). Exploitative and hierarchical antagonism in a cooperative bacterium. *PLoS Biology*, 3, e370.
16. Foster, K.R., Fortunato, A., Strassmann, J.E. & Queller, D.C. (2002). The costs and benefits of being a chimera. *Proceedings of the Royal Society, Series B: Biological Sciences*, 269, 2357-2362.
17. Fowler, A.C. (2021). Atto-foxes and other minutiae. *Bulletin of Mathematical Biology*, 83, 104.
18. Gardner, A. (2020). Price's equation made clear. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375, 20190361.
19. Govaert, L., Fronhofer, E.A., Lion, S., Eizaguirre, C., Bonte, D., Egas, M. *et al.* (2019). Eco-evolutionary feedbacks—theoretical models and perspectives. *Functional Ecology*, 33, 13-30.
20. Harris, I., Osborn, T.J., Jones, P. & Lister, D. (2020). Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. *Scientific data*, 7, 1-18.
21. Hatfull, G.F., Dedrick, R.M. & Schooley, R.T. (2022). Phage therapy for antibiotic-resistant bacterial infections. *Annual Review of Medicine*, 73, 197-211.
22. Hendry, A.P. (2016). *Eco-evolutionary Dynamics*. Princeton University Press.
23. Hersbach, H., Bell, B., Berrisford, P., Hirahara, S., Horányi, A., Muñoz-Sabater, J. *et al.* (2020). The ERA5 global reanalysis. *Quarterly Journal of the Royal Meteorological Society*, 146, 1999-2049.
24. Hilbe, C., Šimsa, Š., Chatterjee, K. & Nowak, M.A. (2018). Evolution of cooperation in stochastic games. *Nature*, 559, 246-249.
25. Hiltunen, T., Hairston Jr, N.G., Hooker, G., Jones, L.E. & Ellner, S.P. (2014). A newly discovered role of evolution in previously published consumer–resource dynamics. *Ecology Letters*, 17, 915-923.
26. Hölldobler, B. & Wilson, E.O. (1990). *The ants*. Harvard University Press.
27. Horiuchi, K. (1983). Co-evolution of a filamentous bacteriophage and its defective interfering particles. *Journal of Molecular Biology*, 169, 389-407.
28. Inglis, R.F., Biernaskie, J.M., Gardner, A. & Kümmerli, R. (2016). Presence of a loner strain maintains cooperation and diversity in well-mixed bacterial communities. *Proceedings of the Royal Society, Series B: Biological Sciences*, 283, 20152682.
29. Kaiser, D. (2004). Signaling in myxobacteria. *Annual Review of Microbiology*, 58, 75-98.
30. Kefford, B.J., Ghalambor, C.K., Dewenter, B., Poff, N.L., Hughes, J., Reich, J. *et al.* (2022). Acute, diel, and annual temperature variability and the thermal biology of ectotherms. *Global Change Biology*, 28, 6872-6888.

31. Konovalova, A., Petters, T. & Sogaard-Andersen, L. (2010). Extracellular biology of *Myxococcus xanthus*. *FEMS Microbiology Reviews*, 34, 89-106.
32. Laue, H., Schenk, A., Li, H., Lambertsen, L., Neu, T.R., Molin, S. *et al.* (2006). Contribution of alginate and levan production to biofilm formation by *Pseudomonas syringae*. *Microbiology*, 152, 2909-2918.
33. Lawrence, J.R., Swerhone, G.D.W., Kuhlicke, U. & Neu, T.R. (2007). In situ evidence for microdomains in the polymer matrix of bacterial microcolonies. *Canadian Journal of Microbiology*, 53, 450-458.
34. Lion, S. (2018). Theoretical approaches in evolutionary ecology: environmental feedback as a unifying perspective. *The American Naturalist*, 191, 21-44.
35. Lowen, A.C. (2018). It's in the mix: reassortment of segmented viral genomes. *PLoS Pathogens*, 14, e1007200.
36. MacArthur, R.H. (1972). *Geographical Ecology: Patterns in the Distribution of Species*. Princeton University Press.
37. Mollison, D. (1991). Dependence of epidemic and population velocities on basic parameters. *Mathematical Biosciences*, 107, 255-287.
38. Nikel, P.I., Silva-Rocha, R., Benedetti, I. & de Lorenzo, V. (2014). The private life of environmental bacteria: pollutant biodegradation at the single cell level. *Environmental Microbiology*, 16, 628-642.
39. Post, D.M. & Palkovacs, E.P. (2009). Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, 1629-1640.
40. Price, G.R. (1970). Selection and covariance. *Nature*, 227, 520-521.
41. Siepielski, A.M. & McPeck, M.A. (2010). On the evidence for species coexistence: a critique of the coexistence program. *Ecology*, 91, 3153-3164.
42. Strassmann, J.E. & Queller, D.C. (2011). Evolution of cooperation and control of cheating in a social microbe. *Proceedings of the National Academy of Sciences*, 108, 10855-10862.
43. van Gestel, J., Vlamakis, H. & Kolter, R. (2015). From cell differentiation to cell collectives: *Bacillus subtilis* uses division of labor to migrate. *PLoS Biology*, 13, e1002141.
44. Wahl, L.M. (2002). The division of labor: genotypic versus phenotypic specialization. *The American Naturalist*, 160, 135-145.

Appendices

A1

Supplementary information of Chapter 2

Supplementary Information

1. Further analyses of the analytical game theory model

In this section, we provide more details for math derivations as well further analyses of the results. In section 1.1, we recall the model designs, in particular the life cycles, population structures, and social interactions of the analytical model. Section 1.2 shows the derivation of fitness of fully random specialisation. In sections 1.3-1.4, we derive and analyse the evolutionarily stable strategies (ESSs) of the target proportion of helpers for fully random and coordinated specialisation. Section 1.5 shows that the ESS target proportion of helpers for fully coordinated specialisers is always equal to or greater than the ESS target proportion of helpers for fully random specialisers. In section 1.6, we rearrange the fitness functions to compare the cost of the two specialising mechanisms. Section 1.7 illustrates the effect of the cost of coordination on the invasion conditions.

1.1 Population, life cycle and social interactions

We assume that the population is structured into distinct social groups that each start with l founding cells, which synchronously replicate to form a lineage of cells in the group. The last generation of the group life cycle occurs when the group has reached the size of lm cells (l lineages, m cells per lineage), at which point fecund cells produce a large number of offspring that disperse globally to found new groups. All cells from the previous groups then die (non-overlapping generations) and the life cycle begins again.

We assume that cells in the last generation of the group life cycle can become either sterile helpers that produce a public good for the group, or pure reproductives that do not produce a public good but use the good to produce a larger number of dispersing offspring. We assume that the fecundity of a reproductive is equal to $(1 - \epsilon) + \epsilon P$, where the parameter ϵ is the essentiality of cooperation, and the variable P is the proportion of helpers produced in the group in the last generation.

Whether and how cells in the last generation become helpers or reproductives depends on the division of labour mechanism of the cells' lineage founders. Our analytical model focus on two special cases: fully random specialisation and fully coordinated specialisation. We denote q as the target proportion of helpers of the founder of a focal lineage and Q as the target proportion of helpers of all other lineage founders in the group. When the focal lineage employs fully random specialisation, each cell in the lineage may become a helper independently of all other cells in the group and with probability equal to q . When the lineage employs fully coordinated

specialisation, cells of the lineage interact with other cells from the same lineage to ensure that the proportion of helpers produced by the lineage is exactly equal q .

1.2 The fitness of fully random specialisers

We can write the fitness of a focal founder in a population where all cells employ random specialisation as:

$$w_{FR}(q, Q) = \sum_0^{(l-1)m} \binom{(l-1)m}{j} Q^j (1-Q)^{(l-1)m-j} \sum_0^m \binom{m}{i} q^i (1-q)^{m-i} (1 - i/m)(1 - \epsilon + \epsilon(i+j)/lm) \quad (S1)$$

where the inner sum iterates over the number of helpers produced in the focal lineage, i , and the outer sum iterates over the number of helpers produced in the other lineages, j . In each case, the probability of producing that number of helpers is equal to the associated probability in the binomial distribution. These probability terms can be simplified with new notation: $f(j) = \binom{(l-1)m}{j} Q^j (1-Q)^{(l-1)m-j}$; $g(i) = \binom{m}{i} q^i (1-q)^{m-i}$. We can simplify Equation (S1) by noting that $\sum_0^m g(i)i = mq$, $\sum_0^m g(i)i^2 = mq(1-q) + m^2 q^2$ and $\sum_0^{(l-1)m} f(j)j = (l-1)mQ$. This gives Equation (1) in main text:

$$w_{FR}(q, Q) = (1-q)(1 - \epsilon + \epsilon q/l + \epsilon(l-1)Q/l - \epsilon q/lm). \quad (S2)$$

Let us consider another case: the fitness of a fully random founder in a population where all other group founders are fully coordinated specialisers. Because fully coordinated founders produce an exact proportion of helpers, Q , there is only one summation in the fitness expression:

$$w_{FR}(q, Q) = \sum_0^m g(i)(1 - i/m)(1 - \epsilon + \epsilon(i + (l-1)mQ)/lm). \quad (S3)$$

By applying the properties of $\sum_0^m g(i)i$ and $\sum_0^m g(i)i^2$, we get Equation (1) in main text again. This is because the lack of higher order terms of j in Equation (S1), and the first-order summation of random founders, $\sum_0^{(l-1)m} f(j)j = (l-1)mQ$, gives the same results of fully coordinated founders (the last term of Equation (S3)).

1.3 Derivation and analysis of the ESS for the target proportion of helpers of fully random specialisers

Since Equation (S2) is a convex function, we solved $\frac{dw_{FR}(q,Q)}{dq} \big|_{q=Q=q^*} = 0$ to find

the q where fitness is maximised,

$$q_{FR}^* = \frac{lm(\epsilon-1)+\epsilon(m-1)}{\epsilon(lm+m-2)}. \quad (S4)$$

By taking partial derivatives of Equation (S3), we find that: increasing essentiality (higher ϵ), and smaller lineages sizes (smaller m) produce a higher target proportion of helpers (larger q_{FR}^*).

We show below the derivative of the ESS proportion of helpers with respect to the number of lineages, l :

$$\frac{\partial(q_{FR}^*)}{\partial l} = \frac{-m(\epsilon+m-2)}{\epsilon(lm+m-2)^2}. \quad (S5)$$

Given the biological meaningful range of the parameters, $0.5 \leq \epsilon \leq 1$, $l \geq 1$, and $m \geq 1$, the denominator is always positive and increase with increasing l . Thus, the sign of the first order derivative depends on $(\epsilon + m - 2)$, which is positive because $m \geq 2$ is the necessity condition for division of labour to take place. In other words, whenever division of labour is favoured ($q_{FR}^* > 0$), a higher relatedness (smaller l) leads to a higher target proportion of helpers (larger q_{FR}^*).

1.4 The fitness of fully coordinated specialisers

The fitness of founders taking fully coordinated specialisation is relatively straightforward, because the exact proportions of helpers can be reached through coordination:

$$w_{FC}(q, Q) = (1 - \theta)(1 - q)(1 - \epsilon + \epsilon q/l + \epsilon(l - 1)Q/l), \quad (S6)$$

where the first part is the cost of coordination, the second part is the loss from producing helpers, and the third part is the baseline fitness and the total amount of help received from the social group. Note that the fitness of coordinated founder in a population where all other founders take random specialisation is the same, since $\sum_0^{m(l-1)} f(j)j/lm$ is equal to the last term of Equation (S6), $\epsilon(l - 1)Q/l$.

1.5 Derivation and analysis of the ESS for the target proportion of helpers of fully coordinated specialisers

Similar to the optimal target proportion of helpers of fully random specialisers, the

ESS of fully coordinated specialisers can be found by solving $\frac{dw_{FC}(q, Q)}{dq} \big|_{q=Q=q^*} = 0$

from Equation (S6),

$$q_{FC}^* = \frac{\epsilon l + \epsilon - l}{\epsilon(l+1)}, \quad (S7)$$

By taking partial derivatives of Equation (S7), we find the same results as q_{FR}^* that: increasing essentiality (higher ϵ), and increasing relatedness (smaller l) produce a higher target proportion of helpers (larger q_{FC}^*). Yet, the target proportion of fully coordinated specialisers is independent with lineage size (m).

1.6 Analysing the difference in ESS target proportions of helpers

From Equation (2) and (4), we can write the difference in target proportions as $q_{FC}^* - q_{FR}^* = (\epsilon l + \epsilon - l)/(\epsilon(l+1)) - (lm(\epsilon - 1) + \epsilon(m - 1))/(\epsilon(lm + m - 2))$. By assuming $lm + m - 2 > 0$, the condition for division of labour to exist, we can simplify the expression as

$$q_{FC}^* - q_{FR}^* = \frac{2l - \epsilon(l+1)}{(\epsilon(l+1))(\epsilon(lm + m - 2))}, \quad (S8)$$

where the denominator is positive, and the numerator is never negative because $\epsilon < 1$ and l is at least 1. As a result, the ESS proportion of fully coordinated specialisers is greater or equal to which of fully random specialisers ($q_{FC}^* \geq q_{FR}^*$). Note that our assumption, $lm + m - 2 > 0$, is required for division of labour to take place, because it is only violated when $l = 1$ and $m = 1$, where there is only one individual in each social group.

The result that $q_{FC}^* \geq q_{FR}^*$ helps to explain the existence of places where neither mechanism can invade the other in Fig. 2 of the main text (black regions). The boundaries of successful invasion (blue and orange regions) are described in Expressions (5) and (6) in main text. Considering the case where coordinated mechanism could be selected ($\theta lm < 1$), the right-hand side of Expression (5) is smaller than or equal to which of Expression (6) by applying $q_{FC}^* \geq q_{FR}^*$. Hence, the blue and orange regions in Fig. 2 never overlap.

1.6 Comparing the costs of fully random mechanism and fully coordinated mechanism

From the previous derivation, we can rearrange the fitness function of random specialisation as

$$w_{FR}(q, Q) = (1 - q)(1 - \epsilon + \epsilon q/l + \epsilon(l - 1)Q/l) - (\epsilon/l)q(1 - q)/m. \quad (S9)$$

Then, letting $G(q, Q) = (1 - q)(1 - \epsilon + \epsilon q/l + \epsilon(l - 1)Q/l)$ and $Var(q) = q(1 - q)/m$, we can simplify the fitness functions,

$$w_{FR}(q, Q) = G(q, Q) - \epsilon/l Var(q). \quad (S10)$$

Since $\frac{\partial^2 G(q, Q)}{\partial q^2} = -\frac{2\epsilon}{l}$, we further simplify the fitness function of random specialisation as,

$$\begin{aligned} w_{FR}(q, Q) &= G(q, Q) + \frac{\frac{\partial^2 G(q, Q)}{\partial q^2} Var(q)}{2}, \\ &= \left(1 + \frac{\frac{\partial^2 G(q, Q)}{\partial q^2} Var(q)}{2G(q, Q)} \right) G(q, Q), \\ &= (1 - \varphi)G(q, Q), \end{aligned} \quad (S11)$$

where $\varphi = -\frac{\frac{\partial^2 G(q, Q)}{\partial q^2} Var(q)}{2G(q, Q)}$ is the cost of random specialisation. By using $G(q, Q)$, we can also rewrite the fitness of fully coordinated specialisers as,

$$w_{FC}(q, Q) = (1 - \theta)G(q, Q), \quad (S12)$$

where the cost of coordination is θ . Note that the two fitness functions may have different optimal q . Using (S7) and (S8), we can solve for when a coordinated specialiser can invade a population of random specialisers by evaluating: $w_{FC}(q_{FR}^*, q_{FR}^*) > w_{FR}(q_{FR}^*, q_{FR}^*)$, giving the condition:

$$\varphi > \theta, \quad (S13)$$

which states that the relative cost of random specialisation (φ) must be larger than the relative cost of coordination (θ).

1.7 Effects of the cost of coordination on invading conditions

The effect of the cost of coordination (θ) on the invasion of coordinated specialisers is shown in Fig. S1. When the cost of coordination is larger (higher θ), the parameter spaces where fully coordinated specialisation invades becomes smaller and more

sensitive to group size (slope decreased); when the cost of coordination becomes smaller (lower θ), coordinated specialisers can invade in a broader parameter space and the boundary is more sensitive to essentiality of cooperation. Yet, qualitatively coordinated specialisation is still favoured when essentiality is high and when groups are small (lower right corners of Fig. S1).

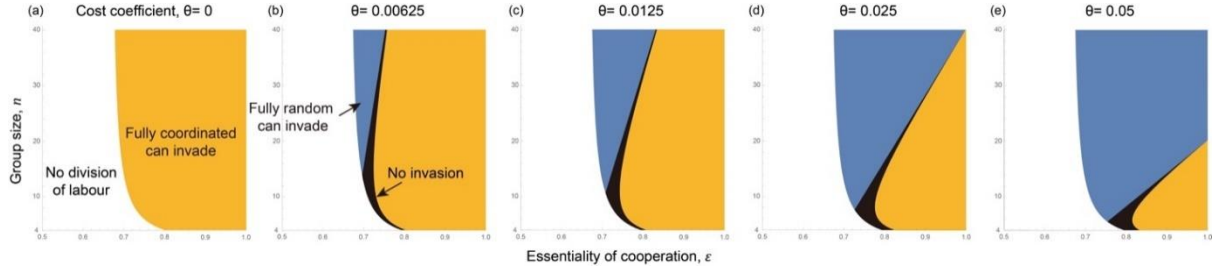


Figure S1. The effects of cost coefficient of coordination (θ) on the invasion conditions in the analytical model, where the blue region is the spaces where random specialisation can invade coordinated specialisation, the orange region is coordinated specialisation invading random, the black region is spaces that neither can invade, and the white region is no division of labour (same as Fig. 2). Each panel shows the conditions of a coefficient value. In the main figures, the cost of coordination is set to $\theta = 0.025$ (panel d). Though we only show the analyses of non-clonal groups here, $R = 0.5$, the qualitative effect of the cost of coordination is not affected by the relatedness of the population.

2. A more complicated analytical model: Mutant founders invade with their own optimal target proportions of helpers

Here, we derive another analytical model where the invading founder does not take the same strategy (i.e., proportion of helpers) as the resident founders. Since the fitness functions remain the same, we only show the derivation of invading conditions. The main result of this model is presented in Fig. 3 of main text.

2.1 Invasion condition for fully coordinated specialisers invading fully random specialisers

Assuming the mutant and fully coordinated founder is not taking q_{FR}^* , we can find the optimal target proportion of helpers of the mutant founder by solving $\frac{dw_{FC}(q, Q=q_{FR}^*)}{dq} = 0$,

$$\hat{q}_{FCInv} = (l(2m(\epsilon - 1) - \epsilon + 2) + \epsilon(2m - 3)) / (2\epsilon(lm + m - 2)). \quad (S14)$$

After substitution, we have

$$w_{FC}(\hat{q}_{FCInv}, q_{FR}^*) = -(\epsilon - l(\epsilon + 2m - 2))^2 (\theta - 1) / 4\epsilon l(lm + m - 2)^2, \quad (S15)$$

and

$$w_{FR}(q_{FR}^*, q_{FR}^*) = (m-1)(-lm + \epsilon)^2 / \epsilon lm(lm + m - 2)^2. \quad (S16)$$

By assuming division of labour occurs, $lm + m > 2$ (see section 1.4 for more details), we can simplify the inequality $w_{FC}(\hat{q}_{FCInv}, q_{FR}^*) > w_{FR}(q_{FR}^*, q_{FR}^*)$ as $\frac{-m(\theta-1)(\epsilon-l(\epsilon+2m-2))^2}{4(m-1)(\epsilon-lm)^2} > 1$. We solve the roots of ϵ ,

$$\epsilon < \frac{2(\sqrt{-l^2m(m-1)(lm+m-2)^2(\theta-1)} + lm(m-1)(\theta+1) + l^2m(m+\theta-\theta m-1))}{m(\theta+l(l-2)(\theta-1)+3)-4}, \quad (S17.1)$$

$$\epsilon > \frac{2(\sqrt{-l^2m(m-1)(lm+m-2)^2(\theta-1)} - lm(m-1)(\theta+1) + l^2m(m+\theta-\theta m-1))}{m(\theta+l(l-2)(\theta-1)+3)-4}, \quad (S17.2)$$

where Equation (S17.2) is in biologically meaningful parameter space and plotted in Fig. 3.

2.2 Invasion condition for fully random specialisers invading fully coordinated specialisers

Similar with the previous section, we find the optimal strategy of the mutant founder

from solving $\frac{dw_{FR}(q, Q=q_{FC}^*)}{dq} = 0$,

$$\hat{q}_{FRInv} = (2\epsilon m - \epsilon - l(\epsilon - 2m(\epsilon - 1))) / (2\epsilon(l+1)(m-1)). \quad (S18)$$

After substituting $w_{FR}(\hat{q}_{FRInv}, q_{FC}^*) > w_{FC}(q_{FC}^*, q_{FC}^*)$, we have $\frac{(\epsilon+l(\epsilon-2m))^2}{4l^2m(m-1)(\theta-1)} < 1$.

Then we find the roots for ϵ ,

$$\epsilon < \frac{2(l^2m + lm + \sqrt{-m(m-1)(l^2+l)^2(\theta-1)})}{(l+1)^2}, \quad (S19.1)$$

$$\epsilon > \frac{2(l^2m + lm - \sqrt{-m(m-1)(l^2+l)^2(\theta-1)})}{(l+1)^2}. \quad (S19.2)$$

where Equation (S19.1) is in biologically meaningful parameter space and plotted in Fig. 3.

3. Additional simulations

We run a series of supplementary simulations to help understand the behaviour of simulations. In section 3.1, we examine the proportion of helpers without the presence of coordination and show it is very close to analytical results. Section 3.2 shows the difference in analytical and simulative results is resulted from interactions between coevolving traits. Section 3.3 tests the robustness of simulations by initiating with different proportions of helpers. Section 3.4 uses the same assumption as the analytical model that coordination only operates within the same lineage. We explain why coordination is less favoured in these simulations. In section 3.5, we test an alternative coordination cost design: linear cost and find the results remain qualitatively the same. Section 3.6 explains some unexpected patterns of section 3.5. The biological details of simulation are described in main text, we also provide pseudocode for the simulations run in the main text in section 3.7.

3.1 Evolved proportion of helpers under fully random specialisation

As additional verification between the analytical model and the simulations, we ran simulations where no coordination takes place. In this single-trait evolving scenario, we found the evolved proportion of helpers ($\bar{P}_{Rand}$) is almost identical with the ESS target proportion of helpers (q_{FR}^*) in the analytical model (Fig. S2e-h and Fig. 2i-l).

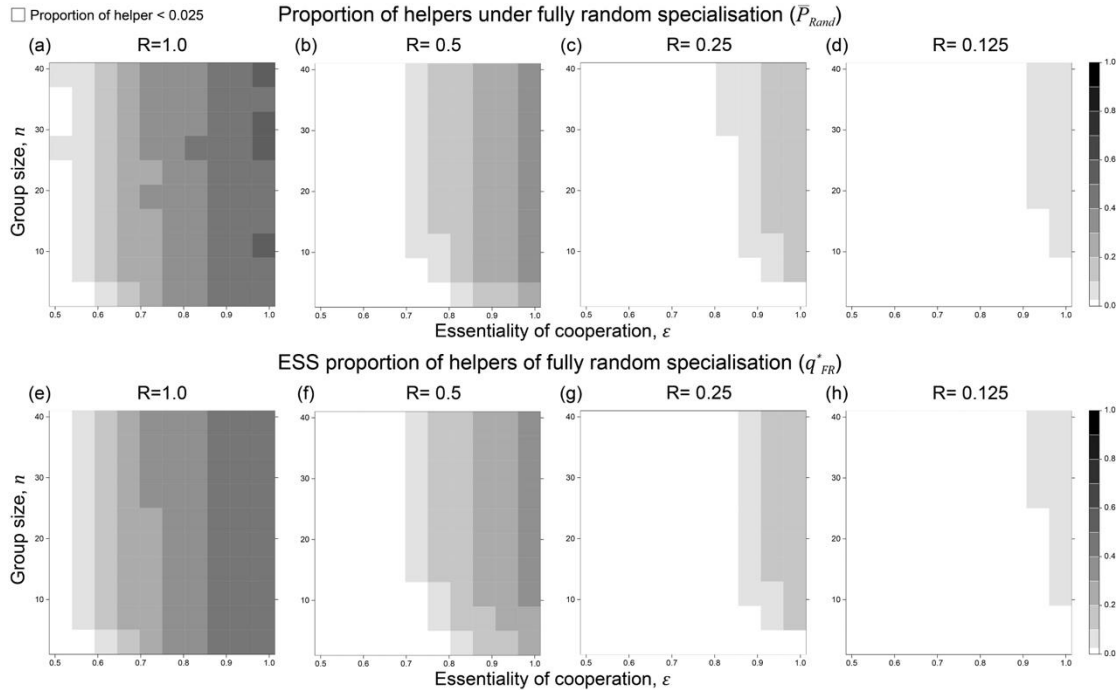


Figure S2. The optimal proportion of helper, or level of cooperation, of fully random specialisers in simulations and the analytical model. (a-d) We run single-trait evolving simulation where only target proportions of helpers are evolving, while coordination level is fixed at 0. Darker shades mean there are more helpers in the steady-state populations. All data are extracted in the last 10% of 10^5 generation simulations with 10 repetitions for each parameter combination and group size is equal to number of

lineages times lineage sizes ($n = lm$). (e-h) The corresponding proportion of helpers in analytical model. We plot the same q_{FR}^* as in Fig. 2i-l but in identical way as simulations for better comparison.

3.2 Interactions between coevolving traits explain some discrepancies between simulations and analytical results in non-clonal groups

In main text we found the analytical model and simulations have the same qualitative results on optimal mechanisms when groups are clonal (Fig. 2a, Fig. 5a), but there are some disagreements when relatedness is 0.5 (Fig. 2b; Fig. 5b). These differences can arise from potential interactions between the coevolving helper proportion and the level of coordination, because one trait controls the average number of helper whereas the other trait controls the variation in number across groups. Thus, we run a set of simulations with the proportion of helpers fixed at the ESS of fully random specialisers (q_{FR}^*), and only evolving levels of coordination.

The single-trait simulations have very similar pattern to which in analytical models (Fig. S3b; Fig. 2b; Fig. 3b). In particular, high levels of coordination ($\bar{S}$) are only evolved in small groups (n) (Fig. S3b), whereas the coevolved simulations show high coordination when essentiality (ϵ) is high, regardless of group size (Fig. S3a). In addition, we also found the proportion of helpers ($\bar{P}$) in single-trait simulations are higher than which in coevolving simulations (Fig. S3c-d). Altogether, these results suggest interactions between coevolving traits are likely to be the cause of different patterns.

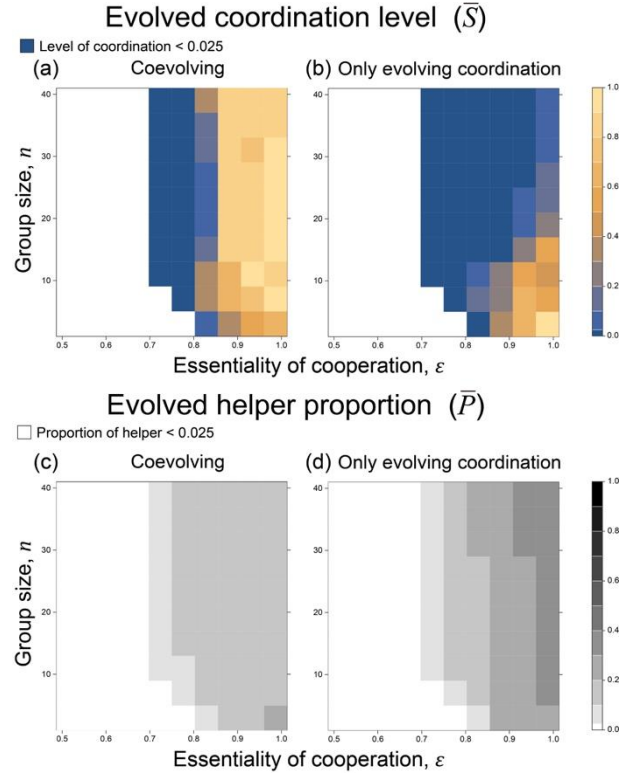


Figure S3. Comparison of coevolving and single-trait-evolving simulations in non-clonal population ($R=0.5$). The first row is the coevolved or evolved level of coordination (a-b) and the second row is the coevolved or phenotypically expressed proportion of helpers (c-d). Importantly, the right column shows the result where target proportion of helper (q) is set at the ESS of random specialisers ($q = q_{FR}^*$) and helper proportion is not evolving. On the other hand, panel a and c are identical with panel b and f of Fig. 5, where both coordination and proportion are evolving. Similar to Fig. 5, coordination levels with proportion of helper less than 0.025 are shown in white as we regard it as no division of labour being favoured and group size, n , is equal to number of lineages (l) times lineage size (m).

3.3 Sensitivity analysis of initial target proportion of helpers

Simulations of various initial target proportions of helpers (q) all show very similar patterns in both coevolved proportions of helpers as well as coevolved coordination levels (Fig. S4). These results suggest the initial proportions do not change the outcome of coevolving traits.

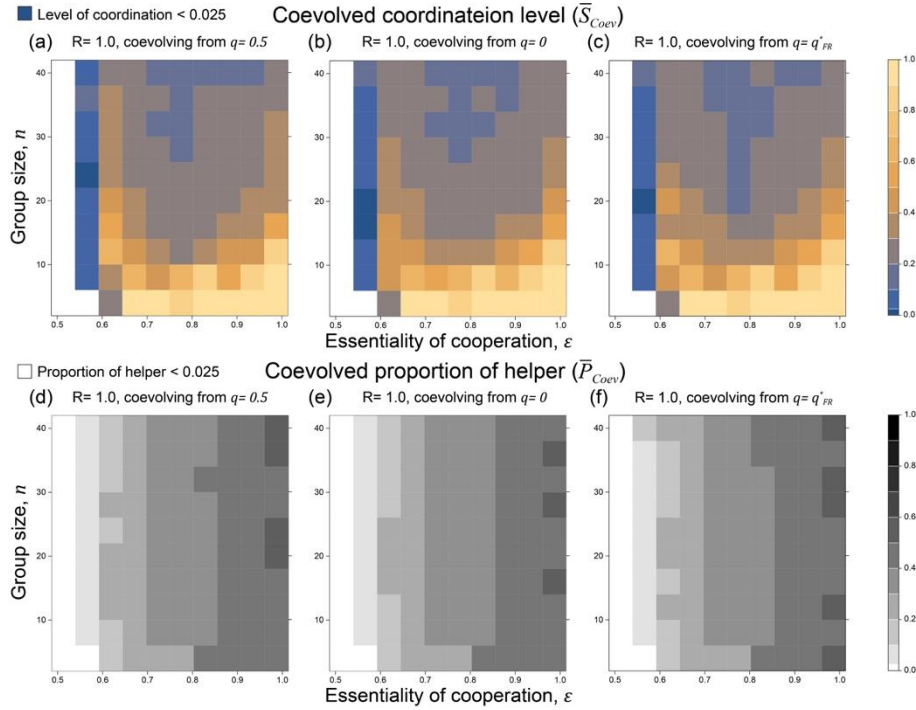


Figure S4. The coevolved coordination level and proportion of helpers under various initial states in clonal population ($R=1$). Simulations are initiated with target proportion of 0.5 (a), 0 (b), and ESS proportions of fully random specialisers (c). All coevolving simulations in the main text and supplementary information have the initial proportion of helpers is 0.5 if not stated otherwise.

3.4 Simulations where individuals only coordinate with clonemates

Let us consider a simulation closer to our analytical model: group members can only be coordinated with individuals of the same lineage (from the same founder). The simulations show both coordination level ($\bar{S}_{Coev}$) and proportion of helpers ($\bar{P}_{Coev}$) are lower (Fig. S5) comparing to the cases where individuals can be coordinated with the entire group (Fig. 5).

This difference happens because the analytical model assumes group size is large and coordinated specialisation can reach exact optimal proportion of helpers. However, the simulations the available proportion of helpers becomes more discrete as group size becomes smaller. That is, for a given group size n , there are only $(n + 1)$ achievable proportions of helpers (x/n ; $\{x \in \mathbb{N} | 0 \leq x \leq n\}$). If the group size is held constant, the minimal non-zero coordinated proportion of helpers increases ($1/n$) with decreasing relatedness. Thus, given that the ESS proportion of helpers is known to decrease with decreasing relatedness (section 1.6) whereas the minimal possible level of coordination is increasing, coordinated specialisation is less favoured because desired proportion cannot be reached through coordination.

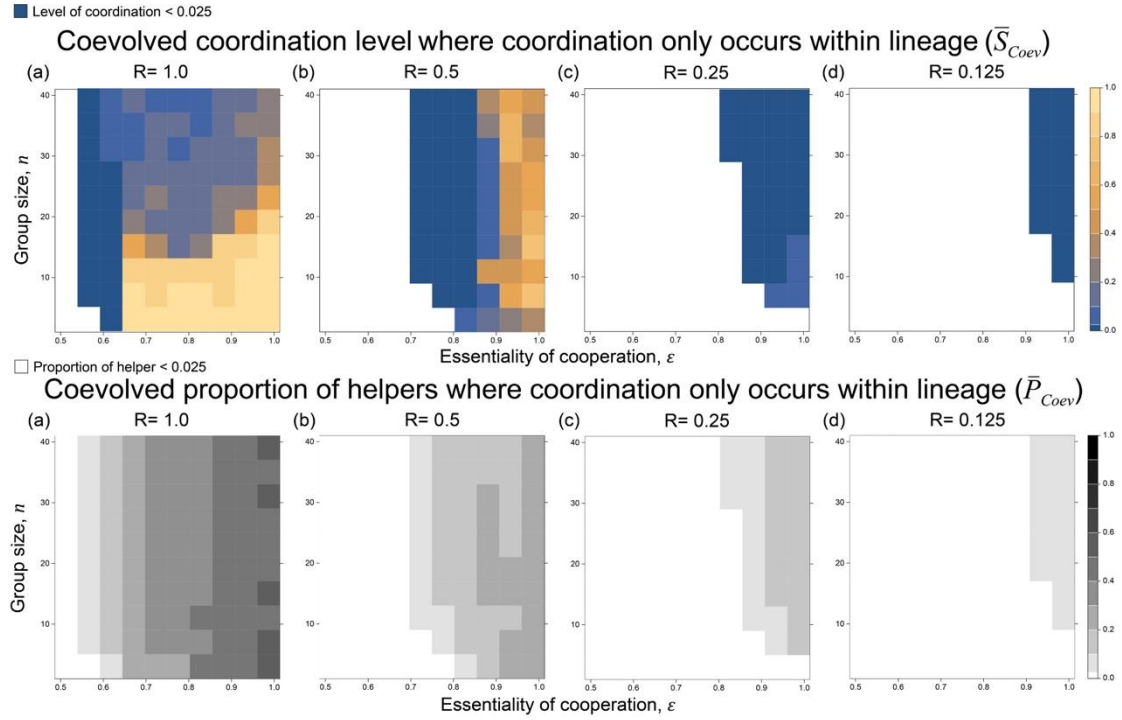


Figure S5. The coevolved coordination level and proportion of helpers in the simulations when coordination only takes place within lineage, which contains the individuals originated from the same founder in the social group. (a-d) The evolved coordination level plotted against the essentiality of cooperation (x-axis) and group size (y-axis). Each panel represents the results of different relatedness setting. The blueish colours represent the evolved mechanism of division of labour is closer to random specialisation, whereas the orangish colours represent the evolved mechanism is closer to coordinated specialisation. Each grid is an average of 10 repeated simulations where the average is taken from the last 10% of 10^5 generations. (e-h) The evolved proportion of helpers. Darker shades mean there are more helpers in the population. Coordination levels when proportion of helpers is below 0.025 is not plotted as we regard it as no division of labour.

3.5 Linear coordination cost

Here, we apply a simpler cost design where the cost of coordination increases proportionally with coordination level, $cost(s) = \theta s$. In terms of the effects of relatedness, the results (Fig. S6) are generally similar with which of decelerating coordination costs (Fig. 5). Yet, the linear cost simulations have more gradual transitions of coordination levels (Fig. 5a and S6a). In addition, proportion of helpers is lower in the linear cost simulations (Fig. 5e-h and S6e-h).

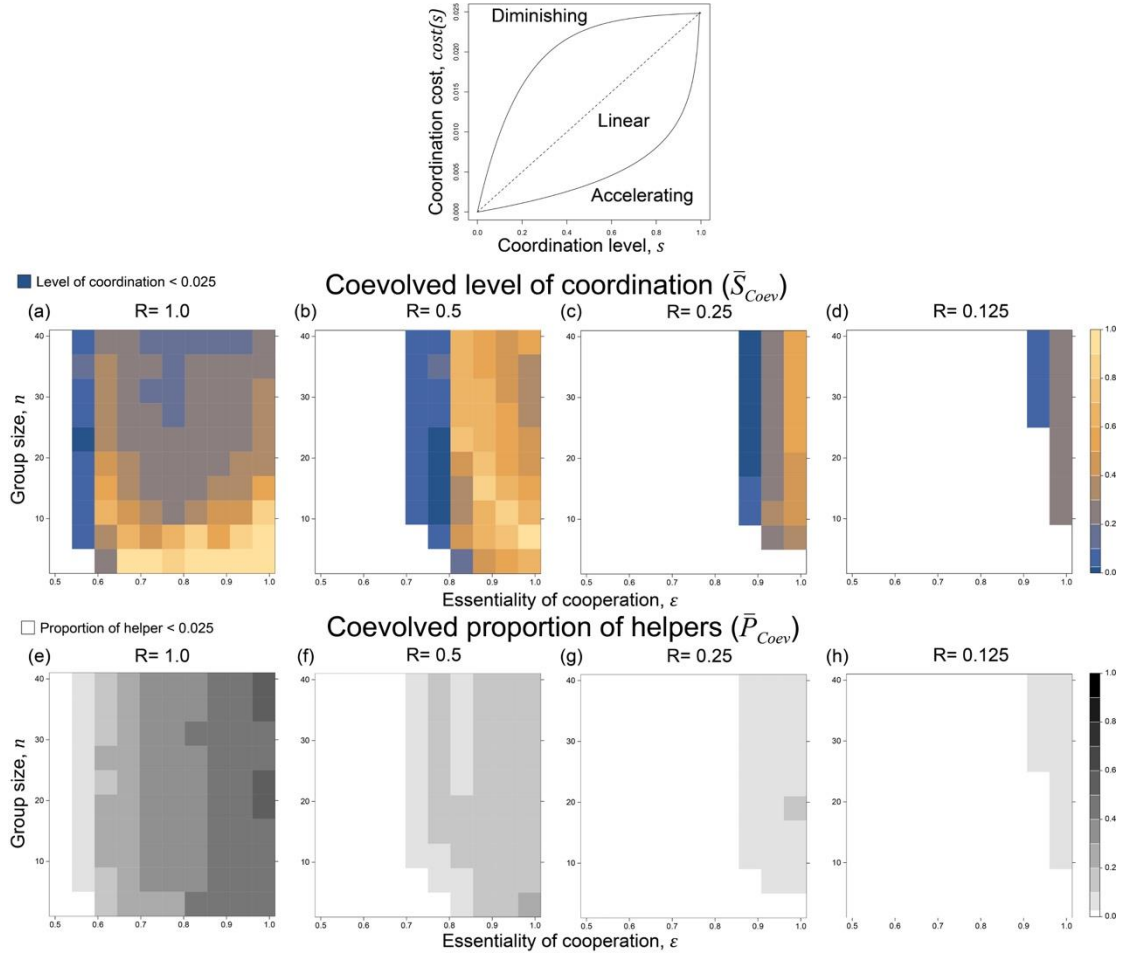


Figure S6. The coevolved level of coordination and proportion of helpers in the simulations when coordination cost is linear. The top panel shows the comparison of cost functions for coordination, where the linear cost is used in SI section 3.5-3.6; the accelerating cost is used in SI section 3.7; and the decelerating cost is used in all other simulations.

3.6 Single trait evolution versus two traits coevolution under linear cost

Despite the similarity, there are some qualitative discrepancies between the two simulations of linear and decelerating coordination cost. That is, we found high levels of coordination ($\bar{S}$) when essentiality (ϵ) is around 0.6 in clonal groups (Fig. S7a; identical to Fig. S6a), which is not found in simulations with decelerating cost of coordination (Fig. 5a).

Similar to section 3.2, we suspected this unexpected pattern arises because of the potential interactions between the coevolving traits and ran another set of simulation where proportion of helpers is not evolving. Since we are interested in the cases where coordinated specialisation evolving from random specialisation, we fix the target proportion of helpers at the ESS proportion of random specialisers (Fig. S2) and let coordination level be the only evolving trait. The single-trait simulations show similar pattern to coevolving simulations with decelerating cost of coordination (Fig. S7b; Fig. 5a). On the other hand, there is no big difference in proportion of helpers between

single-trait and coevolving simulations (Fig. S7c-d). This is because proportion of helpers has a larger impact on the individual fitness and thus is less likely to deviate from analytical results (Equation (10) and Fig. 4). All in all, our results of evolving coordination simulations suggest interactions between traits are likely to be the cause of the unexpected pattern.

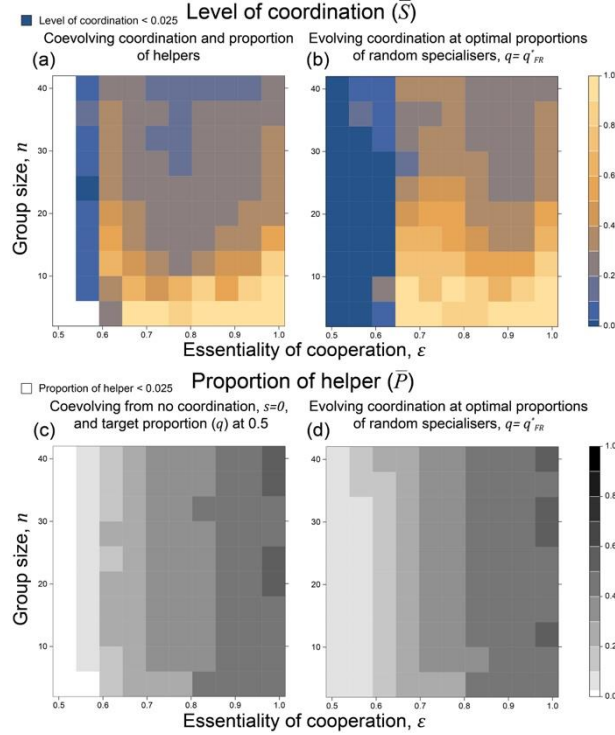


Figure S7. Comparison of dynamics between coevolving traits and single-evolving trait when the cost of coordination is linear. Similar to Fig. S3, the first row is the level of coordination (a-b) and the second row is the proportion of helpers (c-d). Importantly, the right column shows the result where target proportion of helper (q) is set at the optimum of random specialisers ($q = \bar{q}_{rand}$; Fig. S2a): evolved level of coordination is shown in panel b and the phenotypic proportion of helper from fixed target proportion is shown in panel d. On the other hand, panel a and c are identical with panel a and e in Fig. S6, where both coordination and proportion are evolving.

3.7 Single trait evolution versus two traits coevolution under accelerating cost

When the cost for coordination is accelerating, it implies low levels of coordination would have a very similar cost to no coordination and reaching perfect coordination is very costly. As a result, we see coordination level is higher than which in linear or decelerating cost in general, but regions with full coordination is fewer (Fig. S8; in comparison with Fig. S3 and S7). Nevertheless, the parameter spaces with small group size and high essentiality still have greatest level of coordination (group size of 4 and essentiality of 1). In addition, we found interactions between coordination and target proportion of helpers affect the evolved level of coordination, and the unexpected pattern disappears when only coordination is evolving (Fig. S8a-b).

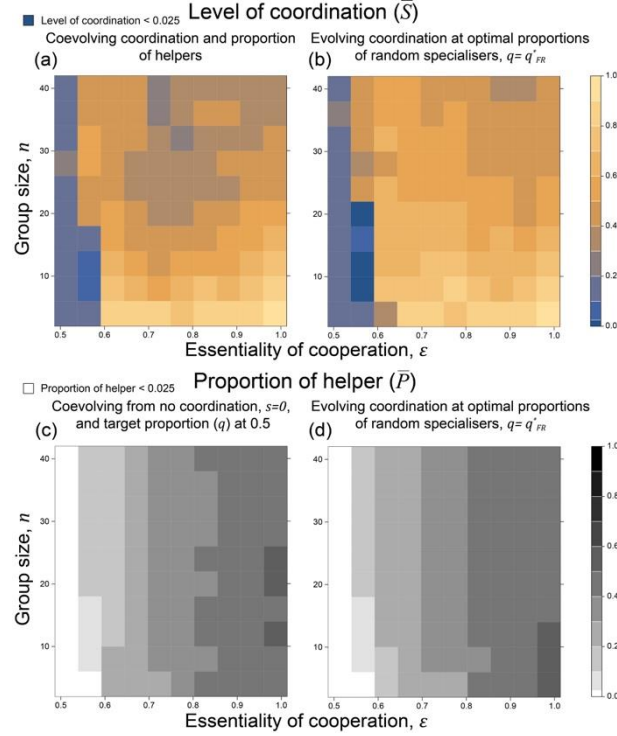


Figure S8. Comparison of dynamics between coevolving traits and single-evolving trait when the cost of coordination is accelerating. See Fig. S3 for plotting details.

3.8 Pseudocodes of the simulations

Pseudocode is provided below, please check data availability section of main text for the link to source codes.

// Initialisation

Define the essentiality of cooperation (ϵ), number of lineages (l), group size (lm), cost coefficient of coordination (θ), mutation rate (p_{mut}), and the distribution of the deviations of mutated traits;

Population size= $lm[10^4/lm]$; Number of groups= $[10^4/lm]$;

Create the population matrix [number of groups, group size, list of individual properties];

Create the coordination matrix [group size, group size];

Create the list for storing fitness [population size];

Initialise each individual with desired values in the list of individual properties: target proportion of helper (q), coordination level (s), and division type (h). Initial population has uniform trait values;

// Main iteration

For each generation (1 till 10^5) {

For each group (1 till number of groups) {

// Coordination

Appendix 1: supplementary information of chapter 2

```
For each individual (1 till group size) {
    Determine whether other individuals are coordinated with focal individual,
    probability is the coordination level of focal individual;
}
// Dividing labour
For number of sampling (1 till 5* group size) {
    Sample one individual from the group;
    Observe the developmental plans of coordinated group members of the focal
    individual and get its observed proportion of 'intended helper';
    If (observed proportion is lower than target) change developmental plan to helper;
    If (observed proportion is higher than target) change developmental plan to
    reproductive;
}
// Fitness calculation
Get the number of helpers of the group;
Calculate the fitness of each individual in the focal group;
}
// Reproduction and mutation
For each group (1 till number of groups) {
    For number of founders {
        Sample one individual from current population, probability is weighted by relative
        fitness;
        If (mutation of a trait, target proportion of helpers or level of coordination, occurs){
            Sample the size of mutation from the distribution;
            Change the trait value;
            Boundary check and fix values at 0 or 1 if exceeded;
        }
        Paste trait value to the future founder;
    }
    // Initialising the next population
    Create the future group of individuals from the founders: if there is no remainder for group
    size divided by the number of founders, each proportion of individuals inherit the traits
    from one founder. If there is a remainder, the remainders' traits are obtained from randomly
    sampled founders;
}
// Recording
Calculate the average trait values and phenotype values of the population;
// Replacing the population
```

Appendix 1: supplementary information of chapter 2

```
    Overwrite the population matrix with the created population from reproduction section;  
}  
Clear memory;  
End of simulation;
```

A2

Supplementary information of
Chapter 3

Electronic supplementary materials

1. Supplementary Figures

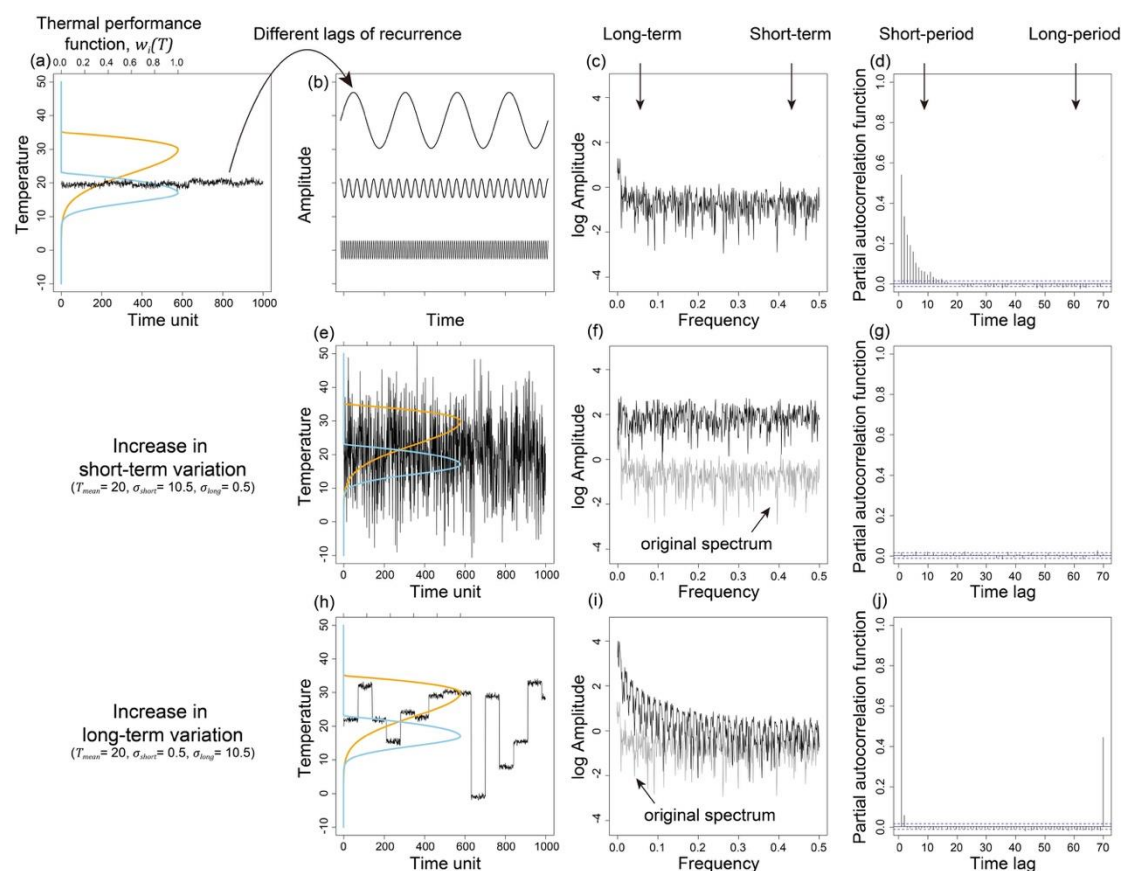


Fig. S1. Additional analysis of temperature time series in Fig. 1, where panels a, e, h are identical to panel a, d, g in Fig. 1. b, Another way of analyzing time series is that the temperature time series can be decomposed into waves of different frequencies through fast Fourier transformation. **c,** For each wave, the amplitude and frequency are plotted as points on a continuous spectrum that characterizes the temperature time series. **d,** We also provide partial autocorrelation functions, a different way of calculating autocorrelations. **e-j,** The same analyses for the other two time series. In the fast Fourier transformations, the spectrum of the first time series (c) is shown in grey for comparison. In the last partial autocorrelation function (j), we show time lags up to 71 time-units need to be included in the analysis; because 71 is the biggest lag that could have substantial autocorrelation, and it also correspond to the duration of updating long-term variation ($\delta = 70$, see Method for more information).

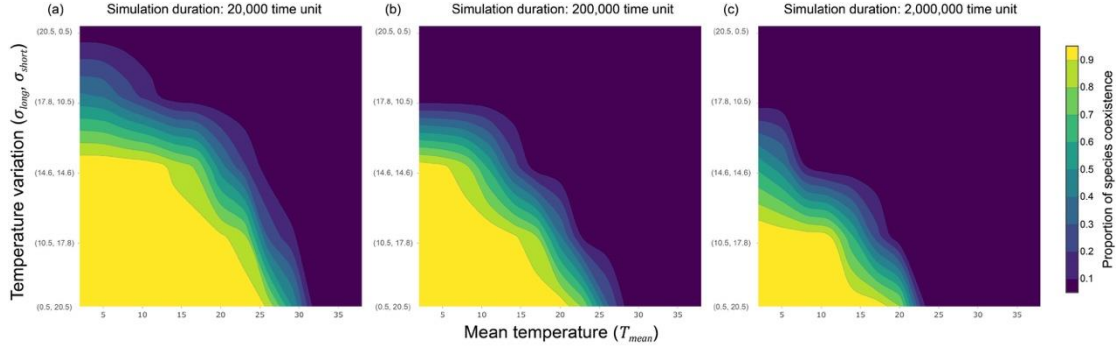


Fig. S2. The effects of simulation durations on species coexistence. We increase the durations in log scales, where the simulation lasts from the same as the main figures (20,000 time units), to 10 times longer, and to 100 times longer from left to right. Brighter color indicates more proportion of the simulations show species coexistence. Note that the y-axis does not represent different size of variability, but different ratios of long- and short-time variations. Hence, the variability is held the same, $(\sigma_{long} - 0.5)^2 + (\sigma_{short} - 0.5)^2 = 400$ (same as Fig. 3).

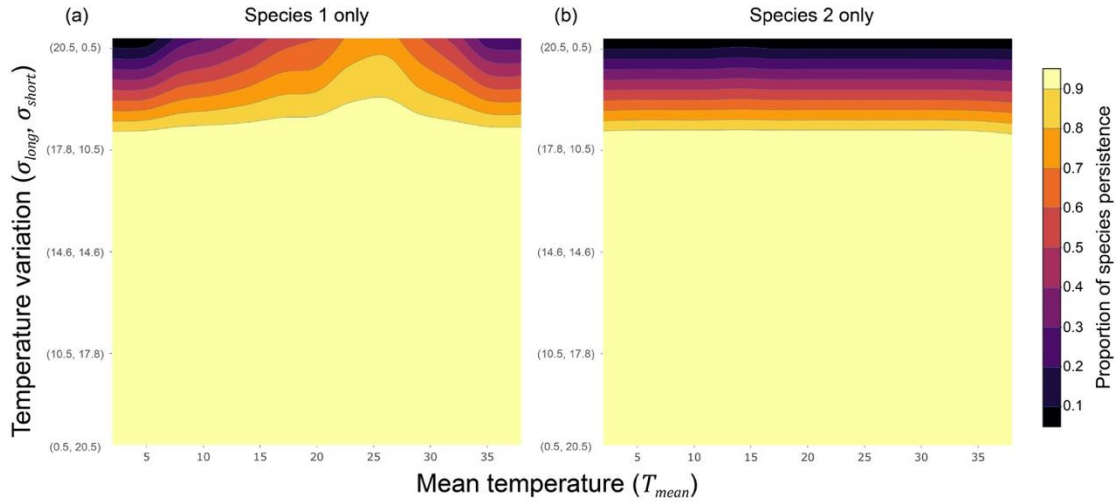


Fig. S3. The persistence probability of single species when there is only one species present in simulations. Brighter colors indicate higher ratio of persisting to the end of simulation (20,000 time units). Parameter settings are the same as Fig S2a.

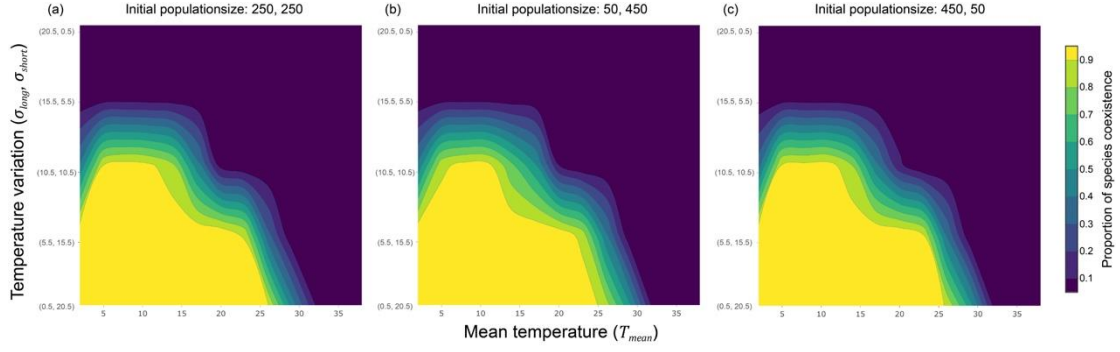


Fig. S4. The invasion analysis. We initialized simulations with different combinations of population sizes: **a**, Both species have the same initial population size, as in main figures. **b**, Species 1 comprises of 10% of the initial population. **c**, Species 2 comprises of 10% of the initial population. Note that the y-axis is different combinations of long- and short-term variations where the sum of standard deviation is held constant. These changes in initial population does not change patterns of coexistence.

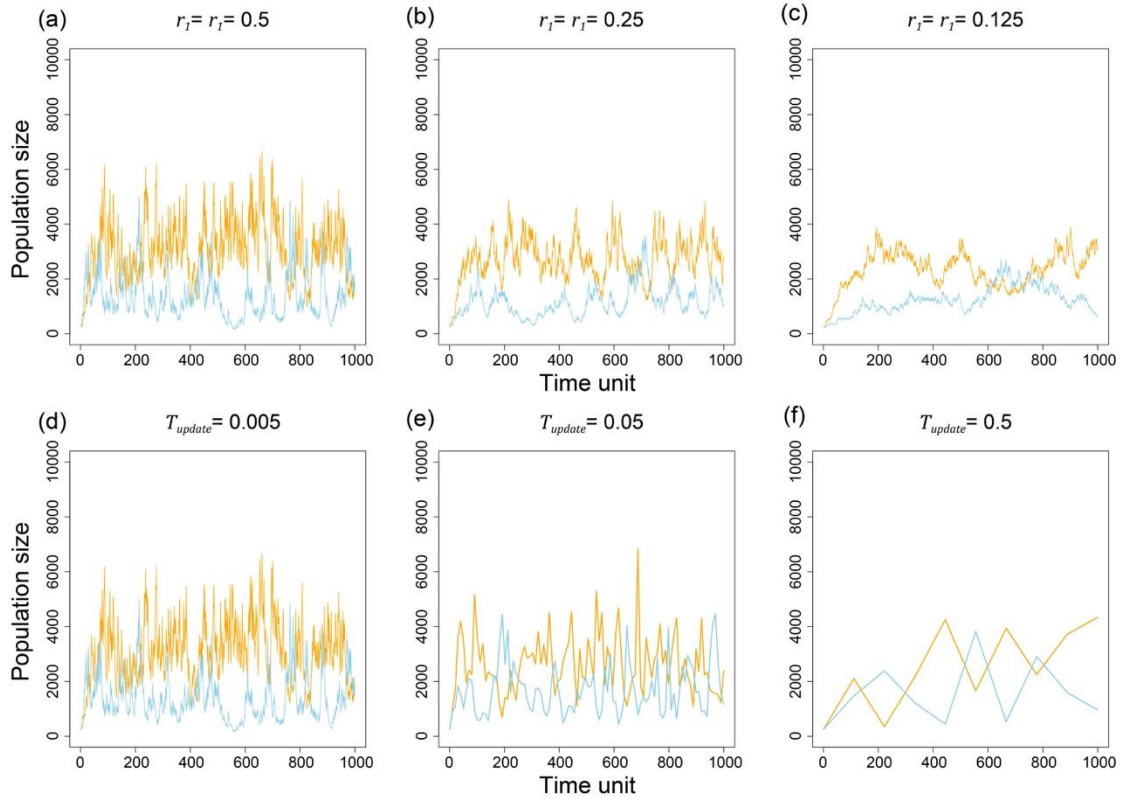


Fig. S5. The potential effects of generation time on population dynamics. We use two methods to evaluate the possible outcome: **(a-c)** the scale of intrinsic growth rate, and **(d-f)** the frequency of updating population size.

A3

Supplementary information of Chapter 4

Appendix 1

Our appendix contains seven sections. In section 1, we formally analyse our model with single mechanisms to manipulate and suppress manipulation. Section 2 contains the non-suppression model, which is a reduced model without suppressive traits. This model bridges the gap between the single-mechanism model and the classic tragedy of the commons (*Frank, 1994; Frank, 1996; Taylor and Frank, 1996; Frank, 1998; Dionisio and Gordo, 2006; Frank, 2010a; Frank, 2010b*). Further, the non-suppression model also illustrates a biologically possible case where manipulation cannot be suppressed. We consider the effects of personal cost in manipulation and suppression in section 3. Section 4 provides additional analyses for the scenario with two mechanisms to manipulate and suppress, where we found oscillations in selfishness and manipulation (*Figure 4*). We examine the origin of the oscillation dynamics and compare the results with game-theoretical analysis. We subsequently present similar arms race dynamics in section 5 for the scenario with multiple mechanisms introduced at different time points (*Figure 5*), with different biological settings assumed in each case. In section 6, we discuss some modelling details and justification. Lastly, section 7 has the details for the individual-based simulations.

1. Formal analysis of the scenario with a single mechanism to manipulate and suppress manipulation

1.1 Derivation of the fitness equation

We track a population of haploid asexual individuals by observing it at discrete points spaced evenly in time. The model population itself is subdivided into groups of size n . Groups are re-constituted at the very end of a given time step, through among group competition. Individuals compete within their own group for their share of the group's productivity.

We use y_i to denote the (absolute) competitive effort realised by focal individual i . We build up the fitness by assuming that the focal individual initially commits to a within-group level of competition equal to z_i . Likewise, each non-focal individual $k \neq i$ commits to a within-group level of competitive effort equal to z_k .

Once each group member has committed to an initial level of competitive effort, the group enters a phase in which members can manipulate effort (i.e., investment) from one another. We model the manipulation phase by assuming that the focal group member is assigned a non-self partner, uniformly at random with replacement. The assumption is equivalent to letting the focal individual manipulate every neighbour to the same degree and we use a probabilistic sampling narrative for the ease of explaining each process (Wild, 2011). When manipulation takes place, the focal individual attempts to manipulate a fraction x_i^a of the competitive effort z_k committed to by the partner. Here, we are treating x_i as a new trait (i.e., effort devoted to manipulation), and a as a constant that modulates the efficacy of the trait (i.e., determines how effective the effort is). The partner individual can also suppress manipulation. Specifically, we assume that the partner individual prevents a fraction $(1 - u_k^b)$ of manipulation that would have otherwise occurred. Here, we are thinking of u_k as a trait, and b as a constant that modulates the efficacy of the trait.

The focal individual is not only a manipulator, it can also be a the target of manipulation. In keeping with the description above, the focal individual is chosen to be manipulated by each of its non-self neighbours with probability $1/(n-1)$. When chosen, the focal individual loses $x_k^a (1 - u_i^b)$ of the competitive effort z_i committed to by itself.

Putting the details together, we arrive at the following equation for the competitive effort realised by focal individual i :

$$\begin{aligned} y_i &= z_i + x_i^a \frac{1}{n-1} \sum_{k \neq i} (1 - u_k^b) z_k - (1 - u_i^b) z_i \frac{1}{n-1} \sum_{k \neq i} x_k^a \\ &= z_i + x_i^a (1 - \bar{u}_{-i}^b) \bar{z}_{-i} - \bar{x}_{-i} (1 - u_i^b) z_i \end{aligned} \quad (\text{A1})$$

where a bar paired with a $-i$ subscript denotes the arithmetic mean of non-focal individuals in the focal group. It is important to note that the share of group productivity of the average non-focal individual can be expressed as

$$\begin{aligned} \bar{y}_{-i} &= \frac{1}{n-1} \sum_{k \neq i} \left(z_k + x_k^a \frac{1}{n-1} (1 - u_i^b) z_i - \frac{1}{n-1} x_i^a (1 - u_k^b) z_k + x_k^a \frac{1}{n-1} \sum_{j \neq i, k} (1 - u_j^b) z_j \right. \\ &\quad \left. - \frac{1}{n-1} (1 - u_k^b) z_k \sum_{j \neq i, k} x_j^a \right) \\ &= \bar{z}_{-i} + \frac{1}{n-1} \bar{x}_{-i} (1 - u_i^b) z_i - \frac{1}{n-1} x_i^a (1 - \bar{u}_{-i}^b) \bar{z}_{-i} + \frac{1}{(n-1)^2} \left(\sum_{k \neq i, j \neq i, k} x_k^a (1 - u_j^b) z_j \right. \\ &\quad \left. - \sum_{k \neq i, j \neq i, k} x_j^a (1 - u_k^b) z_k \right) \end{aligned}$$

Further simplification can be achieved by recognising that the term contained in parenthesis immediately above is zero. To see this let $\delta_{kj} = \delta_{jk}$ take a value of 1 if $k = j$ and a value of 0 otherwise. Then,

$$\begin{aligned} \sum_{k \neq i, j \neq i, k} x_k^a (1 - u_j^b) z_j - \sum_{k \neq i, j \neq i, k} (1 - u_k^b) z_k x_j^a = \\ \sum_{k, j \neq i} x_k^a (1 - u_j^b) z_j (1 - \delta_{kj}) - \sum_{k, j \neq i} x_j^a (1 - u_k^b) z_k (1 - \delta_{jk}) = 0 \end{aligned} \quad (\text{A3})$$

In words, the term in question simplifies to zero because the double sums both represent the total sum of all entries of the same $(n-1) \times (n-1)$ matrix. Therefore, the average competitive effort in the focal group is:

$$\begin{aligned} \bar{y} &= \frac{1}{n} y_i + \frac{n-1}{n} \bar{y}_{-i} \\ &= \frac{1}{n} z_i + \frac{n-1}{n} \bar{z}_{-i} + \frac{1}{n} x_i^a (1 - \bar{u}_{-i}^b) \bar{z}_{-i} - \frac{n-1}{n} \frac{1}{n-1} x_i^a (1 - \bar{u}_{-i}^b) \bar{z}_{-i} \\ &\quad - \frac{1}{n} \bar{x}_{-i} (1 - u_i^b) z_i + \frac{n-1}{n} \frac{1}{n-1} \bar{x}_{-i} (1 - u_i^b) z_i \\ &= \bar{z} \end{aligned} \quad (\text{A4})$$

Where bars with no subscript denote an arithmetic mean taken over the entire group of n individuals. The average competitive effort in the group is not changed by the manipulation phase. Manipulation only serves to redistribute the initial commitment to competitive effort within a given group. It follows that the relative share of group productivity of the focal individual can be expressed as $y_i/\bar{z}$.

We next assume, for simplicity, that all traits impose a cost on group performance at the same rate, and we propose that group productivity is proportional to $1 - \bar{z} - \bar{x} - \bar{u}$. Thus, we express fitness of the focal individual as

$$w_i = \frac{y_i}{\bar{z}} (1 - \bar{z} - \bar{x} - \bar{u}) \quad (\text{A5})$$

Technically, w_i in the previous line is more accurately described as an inclusive-fitness-generating function, because differentiating it following the steps outline in *Taylor and Frank, 1996* will lead to the correct generalisation of Hamilton's rule (*Hamilton, 1964a; Hamilton, 1964b*).

1.2 Kin selection analysis

Following *Taylor and Frank, 1996*, we assume that traits z , x , and u are controlled by a separate locus. We use, g_z , g_x , and g_u , respectively, to denote the genotypic value of the focal individual at the z , x , and u loci.

To capture the effect of competition on an individual's inclusive fitness we treat the traits z_i , $\bar{z}_{-i}$, and $\bar{z}$ as a function of g_z . We then differentiate w_i with respect to g_z and evaluate the result at a point where all individuals in the population have the same genotypic value at every locus. Evaluating at such a point allows us to assert that $z_i = \bar{z}_{-i} = \bar{z} = z$, $x_i = \bar{x}_{-i} = \bar{x} = x$, and $u_i = \bar{u}_{-i} = \bar{u} = u$. It also justifies us replacing $d z_i / d g_z$ with relatedness between the focal individual and itself ($= 1$), replacing $d \bar{z}_{-i} / d g_z$ with relatedness between the focal individual and its average non-self group mate ($= R_{-i,z}$, where the subscript reminds us that relatedness is assessed at the z locus), and replacing $d \bar{z} / d g_z$ with relatedness between the focal individual and the average member of its group, self-included ($= R_z$). Following through this entire multi-step procedure, we arrive at

$$\Delta W_z = \left(\bar{R}_{-i,z} \frac{-\left(\frac{n-1}{n} - x^a (1 - u^b)\right)}{z} + \frac{n-1}{n} \frac{-x^a (1 - u^b)}{z} \right) (1 - z - x - u) - \bar{R}_z \quad (\text{A6})$$

as the inclusive fitness effect of a small increase in selfishness, z . When ΔW_z is zero, z is at equilibrium with respect to the action of selection (ESS).

Treating traits x_i , $\bar{x}_{-i}$, and $\bar{x}$ as a function of g_x we can go through analogous steps to obtain

$$\Delta W_x = \left(\bar{R}_{-i,x} (-a x^{a-1}) (1 - u^b) + a x^{a-1} (1 - u^b) \right) (1 - z - x - u) - \bar{R}_x \quad (\text{A7})$$

where the subscript reminds us that relatedness is now assessed at the x locus. Treating traits u_i , $\bar{u}_{-i}$, and $\bar{u}$ as a function of g_u , we get

$$\Delta W_u = \left(\bar{R}_{-i,u} (-x^a) b u^{b-1} + x^a b u^{b-1} \right) (1 - z - x - u) - \bar{R}_u \quad (\text{A8})$$

as the inclusive fitness effect of suppression, where relatedness is assessed at the u locus.

1.3 Mathematical criterion of manipulation in other theoretical papers

We compare our model with other theoretical papers, which examine related concepts. We start with Frank's (1995) policing model:

$$w_i = \left(\bar{a} - c a_i + (1 - \bar{a}) z_i / \bar{z} \right) \left(1 - (1 - \bar{a}) \bar{z} \right), \quad (\text{A9})$$

where a_i and z_i are the degree of policing and selfishness of the focal individual, $\bar{a}$ and $\bar{z}$ are the averages in the focal group. c is the cost of policing. Policing changes the benefit and cost of selfishness for all group members, with each multiplied by $1 - \bar{a}$. In contrast, our manipulative cheating involves the actor coercing the recipient into being less selfish (more cooperative).

González-Forero and Gavrillets, 2013 examined a different form of 'manipulation'. In their model, fitness is depending on the behaviour of three types: manipulator that performs manipulation, recipient of manipulation, and a benefactor that is not involved in manipulation but benefit from its social partners' behaviour:

$$w = 1 - p c_{\text{manipulate}} k_{\text{manipulator}} - (c_{\text{acquiesces}} P (1 - q) - c_{\text{resistance}} P q) k_{\text{recipient}} + b \Pi (1 - Q) k_{\text{benefactor}}, \quad (\text{A10})$$

where k are the reproductive values, c are the cost for taking each option, p is the probability for a manipulator to act on a reachable subject, q is the probability for a subject to resist, P is the average manipulation probability, Q is the average resistance probability, and Π is the average manipulation probability that manipulation occurs and successfully reaches a recipient. In addition to the more complicated pay-off setting, their model assumes that social interactions are triggered by manipulation. They are examining when a manipulator can be favoured to manipulate a related recipient, to behave in a way that provides an indirect benefit to the recipient, and that this benefit is sufficient that the recipient is not favoured to resist the manipulator. This contrasts with our model, where manipulative cheating involves manipulation of selfishness (cooperation) – manipulation of an already occurring social behaviour to provide a selfish benefit. Therefore, although both models use the term 'manipulation', they are modelling very different biological scenarios.

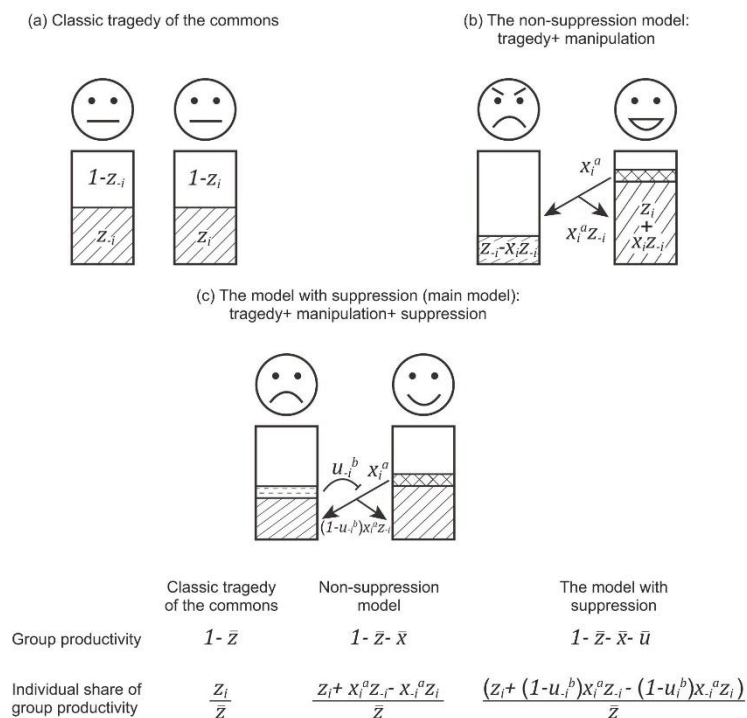
2. The non-suppression model: model with only selfishness and one mechanism for manipulation

We assume suppression is absent in this model, but manipulation can occur. Consequently, this gives:

$$w_i = \frac{z_i + z_{-i} X_i^a - z_i X_{-i}^a}{\bar{z}} (1 - \bar{z} - \bar{x}), \quad (\text{A11})$$

where x_i is the level of manipulative cheating of the focal individual, z_{-i} is the average level of selfishness (excluding the focal individual), a is the shape parameter for manipulative cheating, and $1 - \bar{z} - \bar{x}$ is the group's productivity. As above, the parameter a determines whether the benefit from increased investment into manipulation is decelerating ($a < 1$), linear ($a = 1$) or accelerating ($a > 1$).

To facilitate comparison with previous work, the relations between the classic tragedy of the commons, the non-suppression model, and the model with suppression are illustrated in **Appendix 1—figure 1**. The first model is the classic tragedy of the commons model, which predicts a single optimal level of investment into cooperation / selfish reproduction (**Appendix 1—figure 1a**; Frank, 2010a). The next model is the non-suppression model, which allows for manipulative cheating, where individuals increase their own investment into reproduction, by reducing that of others (**Appendix 1—figure 1b**). Lastly, in the model with suppression, we allow suppression as a defensive mechanism against manipulative cheating (**Appendix 1—figure 1c**).

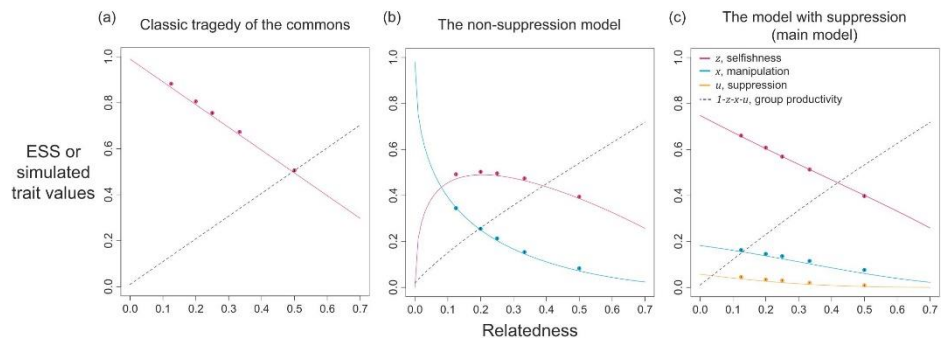


Appendix 1—figure 1. Comparison between the classic tragedy of the commons, the non-suppression model, and the model with suppression. Here, we illustrate three models with gradually increasing complexity. (a) In the classic tragedy of the commons, individuals can choose to invest resources into reproduction (z_i) or group productivity (cooperation; $1 - \bar{z}$). (b) In the non-suppression model, individuals can also manipulate others (x_i), to make them invest more in group productivity. (c) In the model with suppression, individuals can also suppress (block) the manipulation by others (u_i). Within each model, arrows indicate manipulation, the blockings indicate suppression, and the outcome of the two actions are expressed below the arrows. The fitness of an individual is divided into two components: group productivity and share of group productivity, both shown at the bottom of the figure.

We find the change of inclusive fitness effect for selfishness, and manipulation:

$$\begin{cases} \Delta W_z = \left(\bar{R}_{-i,z} \frac{-\left(\frac{n-1}{n} - x^a\right)}{z} + \frac{\frac{n-1}{n} - x^a}{z} \right) (1 - z - x - u) - \bar{R}_z \\ \Delta W_x = \left(\bar{R}_{-i,x} (-ax^{a-1}) + ax^{a-1} \right) (1 - z - x - u) - \bar{R}_x \end{cases} \quad (\text{A12})$$

Equation A12 could not be solved analytically, and so we found solutions with an iterative numerical analysis. We also tested the predictions of our analytical model with an individual-based simulation, which showed close agreement (**Appendix 1—figure 2b**).



Appendix 1—figure 2. Selfishness, manipulation, and suppression of manipulation. The panels show the ESS predictions and simulation results for: (a) tragedy of the commons (only selfishness evolves); (b) the non-suppression model (selfishness and manipulation evolve); (c) the model with suppression (selfishness, manipulation, and suppression of manipulation evolve). The solid lines show the ESS predictions, and the dots show the results of our individual-based simulations ($a = b = 0.5$).

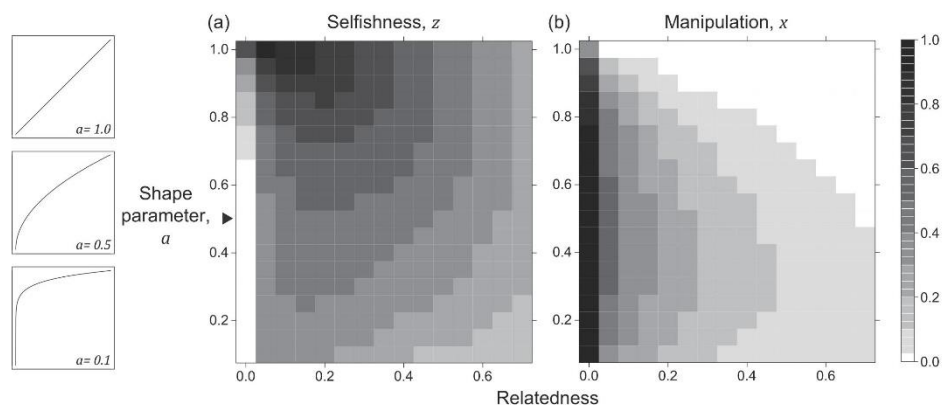
The non-suppression model predicts that manipulation can be favoured, and that greater levels of manipulation will be favoured when relatedness is lower (**Appendix 1—figure 2b**). This prediction arises because there is an indirect fitness cost to manipulating relatives and reducing group productivity. Further, since a function with smaller shape parameter means the manipulative effective is higher for a given trait value, as shown in the three panels on the left of **Appendix 1—figure 2**, we find greater intermediate values of manipulation when the shape is more decelerating (smaller a has more shaded area, **Appendix 1—figure 3b**).

This model also predicts a very different relationship between selfishness and relatedness, compared with the classic tragedy of the commons model and the model with suppression (compare **Appendix 1—figure 2b** with other panels). Specifically, rather than selfishness increasing as relatedness decreases, the non-suppression model predicts that selfishness shows a non-monotonic (domed) pattern, peaking at intermediate relatedness (**Appendix 1—figure 2b**). The difference in the non-suppression model is that, relative to tragedy of the commons, selfishness is not as advantageous when relatedness is low. At low relatedness, there is selection for high levels of manipulation – this dilutes the benefit of selfish reproduction because that effort will be taken away by manipulation. Within our lottery ticket metaphor in the main text, the benefit of buying tickets is reduced if those tickets are just going to be stolen.

Comparing the non-suppression model and the model with suppression, a consequence of adding in suppression is that lower levels of manipulation are selected for, because the benefit of increased manipulation is reduced if it will be suppressed (compare **Appendix 1—figure 2b & c**). This lower level of manipulation, and the fact that some of it is suppressed, lead to selfishness being beneficial at low relatedness, as in the classic tragedy of the commons model (compare **Appendix 1—figure 2a & c**), and in contrast to when there is no suppression (**Appendix 1—figure 2b**).

By comparing all three models, we also found the group productivity did not vary depending upon whether or not manipulation and suppression were included in our model, compared to the classic tragedy of the commons model (**Appendix 1—figure 2**). This is because adding manipulation and suppression does not change the basic structure of the model: share of productivity multiplied

by group productivity. Thus, the relation between relatedness and group productivity is not affected: the two additional traits are sharing the 'loss' in group productivity with selfishness, where the sum of loss is the same for a given relatedness. In other words, manipulation and suppression do not change the degree of cooperation across the group and this finding confirms the design in **equation A4**.



Appendix 1—figure 3. Nonlinear returns in the non-suppression model. Panels (a) and (b) show the predicted ESS level of selfishness (z^*) and manipulation (x^*) respectively, for different values of relatedness (R) and the shape parameter (a). The black triangle indicates the horizontal row where the parameter settings are identical to each other and to **Appendix 1—figure 2b**.

3. The models with personal costs: adding cost to relative shares of group productivity

In the main models, investment in manipulation or suppression led to reduced investment in group productivity. As an alternative, we also investigated a scenario where manipulation and selfishness have a personal cost, reducing the share that an individual obtains of the group productivity, but not influencing the overall success of the group. In this case, manipulative cheating and suppression of cheating now have impacts on the average level of competition, and they are not just redistributing the share of productivity. We first consider the case where suppression is absent and manipulation has a personal cost, and then we move on to the case where both manipulation and suppression are allowed.

3.1 The non-suppression model with personal costs

The personal cost of manipulation can be added in many ways, and one easy way is to multiply the benefit of selfishness by $(1 - x)$, where x is the level of manipulation. For simplicity, we assume cost is multiplicative and there is no coefficient before x . This modification changes the competitive effort through selfishness from z_i to $(1 - x_i)z_i$, and changes the competitive effort gained through manipulation from $z_{-i}x_i^a$ to $(1 - x_{-i})z_{-i}x_i^a$. Similarly, the average effort of the group is $(1 - \bar{x})\bar{z}$, making the fitness of the focal individual,

$$w_i = \frac{(1 - x_i)z_i + (1 - x_{-i})z_{-i}x_i^a - (1 - x_i)z_{-i}x_{-i}^a}{(1 - \bar{x})\bar{z}} (1 - \bar{z} - \bar{x}), \quad (\text{A13})$$

where $(1 - \bar{z} - \bar{x})$ is the group productivity and the terms before it is the relative share of group productivity. By comparing **equations A13** and **A11**, it is apparent that we are multiplying $(1 - x)$ for both the numerator and denominator in **equation A11**. However, it is meaningful because each term is multiplied by different x : some are the level of manipulation of the focal individual, while others are the average of the social neighbours or the average across the entire group.

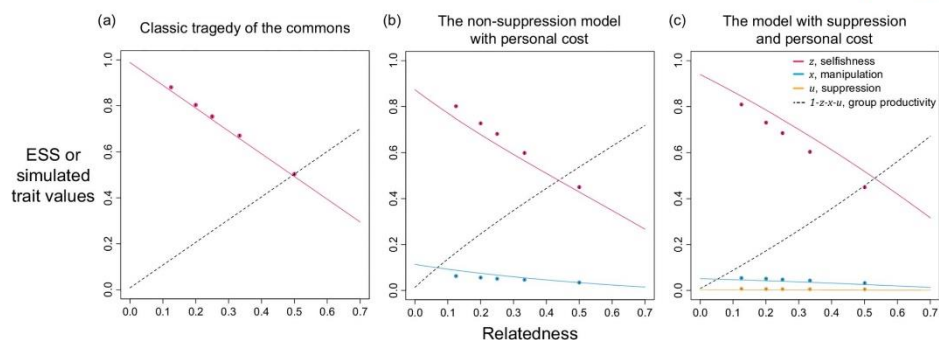
We found that adding a personal cost of manipulation leads to a reduced level of manipulation, especially when $R < 0.1$ (compare **Appendix 1—figure Appendix 1—figures 2b and 4b**). Furthermore, because both selfishness and manipulation have a personal cost, the net benefit obtained from investing in manipulation is reduced, and so we found no dramatic increase in the predicted level of manipulation when $R < 0.1$ (**Appendix 1—figure 4b**). In contrast, when manipulation is cost-free at the individual level, the predicted strategy is to put all selfish effort into manipulation when relatedness of the population is very low (**Appendix 1—figure 2b**).

3.2 The model with suppression and personal cost

We then allow suppression, with a personal cost, that reduces the share of group productivity obtained. We multiply each term in the relative share of productivity in **Equation 1** with $(1 - x - u)$, where x and u are the ones with same identity as z they are multiplying e.g., z_i multiplied by $(1 - x_i - u_i)$. This gives:

$$w_i = \frac{(1 - x_i - u_i)z_i + (1 - u_{-i})(1 - x_{-i} - u_{-i})z_{-i}x_i^a - (1 - u_i)(1 - x_i - u_i)z_{-i}x_{-i}^a}{(1 - \bar{x} - \bar{u})\bar{z}} (1 - \bar{z} - \bar{x} - \bar{u}). \quad (\text{A14})$$

We found the predicted level of suppression is lower when a personal cost of suppression is also included to the model (compare **Appendix 1—figure 2c& Appendix 1—figure 4c**). Because there is (i) a lower need for suppression from a lower predicted level of manipulation, and (ii) a more costly design of suppression, we only find small levels of suppression when relatedness is low (**Appendix 1—figure 4c**). In addition, given the presence of suppression, the predicted level of manipulation is lower than the model without suppression (compare **Appendix 1—figure 4b& c**). Despite the lower predicted ESS values, these models with personal cost for manipulation and suppression still predict both traits can be favoured, especially when relatedness is low.

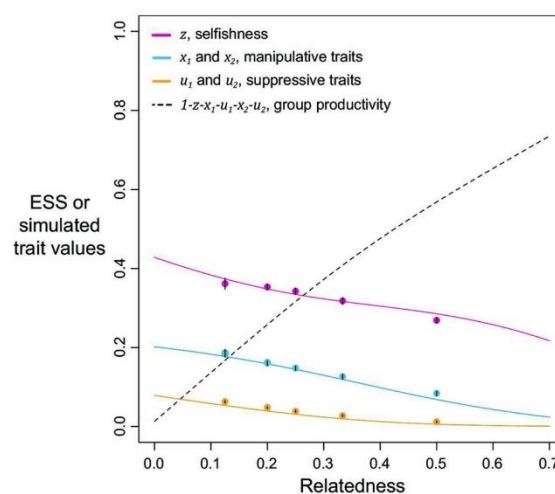


Appendix 1—figure 4. Selfishness, manipulation, and suppression of manipulation. The panels show the ESS predictions and simulation results for: (a) tragedy of the commons (only selfishness evolves); (b) the non-suppression model with personal cost (both selfishness and manipulation evolve); (c) the model with suppression and personal cost (selfishness, manipulation, and suppression evolve). All results are generated with the same shape parameters as other figures ($a = b = 0.5$).

4. Additional analysis of the scenario with two mechanisms where all traits are simultaneously introduced

4.1 The relation between ESS and relatedness

In order to understand the oscillating dynamics (**Figure 4**) better, we perform game-theoretical analysis for the two-mechanisms case and examine when does the game theoretical analysis disagree with simulations. We begin by analysing the model along relatedness and found it gives very similar predictions to the scenario with only one mechanism to manipulate (compare **Figure 2** & **Appendix 1—figure 5**). Selfishness, manipulation, and suppression are all greater when relatedness is lower. Both the two manipulative traits, and the two suppression traits converge to the same ESS values. Notably, because of the constraints on group productivity, where the sum of traits cannot exceed 1, a lower level of selfishness is selected in the scenario with two mechanisms while the levels of manipulation and suppression is roughly the same between the scenario with single mechanism and two mechanisms. In other words, because the additional manipulative mechanism is the same worthy to invest as the other one, we find a lower level of selfishness in the scenario with two mechanisms.



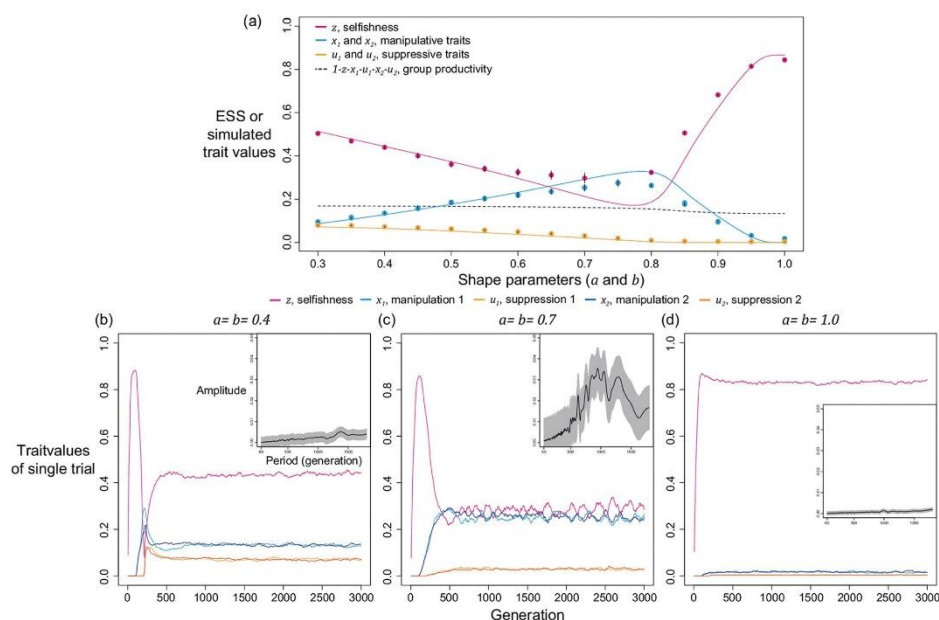
Appendix 1—figure 5. Selfishness, manipulations, and suppressions of manipulation in the scenario with two mechanisms to manipulate and suppress manipulation, where all traits are introduced at the same time point. Solid lines show the ESS predictions, and dots show the results of our individual-based simulations ($a = b = 0.5$).

4.2 The occurrence of oscillation is related to shape parameters

In contrast to the close agreement shown above, we found some disagreement between simulation and game-theoretical analyses when we analyse along shape parameters (**Appendix 1—figure 6**). We used harmonic regression to test for oscillating dynamics in our simulation data. In a harmonic regression, white noise (randomly time series) would be a flat line because all frequencies have similar predicted amplitude; but an oscillating pattern would lead to peak or peaks. For example, a harmonic regression of sine wave would be mainly a single peak because it only consists of a wave at single period. From the time series of selfishness, we found oscillating dynamics at intermediate shape parameters ($a = b = 0.7$; **Appendix 1—figure 6c**), but not at low and high shape parameters (**Appendix 1—figure 6b&d**; $a = b = 0.4$ or 1.0). Specifically, a harmonic regression of selfishness under low and high shape parameter values gave almost horizontal lines, whereas intermediate values showed several peaks at periods around 500–1500 generations (insets of **Appendix 1—figure 6**). These peaks suggest that there is a periodic component to the evolutionary dynamics, with stochastic cycle that repeats about every 500–1500 generations, and the dynamic is not random fluctuations.

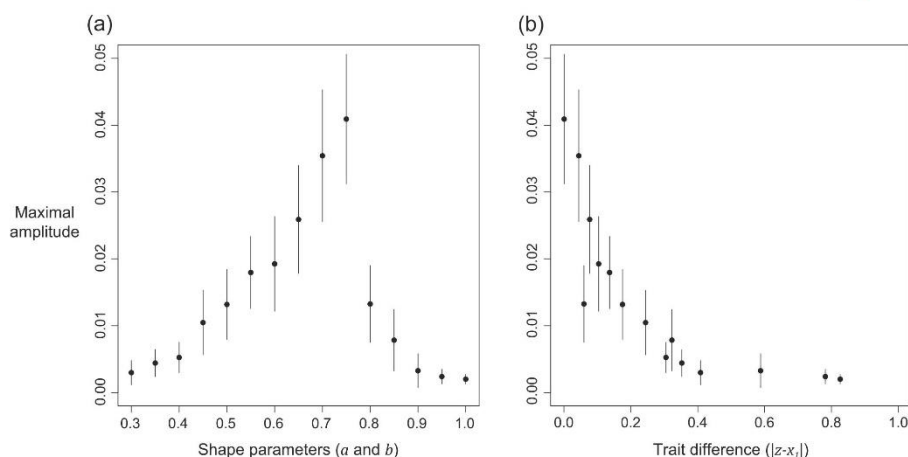
The oscillations could occur because the benefits of manipulation and selfishness become similar when shape parameters are at intermediate values, which subsequently can be influenced by the

interactions between traits. The decreased difference in benefits is supported by a broader analysis, showing the discrepancy between game-theoretical and simulated results only occurs when the former predicts selfishness is lower than the level of manipulative cheating (**Appendix 1—figure 6a**; $0.6 < a, b < 0.8$). When shape parameters are at these intermediate values, the level of selfishness in simulations does not go below the level of manipulation, as predicted by game-theoretical analysis (**Figure 5a**, dots & curves). Moreover, the simulated trait values are a lot more variable as the error bars become visible (**Figure 5a**; black vertical lines over dots).



Appendix 1—figure 6. Oscillation dynamics of selfishness and manipulation occur in some ranges of shape parameters in the scenario with two mechanisms when relatedness is low ($R = 1/8$). (a) ESS predictions and simulation results from the model, where disagreement between two approaches is found when shape parameters are between 0.6 and 0.8. (b–d) Time series of simulations under various relatedness settings. Each panel shows the time series of traits, and the analysis from harmonic regression is shown in the inset (same as **Figure 4**). Harmonic regression found the greatest amplitude in the period of 1385 generations and p-value of 1.5333×10^{-2} when shape parameters are 0.4; period of 815 generations and p-value of 1.8035×10^{-5} when shape parameters are 0.7; period of 1760 and p-value of 2.5202×10^{-3} when shape parameters are 1.0.

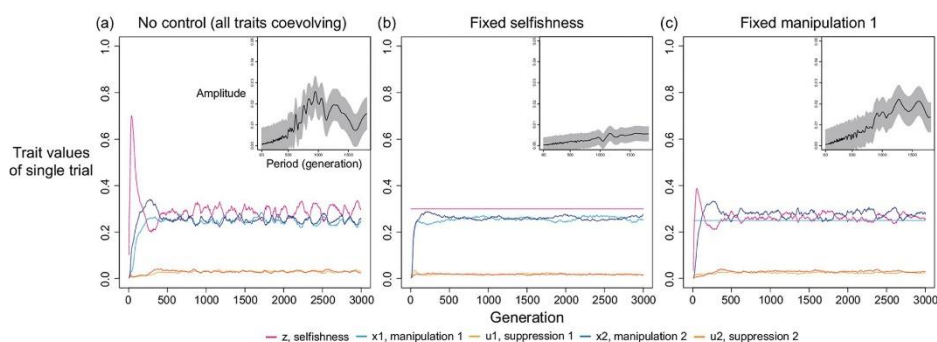
We can further examine the oscillations by checking the changes in maximal amplitude along with shape parameters or evolved trait values (**Appendix 1—figure 7**). In agreement with our previous results (**Appendix 1—figure 6b–d**), the maximal amplitude shows a peak when shape parameters are at intermediate values and amplitude is low when the shape is either high or low (> 0.8 or < 0.6). We are interested in the difference in evolved trait values because oscillation seems to take place when the evolved level of selfishness is close to the level at which manipulation is expressed (**Appendix 1—figure 6a**). After we visualised the relationship between trait difference and the maximal amplitude of oscillation, we found a consistent negative correlation between the difference and amplitude. This finding suggests oscillation might have resulted from interactions between traits when their evolved levels are similar.



Appendix 1—figure 7. The relation of maximal amplitude from harmonic regression in response to (a) shape parameters, and (b) distance in evolved levels of selfishness and manipulation. Relatedness is set to 1/8 and the error bars are the 95% confidence intervals. Details of the analysis are described in Appendix 1—7.5.

4.3 Oscillating trait dynamics disappear when selfishness is not coevolving with manipulation

In order to better understand the origin of oscillation, we reduce the complexity in simulations by reducing the number of coevolving traits. In **Appendix 1—figure 6** and **Appendix 1—figure 7**, there are 5 traits coevolving: selfishness, manipulation 1, suppression 1, manipulation 2, and suppression 2. We make 1 of the 5 traits non-mutable at a time and analyse the evolving traits through harmonic regression. We found oscillation disappears when selfishness is held constant, while holding one of the two manipulative traits constant can still produce oscillating dynamics (**Appendix 1—figure 8b&c**). Moreover, the results are consistent regardless of whether the traits are fixed at the average of coevolving dynamics or the prediction from game-theoretical analysis (only the latter is plotted in **Appendix 1—figure 7** to avoid redundancy).



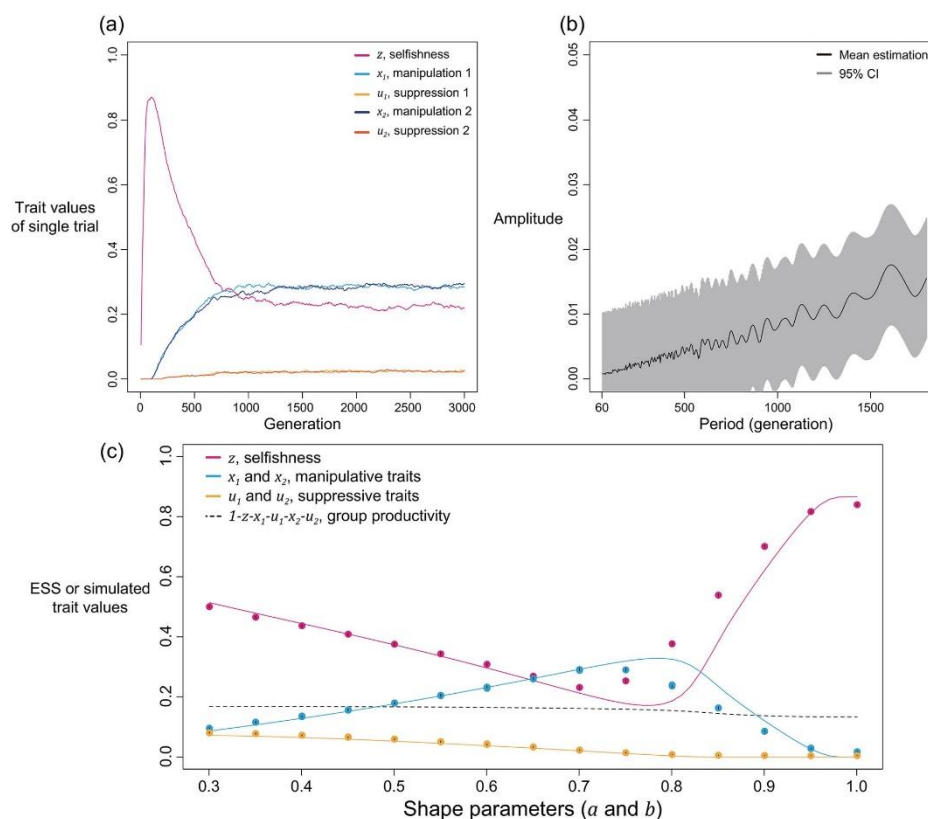
Appendix 1—figure 8. Oscillation disappears when selfishness is controlled and fixed at a value. We use harmonic regression to instead analyse the dynamics of manipulation 2 in this figure, because selfishness and manipulation 1 are fixed in some panels. The level of fixed selfishness and manipulation are determined by the ESS predicted in game-theoretical analysis, where relatedness is 1/8 and shape parameters are 0.7. Harmonic regression found greatest amplitude in the period of 825 generations and p-value of 2.1045×10^{-4} when all traits coevolve; period of 1450 generations and p-value of 2.5239×10^{-4} when selfishness is fixed; period of 1575 and p-value of 5.5099×10^{-4} when manipulation 1 is fixed.

The above findings suggest the oscillation is likely to be an outcome from the linkage (interaction) between selfishness and manipulation. That is, some stochasticity may lead to deviations in trait values, for

instance, increased selfishness and decreased manipulation. This perturbation would generate selective forces that favours decreased level of selfishness and increased level of manipulation. However, because the optimal trait values of both traits are similar to each other, the selective force would be weak, which allows trait values to fluctuate within a range of levels. Finally, when the optimal trait values are more differed and selective forces are strong, oscillation would only take place in a small range of parameter or even disappear. Such effects of linkage (interactions) are not discovered in game-theoretical analysis because inclusive fitness effects are estimated as independent factor (i.e., no linkage between traits). Similar complication in coevolving traits have also been observed in other studies involving individual-based simulations (Tibbetts *et al.*, 2020; Liu *et al.*, 2021).

4.4 Oscillation also disappears when traits are independently inherited without linkage

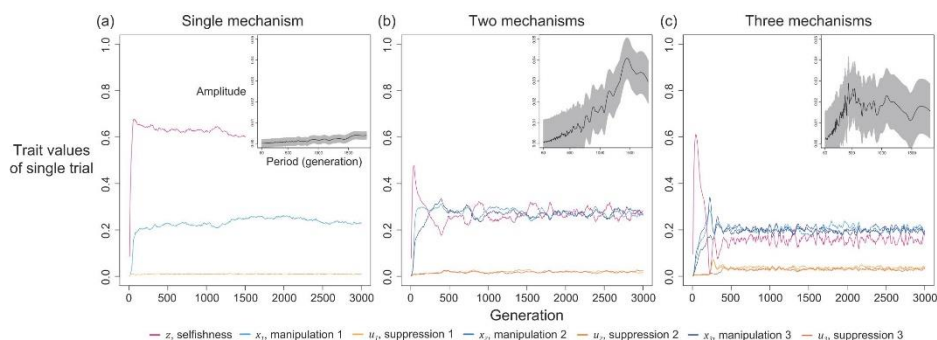
To further test our linkage hypothesis for the oscillations, we examined an artificial scenario where there could be no possible linkage. Specifically, we assumed that each offspring inherits each trait independently, possibly from different individuals (multiple asexual parents, a different one for each trait). In support of our hypothesis, we found that when there can be no linkage, that oscillations do not occur (Appendix 1—figure 9a & b). In addition, in this scenario, where there can be no linkage, the results of the simulation were in quantitative agreement with the analytical prediction (Appendix 1—figure 9c). This contrasts with our simulations results in Appendix 1—figure 6a, where oscillations occurred, and there was a greater quantitative disagreement between our analytical and simulation results. It also suggests that some disagreement between analytical and simulation results were due to the simulation allows for correlation among inherited traits to build over time.



Appendix 1—figure 9. Oscillation does not occur when traits are inherited independently without linkage. (a) Time series of coevolving traits when shape parameters are intermediate, and relatedness is low ($R = 1/8$; $a = b = 0.7$). (b) The estimated amplitude of the time series of selfishness by harmonic regression. (c) Analytical predictions and simulation results from the model without linkage of traits.

4.5 Can oscillation happen when there are fewer or more mechanisms?

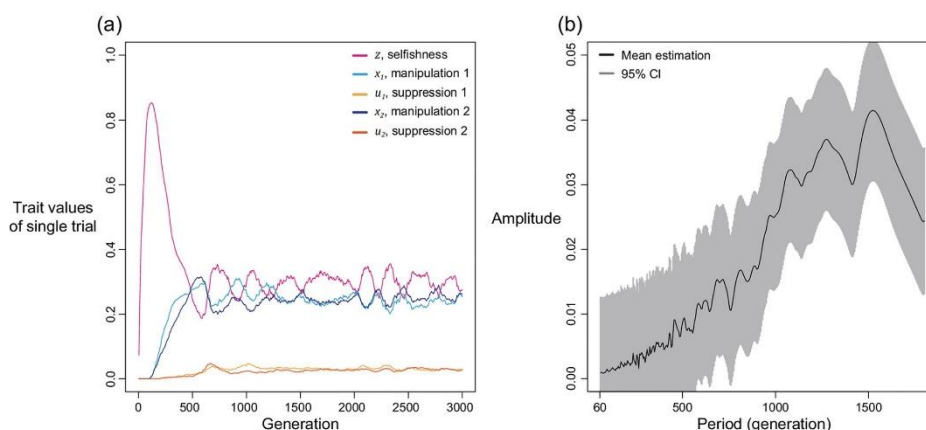
We then analyse whether oscillation can occur in cases with different number of mechanisms. The idea is if the oscillation was caused by interacting selfishness and manipulation, can oscillation still occur when there is only one mechanism to manipulate, or when there are 3 mechanisms. As shown in **Appendix 1—figure 10**, we found single-mechanism simulation cannot generate oscillation while the three-mechanism simulation still has oscillations. This makes sense because multiple mechanisms of manipulation can amplify the initially small perturbations and help oscillation to be maintained in the trait dynamics.



Appendix 1—figure 10. Oscillation happens when there is more than one mechanism to manipulate and suppress. Here, relatedness is $1/8$ and shape parameters are 0.75 . Harmonic regression found greatest amplitude in the period of 1800 generations and p-value of 4.7899×10^{-3} in single-mechanism simulations; period of 1475 generations and p-value of 2.7475×10^{-5} in two-mechanism simulations; period of 390 and p-value of 1.4544×10^{-2} in three-mechanism simulations.

4.6 Does changing mutation rate lead to a different oscillation period?

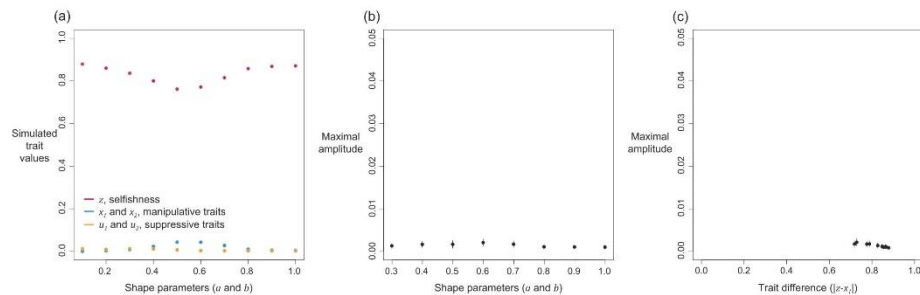
In section 4.3 and 4.4 we have showed that the oscillation results from interactions and linkages between traits, consequently, we expect mutation rate should change the behaviour of oscillation. This is because mutation rate controls the speed of introducing new genotype to the population and the oscillation can be slower if mutation rate is lower. We verify this thought by running simulation with half of the original mutation rate (i.e., 5×10^{-3} , Appendix 1—7). We found supporting evidence as the oscillation of traits last longer per each cycle and have the greatest amplitude at a larger period close to 1500 generations (compare **Appendix 1—figure 11** and **Figure 4**).



Appendix 1—figure 11. Selfishness and manipulative cheating traits oscillate slower when mutation rate is lower. In this figure, we set mutation rate is half the size of which in all other simulation ($R = 1/8$; $a = b = 0.7$).

4.7 Can we find oscillation in the personal cost model?

In section 3, we have discussed that implementing personal cost to manipulation and suppression would not qualitatively change the predictions, but can we still get oscillation in these models? We made a two-mechanism model with personal cost and found out there is no oscillation (**Appendix 1—figure 12b**). The underlying reason is the evolved level of selfishness is much higher than the level of manipulation (**Appendix 1—figure 12**). Despite the model design is different, the relation between maximal amplitude and trait difference seems to remain unchanged by comparing **Appendix 1—figure 12** and **Appendix 1—figure 7b**.

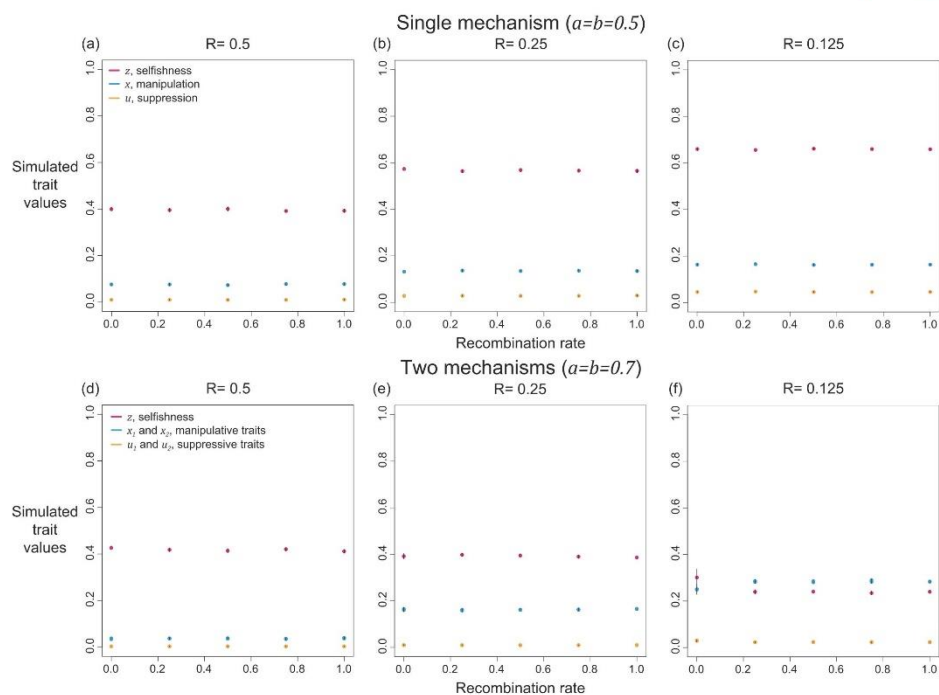


Appendix 1—figure 12. The properties of personal cost model with two mechanisms to manipulate and suppress. (a) The simulated level of selfishness, manipulative cheating, and suppression, (b) the relation between maximal amplitude from harmonic regression and shape parameters ($a = b$), and (c) the relation between maximal amplitude and trait difference in evolved selfishness and evolved manipulation.

4.8 Does recombination change the simulated results?

So far, we have assumed that each set of trait values are carried by single individual across generations (except Appendix 1—4.4), but it might not be the case when recombination take place, where genes may be exchanged between individual cells or segments of chromosomes swapped between two haploid individuals. We address this potential biological complexity by setting an additional recombination phase in the life cycle of individual-based simulations. During the recombination phase, individuals are selected according to relative fitness in the previous generation, paired with another, and then recombine one trait value depending upon the recombination probability (externally set parameter). Only one trait value is exchanged, and an individual recombines only once. After the possible recombination, the two individuals are put into a pool of founders. Once all individuals are in the founder pool, and a specific number of individual (m , Appendix 1—7) are sampled as founder to start the new population.

The effect of recombination depends on how many mechanisms of manipulation and suppression are considered in the simulation. When there is only one mechanism, we found recombination does not affect the results (**Figure 2** and **Appendix 1—figure 13a–c**). This is likely because all social interactions, including manipulation and suppression, are reset at the beginning of each generation that nothing in the past generations is passed on the future generation. When there are multiple mechanisms, recombination also plays minimal role in most cases (**Appendix 1—figure 13d–f**). However, as we have found in Appendix 1—4.4, the oscillating dynamics stop when recombination rate is above 0 (**Appendix 1—figure 13f**). Similar to our finding, recombination could matter in scenarios where associations between traits can be important and potentially build up over time, such as whether punishment can favour cooperation (**Gardner and West, 2004; Gardner et al., 2007; Lehmann et al., 2007; Hilbe et al., 2013**).

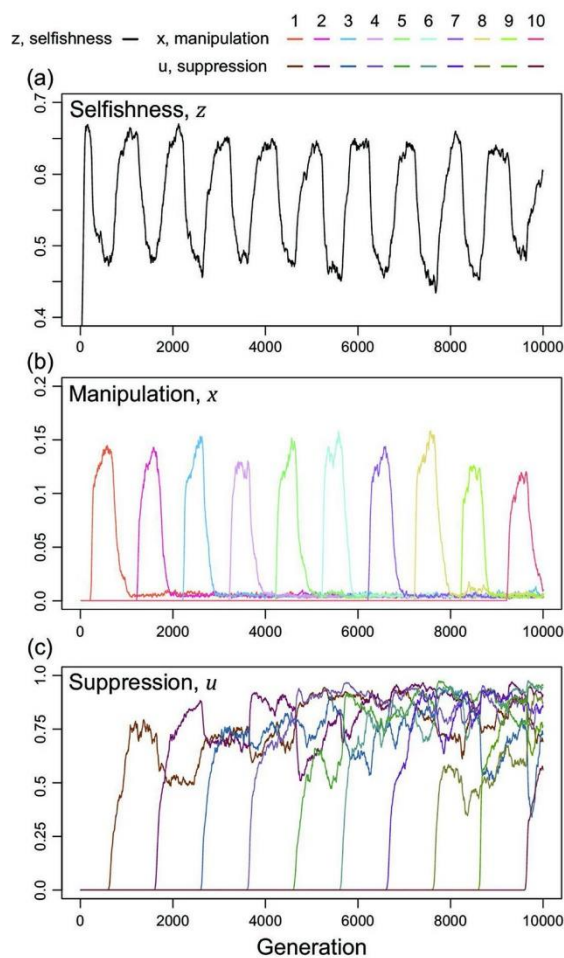


Appendix 1—figure 13. Effects of recombination on the simulated level of selfishness, manipulation, and suppression. Each panel has a specific relatedness setting ($R=0.5$, 0.25 , or 0.125), and various recombination rates (0 , 0.25 , 0.5 , 0.75 , and 1). Two scenarios are simulated: (a–c) single mechanism to manipulate and suppress, (d–f) two mechanisms. The single mechanism results, where shape parameters are 0.5 , extended the discussion in **Figure 2** (no recombination). The two mechanisms results, where shape parameters are 0.7 , bridged the gap between **Appendix 1—figure 6** and **Appendix 1—figure 9** of this appendix as the former is equal to recombination rate of 0 and the latter is equal to 1 .

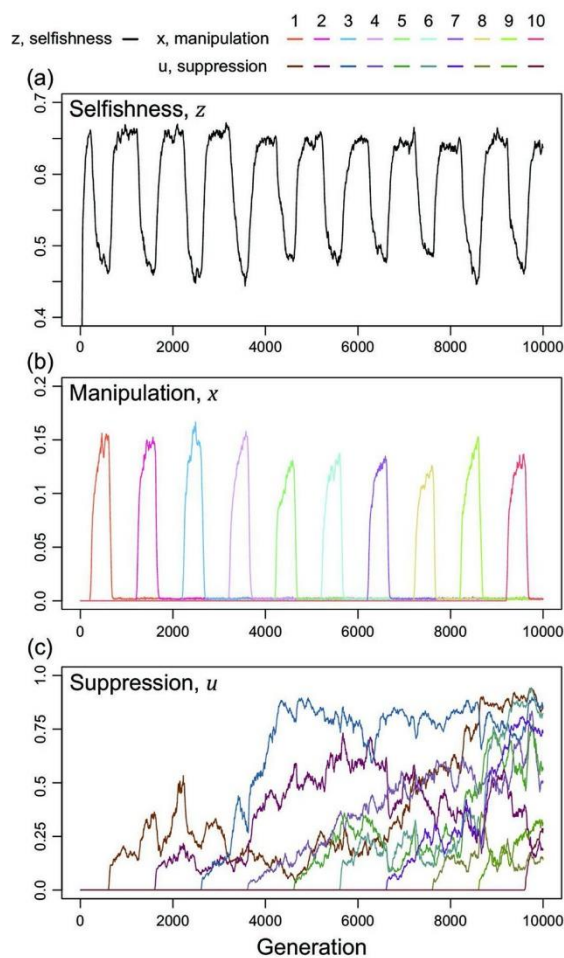
5. Arms race under different biological settings

We construct two additional scenarios to understand if alternative cost design can still generate arms-race-like dynamics. In particular, we modified group productivity so that the cost of suppression is closer to a switch-on/switch-off design. We assume suppression becomes costly only when the group average is above 0.05, and the cost is the same for all suppression above 0.05. This modification allows the share of group productivity and group productivity, to disentangle with each other and relax the linkage assumed in the main model. On top of whether the cost depends continuously on the degree of suppression, the cost can be conditional and only present when there are sufficient levels of manipulation. Thus, we assume the cost of suppression is unconditional in one case, and conditional to the average level of manipulation in the other case. For simplicity, we do not randomly introduce each new mechanism but mutate them at fixed time points with equal intervals.

We found the genetic arms race of manipulation and suppression still occur in both cases, but with different dynamics of suppressive traits. When the cost is unconditional and fixed, we found all of the suppressions go to almost 1 when the shape parameter for manipulation and suppression are equal ($a = b = 0.5$; **Appendix 1—figure 14**). This is because the effect of suppression is relatively linear and it does not cost extra if suppression has higher values (compare **Appendix 1—figure 14c & Figure 5c**). When the cost is conditional to manipulation, we found a drift-like dynamics for all suppressive traits ($a = 0.5, b = 0.01$; **Appendix 1—figure 15**). The underlying reason is suppressions are cost-free and no longer under selection when manipulations are completely suppressed (**Appendix 1—figure 15b & c**). The cost-free suppressions are support by the dynamics of selfishness, which is not gradually decreasing like other cases (compare **Appendix 1—figure 15a** with **Figure 5a & Appendix 1—figure 14a**). Nevertheless, the dynamics of manipulation are very similar in all cases, thus suggesting genetic arms race is a robust result which can be found in different biological settings (**Figure 5, Appendix 1—figure 14, and Appendix 1—figure 15**).



Appendix 1—figure 14. Arms race with an unconditional and fixed amount of cost for suppressing manipulative cheating. Suppression is costly in group productivity when it exceeds 0.05, and the cost is fixed at 0.01 regardless of the trait value of suppression. Shape parameters are equal for manipulation and suppression ($a = b = 0.5$) and relatedness is $1/3$.



Appendix 1—figure 15. Arms race with a conditional and fixed cost for suppression. Suppression is costly only when both manipulative cheating and suppression are above 0.05, and the cost of suppression is fixed at 0.05 regardless of the trait value. Shape parameters are the same as **Figure 5** ($a = 0.5$ and $b = 0.01$) and relatedness is $1/3$.

6. Additional discussion

6.1 Decelerating return and the nature of communication

Contrary to the linear effects assumed in the classic tragedy of the commons, empirical studies have shown that nonlinear effects might be common in communications between cells and individuals. This is because the limitation of diffusion in communicative molecules, making the benefit of increased production in those molecules diminishes (Müller et al., 2006; Wiley, 2013; Rogers et al., 2017); same principle can be applied to social insects, as increased time in communicating would also lead to increased possibility in encountering previously-interacted individuals (Gill and Johnson, 2002). As manipulation and suppression require such short-distance interaction, we have considered the nonlinear impact of traits on fitness to make the model more realistic. In particular, manipulative, and suppressive traits may have larger gains when they evolved from absence to some intermediate extent of effects, whereas the benefit of complete manipulation or suppression is diminishing. Similar saturating property has been found in other social traits, such as the benefit in increased stalk/spore ratio in slime moulds (Bonner, 1982; Hudson et al., 2002). Thus, we focus on cases where nonlinear effects have linear or diminishing returns ($0 < a \leq 1$ and $0 < b \leq 1$). For simplicity, we only add nonlinearity effect to individual reproduction component and not the group productivity component (i.e., $1 - \bar{z} - \bar{x} - \bar{u}$).

6.2 Methodological details of numerical analysis in game theory results

For a given number of iteration t , we update trait values by $\{z_{t+1}, x_{t+1}, u_{t+1}\} = \{z_t + c_{scale} \Delta W_z, x_t + c_{scale} \Delta W_x, u_t + c_{scale} \Delta W_u\}$ and find the trait values that converge to fixed values. All traits are initialised at 0.1. We set the scaling coefficient c_{scale} to 2.5×10^{-3} for better precision. Finally, due to slow convergence when relatedness is low, we iterated 4×10^6 times for $0.1 \geq R$ and 4×10^5 times for the rest of relatedness values.

7. Individual-based simulations

7.1 Overview

We use individual-based simulations following genetic algorithm to understand the evolution of selfishness, manipulative cheating, and suppression of manipulation. In contrast to the game-theoretical analysis, individual-based simulations allow us to explicitly model mutations and trait dynamics under a continuous trait space. Following the formal analysis (section 1), we employ similar assumptions that the population size is constant, and generations do not overlap.

After initialisation, the biological processes in each generation take place in the following order: First, groups are formed from several (l) founders where the number of founders determines the relatedness ($R = 1/l$). Each group has the same number of founders, and it is a parameter for population structure. Second, the founders expand the social group to a size that social interactions take place, where each founder replicate the same number of times. Third, we calculate the group averages of trait values and the relative fitness of each individual. Fourth, the new-born founders are randomly drawn from the current population according to the relative fitness. Each new-born founder inherits its parent's trait values with independent chances of mutation for each of the three traits.

We use the design with founders and group growth to avoid the potential effects of small integers and small group sizes (i.e., demographic stochasticity in social groups). This simulation framework is known as haystack models (Maynard Smith, 1964).

7.2 Initialisation

We initialise the population by creating a population with uniform group size (n) and uniform properties of no selfish traits (i.e., $z = x = u = 0$). In order to reduce the effect of demographic stochasticity, we hold the size of the whole population and let the number of groups change because group size (n) is explicitly modelled in the model. Specifically, we set population size parameter (N) and obtain the number of groups as $M = \lceil N/n \rceil$, the least integer larger than or equal to N/n . Thus, the population size, Mn , is relatively constant while the number of groups increases as the group size decreases.

Each individual has three variables: the degree of selfishly increase own fitness share at the cost of entire group (z), the degree of manipulative cheating (x) and the degree of suppressing manipulative cheating (u). Within one group, there are $m = n/l$ individuals that have identical trait values because they are replicated from the same founder. Further, trait values could only be changed in *mutation* process.

7.3 Fitness calculation and the coevolving traits

We perform two levels of selection to choose founders for the next generation: (i) group selection according to group productivity from average trait values of all group members, and (ii) individual selection according to each group members' relative fitness (Figure 1). In other words, the probability that a founder comes from a group depends on the group's productivity, $1 - \bar{z} - \bar{x} - \bar{u}$, and the chance that it is an offspring of the focal individual is determined by its relative fitness in the group (or share or productivity), $\frac{z_i + (1 - u_i)z - x_i - (1 - u_i)z_i x - i}{\bar{z}}$. Although the simulations calculate fitness in the same way as the game-theoretical analysis, the process for each event is explicitly modelled in the simulations and individuals can have any trait values, whereas the game-theoretical analysis tracks on the trait dynamics of the entire population under weak selection assumption.

7.4 Mutation of the coevolving traits

We assumed the traits can mutate between generations. For each trait of a new founder, the mutation rate is $p_{mut} = 0.01$. If a mutation occurs, we perturb the parental trait value by a normally distributed deviation with mean of 0 and standard deviation of 0.1, constrained at the boundaries [0,1].

7.5 Analysis of the simulations

In all cases, we repeated the simulation 10 times, and let social interactions take place at the population around 10^5 individuals (i.e., $lm \lceil 10^5/lm \rceil$) to ensure that the trait values converged to their evolutionary optima. We introduced mutation to selfishness from the start of simulation, manipulative

cheating from 100 generation, and suppression from 200 generation to make sure the latter traits are not evolved in the absence of selfishness. We tracked the trait dynamics for 3×10^3 generations (unless specified elsewhere) and average over the last 10% to calculate the average and standard deviation of trait values. Except for the ones with oscillation dynamics, the coevolving traits usually stabilise within 500 generations (e.g., **Appendix 1—figure 6b & d**).

A4

Supplementary information of
Chapter 5

Equilibria and oscillations in cheat-cooperator dynamics

Supplementary Information

Ming Liu, Geoff Wild, and Stuart A. West

June 19, 2023

This document is the supplementary information for the **Equilibria and oscillations in cheat-cooperator dynamics** and contains four sections: The first section has the list of all parameters used in the model; the second section is a broader investigation for frequency and density dependence in scenario 1; the third section contains additional results and checking related to periodic population bottlenecks in scenario 2; the fourth section has the additional results related stochasticity in scenario 3.

Contents

1	General	2
1.1	Table of all model parameters	2
2	Frequency and density dependence (scenario 1)	3
2.1	Dynamics of various weightings of density and frequency dependencies	3
2.2	Analytical stability analysis	3
3	Periodic population bottlenecks (scenario 2)	5
3.1	Parameters in density and frequency dependence function	5
3.2	ODE solver checking	6
3.3	Intrinsic growth rate checking (r)	7
3.4	Effects of the costs of cooperation (h)	7
3.5	Relative fitness of cheats	8
3.6	Effects of dilution ratio (D)	10
3.7	Effects of growing time (T_{grow})	10
3.8	Effects of shape parameter (s_d, s_f)	11
3.9	Effects of threshold parameter (t_d, t_f)	13
4	Stochastic group formation (scenario 3)	15
4.1	Simulation setup of scenario 3	15
4.2	Reducing stochasticity through increasing carrying capacity (K)	16
4.3	Relative fitness of cheats on a board scale	17
4.4	Effects of mixing coefficient (F)	18
4.5	Effects of mixing coefficient when cheats are obligate (F, a or $b=1$)	19

1 General

1.1 Table of all model parameters

Table S1: The list of parameters in the model. Sections column provides the relevant section numbers in this document for each parameters.

Name	Value	Description	Sections
General variables used in all scenarios			
r	2	Intrinsic growth rate	3.3
K	10	Carrying capacity (set to 100 in scenario 3)	4.2
h	4	Benefit coefficient of cheats	3.4
s_d	3	Shape coefficient of density dependence function	3.8
t_d	1.5	Threshold of density dependence function	3.9
s_f	10	Shape coefficient of frequency dependence function	3.8
t_f	0.5	Threshold of frequency dependence function	3.9
For growth cycles (scenario 2& 3)			
D	10	Dilution ratio (set to 5 in scenario 3)	3.6
T_{grow}	4	Duration of growth phase in each growth cycle	3.7
For stochasticity (scenario 3)			
F	1.3	Mixing coefficient	4.4, 4.5
M	4000	Number of subpopulation (social groups)	Fig. 4

2 Frequency and density dependence (scenario 1)

2.1 Dynamics of various weightings of density and frequency dependencies

In Fig. 2 of the main text, we showed the population dynamics quickly become static after carrying capacity has been reached, we tested whether the result was just a special case in this section. As shown in Fig. S1, we found the same dynamics across all combinations of weighting coefficients. These results suggest a relatively static dynamic is prevalent.

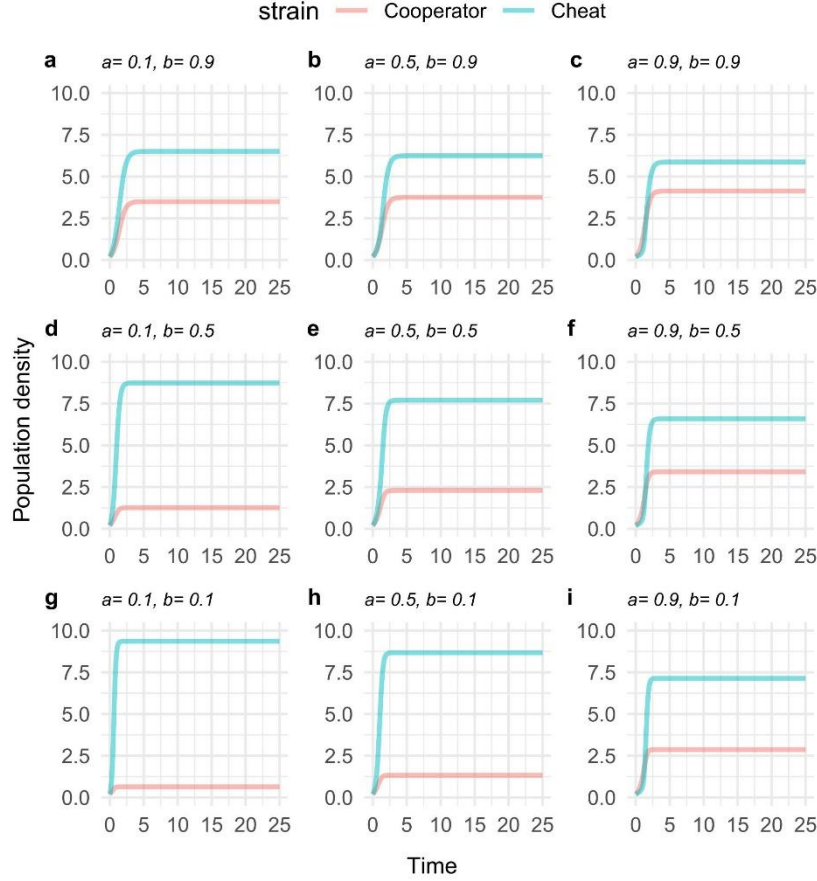


Figure S1: Population dynamics across various combinations of weightings in the baseline setting.

2.2 Analytical stability analysis

We re-write Eq (2) of the main text as

$$\begin{aligned} N'_{co} &= F(N_{co} + \alpha N_{ch}; K) N_{co} \\ N'_{ch} &= h G(N_{co}; a, s_d, t_d) G\left(\frac{N_{co}}{N_{co} + N_{ch}}; b, s_f, t_f\right) F(\alpha N_{co} + N_{ch}; K) N_{ch} \end{aligned} \quad (S1)$$

where

$$F(N; K) = 1 - \frac{N}{K}, \quad \text{and} \quad G(N; a, s, t) = 1 - a + a \frac{1}{1 + \exp\{-s(N - t)\}} \quad (S2)$$

and $0 < \alpha \leq 1$ is constant.

If α is strictly less than 1, then

$$N_{co}^* = N_{ch}^* = \frac{K}{1 + \alpha} \quad (S3)$$

is a unique, positive equilibrium; it is locally asymptotically stable when all eigenvalues of

$$J = \begin{bmatrix} F'(K) N_{co}^* & \alpha F'(K) N_{co}^* \\ \alpha h G(N_{co}^*) G\left(\frac{N_{co}^*}{N_{co}^* + N_{ch}^*}\right) F'(K) N_{ch}^* & h G(N_{co}^*) G\left(\frac{N_{co}^*}{N_{co}^* + N_{ch}^*}\right) F'(K) N_{ch}^* \end{bmatrix} \quad (S4)$$

have negative real part (parameters of G and F have been omitted, for simplicity). The Routh-Hurwitz criteria for two-dimensional systems tell us that the eigenvalue condition on J is satisfied when both its trace is negative and its determinant is positive. Direct calculation shows

$$\text{trace } J = F'(K) \left(N_{co}^* + h G(N_{co}^*) G\left(\frac{N_{co}^*}{N_{co}^* + N_{ch}^*}\right) N_{ch}^* \right) < 0 \quad (S5)$$

and

$$\det J = h G(N_{co}^*) G\left(\frac{N_{co}^*}{N_{co}^* + N_{ch}^*}\right) (F'(K))^2 N_{ch}^* N_{co}^* (1 - \alpha^2) > 0 \quad (S6)$$

which implies the equilibrium is locally asymptotic stable. A sketch of the phase plane shows not only that the equilibrium is globally stable but also that periodic (oscillating) trajectories do not occur (Fig S). We formalize the line of reasoning, here, using Dulac's criterion. Define $H(N_{co}, N_{ch})$ as $\frac{1}{N_{co} N_{ch}}$ and consider the vector field, V , defined (strictly inside the first quadrant of the plane) as

$$V(N_{co}, N_{ch}) = \left(\frac{F(N_{co} + \alpha N_{ch})}{N_{ch}}, h G(N_{co}) G\left(\frac{N_{co}}{N_{co} + N_{ch}}\right) \frac{F(N_{ch} + \alpha N_{co})}{N_{co}} \right) \quad (S7)$$

where, again, we have omitted the parameters of F and G for simplicity. We calculate the divergence of V to be

$$\begin{aligned} \nabla \cdot V = & \frac{F'(N_{co} + \alpha N_{ch})}{N_{ch}} + h G(N_{co}) G'\left(\frac{N_{co}}{N_{co} + N_{ch}}\right) (-1) \frac{N_{co}}{(N_{co} + N_{ch})^2} \frac{F(N_{ch} + \alpha N_{co})}{N_{co}} \\ & + h G(N_{co}) G\left(\frac{N_{co}}{N_{co} + N_{ch}}\right) \frac{F'(N_{ch} + \alpha N_{co})}{N_{co}}. \end{aligned} \quad (S8)$$

Because $F' < 0$ and $G' > 0$ we can be sure that $\nabla \cdot V < 0$. Following Dulac's criterion, then, we formally rule out the existence of periodic solutions to Eq (S1), because $\nabla \cdot V$ neither changes sign nor is identically zero. Because trajectories in the planar system, represented by Eq (S1), are bounded (Fig. S2), the Poincaré-Bendixson Theorem tells us that they must either tend to an equilibrium or a periodic orbit thereof. Given that we have ruled out the latter, it must be true that solutions tend to the only equilibrium we have identified inside the first quadrant.

If $\alpha = 1$, then we can replace the variables N_{co} and N_{ch} with $N = N_{co} + N_{ch}$ and $P = \frac{N_{co}}{N}$. With these new variables the dynamics become

$$\begin{aligned} N' &= (P + h G(PN) G(P) (1 - P)) F(N) N, \\ P' &= P(1 - P) (1 - h G(PN) G(P)) F(N). \end{aligned} \quad (S9)$$

As an aside, we note that the rate of change in the proportion of cooperators is the product of the trait variance (namely, $P(1 - P)$) and the change in fitness experienced by an individual who switches from cheating to cooperation (namely, $F(N) - h G(PN) G(P) F(N)$). The sign of N' is solely determined by $F(N)$. Therefore, over time, N converges monotonically to K (the line $N_{co} + N_{ch} = K$ in Fig 2c). The sign of P' is also solely determined by $F(N)$, and so the evolution of P will follow that of N (P increases when N increases and decreases when N decreases). It follows that P will also tend to some equilibrium in a monotonic fashion. Overall, we rule out the existence of periodic solutions when $\alpha = 1$.

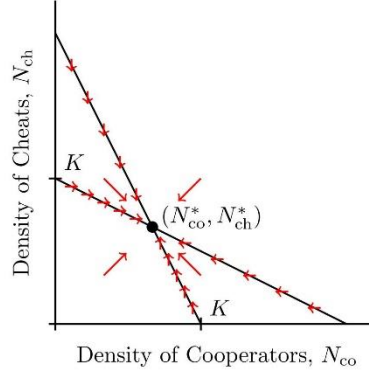


Figure S2: A sketch of the phase plane associated with Eq (S1). Red arrows show the direction followed by trajectories over time. Solid lines show the set of states for which $N'_{co}=0$ and $N'_{ch}=0$, respectively. For the former set, trajectories are instantaneously moving in a purely vertical direction; for the latter set, trajectories are instantaneously moving in a purely horizontal direction. Solid lines intersect at the equilibrium (N^*_{co}, N^*_{ch}) , and this equilibrium is asymptotically stable.

3 Periodic population bottlenecks (scenario 2)

3.1 Parameters in density and frequency dependence function

Our density and frequency dependence functions share the same the basic structure of

$$f(x) = h(1 - a + \frac{a}{1 + e^{-s(x-t)}}), \quad (\text{S10})$$

where x is density (or frequency) of cooperators in the population, h is the coefficient of relative growth advantage of cheats, which is a proxy for the costs of cooperation, a (or b) is the weighting coefficient of the dependence, s is the shape coefficient adjusting the steepness, and t is the threshold, in which the steepest change in response takes place. The effects of each parameter is shown in Fig. S3: increasing a results in a lower response at low cooperator density (or frequency; Fig. S3a), increasing h results in greater response after threshold is passed (Fig. S3b); increasing s results in steeper response, making cheat's dependence more restricted to a specific range (Fig. S3c); increasing t horizontally shifts the response of cheats (Fig. S3d).

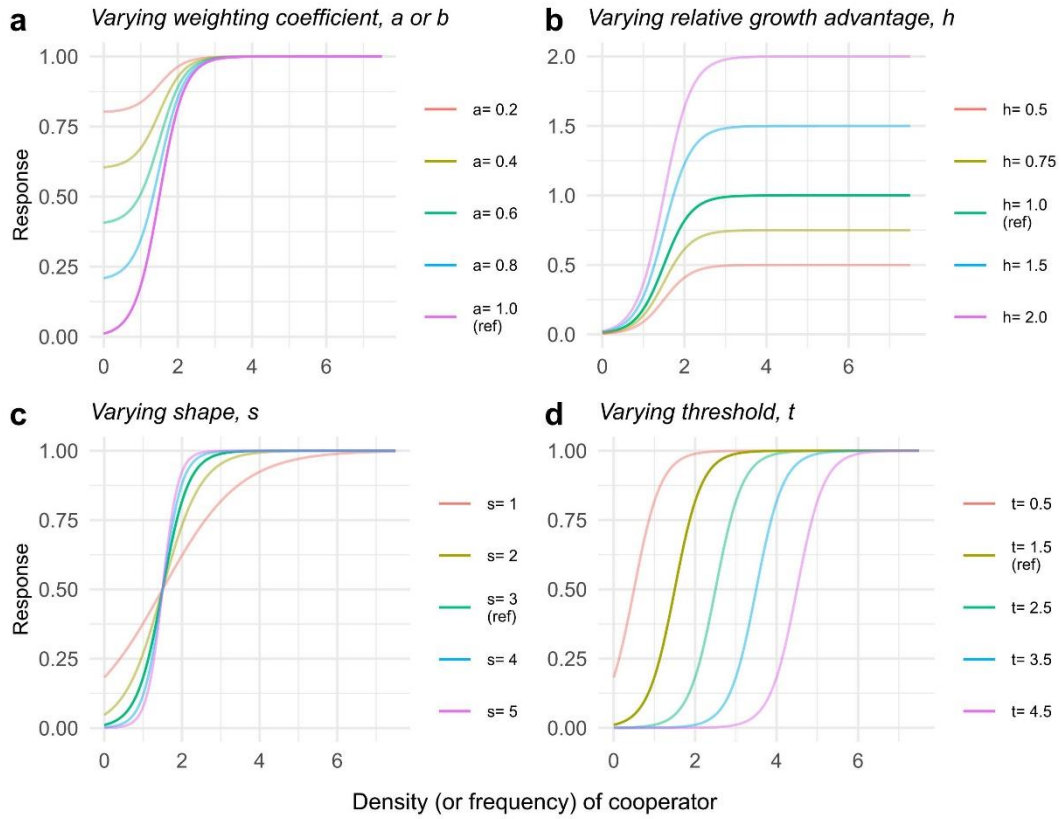


Figure S3: Parameters of logistic function used in density and frequency dependence terms.

3.2 ODE solver checking

We set up the ODE solver (the standard package in Python3: `scipy.integrate.odeint`) to numerically track the population dynamics. The time in each iteration of the solver is adjustable that bigger leap means less precision but less calculation time. Thus, we checked the precision setting to make sure we are not generating numerical artifacts in our result. As shown in Fig. S4, the dynamics in all panels are close to identical, suggesting all settings have reasonable precision. Thus, we use 20 iterations per time unit for all analysis (Fig. S4b).

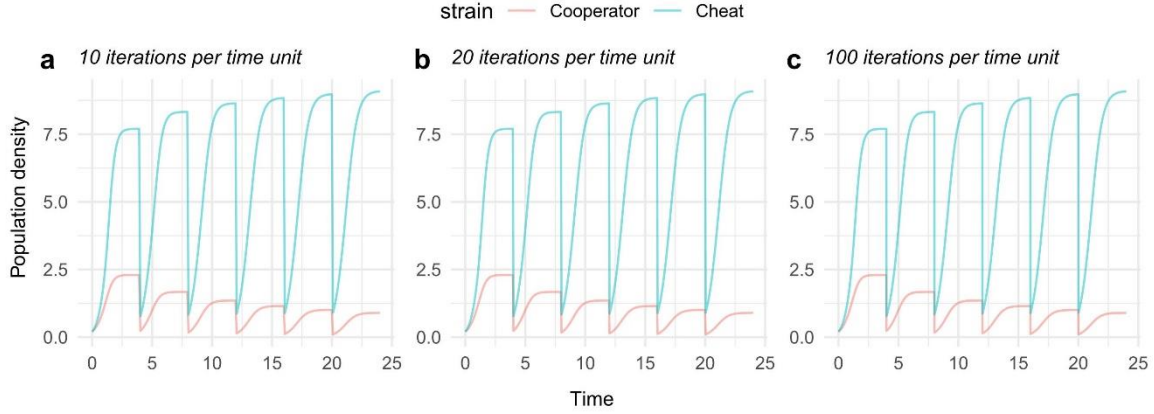


Figure S4: Time series of population dynamics under different precisions ($a=0.5$, $b=0.5$).

3.3 Intrinsic growth rate checking (r)

We then checked the effects of intrinsic growth rate to make sure the oscillation is not an artifact from special combinations of growth cycle (i.e., periodic population bottleneck) and growth rate (r). We found the pattern of oscillation is robust as long as $r > 1.5$ in Fig. S5. Hence, we have been using $r = 2$ to make sure the results are good representations for most ranges of the intrinsic growth rate.

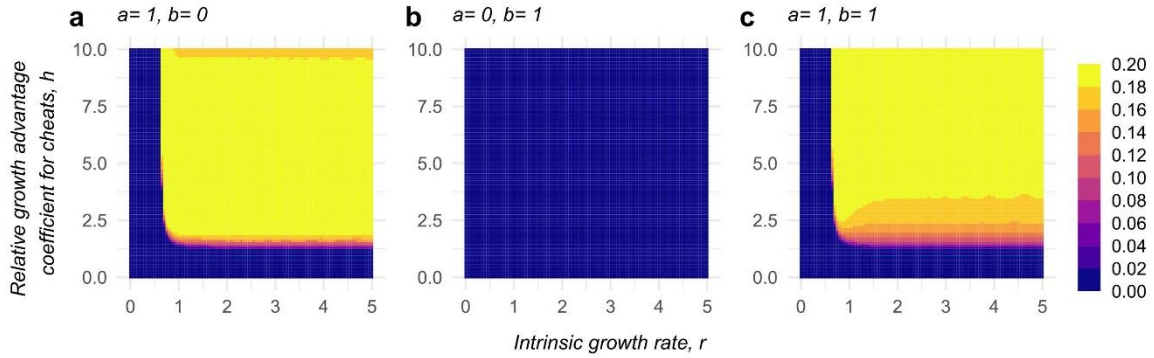


Figure S5: Amplitude of oscillation in proportion of cheats across various intrinsic growth rates, and relative growth advantage coefficient of cheat. Brighter colour indicates larger oscillation and the amplitude is calculated from the maximal difference in proportion of cheats in each growth cycle after the dynamics become stabilised.

3.4 Effects of the costs of cooperation (h)

We use the coefficient of relative growth advantage of cheats, h , as a proxy for the cost of performing cooperation and we found as h increases, a region of no oscillation expands (arrows in Fig. S6b-d). The region is where both weightings are small, meaning cheats do not depend as much on the density or frequency (proportion) of cooperators. The underlying reason is as h is at a larger value, cheats can out-compete cooperators in a wider range of parameters. In contrast, the result when weighting is at 1 does not change with h , as cheats cannot eliminate cooperators when there is complete dependence in one of the two weightings. Lastly, we found no oscillation at all when $h = 1$ as the fitness of cheats never exceeds cooperator in such setting (i.e., cheats are eliminated).

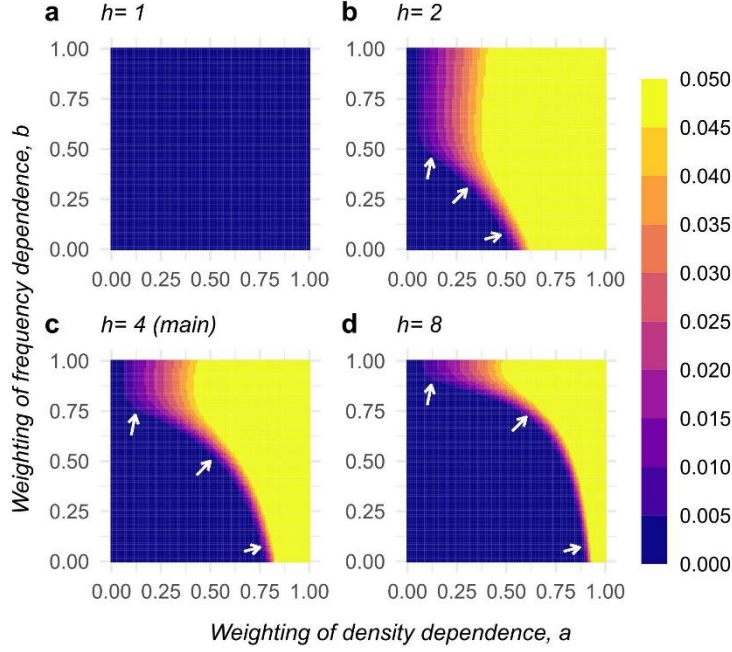


Figure S6: Amplitude of oscillation across various relative growth advantage coefficient for cheats. Higher h means higher benefit of cheats and brighter colour indicates larger oscillation.

3.5 Relative fitness of cheats

One important factor that determines whether cheats or cooperators win is the relative fitness, which is defined as

$$w_{rel,ch} = \frac{w_{ch}}{p_{ch}w_{ch} + (1 - p_{ch})w_{co}}, \quad (S11)$$

where p_{ch} is the proportion of cheats in the population, w_{ch} is the per capita growth rate of cheats, $\frac{dN_{ch}}{N_{ch}dt}$, and w_{co} is the per capita growth rate of cooperator, $\frac{dN_{co}}{N_{co}dt}$. When $w_{rel,ch} > 1$, cheats can outgrow cooperators and potentially eliminate cooperators; when $w_{rel,ch} < 1$, cooperators grow better than cheats and can potentially eliminate cheats.

We looked into two quantities of relative fitness: (1) the maximal difference within each growth cycle, and (2) the average relative fitness over a single growth cycle. We found the pattern in differences is similar to the pattern in oscillation amplitudes (Fig. S7 and Fig. S6). The only exception is when $h = 1$ because the brighter colors in Fig. S7a mean cheats are much worse than cooperators, whereas brighter colors other panels mean cheats are doing better than cooperators. This explanation is supported by the average relative fitness (Fig. S8), that we only found relative fitness lower than 1 when $h = 1$. In addition, we found the region of $w_{rel,ch} \approx 1$ expands as h increases because relative fitness is 1 for cheats when there is no cooperator in the population (arrows in Fig. S8b-d).

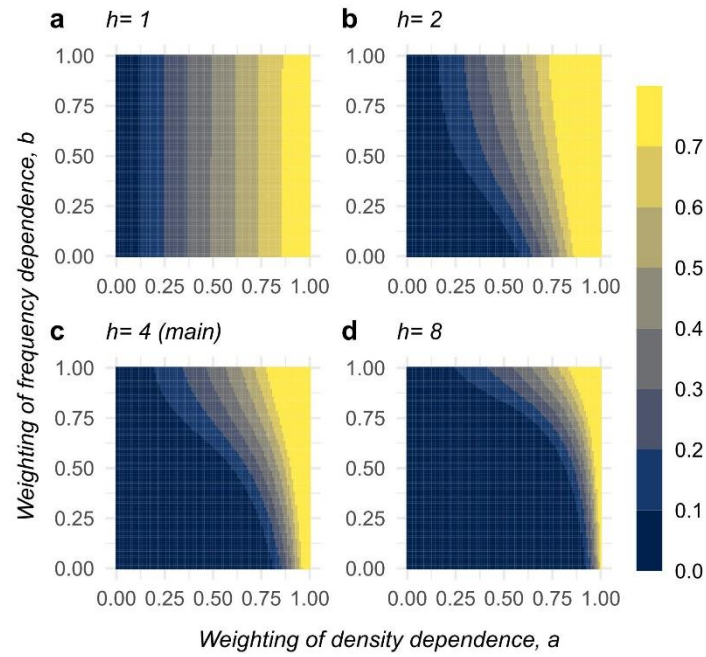


Figure S7: Maximum difference in relative fitness of cheats in each growth cycle.

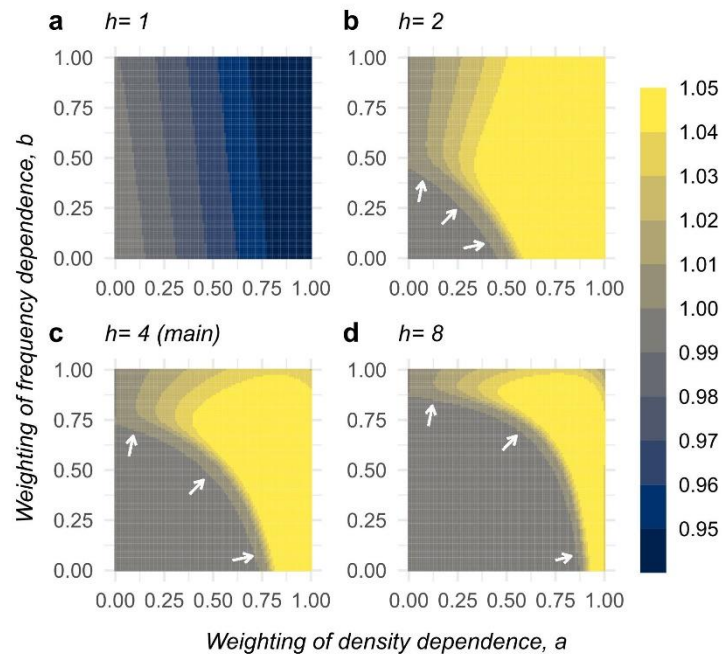


Figure S8: Average of relative fitness of cheats in each growth cycle.

3.6 Effects of dilution ratio (D)

The next factor we investigated is the dilution ratio, which controls the size of population bottleneck between growth cycles. Intuitively, we found when D is too low, there is almost no oscillation (Fig. S9a-b), while the pattern roughly stays the same when D is large (Fig. S9c-d). The reason is when there is insufficient dilution would prevent cooperators from recovering their proportion in the population and eventually lead to extinction (see also Fig. 6a-e in main text).

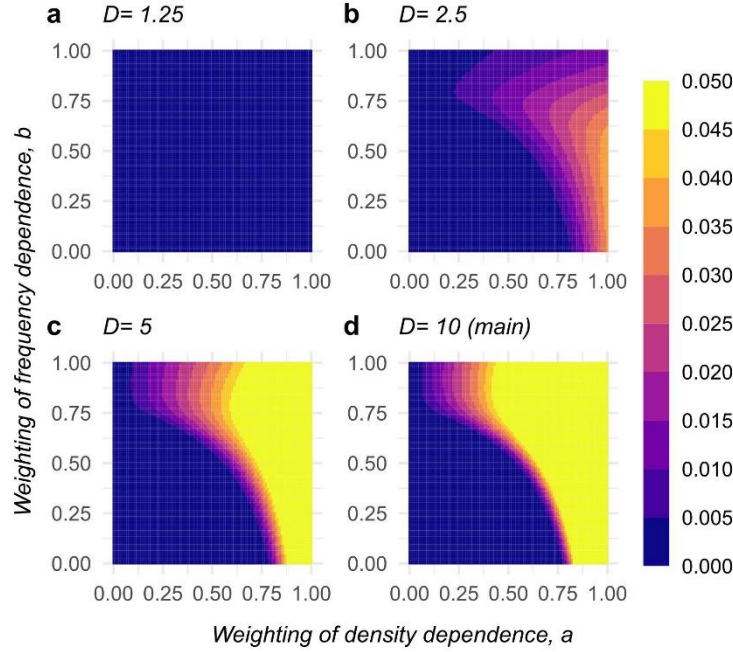


Figure S9: Amplitude of oscillation across various dilution ratios.

3.7 Effects of growing time (T_{grow})

Another growth cycle-related factor is the duration of growth time in each growth cycle, which determines whether dilution takes place at lag, exponential, or stationary phase of the growth curve. We found once T_{grow} is greater than 2, the results is identical regardless of the duration of growing time (Fig. S10b-d). This result is also easy to explain as cheats would not have time to grow and increase their proportion if growing time is too short (Fig. S9a). Yet, once cheats have chances to gain proportion, there is little difference in the results because both strains would saturate in densities as growing time increases.

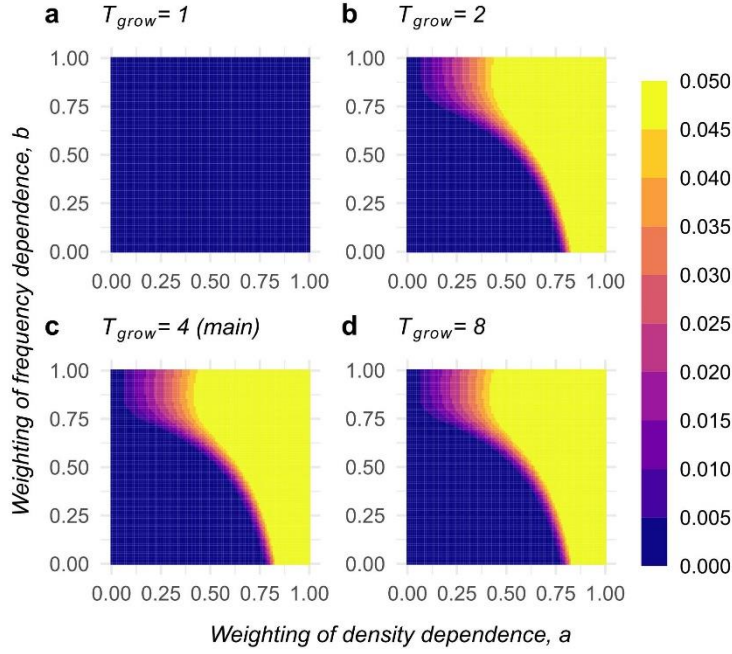


Figure S10: Amplitude of oscillation across various duration of growth phase.

3.8 Effects of shape parameter (s_d , s_f)

Besides the settings in growth cycle, another important aspect of the model is the parameters controlling the density and frequency dependence. We began from the shape parameters, s_d and s_f , where larger s means steeper response (Fig. S3c). We found increasing s_d always help oscillation as yellow area consistently increases as s_d increases (Fig. S11). On the contrary, the effect of s_f is inconsistent as it helps oscillation when $s_f < 10$ but somewhat hinders oscillation when $s_f > 10$ (Fig. S12). This discrepancy happens because (i) density can fluctuate through dilution processes between growth cycle while frequency does not change through dilution, and (ii) increasing s would narrow the range where response can be different. In other words, the fitness landscape becomes more binary when s increases, while density dependence still have the drive to bounce between lower and higher responses (through dilution), frequency dependence completely relied on relative fitness to oscillate and it is harder to move around when the response is too steep. One final note is we set s_d and s_f to different values because the range of density is larger than the range of frequency, which is confined in $\{0, 1\}$.

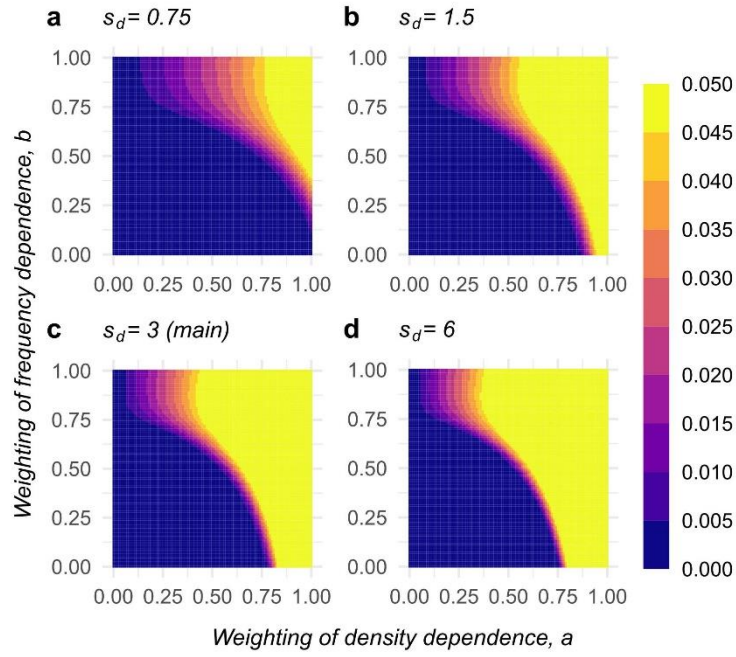


Figure S11: Amplitude of oscillation across various shape parameter of density dependence.

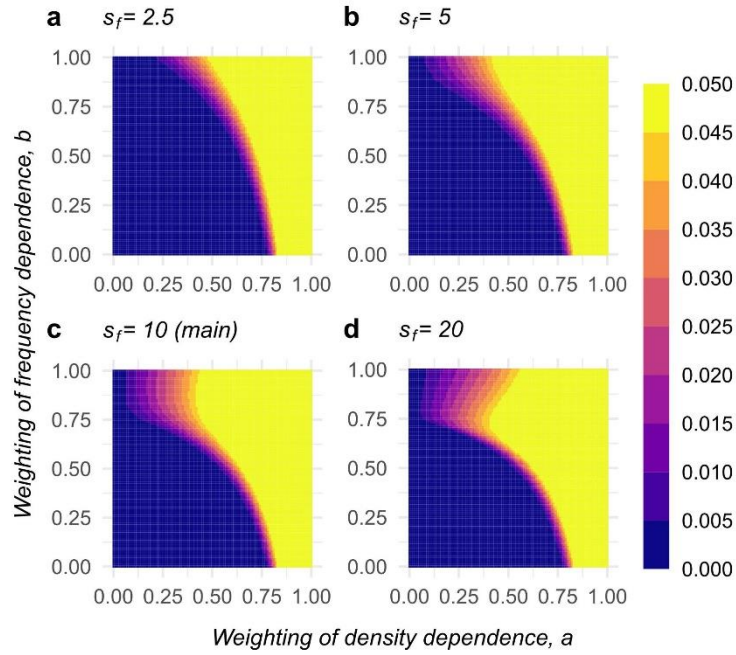


Figure S12: Amplitude of oscillation across various shape parameter of frequency dependence.

3.9 Effects of threshold parameter (t_d , t_f)

The last parameter we examined in scenario 2 is the threshold of the response, t_d and t_f , which horizontally move the response to different ranges (Fig. S3d). We found changing thresholds result in diverse outcomes. Specifically, increasing t_d initially increases the region of oscillation, but when $t_d > 1.5$, increasing threshold hampers oscillations (Fig. S13). This is likely because when threshold is too high or too low, the majority of time in the growth cycle would favour one of the two strains (favouring cheats when threshold is low and cooperators when threshold is high), and prevent oscillation. In one extreme case, when $t_d = 6$ and $a > 0.9$, there is no oscillation as cooperator density hardly exceeds 6 and cheats cannot grow (Fig. S13d) On the other hand, the pattern of changing thresholds in frequency is similar to changing shapes in frequency dependence (Fig. S14).

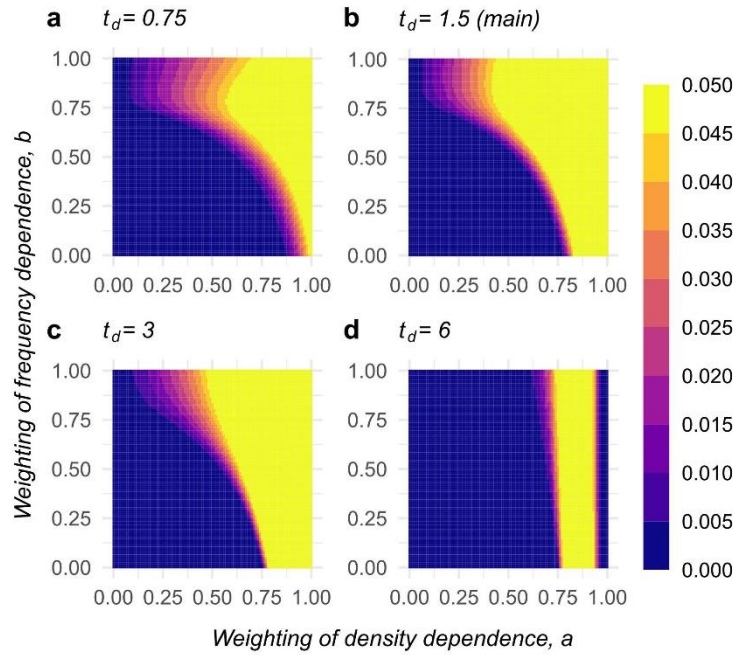


Figure S13: Amplitude of oscillation across various threshold of density dependence.

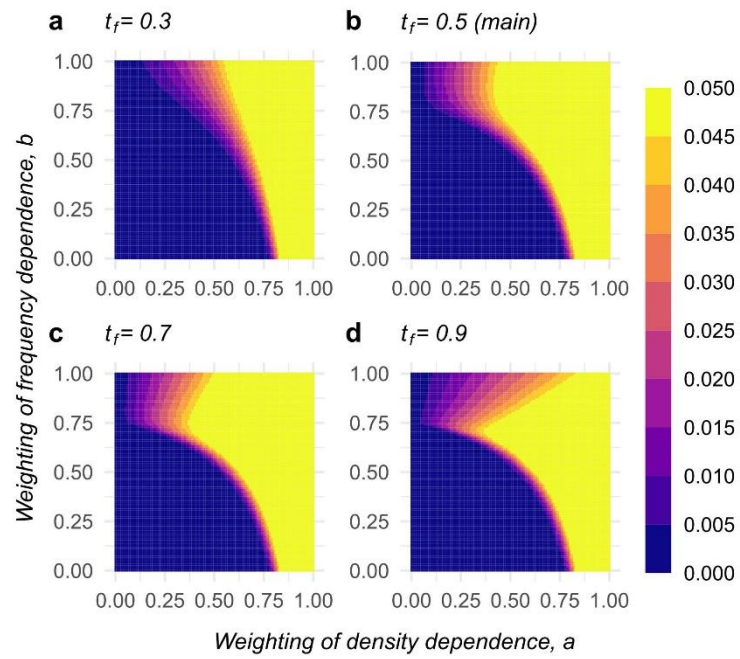


Figure S14: Amplitude of oscillation across various threshold of frequency dependence.

4 Stochastic group formation (scenario 3)

4.1 Simulation setup of scenario 3

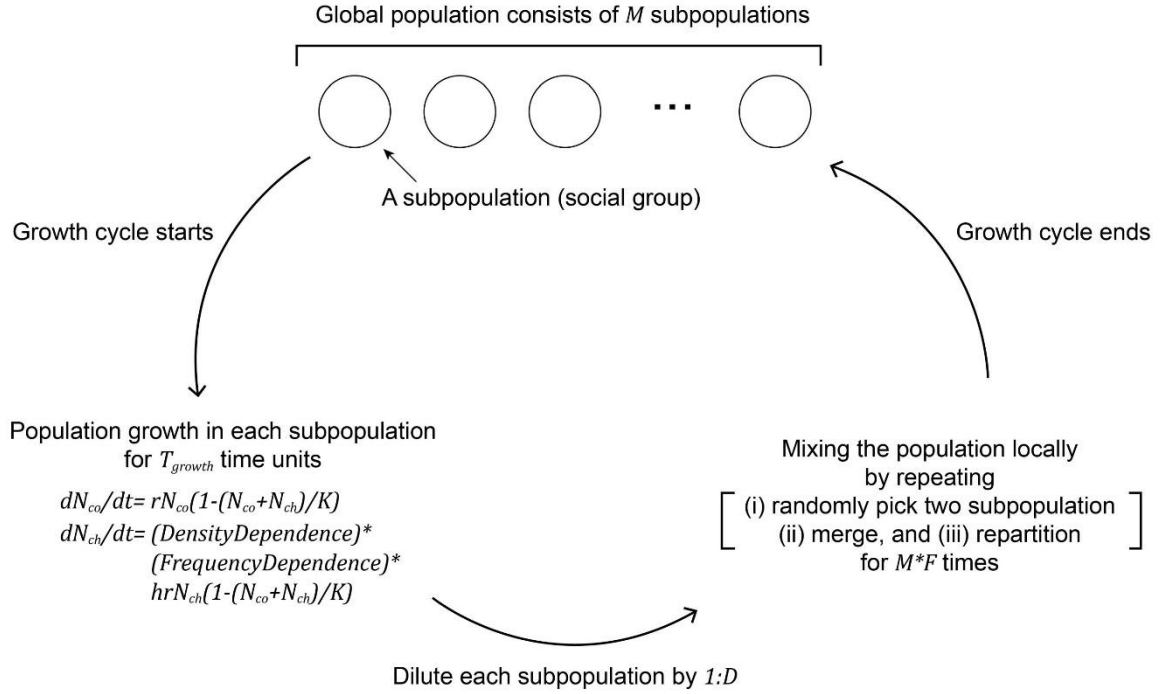


Figure S15: Schematic view of simulation steps in scenario 3, where the simulation codes are modified from Muzuuchi et al, 2022. Following their design, we convert the densities of cheaters and cooperators to integer after dilution, and we use binomial process with equal probabilities to re-disperse each unit of cheaters and cooperators during repartitioning process. Both processes can help generate stochasticity to the system.

4.2 Reducing stochasticity through increasing carrying capacity (K)

In addition to Fig. 4 in main text, an alternative way to manipulate stochasticity is to increase the population size within each subpopulation. This can be done through increasing carrying capacity, K . Nevertheless, the duration of growth cycle and the threshold of dependence have to change because the density of the two strain is going to be at a different level (see figure caption of Fig. S16 for more information). We found the amplitude of oscillation decreases as carrying capacity increases (Fig. S16g-i) and the result is consistent with Fig. 4.

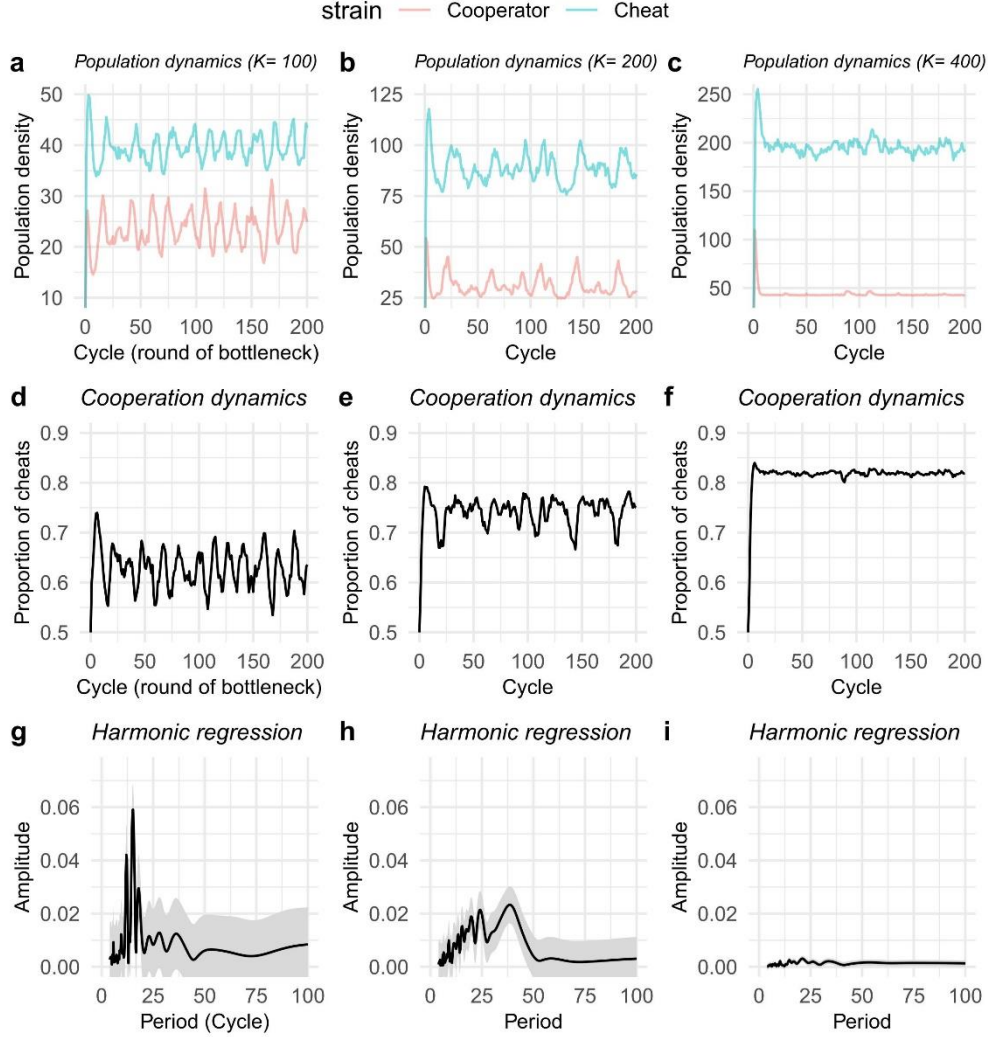


Figure S16: Increasing carrying capacity within each subpopulation decreases stochasticity and produce smaller oscillation in proportion of cheats. Growth cycle and threshold are changed to different values to allow population having sufficient growing time (T_{grow} is 8 and t_d is 6 for $K=200$; T_{grow} is 16 and t_d is 9 for $K=400$; $a=1$ and $b=0$).

4.3 Relative fitness of cheats on a board scale

In Fig. S17, we found the average relative fitness of cheats is above 1 but close to 1 at the regions where oscillating dynamics take place (Fig. 5). Together with Fig. 5, these results suggest oscillation happens at the parameter space (1) where cheats are slightly fitter than cooperators and (2) cheats' fitness is highly variable between subpopulations.

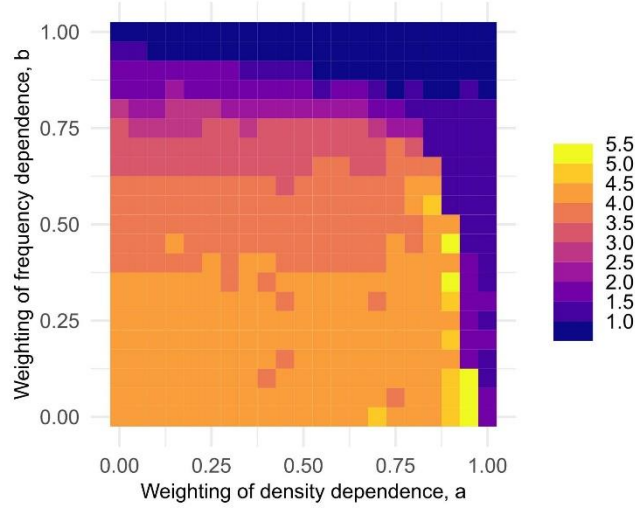


Figure S17: Relative fitness of cheats. Brighter color means cheats having greater fitness over cooperators. Relative fitness is calculated at subpopulation level, based on the proportion of cheats within each subpopulation at the beginning of focal growth cycle. The absolute fitness used for relative fitness is approximated from per capita growth rate, the change in cheat or cooperator density within subpopulation, divided by T_{grow} and the initial densities. We then averaged the relative fitness over all subpopulations and time, and rescaled the relative fitness by dividing it with the relative fitness of cooperators because the raw values are deflated by empty subpopulations. Each grid is the average of 10 repeated simulations. All simulations last 200 growth cycles and data was collected from cycle 51 to 200. The number of subpopulations is set to 4000.

4.4 Effects of mixing coefficient (F)

We examined the effects of mixing coefficient as it can potentially change the spatial structure of the entire population and help the spread of cheats or cooperators. We found that greater mixing produces a less periodic dynamic - as the mixing coefficient (F) increases, the peak signal in harmonic regression become smaller, while the non-peak signals become larger (Fig. S18d& e). This is because faster mixing makes the spread of cheats and cooperators faster, resulting in less periodic dynamics (Fig. S18c& f).

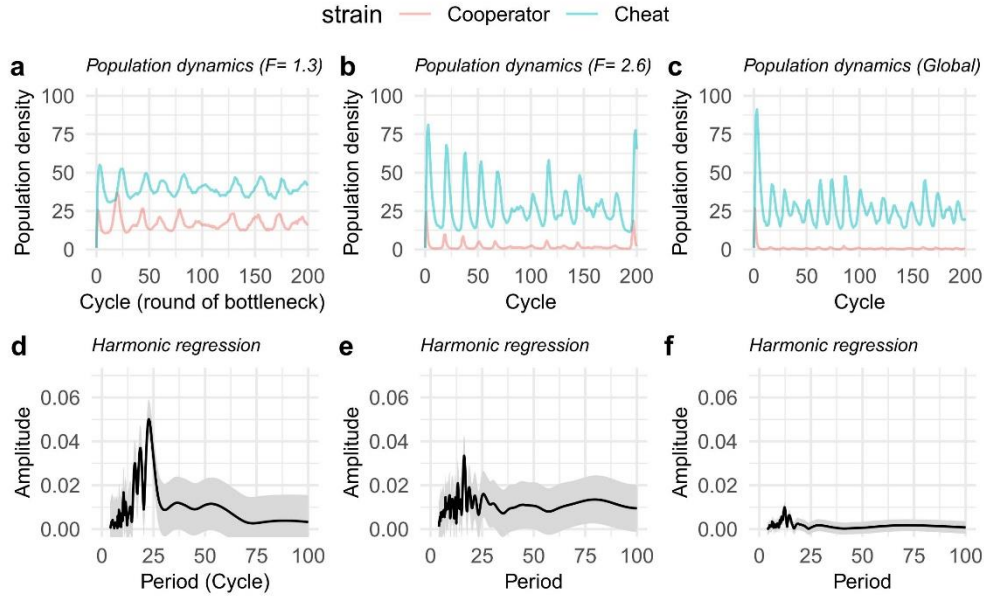


Figure S18: Local mixing is crucial for oscillation across growth cycles. (a-c) Population dynamics under default mixing (a, $F=1.3$), twice times of mixing (b, $F=2.6$), and global mixing after the dilution in each growth cycle. In global mixing setting, subpopulations are re-initialised through Poisson sampling after summing the density of cheat and cooperator and after periodic population bottlenecks, so stochasticity is not reduced to zero. (d-f) The corresponding Harmonic regression on proportion of cheats in each setting. Density and frequency dependence are set to the combination where largest coefficient of variation was observed in Fig. 5 ($a=0.9$, $b=0.25$).

4.5 Effects of mixing coefficient when cheats are obligate (F , a or $b=1$)

In addition to Fig. S18, where cheats are facultative that they can grow in the absence of cooperator, we found some interesting result when cheat is obligate. Specifically, we found increasing mixing results in lower proportion of cheats (Fig. S19). Further, increasing mixing produces more frequent oscillations as the peak in harmonic regression moves to the left when F is larger. This is because of the complication that cheats are obligate cheats in this case and cannot grow when cooperator is absent in the group. These results suggest mixing is a crucial factor of our model and it can determine the extent of periodicity and noise in the dynamics.

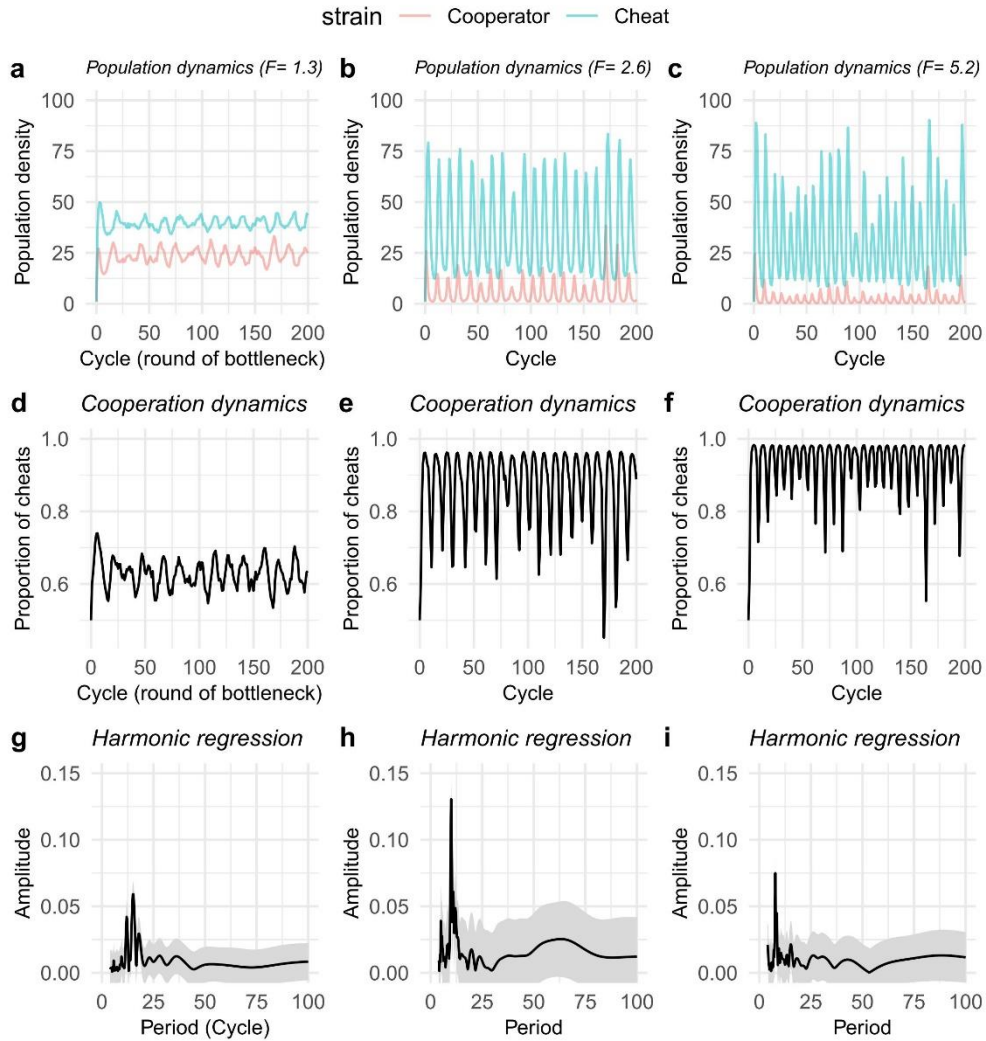


Figure S19: Increasing mixing coefficient results in more frequent oscillation when cheats can only grow when cooperator is present ($a=1, b=0$).

A5

Complex signals alter recognition
accuracy and conspecific
acceptance thresholds

PHILOSOPHICAL TRANSACTIONS B

royalsocietypublishing.org/journal/rstb

Research



Cite this article: Tibbetts EA, Liu M, Laub EC, Shen S-F. 2020 Complex signals alter recognition accuracy and conspecific acceptance thresholds. *Phil. Trans. R. Soc. B* **375**: 20190482.
<http://dx.doi.org/10.1098/rstb.2019.0482>

Accepted: 9 February 2020

One contribution of 22 to a theme issue 'Signal detection theory in recognition systems: from evolving models to experimental tests'.

Subject Areas:

behaviour

Keywords:

redundant signals, recognition, multimodal signals, nest-mate recognition

Author for correspondence:

Elizabeth A. Tibbetts
e-mail: tibbetts@umich.edu

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.4946580>.

THE ROYAL SOCIETY
PUBLISHING

Complex signals alter recognition accuracy and conspecific acceptance thresholds

Elizabeth A. Tibbetts¹, Ming Liu², Emily C. Laub¹ and Sheng-Feng Shen²

¹Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA

²Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan, Republic of China

EAT, 0000-0002-5625-892X; ML, 0000-0002-5170-8688; S-FS, 0000-0002-0631-6343

Many aspects of behaviour depend on recognition, but accurate recognition is difficult because the traits used for recognition often overlap. For example, brood parasitic birds mimic host eggs, so it is challenging for hosts to discriminate between their own eggs and parasitic eggs. Complex signals that occur in multiple sensory modalities or involve multiple signal components are thought to facilitate accurate recognition. However, we lack models that explore the effect of complex signals on the evolution of recognition systems. Here, we use individual-based models with a genetic algorithm to test how complex signals influence recognition thresholds, signaller phenotypes and receiver responses. The model has three main results. First, complex signals lead to more accurate recognition than simple signals. Second, when two signals provide different amounts of information, receivers will rely on the more informative signal to make recognition decisions and may ignore the less informative signal. As a result, the particular traits used for recognition change over evolutionary time as sender and receiver phenotypes evolve. Third, complex signals are more likely to evolve when recognition errors are high cost than when errors are low cost. Overall, redundant, complex signals are an evolutionarily stable mechanism to reduce recognition errors.

This article is part of the theme issue 'Signal detection theory in recognition systems: from evolving models to experimental tests'.

1. Introduction

Recognition is important in many contexts, including identifying species, kin, mates, individuals and nest-mates [1,2]. Much recognition involves phenotype matching, where receivers assess sender phenotypes, compare sender phenotypes to a template of expected phenotypes, then make a decision about how to treat senders based on the match between the template and phenotype [3,4]. For example, during nest-mate recognition, guards smell conspecifics and compare conspecific odour to the expected odour of nest-mates. If the individual smells enough like the expected odour of nest-mates, the guard will accept the individual as a nest-mate. If the individual does not smell enough like a nest-mate, the guard will reject the individual and treat it as a non-nest-mate [5–7].

Recognition typically involves signals that coevolve between senders and receivers. Signals used during recognition usually benefit senders (i.e. the individuals displaying the trait) by influencing the behaviour of receivers (i.e. the individuals perceiving the trait). Because senders benefit from receiver responses, selection favours sender phenotypes that effectively convey information to receivers [8]. For example, both senders and receivers in a social insect colony benefit when nest-mate recognition is accurate. The benefits of accurate recognition influence the evolution of odours that signal nest-mate identity and the perceptual and cognitive processes that facilitate accurate recognition. Recognition can occur without senders benefitting or sender/receiver coevolution. For example, humans can recognize a squirrel based on its appearance. However, squirrel evolution is not influenced by how well humans recognize squirrels. Our

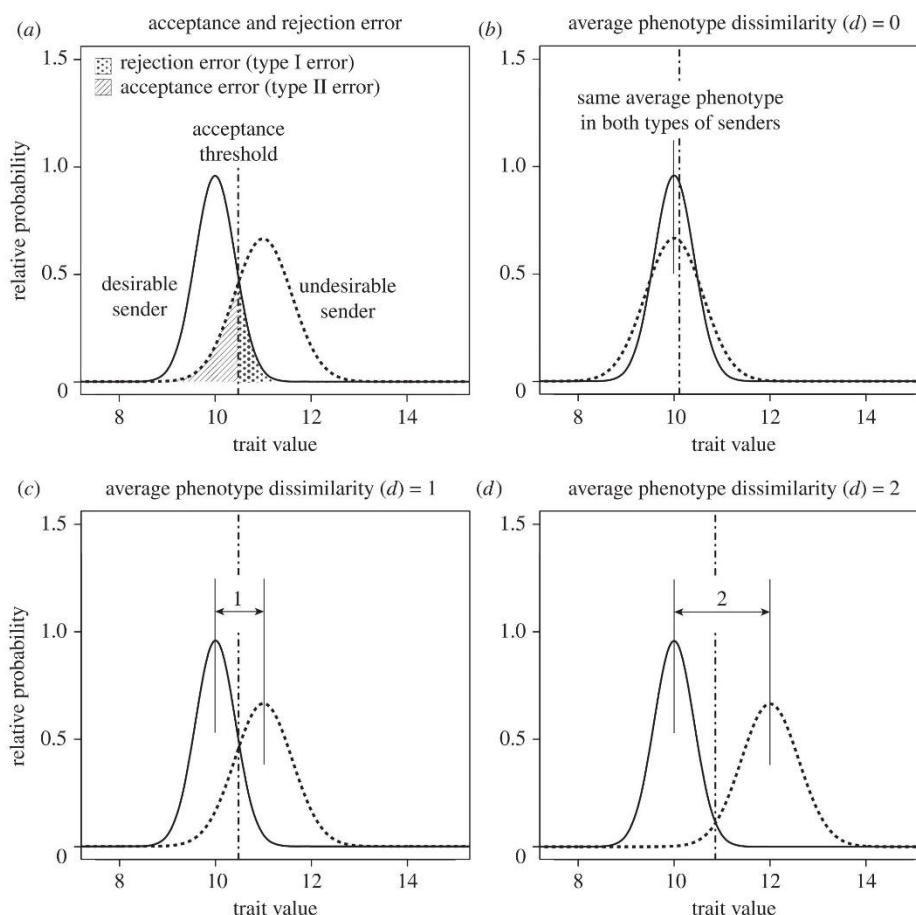


Figure 1. The phenotypic distributions of senders and their relationship with acceptance threshold and phenotypic dissimilarity. (a) The schematic view of acceptance and rejection errors (modified from [9]). The probability distribution of a desirable sender's trait value is depicted by the solid curve. The probability distribution of an undesirable recipient's trait value is depicted by the dashed curve. The threshold of acceptance or rejection is the dot-dashed line. The dotted area above the threshold shows rejection errors (type I error). These are trait values for which desirable senders are rejected. The striped area below the threshold shows acceptance errors (type II errors). These are trait values for which undesirable senders are accepted. (b–d) Examples of the sender traits and the threshold at the end of simulations. The acceptance threshold (broken lines) is optimized according to the phenotype dissimilarity between the average trait value of desirable and undesirable senders. If the phenotypic dissimilarity is 0, the undesirable senders can have the same average trait value as the desirable senders (b). If the phenotypic dissimilarity is 1 or 2, respectively, then the traits of undesirable are always higher than desirable senders (c,d).

model focuses on recognition that involves coevolution between senders and receivers.

A major challenge of recognition is that it is not possible for receivers to make perfect recognition decisions. Instead, the phenotypes used as recognition signals have broad and overlapping distributions, so errors are common. Receivers can make two types of errors (figure 1a) [9]. (i) Rejection errors occur when receivers reject individuals that should not be rejected (e.g. guards prevent nest-mates from entering their own nest). Rejection errors are similar to type I errors in statistics, when a true hypothesis is rejected. (ii) Acceptance errors occur when receivers accept individuals they should not accept (e.g. guards allow non-nest-mates to enter the nest). Acceptance errors are similar to type II errors in statistics, when a false hypothesis is accepted.

Conspecific acceptance threshold theory [9] makes predictions about how receivers optimize recognition behaviour in different situations. Reeve [9] proposed that receivers have an 'acceptance threshold' that is the maximum amount of dissimilarity between template and trait that receivers will

tolerate without rejecting an individual. The probabilities of acceptance and rejection errors change based on the location of the acceptance threshold. If the receiver reduces one type of error, it is inevitable that the other type of error will increase (figure 1a). For example, if the receiver alters the acceptance threshold to reduce rejection errors, acceptance errors will increase. As a result, there is a trade-off to balance both error types. The placement of the optimal acceptance threshold that maximizes receiver fitness varies based on multiple variables [6,7,10].

Reeve [9] illustrated that the acceptance threshold is influenced by factors like the cost of making mistakes, the frequency of desirable and undesirable senders, and the phenotypes of desirable and undesirable senders. For example, if accepting undesirable senders is very low cost (e.g. non-nest-mates do not do any damage to a nest), receivers are expected to have a very permissive acceptance threshold (see electronic supplementary material, S52 for related discussion in complex signals). They will allow many non-nest-mates on the nest and will not accidentally reject nest-

mates [7]. On the other hand, if accepting undesirable senders is very costly (e.g. non-nest-mates destroy the nest), receivers will have a very restrictive acceptance threshold. They will allow few non-nest-mates onto the nest, but will also reject many nest-mates.

The acceptance threshold model focused on recognition based on a single trait. However, over the last decades, there has been a growing appreciation that most animal signals are 'complex' [11–14], involving multiple signal components in a single sensory modality (multi-component signalling) or in multiple sensory modalities (multimodal signalling) [12]. Many complex signals are thought to be 'redundant', in that multiple signals convey similar information [15,16]. For example, egg recognition can be based on egg size, shape, colour and pattern [17,18]. Species recognition may be based on multiple aspects of coloration, odour, and auditory signals like songs. Female swordtail fish distinguish between heterospecific and conspecific males more accurately based on both chemical and visual traits than based on one alone [19]. Even when recognition occurs in a single sensory modality, traits often have multiple components. For example, nest-mate recognition is based on the amount and distribution of multiple chemicals [5]. Thus far, it is not clear how redundant complex signals alter the evolution of optimal acceptance thresholds or error rates.

Here, we update the acceptance threshold model to test how multi-component traits influence the evolution of acceptance thresholds. We test how the number of traits affects the evolution of the optimal acceptance threshold, acceptance errors and rejection errors. We construct individual-based models that vary in population properties, including phenotypic similarity between desirable and undesirable senders, as well as the cost of making recognition errors. We allow the phenotypes of desirable and undesirable senders to evolve, so the model also provides insight into how sender phenotypes evolve in response to receiver recognition behaviour thresholds. The goal of the models is to find the threshold that optimizes receiver fitness by allowing receivers to reject undesirable senders and accept desirable senders.

2. Methods

We use individual-based models following a genetic algorithm to understand the evolution of acceptance thresholds. All simulated data were generated using the C language. The code used for this study is available at https://github.com/mingpapilio/Codes_ComplexSignal. This method explicitly models the reproductive events, the process of acceptance or rejection, and the mutations within populations. In other words, each individual has its own trait values and acceptance thresholds. There are two categories of individuals in our models: (i) receivers make decisions about whether to accept/reject other individuals and also act as desirable senders (hosts, nest-mates), and (ii) undesirable senders (brood parasites, non-nest-mates).

The receiver makes the acceptance/rejection decision based on its acceptance threshold and the trait values of the desirable and undesirable senders. Senders with trait values above threshold are rejected, whereas senders with values below or equal to the threshold are accepted (figure 1a). Senders are accepted when both traits (traits 1 and 2) meet the receiver's threshold and are rejected when either trait 1 or trait 2 does not meet the receiver's threshold. There are no interactive effects between traits. For example, the phenotypes of trait 2 does not

influence decisions about trait 1, consistent with empirical work on assessment of redundant complex signals [12,20].

In the model, traits provide information to receivers and reduce receiver uncertainty [21]. More informative traits produce a greater reduction in uncertainty. For example, traits that have a greater phenotypic dissimilarity between desirable and undesirable recipients are more informative and decrease receiver uncertainty more than traits for which the desirable and undesirable recipients have very similar phenotypes.

Only the accepted senders survive to establish future populations. Specifically, the surviving desirable senders produce the desirable senders of the next generation. During reproduction, desirable senders act as receivers because they assess the trait values of all offspring, compare the trait values with their acceptance threshold, then choose which offspring to raise. Receiver reproductive success is reduced if they reject their own offspring (termed desirable senders) or accept foreign offspring (termed undesirable senders). Undesirable senders only survive to reproduce if they are accepted by a receiver. Surviving undesirable senders die after producing undesirable senders of the next generation.

The consequences of recognition errors vary across models. For simplicity, we first assume that accepting an undesirable sender kills all desirable senders produced by the focal receiver. This is similar to a brood parasitism scenario where accepted brood parasites kill the host's offspring. In subsequent models, we relax this assumption by varying levels of cost from accepting an undesirable sender (§3c and electronic supplementary material, §S2). The genotypes, including the acceptance thresholds and the trait values of the desirable and undesirable senders, are heritable with a slight chance of mutation. The model simulates small magnitude mutations for the traits of the undesirable senders. The magnitude of the mutations is determined by drawing random numbers from a normal distribution with average of 0 and standard deviation of 0.25. The initial difference between desirable and undesirable senders is 5.

The genetic algorithm finds the optimum genotype that maximizes reproductive success. For desirable senders, successful genotypes have acceptance thresholds and trait values that reduce the probability of recognition errors. For undesirable senders, successful genotypes have trait values that 'trick' receivers into accepting them as offspring. Successful genotypes will increase in frequency in the population through generations. Model results reflect equilibrium trait values that have been stable for generations.

We begin with the most basic scenario, where the acceptance threshold(s) is evolvable, but the two senders have fixed phenotypic variability. That is, the average phenotypic dissimilarity (d) between desirable and undesirable senders as well as the distributions of phenotypes are constant (e.g. figure 1b–d). For simplicity, we allow the acceptance thresholds in different traits to evolve independently and do not impose a cost related to the number of traits used during recognition. Under these assumptions, we explore difference in the optimal threshold(s) between using one trait and two traits (figures 2 and 3).

We then examine the more realistic case, where both the acceptance threshold(s) and the trait values of the undesirable senders are changeable. Because the trait values of the undesirable senders can evolve, the average phenotypic dissimilarity (d) between desirable and undesirable senders is also evolvable. The distributions of trait values of desirable senders are fixed, which simplifies the model but does not lose much biological realism. For simplicity, we also set an additional non-evolvable threshold at the lower point of the desirable senders' trait distribution. Senders are only accepted when their values are above this additional threshold. This assumption is biologically realistic because receivers are unlikely to accept the senders with trait

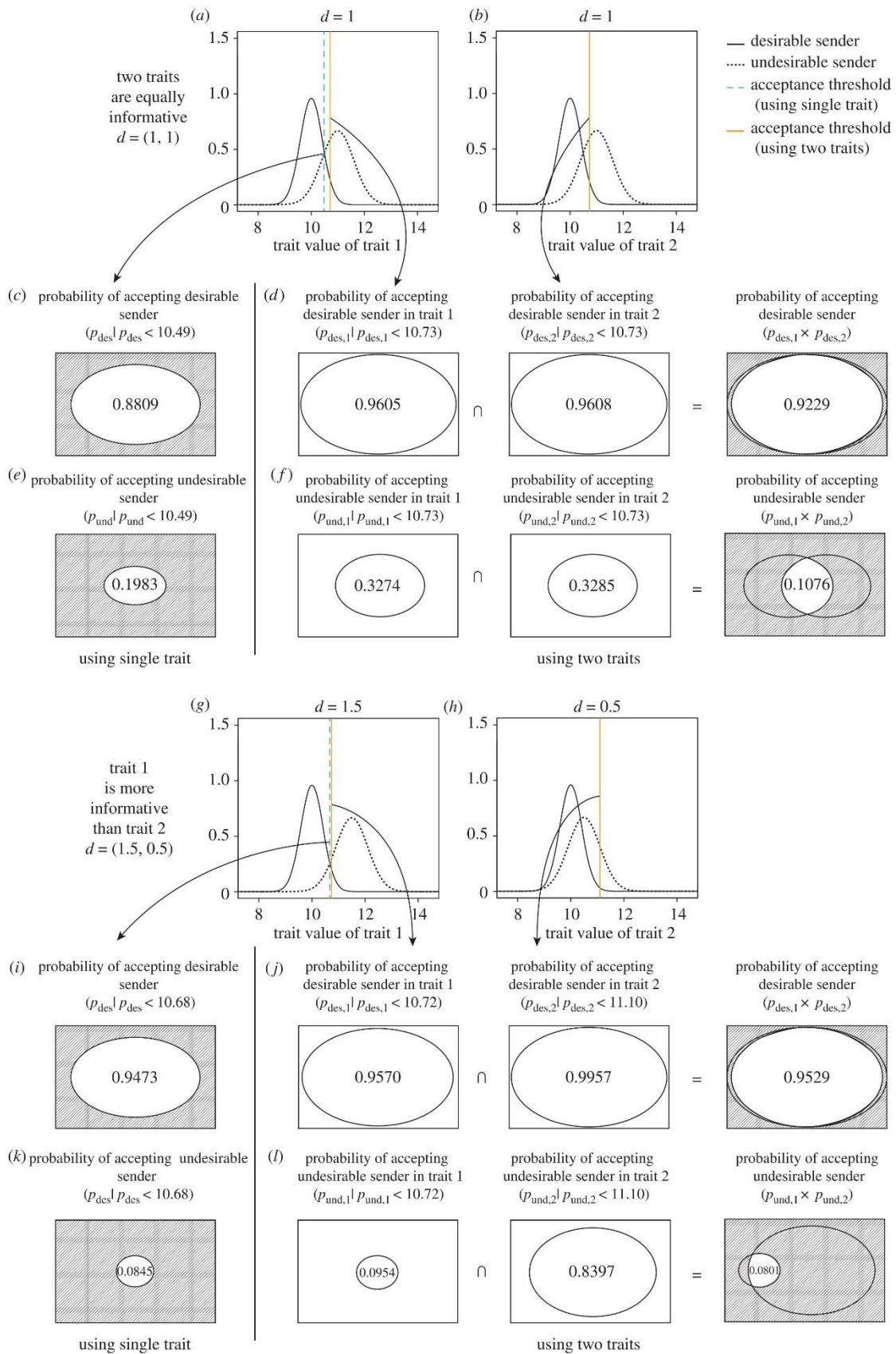


Figure 2. (Caption overleaf.)

Figure 2. (*Overleaf.*) Threshold and error rate comparison between using a single trait or two traits for recognition when phenotypic dissimilarities between desirable and undesirable senders are fixed. (*a,b*) The optimal acceptance threshold when using a single trait (vertical blue dashed line) or two traits (vertical orange solid lines). (*c,e*) Based on the acceptance threshold and the phenotype of senders, we calculate the probability of accepting a desirable sender (*c*) and the probability of accepting an undesirable sender (*e*) when only one trait is used. (*d*) Similarly, when two traits are used, the probability of accepting a desirable sender can be calculated from the intersection of acceptance probabilities for each trait. (*f*) The probability of accepting undesirable senders is also obtained through the intersection of acceptance probabilities for each trait. (*g–i*) Each panel corresponds to (*a–f*), but the phenotypic dissimilarities are different in the two traits. Owing to limited space, we only compare here the results of using two traits with the results of using the trait with greatest dissimilarity. The error rate comparison for other phenotypic dissimilarities ($d = (2, 0)$) is shown in electronic supplementary material, figure S1. Finally, note that the rejection error is equal to $(1 - \text{probability of accepting desirable senders})$, whereas the acceptance error is equal to the probability of accepting undesirable senders. (Online version in colour.)

values much lower than the trait values of desirable senders. We analyse the probability of making errors through time (figure 4), the final trait distributions (figure 5) and the average error over repetitions (figure 6) under this scenario (see electronic supplementary material, table S1 for summary of parameter values of the model).

3. Results

(a) Acceptance threshold can evolve, but phenotypic traits cannot evolve

We explore how trait dissimilarity influences the evolution of acceptance thresholds when one versus two traits are used during recognition. We vary the trait dissimilarity and test how acceptance threshold(s) evolve and how error rates change. We first model the case where both traits have the same phenotypic dissimilarity ($d = (1, 1)$) between the average phenotype of desirable and undesirable senders (figure 2*a,b*). When both traits are similarly informative, using two traits during recognition results in more accurate recognition than using one trait. Both rejection errors and acceptance errors are lower when two traits are used than when one trait is used (figure 2*c–f* and the arrows in figure 3).

Using two traits reduces recognition errors. One key reason for the increased accuracy is that if senders are to be accepted, both traits must meet the receivers' thresholds. However, the senders will be rejected if either trait 1 or trait 2 does not meet the thresholds of the receivers. When receivers use two traits during recognition, receivers move the threshold for each trait to be less restrictive. The less restrictive threshold reduces the probability that receivers will reject desirable senders (rejection errors). If receivers used a single trait, a less restrictive threshold would automatically increase acceptance errors. However, because acceptance is based on the intersection of acceptance in trait 1 and trait 2, acceptance errors are reduced despite the less restrictive threshold for each trait. Using two traits both increases the probability of accepting desirable senders and decreases the probability of accepting undesirable senders (figure 2*d,f* and electronic supplementary material, figure S2).

We also model the case where traits have different phenotypic dissimilarities between desirable and undesirable senders. In these simulations, one trait has relatively lower phenotypic dissimilarity ($d = 0.5$), while the other trait has higher phenotypic dissimilarity ($d = 1.5$) (figure 2*g,h*). Notably, using two traits does not significantly alter error rates when traits have different phenotypic dissimilarities between desirable and undesirable senders (figure 3). Instead, receivers make decisions using only the trait with higher phenotypic dissimilarity and ignore the trait with lower phenotypic dissimilarity (figure 2*g,h*). The trait with higher

dissimilarity ($d = 1.5$) between desirable and undesirable senders allows receivers to more accurately discriminate between desirable and undesirable senders. Paying attention to the less informative trait ($d = 0.5$) does not increase recognition accuracy or influence probability of accepting undesirable senders (figure 2*i–l*).

(b) Acceptance threshold and phenotypic dissimilarity between desirable and undesirable senders can evolve

In this section, we allow the trait values of undesirable senders to evolve, which can alter the phenotypic dissimilarity between desirable and undesirable senders. One key result of this model is that using two traits during recognition is more effective than using a single trait because using two traits reduces acceptance errors (figure 4*b*). Receivers that use two traits during recognition are less likely to accept undesirable senders. However, using two traits during recognition does not change the probability of making rejection errors (figure 4*a*). This result differs from the fixed trait scenario where using two traits is only beneficial when the two traits provide similar information quality (figure 3, $d = (1, 1)$). Using two traits reduces recognition errors because it is more difficult for senders to perfectly match two different traits simultaneously than a single trait.

The evolutionary dynamics of multiple traits are a key reason why using two traits for recognition decreases errors. When there are two traits that could be used for recognition, natural selection favours receivers that ignore the less informative trait (i.e. smaller trait dissimilarity, d , between desirable and undesirable senders) and pay attention to the more informative trait (larger trait dissimilarity, d , between desirable and undesirable senders). Receiver behaviour exerts a strong selective pressure on the trait values of undesirable senders to decrease phenotypic dissimilarity with desirable senders. When a trait becomes less informative to receivers, receivers will switch their attention to the more informative trait. These coevolutionary dynamics lead to trait values and acceptance thresholds that either reach a stable state (e.g. figure 6) or continue cycling (see electronic supplementary material, figure S3 for more examples of the evolutionary dynamics among trait values and acceptance thresholds). In summary, using two traits allows receivers to reduce acceptance error because receivers can use the more informative trait. If the focal trait becomes less informative, receivers can switch their attention to the other trait.

(c) Different costs of acceptance errors

Finally, we test how reducing the costs of an acceptance error influences the evolution of acceptance thresholds and error

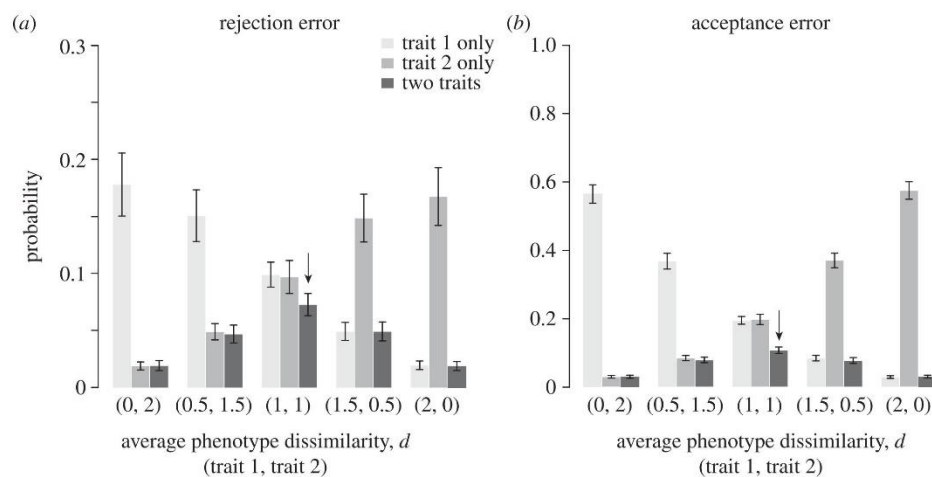


Figure 3. Error comparisons of using one trait and two traits when phenotypic dissimilarities are fixed. Each set of three bars, from left to right represent (i) light grey—using trait 1 with the first phenotype dissimilarity value, (ii) grey—using trait 2 with the second phenotype dissimilarity value, and (iii) dark grey—using both trait 1 and trait 2. Each error bar shows the standard deviation of the error probabilities from 100 simulation repetitions. Each simulation lasted 3000 generations and we took the average error of the last 300 generations as one repetition. The arrows indicate places where using two traits has a lower error than using a single trait. Note that the potential probability range of rejection and acceptance errors is different, with rejection error probability (0, 0.3) and acceptance error probability (0, 1).

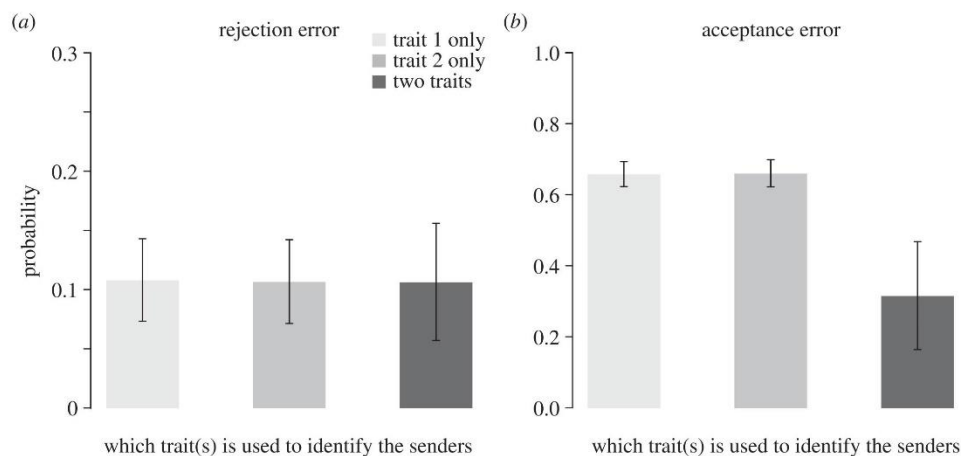


Figure 4. Error comparisons of using one trait and two traits when both acceptance thresholds and phenotypic dissimilarities are allowed to evolve. The simulation was 500 repetitions, with the error bars showing the standard deviation of the final 50 repetitions. Each set of three bars, from left to right represent (i) light grey—using trait 1, (ii) grey—using trait 2, (iii) dark grey—using both trait 1 and trait 2. In the single trait scenario, the trait values of the two traits converge, presumably because they have the same initial conditions. The potential probability range of rejection and acceptance errors (y-axis scales) is different, with rejection error probability (0, 0.3) and acceptance error probability (0, 1).

rates. In previous models, receivers have zero reproductive success if they accept an undesirable sender. In this new model, the cost of making an error is much lower (e.g. 0.25–0.75 reduction in offspring production). Notably, the use of two traits for recognition is beneficial regardless of the precise cost of errors, though the benefit of using two traits decreases as the cost of errors decreases.

Consistent with previous models, our results indicate that a higher cost of accepting undesirable senders favours more restrictive thresholds that reduce acceptance errors (figure 7a). However, a more restrictive threshold will inevitably cause higher rejection errors (figure 7b). In other words, the relative costs of making rejection and acceptance errors influence the evolution of acceptance threshold in a straightforward manner consistent with previous work [9].

4. Discussion

Our simulation has three major results. First, using complex signals during recognition improves recognition accuracy. Receivers that use complex signals make fewer acceptance and rejection errors. For example, birds that use two traits to recognize their eggs are less likely to accidentally accept brood parasitic eggs and less likely to reject their own eggs than birds that use one trait for recognition. One key reason that complex signalling decreases recognition errors is that using multiple traits provides flexibility. When a particular trait becomes less informative (i.e. lower trait dissimilarity), receivers switch their attention to a more informative trait. Over time, the signalling system either reaches a stable state or cycles between multiple traits. Second, when two signals

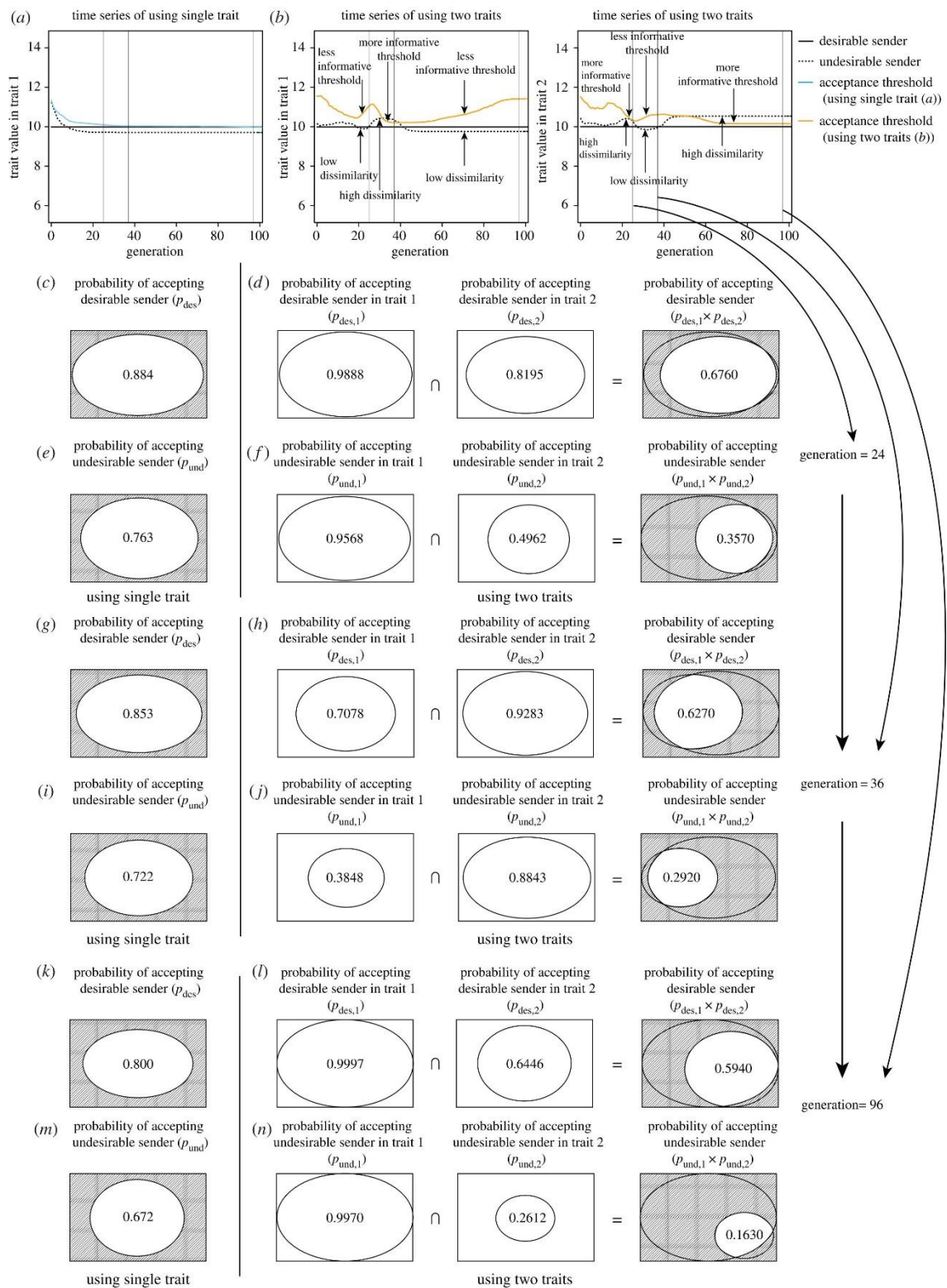


Figure 5. Threshold and error rate comparison between using a single trait and two traits when both acceptance thresholds and phenotypic dissimilarities are allowed to evolve. (a) The time series of the average acceptance threshold and average genotype of undesirable senders when a single trait is used. Acceptance threshold is shown by the blue curve. (b) The time series for using two traits, where acceptance thresholds are presented by the solid orange curves. (c–f) The error rate of using a single trait and two traits at generation 24. Similarly, the error rates at generation 36 (g–j) and 96 (k–n) are analysed. See figure 2 for more explanation of probabilities in each panel, and electronic supplementary material, figure S2 for more time series of the simulations. (Online version in colour.)

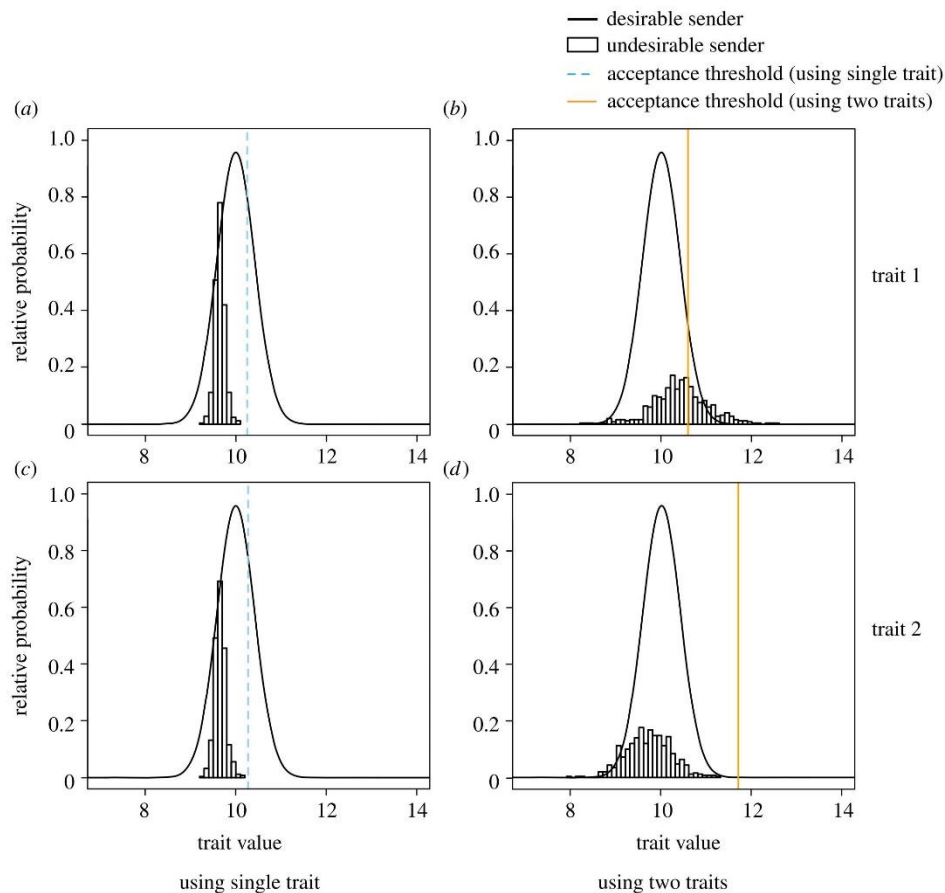


Figure 6. Acceptance threshold comparison between using a single or two traits when both acceptance thresholds and phenotypic dissimilarities are allowed to evolve. Since the trait values of undesirable recipients are evolvable, we use histogram to represent the average trait value of each simulation (500 repetitions for each panel). Note that the simulations using a single trait are data from two separate simulations (*a,c*), while the simulations using two traits are from the same sets of data (*b,d*). (Online version in colour.)

provide different amounts of information, receivers will rely on the more informative signal to make recognition decisions. When one signal is much more informative than the other, receivers will use the more informative signal alone and ignore the less informative signal. As a result, the particular signals used for recognition are not fixed over evolutionary time. Instead, it is more accurate to think of recognition as an evolutionary arms race where the particular signals used for recognition change as undesirable senders evolve more effective mimicry. Third, complex signals are most beneficial when making recognition errors is very costly. When recognition errors are not very costly, receivers may use a single trait for recognition.

(a) Complex signals improve recognition accuracy

The model illustrates that using two traits improves recognition accuracy because undesirable senders are less able to accurately match two traits simultaneously than to match a single trait. Using multiple traits for recognition creates more moving targets for undesirable senders to match. As a result, it is more difficult for undesirable senders to ‘catch up’ and match the phenotype of both traits. In the simulation, undesirable senders end up with one trait that accurately mimics the phenotypes of desirable senders and one trait that does not or recognition cycles between multiple traits (figure 5).

One limitation of the simulation is the populations reproduce asexually. Sexual reproduction could allow undesirable senders to more rapidly mimic complex signals; sexual reproduction allows genetic exchange between diverse lineages so beneficial alleles are more rapidly assembled into the same genome [22]. Future work that models a sexually reproducing population would be useful to test the effect of sexual reproduction on the evolution of complex recognition. Overall, our results illustrate that accurate mimicry in many traits is more difficult than accurate mimicry in a single trait. As a result, complex signals improve recognition accuracy.

Empirical work supports the prediction that signal receivers make more accurate recognition decisions when they use complex signals. Bees discriminate more accurately between rewarding and non-rewarding flower types when the flowers differ in both appearance and odour than when the flowers differ in only one sensory modality [23]. Complex signals may improve foraging by providing bees with additional sources of information. Mosquitofish identified predators more quickly and accurately when they were provided with both visual and olfactory traits than when either visual or olfactory traits were provided separately [24].

Comparative analyses also suggest that complex signals are more likely to evolve when accurate recognition is beneficial. For example, bird species that experience brood

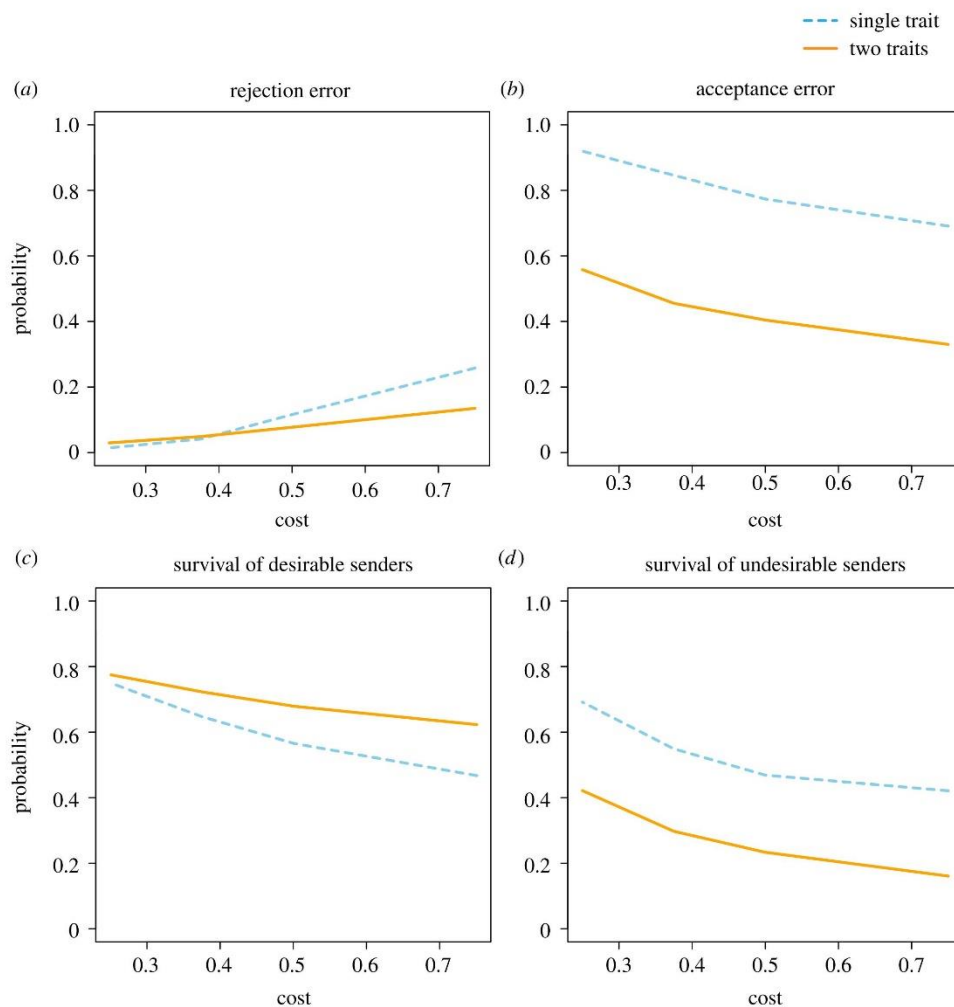


Figure 7. The errors and survival rates of senders in response to various costs when both acceptance thresholds and phenotypic dissimilarities are allowed to evolve. Each line shows the average error or survival at given cost values, where blue dashed lines represent recognition using a single trait and orange solid lines represent recognition using two traits. See electronic supplementary material, S52 for more details and results about the model with variable costs. (Online version in colour.)

parasitism have eggs that are more variable across multiple parameters than bird species that do not experience brood parasitism. The high egg variation is thought to occur because complex signalling across multiple egg parameters reduces recognition errors by hosts [25,26]. Similarly, ant populations that commonly experience social parasitism have greater chemical trait diversity than ant populations that lack social parasitism. Recognition trait diversity may be favoured because it is more difficult for parasites to match complex recognition profiles of hosts [27]. Therefore, complex signals are more likely to arise in taxa with recognition challenges, likely because complex signals improve recognition accuracy.

(b) Receivers use more informative traits when traits differ in information content

The second major result of the simulation is that receivers pay attention to the more informative trait and ignore the less informative trait when traits convey different amounts of information. On the one hand, this result is not surprising. Of course, receivers will make decisions based on the most

informative traits. On the other hand, this result highlights the flexibility of recognition systems. The signals used for recognition are not an evolutionary endpoint. Instead, senders and receivers may frequently change which signals are used for recognition based on the phenotypic dissimilarity between desirable and undesirable senders. Over time, some signals become more informative, while others become less informative. Less informative traits may be lost or they may be maintained through negative frequency selection based on the phenotypes of undesirable senders. Both genetic and non-genetic changes in multi-component recognition signals (e.g. learning, cultural transmission) play a role in evading mimics. Therefore, recognition is often a dynamic and flexible process akin to an evolutionary arms race between undesirable senders and receivers.

Given the evolutionary arms race between undesirable senders and receivers, the signals used for recognition may frequently change over evolutionary time. As a result, animals may often have 'ghosts of signals past', traits that were once used for recognition that no longer provide reliable information and are ignored by receivers [28]. Consistent with this hypothesis, there are many situations where

receivers seem to use only a small fraction of the available information for recognition. For example, some birds identify eggs using multiple egg characteristics, while other species use only a subset of available signals [18]. Insect cuticular hydrocarbon profiles are composed of many different chemicals [5]; however, receivers only use a small fraction of the available chemical information for recognition [29]. Were the other chemicals used for recognition in the past, then ignored when they became uninformative? Comparative analyses of the signals used for recognition across multiple, closely related taxa provide a good opportunity to test the evolutionary flexibility of recognition. We predict that contexts where the fitness of undesirable senders depends on their ability to trick receivers (e.g. mimicry, host–parasite interactions) are especially likely to lead to rapid changes in recognition signals. The changes in recognition signals may be either plastic changes that occur over developmental time or longer-term evolutionary responses.

The simulation also predicts that the particular signals used for recognition may change rapidly based on their information content. Receivers may flexibly change how they use different aspects of a complex signal when the information content varies [30,31]. For example, squirrels living in areas with higher urban noise pay closer attention to the visual aspect of an auditory–visual alarm signal than squirrels from rural areas [32], perhaps because the auditory signal is less informative when it is masked by urban noise. Similarly, female jungle fowl change which male ornament they use for mate choice based on which signals are the most informative [33]. Psychological research indicates that the cognitive system changes how different stimuli are weighted based on the signal-to-noise ratio of each stimulus. For example, humans can use both visual and tactile information to assess object height. When researchers experimentally reduced the reliability of visual information, subjects increased their reliance on tactile information [34]. Therefore, animals may often change how they use complex signals to best match shifting sensory environments. Experimental work in additional contexts and taxa will be useful to test how the phenotypic dissimilarity between desirable and undesirable senders influences how animals use complex traits and how they evaluate different components of signals into their responses.

(c) Complex signals are more common when recognition errors are costly

The final key result of the simulation is that complex signals are most beneficial when recognition errors are very costly. When errors are low cost, receivers may rely on simple signals for recognition. Empirical work largely supports this prediction, as many examples of redundant signals occur in situations where recognition errors are costly [35]. Redundant signals are used during species recognition, during parent–offspring recognition and in taxa with brood parasitism [17]. For instance, Australian sea lion mothers use acoustic, olfactory and visual signals to accurately discriminate their own pup from the many other pups in the colony [36]. Birds often use multiple features to assess whether an egg has been laid by a brood parasite, including factors like egg size, egg shape, eggshell maculation, background colour and ultraviolet reflectance [17]. If a host accepts a brood parasitic egg, the host may lose an entire breeding season's

reproduction. As a result, mechanisms that improve recognition accuracy, like complex signalling, are favoured.

There are relatively few examples of redundant signals in contexts where recognition errors are low cost. For example, although complex sexual signals are common, most do not convey redundant information. Instead, multiple signals are used in different contexts or convey different information to receivers [30,37]. For example, eland antelope have non-redundant sexual signals, as males signal body size via the frequency of knee clicks and age via dewlap size [38]. Models also suggest that redundant sexual signals are rarely evolutionarily stable, especially when producing or assessing signals is costly [15]. Although choosing a lower quality mate may reduce reproductive success, it is unlikely to result in the death of all offspring. Therefore, redundant sexual ornaments may be relatively uncommon because recognition errors are not sufficiently costly.

Additional experimental work will be important to test how the cost of errors influences whether animals use simple or complex signals for recognition. Creative experiments have shown that animals change their optimal acceptance thresholds as the cost of errors changes [6,7]. Our models predict that animals will change whether they use simple versus complex traits for recognition as the costs of making an error change. Specifically, they will be more likely to use complex signals for recognition when the cost of errors is high.

This model focuses on the recognition benefits of complex signals rather than the sender or receiver costs that may disfavour complex signalling. There is much evidence that perceiving, assessing and making decisions about signals is cognitively costly [39–41]. As a result, complex signals that occur across multiple sensory modalities or involve multiple signal components will involve greater cognitive costs for receivers than simple signals [13,42,43]. Further, producing and maintaining signals is costly for senders, with some signals imposing much greater costs than other signals (e.g. body size) [44,45]. Additional empirical research will be important to assess how the costs of perceiving and producing complex signals trade-off with the recognition benefits of complex signals in different contexts.

Overall, our model shows that redundant, complex signals are an evolutionarily stable way to reduce recognition errors. The simulation makes three testable predictions. First, redundant complex signals reduce recognition errors compared with simple signals. Second, redundant signals are most likely to occur when the costs of recognition errors are high. Third, when signals contain different amounts of information, receivers will pay attention to the more informative signal and ignore the less informative signal. Although empirical work generally supports these predictions, there is much room for additional experiments testing how complex signal use and recognition thresholds vary in different contexts. Thus far, our results indicate that signal detection theory provides a useful conceptual framework for understanding the evolution of complex signals.

Data accessibility. All simulated data were generated using the C language. The code used for this study is available at https://github.com/mingpapiio/Codes_ComplexSignal.

Authors' contributions. E.A.T. conceived of the study, coordinated the study and helped write the manuscript. M.L. helped write the model code and results and critically revised the manuscript.

E.C.L. critically revised the manuscript. S.-F.S. helped write the model code and results, coordinated the study and critically revised the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed.

Competing interests. We declare we have no competing interests.

Funding. This material is based in part upon work supported by the National Science Foundation under grant no. IOS-1557564 (to E.A.T.). S.-F.S. was supported by and Investigator Award (AS-IA-106-L01), Academia Sinica and the Ministry of Science and Technology of Taiwan (106-2621-B-001-005-MY3 and 108-2314-B-001-009-MY3).

References

- Bradbury J, Vehrencamp S. 1998 *Principles of animal communication*. Sunderland, MA: Sinauer Associates.
- Maynard Smith J, Harper D. 2003 *Animal signals*. New York, NY: Oxford University Press.
- Hauber ME, Sherman PW. 2001 Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends Neurosci.* **24**, 609–616. (doi:10.1016/S0166-2236(00)01916-0)
- Lacy RC, Sherman PW. 1983 Kin recognition by phenotype matching. *Am. Nat.* **121**, 489–512. (doi:10.1086/284078)
- van Zweden JS, d'Ettorre P, Blomquist GJ, Bagnères AG. 2010 Nestmate recognition in social insects and the role of hydrocarbons. In *Insect hydrocarbons: biology, biochemistry, and chemical ecology* (eds GJ Blomquist, A-G Bagnères), vol. 11, pp. 222–243. Cambridge, UK: Cambridge University Press.
- Starks PT, Fischer DJ, Watson RE, Melikian GL, Nath SD. 1998 Context-dependent nestmate-discrimination in the paper wasp, *Polistes dominulus*: a critical test of the optimal acceptance threshold model. *Anim. Behav.* **56**, 449–458. (doi:10.1006/anbe.1998.0778)
- Downs SG, Ratnieks FW. 2000 Adaptive shifts in honey bee (*Apis mellifera* L.) guarding behavior support predictions of the acceptance threshold model. *Behav. Ecol.* **11**, 326–333. (doi:10.1093/beheco/11.3.326)
- Tibbetts EA, Mullen SP, Dale J. 2017 Signal function drives phenotypic and genetic diversity: the effects of signalling individual identity, quality or behavioural strategy. *Phil. Trans. R. Soc. B* **372**, 20160347. (doi:10.1098/rstb.2016.0347)
- Reeve HK. 1989 The evolution of conspecific acceptance thresholds. *Am. Nat.* **133**, 407–435. (doi:10.1086/284926)
- Hauber ME, Moskat C, Ban M. 2006 Experimental shift in hosts' acceptance threshold of inaccurate-mimic brood parasite eggs. *Biol. Lett.* **2**, 177–180. (doi:10.1098/rsbl.2005.0438)
- Hebets EA, Papaj DR. 2005 Complex signal function: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* **57**, 197–214. (doi:10.1007/s00265-004-0865-7)
- Partan SR, Marler P. 2005 Issues in the classification of multimodal communication signals. *Am. Nat.* **166**, 231–245. (doi:10.1086/431246)
- Rowe C. 1999 Receiver psychology and the evolution of multicomponent signals. *Anim. Behav.* **58**, 921–931. (doi:10.1006/anbe.1999.1242)
- Moller AP, Pomiankowski A. 1993 Why have birds got multiple sexual ornaments? *Behav. Ecol. Sociobiol.* **32**, 167–176. (doi:10.1007/bf00173774)
- Johnstone RA. 1996 Multiple displays in animal communication: 'backup signals' and 'multiple messages'. *Phil. Trans. R. Soc. Lond. B* **351**, 329–338. (doi:10.1098/rstb.1996.0026)
- Hebets EA, Barron AB, Balakrishnan CN, Hauber ME, Mason PH, Hoke KL. 2016 A systems approach to animal communication. *Proc. R. Soc. B* **283**, 20152889. (doi:10.1098/rspb.2015.2889)
- Spottiswoode CN, Stevens M. 2010 Visual modeling shows that avian host parents use multiple visual cues in rejecting parasitic eggs. *Proc. Natl Acad. Sci. USA* **107**, 8672–8676. (doi:10.1073/pnas.0910486107)
- Hanley D, Lopez AV, Fiorini VD, Reboreda JC, Grim T, Hauber ME. 2019 Variation in multicomponent recognition cues alters egg rejection decisions: a test of the optimal acceptance threshold hypothesis. *Phil. Trans. R. Soc. B* **374**, 20180195. (doi:10.1098/rstb.2018.0195)
- Hankison SJ, Morris MR. 2003 Avoiding a compromise between sexual selection and species recognition: female swordtail fish assess multiple species-specific cues. *Behav. Ecol.* **14**, 282–287. (doi:10.1093/beheco/14.2.282)
- Uetz GW, Roberts JA. 2002 Multisensory cues and multimodal communication in spiders: insights from video/audio playback studies. *Brain Behav. Evol.* **59**, 222–230. (doi:10.1159/000064909)
- Seyfarth RM, Cheney DL, Bergman T, Fischer J, Zuberbühler K, Hammerschmidt K. 2010 The central importance of information in studies of animal communication. *Anim. Behav.* **80**, 3–8. (doi:10.1016/j.anbehav.2010.04.012)
- Muller HJ. 1932 Some genetic aspects of sex. *Am. Nat.* **66**, 118–138. (doi:10.1086/280418)
- Leonard AS, Masek P. 2014 Multisensory integration of colors and scents: insights from bees and flowers. *J. Comp. Physiol. A* **200**, 463–474. (doi:10.1007/s00359-014-0904-4)
- Ward AJW, Mehner T. 2010 Multimodal mixed messages: the use of multiple cues allows greater accuracy in social recognition and predator detection decisions in the mosquitofish, *Gambusia holbrooki*. *Behav. Ecol.* **21**, 1315–1320. (doi:10.1093/beheco/arq152)
- Medina I, Troschiano J, Stevens M, Langmore NE. 2016 Brood parasitism is linked to egg pattern diversity within and among species of Australian passerines. *Am. Nat.* **187**, 351–362. (doi:10.1086/684627)
- Caves EM, Stevens M, Iversen ES, Spottiswoode CN. 2015 Hosts of avian brood parasites have evolved egg signatures with elevated information content. *Proc. R. Soc. B* **282**, 20150598. (doi:10.1098/rspb.2015.0598)
- Jongepier E, Foitzik S. 2016 Ant recognition cue diversity is higher in the presence of slavemaker ants. *Behav. Ecol.* **27**, 304–311. (doi:10.1093/beheco/arv153)
- Ryan MJ, Rand AS. 1993 Sexual selection and signal evolution: the ghost of biases past. *Phil. Trans. R. Soc. Lond. B* **340**, 187–195. (doi:10.1098/rstb.1993.0057)
- Dani FR, Jones GR, Destri S, Spencer SH, Turillazzi S. 2001 Deciphering the recognition signature within the cuticular chemical profile of paper wasps. *Anim. Behav.* **62**, 165–171. (doi:10.1006/anbe.2001.1714)
- Bro-Jorgensen J. 2010 Dynamics of multiple signalling systems: animal communication in a world in flux. *Trends Ecol. Evol.* **25**, 292–300. (doi:10.1016/j.tree.2009.11.003)
- Munoz NE, Blumstein DT. 2012 Multisensory perception in uncertain environments. *Behav. Ecol.* **23**, 457–462. (doi:10.1093/beheco/arr220)
- Partan SR, Fulmer AG, Gounard MAM, Redmond JE. 2010 Multimodal alarm behavior in urban and rural gray squirrels studied by means of observation and a mechanical robot. *Cur. Zool.* **56**, 313–326. (doi:10.1093/czoolo/56.3.313)
- Zuk M, Ligon JD, Thornhill R. 1992 Effects of experimental manipulation of male secondary sex characters on female mate preference in red jungle fowl. *Anim. Behav.* **44**, 999–1006. (doi:10.1016/S0003-3472(05)80312-4)
- Ernst MO, Banks MS. 2002 Humans integrate visual and haptic information in a statistically optimal fashion. *Nature* **415**, 429–433. (doi:10.1038/415429a)
- Bretman A, Westmancoat JD, Gage MJG, Chapman T. 2011 Males use multiple, redundant cues to detect mating rivals. *Curr. Biol.* **21**, 617–622. (doi:10.1016/j.cub.2011.03.008)
- Wierucka K, Pitcher BJ, Harcourt R, Charrier I. 2018 Multimodal mother–offspring recognition: the relative importance of sensory cues in a colonial mammal. *Anim. Behav.* **146**, 135–142. (doi:10.1016/j.anbehav.2018.10.019)
- Candolin U. 2003 The use of multiple cues in mate choice. *Biol. Rev.* **78**, 575–595. (doi:10.1017/S1464793103006158)
- Bro-Jorgensen J, Dabelsteen T. 2008 Knee-clicks and visual traits indicate fighting ability in eland antelopes: multiple messages and back-up signals. *BMC Biol.* **6**, 47. (doi:10.1186/1741-7007-6-47)
- Dukas R. 2004 Evolutionary biology of animal cognition. *Annu. Rev. Ecol. Syst.* **35**, 347–374. (doi:10.1146/annurev.ecolsys.35.112202.130152)
- Mendelson TC, Fitzpatrick CL, Hauber ME, Pence CH, Rodriguez RL, Safran RJ, Stern CA, Stevens JR. 2016 Cognitive phenotypes and the evolution of animal

- decisions. *Trends Ecol. Evol.* **31**, 850–859. (doi:10.1016/j.tree.2016.08.008)
41. Guilford T, Dawkins MS. 1991 Receiver psychology and the evolution of animal signals. *Anim. Behav.* **42**, 1–14. (doi:10.1016/s0003-3472(05)80600-1)
42. Halfwerk W, Varkevisser J, Simon R, Mendoza E, Scharff C, Riebel K. 2019 Toward testing for multimodal perception of mating signals. *Front. Ecol. Evol.* **7**, 124. (doi:10.3389/fevo.2019.00124)
43. Dore AA, McDowall L, Rouse J, Bretman A, Gage MJG, Chapman T. 2018 The role of complex cues in social and reproductive plasticity. *Behav. Ecol. Sociobiol.* **72**, 124. (doi:10.1007/s00265-018-2539-x)
44. Cotton S, Fowler K, Pomiankowski A. 2004 Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc. R. Soc. Lond. B* **271**, 771–783. (doi:10.1098/rspb.2004.2688)
45. Searcy WA, Nowicki S. 2005 *The evolution of animal communication*. Princeton, NJ: Princeton University Press.

A6

The evolution of division of
labour in structured and
unstructured groups

The evolution of division of labour in structured and unstructured groups

Guy Alexander Cooper^{1,2*}, Hadleigh Frost³, Ming Liu², Stuart Andrew West²

¹St John's College, Oxford, United Kingdom; ²Department of Zoology, University of Oxford, Oxford, United Kingdom; ³Mathematical Institute, University of Oxford, Oxford, United Kingdom

Abstract Recent theory has overturned the assumption that accelerating returns from individual specialisation are required to favour the evolution of division of labour. Yanni et al., 2020, showed that topologically constrained groups, where cells cooperate with only direct neighbours such as for filaments or branching growths, can evolve a reproductive division of labour even with diminishing returns from individual specialisation. We develop a conceptual framework and specific models to investigate the factors that can favour the initial evolution of reproductive division of labour. We find that selection for division of labour in topologically constrained groups: (1) is not a single mechanism to favour division of labour—depending upon details of the group structure, division of labour can be favoured for different reasons; (2) always involves an efficiency benefit at the level of group fitness; and (3) requires a mechanism of coordination to determine which individuals perform which tasks. Given that such coordination must evolve prior to or concurrently with division of labour, this could limit the extent to which topological constraints favoured the initial evolution of division of labour. We conclude by suggesting experimental designs that could determine why division of labour is favoured in the natural world.

Editor's evaluation

This manuscript presents a theoretical study of the evolution of division of labor, exploring the impact of topology, the convexity and concavity of fitness returns on investment, and different biological modes through which division of labor may arise. This is a difficult topic to study as division of labor evolved long ago, and many theoretical predictions have proven difficult to directly test. The results presented here may provide the next step necessary to produce truly testable hypotheses on how division of labor evolves, and will be of interest to evolutionary biologists, mathematical biologists, and biophysicists.

*For correspondence:
guy.cooper@zoo.ox.ac.uk

Funding: See page 15

Received: 06 July 2021

Accepted: 26 October 2021

Published: 29 October 2021

Reviewing Editor: Raymond E Goldstein, University of Cambridge, United Kingdom

© Copyright Cooper et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Introduction

Division of labour, where cooperating individuals specialise to carry out distinct tasks, plays a key role at all levels of biology (Bourke, 2011; Queller, 1997; Maynard Smith and Szathmáry, 1995; West et al., 2015). Cells are built by genes carrying out different functions (Bourke, 2011; Levin and West, 2017). In clonal groups of bacteria, cells specialise to produce and secrete different factors that facilitate growth (Dragoš et al., 2018a; Veening et al., 2008; West and Cooper, 2016). Pathogens rely on division of labour for protection from the host immune response and competitors (Ackermann et al., 2008; Diard et al., 2013). Multicellular organisms are composed of reproductive germ cells and sterile somatic cells that are not passed to the next generation (Bourke, 2011; Maynard Smith and Szathmáry, 1995). The ecological dominance of the social insects arises from division of

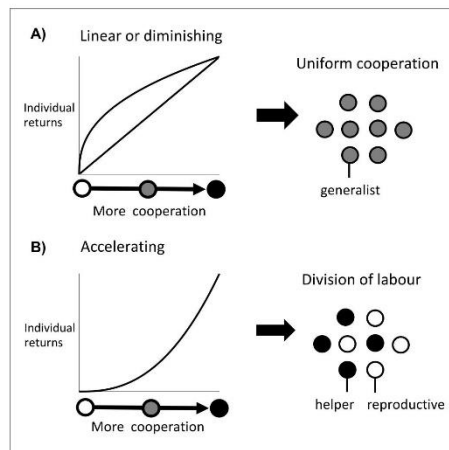


Figure 1. Division of labour is favoured by accelerating returns from individual specialisation. (A) Theory has shown that either a linear or diminishing return from more cooperation (or reproduction) favours uniform cooperation, with all individuals investing the same amount of effort into cooperation and reproduction (i.e. no division of labour) (Cooper and West, 2018; Michod, 2006; Schiessl et al., 2019). (B) In contrast, an accelerating return from more cooperation (or reproduction) favours reproductive division of labour, with some individuals specialising in high levels of cooperation (helpers) and others in low levels of cooperation (reproductives) (Cooper and West, 2018; Michod, 2006; Schiessl et al., 2019).

labour between queens and the different types of workers (castes) (Hölldobler and Wilson, 1990; Oster and Wilson, 1978).

It has long been established that the evolution of division of labour requires an efficiency benefit from individual specialisation (Figure 1A and B for reproductive division of labour) (Bourke, 2011; Cooper and West, 2018; Ispolatov et al., 2012; Michod, 2006; Oster and Wilson, 1978; Schiessl et al., 2019; Biggart, 1776; Maynard Smith and Szathmáry, 1995; Solari et al., 2013). In particular, that there is an accelerating (convex) return when individuals commit more effort to a particular task, such that twice the investment more than doubles the return (Bourke, 2011; Cooper and West, 2018; Ispolatov et al., 2012; Michod, 2006; Solari et al., 2013). An accelerating return from individual investment can exist for several reasons. A task could become more effective as more effort is put into it, or it could be carried out with diminishing costs. This could occur if there are large upfront costs from performing a task. For instance, any reproduction by a cell in Volvocine groups first requires individual growth to the size of a daughter colony (Michod, 2006). Alternatively, there could be a disruptive cost to carrying out multiple tasks at the same time if the tasks do not mix well. For instance, in cyanobacteria the enzymes that fix environmental nitrogen are degraded by oxygen, a bi-product of photosynthesis (Flores and Herrero, 2010).

In contrast, Yanni et al. found that division of labour between helpers and reproductives can

sometimes be favoured even when there are diminishing (concave) returns from individual specialisation (Yanni et al., 2020). Specifically, reproductive division of labour could arise in topologically constrained groups—where each cell in a spatially structured group shares cooperative benefits with only their direct neighbours (Staps and Tarnita, 2020; Yanni et al., 2020). Their analyses suggested that this is particularly likely to occur in sparsely structured groups, where cells have a small number of neighbours (Yanni et al., 2020). This is a novel result. Diminishing returns means that specialised individuals are inefficient, and earlier work suggested that division of labour could not be favoured in this situation (Figure 1A, Cooper and West, 2018; Michod, 2006; Schiessl et al., 2019). Consequently, this result has the potential to overturn our understanding of the factors that favour the evolution of division of labour.

However, there are several issues that still need to be resolved with how topological constraints can favour division of labour. Why exactly do the predictions of this new theory differ from previous theory? Are special group structures the only way to alter the predictions of the previous theory, or is this an example of a more general phenomenon (Rueffler et al., 2012)? Do these findings rely upon implicit assumptions, which may not be reasonable during the initial evolution of division of labour? Answering these questions is not only of theoretical importance: it is also key for planning future empirical studies. Quantifying the shape of the returns from individual specialisation has been assumed to be a fundamental step in determining why division of labour was favoured in some species, but not others (Diard et al., 2013; Dragoš et al., 2018a; Flores and Herrero, 2010; Koufopanou, 1994; Mridha and Kummerli, 2021; Strassmann et al., 2000; Veening et al., 2008).

We first use the methodology developed by Rueffler et al., 2012, to derive the general conditions that favour the initial evolution of reproductive division of labour between helpers and reproductives.

We then use this framework to examine when and why topological constraints can favour division of labour. More specifically, we determine the ultimate cause of division of labour in specific topologically constrained groups, such as filaments and branching growths, as well as in a general analysis of arbitrary group structures. We then ask whether division of labour without an accelerating return from individual specialisation could arise in groups without topological constraints. To test our hypothesis that between-individual coordination is required for division of labour in these cases, we re-examine our models while assuming that cells adopt helper and reproductive roles randomly (no coordination). We finish by suggesting experimental designs for determining why division of labour has evolved in specific species.

Results and discussion

General invasion analysis

We follow previous studies by assuming that individual fitness is the product of individual viability, which is the chance of surviving to maturity, and individual fecundity, which is proportional to the number of offspring if the individual reaches maturity (Cooper and West, 2018; Michod, 2006; Yanni et al., 2020). We examine the specific case of reproductive division of labour between helpers and reproductives, where helpers are more cooperative, contributing to a higher viability for group members, and reproductives are less cooperative, contributing to higher individual fecundity.

We consider an initial population of clonal groups each containing n individuals, in which all individuals cooperate at the evolutionarily stable (ES) level (z^*), which is the level that cannot be outcompeted by a mutant strain that uses a different level of uniform cooperation across the group (Maynard Smith, 1982). We then ask when this population of uniform cooperators can be invaded by a mutant strain that employs a reproductive division of labour.

Without loss of generality, we assume that the mutant strain is composed of n_h helpers that invest $z_h \geq 0$ into cooperation and n_r reproductives that invest $z_r \geq 0$ into cooperation (where $n_h + n_r = n > 2$ and $z_h > z_r$). We set individual fitness as the product of individual fecundity, $F > 0$, and individual viability, where helpers and reproductives may in principle have different viability functions, $V_h > 0$ and $V_r > 0$ (but see Appendix C.2) (Michod, 2006; Yanni et al., 2020). The fitness of the clonal group is given by the sum of individual fitness:

$$W(z_h, z_r) = n_h F(z_h) V_h(z_h, z_r) + n_r F(z_r) V_r(z_h, z_r), \quad (1)$$

where the first term on the right-hand side is the total fitness of the prospective helpers and the second term on the right-hand side is the total fitness of the prospective reproductives.

Fecundity is determined by an individual's investment in cooperation ($F = F(z)$, where z is the focal individual's level of cooperation) and viability is determined by the level of cooperation at the level of the group ($V_h = V_h(z_h, z_r)$ and $V_r = V_r(z_h, z_r)$). We assume that there is a tradeoff between fecundity and viability such that higher individual cooperation leads to lower individual fecundity ($F'(z) < 0$), but that more cooperation leads to a higher viability for all individuals (i.e. $V_h^{z_h}, V_h^{z_r}, V_r^{z_h}, V_r^{z_r} > 0$, where superscripts denote partial derivatives). We assume that viability selection occurs just prior to reproduction. This is consistent with previous models and ensures that there is no feedback between a cell's viability and its ability to produce cooperative benefits for the group (Cooper and West, 2018; Michod, 2006; Yanni et al., 2020).

We determine the invadability conditions that favour reproductive division of labour by applying the general approach of Rueffler et al., 2012. The key step is to approximate the relative fitness of a reproductive division of labour mutant by taking a second-order Taylor expansion of fitness, centred on the resident strategy of uniform cooperation, z^* :

$$W(z^* + \Delta z_h, z^* + \Delta z_r) - W(z^*, z^*) \approx \underbrace{W^{z_h} \Delta z_h}_{2(a)} + \underbrace{W^{z_r} \Delta z_r}_{2(b)} + \underbrace{\frac{1}{2} W^{z_h z_h} \Delta z_h^2}_{2(c)} + \underbrace{\frac{1}{2} W^{z_r z_r} \Delta z_r^2}_{2(d)} + \underbrace{W^{z_h z_r} \Delta z_h \Delta z_r}_{2(e)}, \quad (2)$$

where $\Delta z_h > \Delta z_r$ captures the change in the level of cooperation for mutant helpers and reproductives, respectively, which we assume are small in magnitude. The superscripts represent first- and second-order partial derivatives, where all partial derivatives are evaluated at the resident strategy

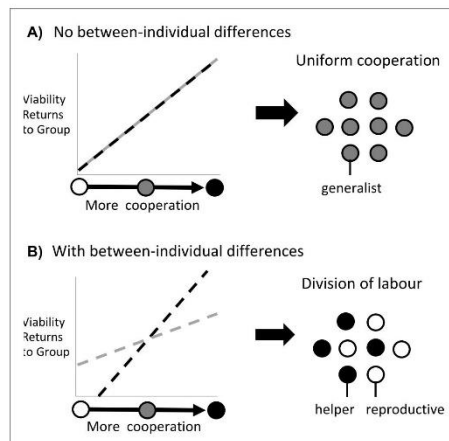


Figure 2. Division of labour is favoured by between-individual differences. Division of labour is favoured if some individuals are predisposed to being reproductives or helpers. (A) In the absence of another mechanism, if there are no differences between individuals (black and grey lines), then division of labour is not favoured. (B) If some individuals can produce larger viability benefits than others (black line), or if some individuals can access greater fecundity benefits than others (grey line), then this predisposition favours division of labour.

of uniform cooperation ($z_h = z_r = z^*$). If a mutant strain exists such that **Equation 2** is positive ($W(z^* + \Delta z_h, z^* + \Delta z_r) > W(z^*, z^*)$), then division of labour between helpers and reproductives is favoured to evolve. Conversely, if for all possible mutant strains, **Equation 2** is negative ($W(z^*, z^*) > W(z^* + \Delta z_h, z^* + \Delta z_r)$), then uniform cooperation is evolutionarily stable.

The three pathways to division of labour

We found that reproductive division of labour could be favoured for three distinct reasons, corresponding to different subsets of terms on the right-hand side of **Equation 2**. Our results for reproductive division of labour, where fitness is partitioned as the product of fecundity and viability, align with those found by *Rueffler et al., 2012*, for division of labour more generally. We now go through these three distinct scenarios.

Scenario 1: Accelerating returns from individual specialisation

The first and most studied scenario that can favour division of labour is when there are accelerating returns from individual specialisation. This occurs if there is an accelerating fitness return from either helper specialisation in cooperation or reproductive specialisation in fecundity (*Figure 1*;

Cooper and West, 2018; Michod, 2006; Oster and Wilson, 1978).

Mathematically, this scenario is a consequence of the third and fourth terms of the Taylor expansion (2c and 2d), which capture the second-order fitness effect of a small, unilateral change in cooperation by either prospective helpers or reproductives, respectively. Division of labour is favoured to evolve whenever at least one of 2c and 2d is greater than zero ($W^{z_h z_h} > 0$ or $W^{z_r z_r} > 0$), and where we have assumed that the first two terms are both zero ($W^{z_h} = 0$ and $W^{z_r} = 0$; see between-individual differences below; *Figure 2A*). In either scenario, an efficiency benefit to group fitness arises from individual specialisation because the more effort that each individual puts into a task, the better they can perform that task. Rueffler et al. termed these kinds of scenario as 'accelerating performance functions' (*Rueffler et al., 2012*).

Scenario 2: Between-individual differences

The second scenario that can favour reproductive division of labour is when there are pre-existing differences between individuals in the group, such that some individuals are predisposed to one task or the other. For example, if some individuals can secure larger viability benefits for the group at the same fecundity cost as others (*Figure 2B*).

This scenario is captured by the first two terms of the Taylor expansion (2a and 2b), which are the first-order fitness effects from a small, unilateral change in the level of cooperation by prospective helpers or reproductives. If the direct fitness effects are non-zero (positive or negative) at the resident strategy of uniform cooperation ($W^{z_h} \neq 0$ or $W^{z_r} \neq 0$), then division of labour can invade independently of any higher-order effects (the remaining terms in **Equation 2**).

We term this scenario 'between-individual differences' because it requires that there is pre-existing phenotypic or environmental variation between individuals in the group. For the within-species case, ancestral groups are usually composed of clonal or highly related individuals, who will be phenotypically similar or identical. Consequently, this mechanism could be less important for the division of labour except when there are consistent differences in the microenvironment experienced by different

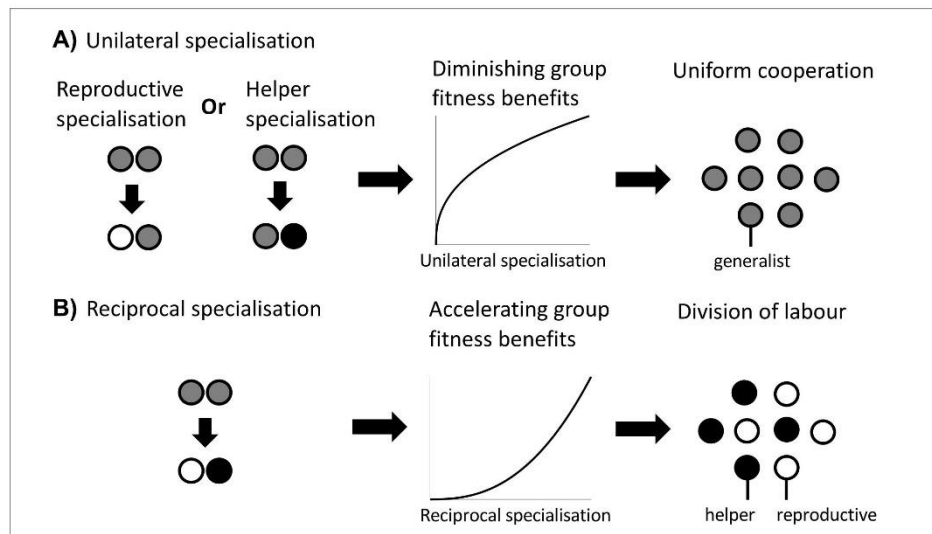


Figure 3. Division of labour is favoured by reciprocal specialisation. We assume that there are diminishing returns from specialisation in either viability or fecundity (**Figure 1A**). **(A)** In this case, a unilateral increase in cooperation by helpers or a unilateral decrease in cooperation by reproductives leads to a diminishing fitness benefits to the group, which favours uniform cooperation (no division of labour). **(B)** In contrast, a reciprocal increase in cooperation by helpers (more viability benefits provided by helpers) and a decrease in cooperation by reproductives (larger reproductive fecundity) can produce an accelerating return to the fitness of the group if the benefits of increased cooperation are preferentially directed to reproductives. Thus, reciprocal specialisation can still favour division of labour, even though the returns from individual specialisation are diminishing. In the middle plots of **(A)** and **(B)**, only the shape of the benefits from increased specialisation is plotted.

individuals (Tverskoi et al., 2018; Tverskoi and Gavrillets, 2021). In contrast, this scenario is likely to be widespread in the evolution of non-reproductive division of labour between species, such as for mutualisms or symbioses (Kiers et al., 2011; Rueffler et al., 2012; Wyatt et al., 2014). Individuals of different species often differ in their abilities to perform certain tasks (Kiers et al., 2011). Rueffler et al. termed this scenario ‘positional’, but we avoid that term to prevent confusion with topological position (Rueffler et al., 2012).

Between-individual differences provide a first-order fitness benefit to dividing labour, and so it does not matter whether the subsequent benefits of increased cooperation or fecundity are accelerating or diminishing (**Figure 1**), so long as these benefits are different for different individuals (**Figure 2**). When some individuals are predisposed to being either helpers or reproductives, then individual specialisation provides an efficiency benefit to group fitness by capitalising on these inherent differences.

Scenario 3: Reciprocal specialisation

The final scenario that can favour division of labour is when reciprocal specialisation by both helpers and reproductives provides a fitness benefit to the group (**Figure 3**). This scenario requires two key conditions. First, simultaneous specialisation, where some individuals invest more in cooperation (more viability benefits for the group), and others invest less in cooperation (greater individual fecundity; but see below). Second, this reciprocal specialisation must provide a group-level fitness benefit, because the increased benefits of cooperation are preferentially directed towards reproductives.

Mathematically, this scenario involves the last term of the Taylor expansion ($2e; W^{z_h z_r} \Delta z_h \Delta z_r$). This term is generated by a between-individual, second-order fitness effect, capturing how increased investment in viability by some individuals affects the returns from increased investment in fecundity by others, and vice versa. Rueffler et al. referred to this as a ‘synergistic benefit’ to division of labour (Queller, 1985; Queller, 2011; Rueffler et al., 2012).

Critically, this scenario still involves an efficiency benefit to specialisation, but at the level of group fitness rather than in each fitness component separately (Appendix C.1). By this we mean that there is an accelerating fitness benefit to the group when helpers and reproductives reciprocally specialise, leading to a higher group fitness than in groups with uniform cooperation (generalists). This occurs if the increased help given to reproductives is sufficiently amplified by the increased fecundity of reproductives (Yanni *et al.*, 2020). This synergistic efficiency benefit can favour division of labour even if there are diminishing returns from individual specialisation.

Division of labour by reciprocal specialisation can also evolve without a joint mutation in the level of cooperation of both helpers and reproductives (no simultaneous specialisation). In this case, the chance invasion (to fixation) of a slightly deleterious mutant that specialises in only one phenotype can destabilise uniform cooperation, creating a selection pressure for the other phenotype to also specialise that is greater than the selection pressure to purge the initial mutant. In this two-step scenario, it is nevertheless the synergistic benefit from reciprocal specialisation that makes division of labour more efficient.

Group structure in the general framework

Our above analysis has shown that reproductive division of labour can be favoured for three reasons: (1) accelerating returns make individual specialisation more efficient; (2) between-individual differences make individual specialisation more efficient; and (3) there is a synergistic efficiency benefit from reciprocal specialisation. These results agree with previous analyses by Rueffler *et al.*, 2012.

We now use this framework to examine how and why topological constraints can favour division of labour in the absence of an accelerating return from individual specialisation (i.e. when scenario 1 does not hold). We ask three questions. First, can topological constraints favour division of labour by between-individual differences (scenario 2), and/or by reciprocal specialisation (scenario 3)? Second, are topological constraints the only way to evolve a division of labour without an accelerating return from individual specialisation? Third, does the evolution of division of labour by between-individual differences (scenario 2) and reciprocal specialisation (scenario 3) require coordination between individuals to determine which cells become helpers or reproductives?

Question 1: How do topological constraints favour division of labour?

We consider two spatial models, based on the group structures proposed by Yanni *et al.*, to examine whether topologically constrained groups favour division of labour by: (a) between-individual differences and/or (b) reciprocal specialisation (Yanni *et al.*, 2020).

Can topological constraints lead to division of labour by between-individual differences?

Consider a group in which cells alternately have either two or three neighbours, in a branching structure (Figure 4). Such a group structure might have occurred for some early forms of multicellular life (Yanni *et al.*, 2020). We term cells with three neighbours 'node' cells and cells with two neighbours 'edge' cells. We assume that cells investing an amount $z \geq 0$ into cooperation produce an amount $H(z)$ of a public good. We assume non-accelerating returns from individual specialisation (i.e. $H''(z) \leq 0$ or $F''(z) \leq 0$). The cell keeps a fraction $1 - \lambda$ of the public good that it produces, and the remaining fraction λ is shared equally between its direct neighbours (the 'shareability' of cooperation: $0 < \lambda \leq 1$). We assume that the viability of a cell is equal to the sum of the public good that it absorbs.

For this model, we find that for all social traits ($\lambda > 0$), reproductive division of labour by between-individual differences can evolve (Figure 4A; Appendix A.1). This occurs because different cells have different viability-fecundity tradeoffs depending on their position in the group. Edge cells receive relatively less public good from their (fewer) neighbours, and so pay a smaller opportunity cost from decreased fecundity (increased cooperation). In contrast, node cells receive relatively more public good from their (more numerous) neighbours, and so pay a larger opportunity cost from decreased fecundity (increased cooperation). Consequently, this between-cell difference favours node cells to specialise in fecundity (reproductives) and edge cells to specialise in increased cooperation (helpers). Importantly, because this pathway to division of labour is driven entirely by a first-order effect (2a and 2b), it does not require a second-order efficiency benefit from specialisation (2c, 2d, or 2e).

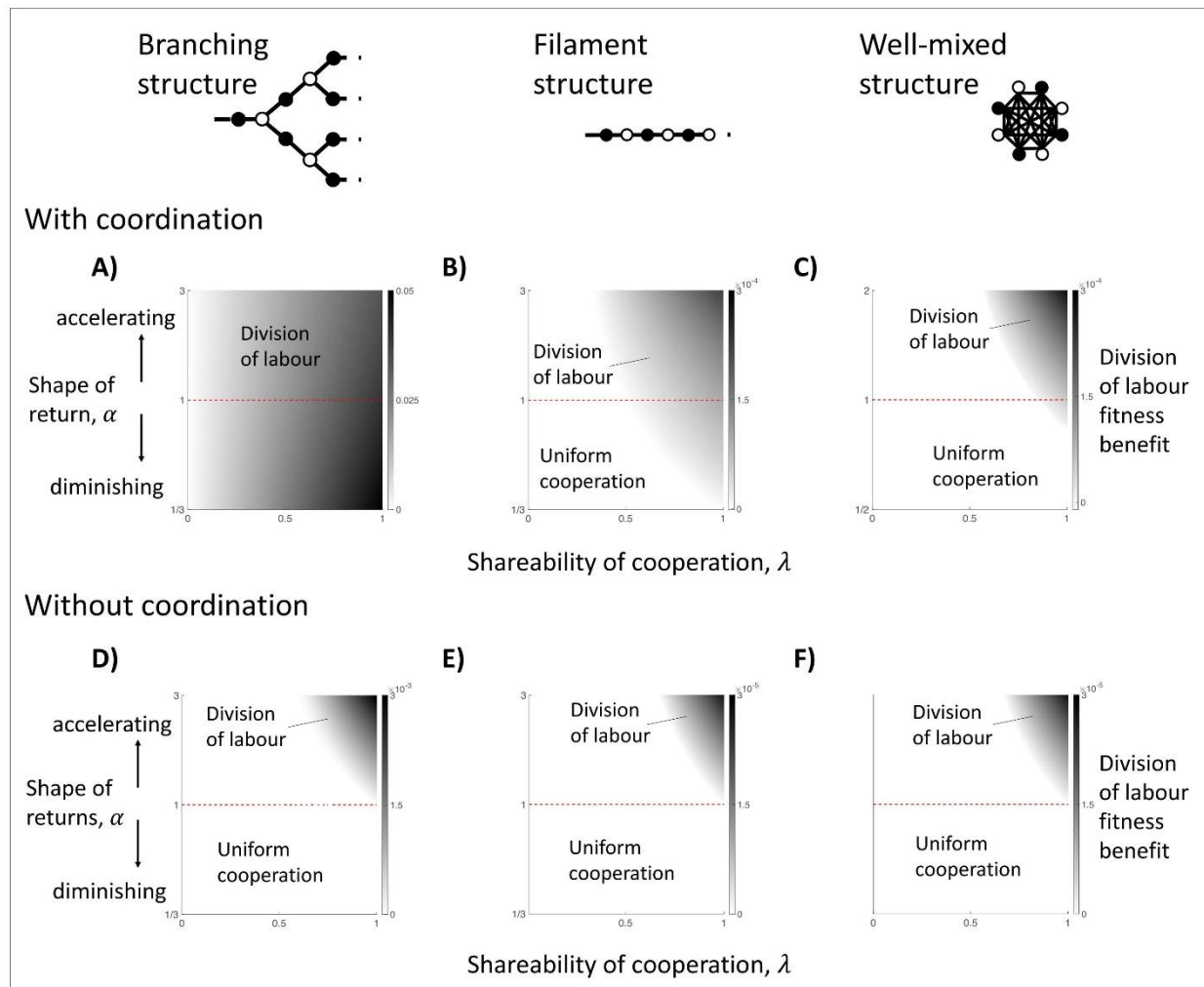


Figure 4. The impact of topological constraints on the division of labour. We show here different scenarios in which division of labour can evolve (non-white shades) and the size of its fitness benefit if so (darker shades). We consider three specific spatial models, including: a branching structure (A and D); a filament structure (B and E); and a well-mixed group (C and F). We consider when cells know their location in the group when specialising (with coordination; A–C) and when they do not (without coordination; D–F), in which case cells specialise randomly. (A) In a branching group structure with coordination, division of labour with diminishing returns from specialisation ($\alpha < 1$) can be favoured by between-individual differences whenever the benefits of cooperation are shared ($\lambda > 0$). (B) In a filament structure with coordination, division of labour with diminishing returns from specialisation ($\alpha < 1$) can be favoured by reciprocal specialisation when cells share a sufficient majority of the public good they produce with neighbours (e.g. when linear returns: $\lambda > \frac{1}{2}$). (C) In a well-mixed group with coordination, division of labour with diminishing returns from specialisation ($\alpha < 1$) can be favoured by reciprocal specialisation if cells share an even larger proportion of the public good they produce with neighbours (e.g. when linear returns: $\lambda > \frac{n-1}{n}$). (D–F) When cells specialise randomly (no coordination) across all three spatial models, then division of labour can only evolve if there is an accelerating return from investment in cooperation, $H(x) = x^\alpha$, where α controls the shape of the return. Throughout, we have assumed a linear return from fecundity specialisation, $F(x) = 1 - x$, and allow for a non-linear return from investment in cooperation, $H(x) = x^\alpha$, where α controls the shape of the return.

More generally, a formal analysis of arbitrary group structures reveals that division of labour by between-individual differences can always evolve whenever the number of neighbours varies for different cells in the group (Appendix A.2).

Can topological constraints lead to division of labour by reciprocal specialisation?

Consider a one-dimensional chain of cells, as examined by Yanni et al. Chains are found in species like cyanobacteria that form filaments of cells, and such structures might have been important at the onset of the evolution of multicellularity (Figure 4, Yanni et al., 2020). We assume arbitrarily that ‘odd’ cells along the filament are putative helpers and ‘even’ cells are putative reproductives. We otherwise make the same assumptions as for the branching structure model: there is a non-accelerating return from individual specialisation (i.e. $H''(z) \leq 0$ or $F''(z) \leq 0$), and the cell keeps a fraction $1 - \lambda$ of the public good that it produces, with the remaining fraction λ being shared equally by its direct neighbours.

If the amount of public good shared with neighbours is sufficiently large (high λ), then we find that division of labour via reciprocal specialisation can evolve (Figure 4B; Appendix A. 3). For instance, in the case of linear fecundity and public good returns ($H''(z) = F''(z) = 0$), division of labour by reciprocal specialisation can evolve if helpers share more of the public good that they produce with their neighbours than they keep for themselves ($\lambda > \frac{1}{2}$). If there are diminishing returns from specialisation ($H''(z) < 0$ or $F''(z) < 0$), then division of labour can still be favoured but then the amount of the public good preferentially shared with neighbours must be even greater still (higher λ ; Figure 4E).

For an arbitrary group structure, our analysis in the previous section implies that division of labour can evolve by between-individual differences, unless every cell in the group has the same number of neighbours. Consider a group in which every cell has exactly d neighbours. In this case, we show (Appendix A.5) that division of labour can still be favoured due to reciprocal specialisation if:

$$\lambda\mu > d \quad (3)$$

λ is the shareability of cooperation as defined previously and μ captures how easily the group can be ‘bi-partitioned’. That is, μ is a measure of the extent that the group can be divided into two classes of cells such that cells are neighbours with many cells of the opposing class and few neighbours of their own class. Thus, reciprocal specialisation can favour division of labour if: (1) groups are more sparse (low d); (2) groups are structured such that helpers can be neighbours with reproductives more than with other helpers, and vice versa (high μ); and/or (3) when the benefits of cooperation are preferentially shared with neighbours (high λ). In combination, these three factors amplify the synergistic benefits of reciprocal helper and reproductive specialisation, which can produce an accelerating fitness return for the group, even when there are non-accelerating returns from individual specialisation (Appendix C.1).

If one or two of these factors are particularly favourable for reciprocal specialisation, then the condition(s) on the remaining factor(s) can be relaxed. For instance, cells in a filament have only two neighbours ($d = 2$), and the potential alternation of helpers and reproductives in the filament means that helpers can share their cooperative public goods with reproductives exclusively (maximal μ). Consequently, reciprocal specialisation is possible even when the shareability of cooperation is reasonably low (e.g. $\lambda > \frac{1}{2}$ for linear benefits).

To conclude, topological constraints are not a single explanation for division of labour, in that they can favour division of labour for two different biological reasons. Different group structures can lead to either between-individual differences favouring division of labour (scenario 2) or reciprocal specialisation favouring division of labour (scenario 3). In all cases, there is an efficiency benefit from specialisation at the level of group fitness even if the returns from individual specialisation are non-accelerating.

Question 2: Are topological constraints required for division of labour without accelerating returns from individual specialisation?

We considered a well-mixed social group of n cells, where all cells share the benefits of cooperation with one another, and so there are no topological constraints (Figure 4). We then examined whether division of labour could be favoured by: (a) between-individual differences; and/or (b) reciprocal specialisation. In both cases, we assume that when a cell invests z into cooperation, it produces

an amount $H(z)$ of a public good. A cell keeps a fraction $1 - \lambda$ of the public good that it produces and the remaining fraction λ is shared by the rest of the social group members equally. We again consider the case where there is a non-accelerating return from individual specialisation ($H''(z) \leq 0$; $F''(z) \leq 0$).

Can between-individual differences favour division of labour without a topological constraint?

In the well-mixed group of identical cells, we find that division of labour cannot arise by between-individual differences (Appendix A.4). This is because all cells have the same number of neighbours, which we have shown more generally can never produce between-individual differences. This prediction could be violated if one of our assumptions do not hold: for instance, if there are consistent differences in the microenvironment that predispose some cells to one task or the other (Tverskoi et al., 2018; Tverskoi and Gavrilets, 2021; Yanni et al., 2020).

Can reciprocal specialisation favour division of labour without a topological constraint?

In the well-mixed group of identical cells, if the amount of public good shared with neighbours is sufficiently large (high λ), then we find that division of labour via reciprocal specialisation can evolve (Figure 4F; Appendix A.4). If there are linear returns from increased specialisation ($H''(z) = F''(z) = 0$), then division of labour can evolve when the public good produced by an individual benefits an average group member more than the producer ($\lambda > \frac{n-1}{n}$; Figure 4C). These results are like those found for a filament of cells (Figure 4B). In both cases, more generous sharing (higher λ) means that the synergistic benefits of reciprocal specialisation can be great enough to compensate for the non-accelerating returns from individual specialisation. In well-mixed groups, very generous sharing ($\lambda \approx 1$) also compensates for the fact that helpers are neighbours with all other helpers (no sparsity and minimally 'bi-partitionable').

To conclude, the well-mixed model shows that a topological constraint is not required for the evolution of division of labour with non-accelerating returns from individual specialisation. This result is in direct contradiction to that of Yanni et al., 2020. This difference arises because helpers in their model always benefit at least as much as any of its neighbours from its own public good production ($\lambda \leq \frac{n-1}{n}$) (Yanni et al., 2020). Our model allows for biological scenarios where the public good benefits an average neighbour more than the producer ($\lambda > \frac{n-1}{n}$). For instance, reproductive cells in cyanobacteria may absorb more of the fixed nitrogen produced by helpers than helpers do to meet the large energetic requirements of cell duplication and division (Flores and Herrero, 2010; Herrero et al., 2016; Meeks and Elhai, 2002). At the extreme, the public good can be an 'others-only' trait that benefits neighbours but not the producer at all ($\lambda = 1$) (Pepper, 2000). An example of this are the dispersal benefits provided by stalk cells in *Dyctiostelium discoideum* fruiting bodies or the self-sacrificing behaviour of helper cells in *Salmonella enterica* infections (Ackermann et al., 2008; Strmecki et al., 2005). Consequently, our model allows for a wider spectrum of biologically realistic scenarios. Critically, division of labour can be favoured in a group of well-mixed cells because it provides an efficiency benefit at the group level, via reciprocal specialisation (scenario 3), in an analogous way to our model with a one-dimensional chain of cells (question 1b).

Question 3: Is coordination required to favour division of labour without accelerating returns from individual specialisation?

We hypothesised that the benefits of between-individual differences (scenario 2) or reciprocal specialisation (scenario 3) rely on the implicit assumption that cells are coordinating which individuals specialise to become reproductive and helpers. This matters because mechanisms for coordinating division of labour, such as between cell signalling, might not be expected to exist before division of labour has evolved (Cooper et al., 2022; Liu et al., 2021). Consequently, if coordination was required, then this could limit the extent to which topological constraints favour the initial evolution of division of labour.

We investigated this hypothesis by repeating our above analyses, while assuming that cells do not have access to information that allows them to coordinate their phenotypes. Specifically, cells do not know if they are 'odd' or 'even', or if they are 'edge' or 'node'. We assumed instead that a reproductive

division of labour mutant induces each cell in the group to adopt the role of a helper or reproductive with a uniform probability (random specialisation). Random specialisation has been observed in a number of microbes (Ackermann et al., 2008; Diard et al., 2013; Veening et al., 2008). For filaments, branching group structures, and well-mixed groups, we found that division of labour can no longer evolve with non-accelerating returns from individual specialisation (Figure 4D–E; Appendices B.1 and B.2). In Appendix B.3, we have shown that this result holds for any group structure.

Consequently, for division of labour to evolve with non-accelerating returns from individual specialisation, there must exist some mechanism to coordinate which cells specialise to perform which tasks. It is possible that the mechanism need not produce a perfect allocation of labour across the group as analysed in our models (Liu et al., 2021). However, because division of labour cannot be favoured to evolve if role allocation is fully random, at least some degree of even imperfect between-cell coordination will be required.

A clear example of coordinated division of labour in topologically constrained groups is the use of between-cell signalling in some cyanobacteria filaments to determine which cells become sterile nitrogen fixing heterocysts and which cells become reproductive photosynthesisers (Flores and Herrero, 2010; Meeks and Elhai, 2002). However, a signal to coordinate distinct phenotypes must exist prior to or concurrently with the emergence of division of labour, and so a topological constraint is less likely to have favoured the initial evolution of division of labour in cyanobacteria. Alternatively, division of labour could have been favoured by an accelerating return from individual specialisation (scenario 1), with coordination only being favoured to evolve subsequently. Empirically, an accelerating return seems likely, as the key tasks performed by reproductives and helpers do not mix well (photosynthesis and nitrogen fixation) (Flores and Herrero, 2010; Meeks and Elhai, 2002).

These analyses do not suggest that topological constraints could never favour the initial evolution of division of labour. For instance, a pre-existing cue could allow division of labour to initially evolve with a metabolically cheaper form of coordination. More specifically, phenotype could be determined in response to the number of neighbours or the local concentration of some resource. Further, if there are pre-existing differences between individuals due to a pre-existing mechanism of coordination, then this mechanism can be co-opted to coordinate division of labour. However, the biological plausibility of any pre-existing mechanism would need to be explicitly justified and modelled on a case-by-case basis (Duarte et al., 2011). This would include modelling the metabolic cost, benefits and effectiveness of the mechanism (Cooper et al., 2022; Duarte et al., 2012; Liu et al., 2021). Empirically, while between-cell coordination has evolved in several labour-dividing microbial species, further studies—such as ancestral-state reconstructions—are needed to show whether coordination evolved prior to, concurrently with, or subsequent to division of labour in individual species.

In contrast, an accelerating return from individual specialisation depends on non-adaptive factors such as the physics, chemistry, or external constraints associated with the public good and its production. For instance, an accelerating return can arise if some intermediate products associated with cooperation and fecundity do not ‘mix-well’ on a chemical level. Consequently, no additional adaptive or pre-adaptation argument is needed to explain this pathway to division of labour.

Distinguishing the ultimate causes of division of labour in the wild

How can we distinguish empirically which of the different scenarios favoured real-world examples of division of labour (Figure 5)? We suggest experimental designs for microbial systems, where mixtures of helper and reproductive cells are grown together, and which make use of methods to genetically manipulate and measure the relative levels of cooperation and reproduction of each phenotype (Ackermann et al., 2008; Diard et al., 2013; Dragoš et al., 2018a; Dragoš et al., 2018b; Mavridou et al., 2018; Mridha and Kummerli, 2021; van Gestel et al., 2015). These are rough suggestions for the kind of experiments required, as details and possibilities will vary system from system, depending upon factors such as the degree of specialisation, the mechanism by which labour is divided, and what manipulations are possible. In addition, these experiments would need to follow from key first steps, such as demonstrating division of labour and a tradeoff between reproduction and cooperation (Diard et al., 2013; Dragoš et al., 2018a; Dragoš et al., 2018b; Veening et al., 2008; Zhang et al., 2020; Figure 5).

Testing for accelerating returns from individual specialisation (scenario 1): In at least three treatments, vary the level of cooperation performed by the helpers (Figure 5A top-left), to test whether the

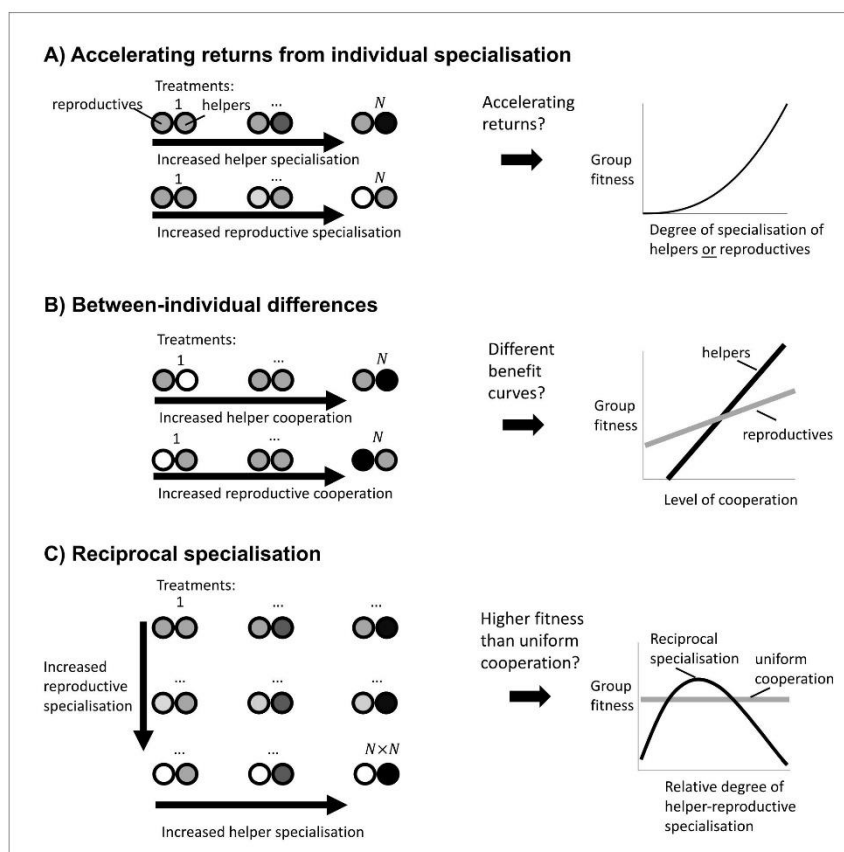


Figure 5. Experimental guidelines to distinguish the causes of division of labour. **(A)** To test whether division of labour is favoured by an accelerating return from individual specialisation, we must separately determine whether an increase in helper cooperation or a decrease in reproductive cooperation leads to an accelerating increase in group fitness. **(B)** To test whether division of labour is favoured by between-individual differences, we must determine whether an increase in cooperation by helpers produces a different group fitness benefit than an increase in cooperation by reproductives. **(C)** To test whether division of labour is favoured by reciprocal specialisation, we must determine whether there exists at least one relative degree of helper-to-reproductive specialisation for which group fitness is greater than the fitness of uniform cooperation.

benefits of increased cooperation are accelerating (**Figure 5A** right). Across at least three other treatments, vary the level of reproduction by the reproductives (**Figure 5A** bottom-left), to test whether the benefits of increased fecundity are accelerating (**Figure 5A** right). At least three treatments are required to be able to test for non-linear (accelerating) benefits.

Testing for between-individual differences (scenario 2): In some treatments, vary the level of cooperation of helpers (**Figure 5B** top-left). In other treatments vary the level of cooperation of reproductives (**Figure 5B** bottom-left). If division of labour evolves in this system by between-individual differences, then we should observe that group fitness varies differently depending on whether it is helpers or reproductives that are cooperating at a higher rate (**Figure 5B** right).

Testing for reciprocal specialisation (scenario 3): Use a classic $N \times N$ factorial experiment where the level of cooperation performed by helpers and the level of reproduction performed by reproductives are both varied (**Figure 5C** left). Division of labour is favoured by reciprocal specialisation if there is a significant interaction term between these two factors, with at least one treatment that produces a group fitness larger than that for uniformly cooperating cells (**Figure 5C** right).

Conclusion

Division of labour can be favoured to evolve without accelerating returns from individual specialisation. Nevertheless, for this to occur requires: (a) between-individual differences in task-efficiency or synergistic benefits from reciprocal specialisation and (b) a mechanism to coordinate which individuals perform which tasks. In contrast, accelerating returns can favour division of labour without a mechanism to coordinate task allocation, possibly making it more likely to favour the initial evolution of division of labour. Ultimately, determining the relative importance of these different pathways to division of labour is an empirical question, requiring experimental studies of the type we have outlined above.

Materials and methods

Resident strategy of uniform cooperation

We start by solving for the ESS strategy where both types of individuals invest the same amount in cooperation ($z_h = z_r = z$; uniform cooperation). This is the level of uniform investment in cooperation, z^* , for which there is no selection for a uniform change in the amount of cooperation by all individuals in the group:

$$\frac{\partial W(z, z)}{\partial z} \Big|_{z=z^*} = 0.$$

More explicitly, we can write this as:

$$n_h F V_h \left(\frac{F'}{F} + \frac{n_h V_h^{z_h} + n_r V_r^{z_h}}{n_h V_h} \right) \Big|_{z_h=z_r=z^*} + n_r F V_r \left(\frac{F'}{F} + \frac{n_h V_h^{z_r} + n_r V_r^{z_r}}{n_r V_r} \right) \Big|_{z_h=z_r=z^*} = 0 \quad (4)$$

where we have suppressed the functional dependencies for ease of presentation. The first term gives the group fitness change due to a marginal increase in ‘helper’ cooperation, and the second term gives the group fitness change due to a marginal increase in ‘reproductive’ cooperation. So, at the uniform strategy, z^* , any increase in the fitness caused by the increased cooperation of one subgroup of the population is balanced by a commensurate decrease in fitness caused by the same increase in cooperation for the other subgroup $\frac{\partial W(z_h, z_r)}{\partial z_h} \Big|_{z_h=z_r=z^*} = -\frac{\partial W(z_h, z_r)}{\partial z_r} \Big|_{z_h=z_r=z^*}$.

The constrained optimum, $(z_h, z_r) = (z^*, z^*)$, computed using **Equation 4** does not necessarily correspond to a critical point of W , that is, the first derivatives $\frac{\partial W(z_h, z_r)}{\partial z_h}$ and $\frac{\partial W(z_h, z_r)}{\partial z_r}$ might not vanish at this point. However, if the functions V_h and V_r are symmetrical, in the sense that

$$n_h V_h(z_h, z_r) = n_r V_r(z_r, z_h), \quad (5)$$

then the fitness function satisfies $W(z_h, z_r) = W(z_r, z_h)$, and it can be seen that this implies that the point $(z_h, z_r) = (z^*, z^*)$ is actually a critical point of $W(z_h, z_r)$.

Division of labour by between-individual differences

Division of labour by between-individual differences occurs if either of the first two terms of the Taylor expansion (**Equation 2**) is non-zero ($W^{z_h} \neq 0$ or $W^{z_r} \neq 0$). In this case, directional selection will increase or decrease the level of cooperation of one of the individual types. We give here the associated partial differentials of fitness (**Equation 1**):

$$\frac{\partial W(z_h, z_r)}{\partial z_h} \Big|_{z_h=z_r=z^*} = n_h F' V_h + n_h F V_h^{z_h} + n_r F V_r^{z_h}. \quad (6)$$

$$\frac{\partial W(z_h, z_r)}{\partial z_r} \Big|_{z_h=z_r=z^*} = n_r F' V_r + n_h F V_h^{z_r} + n_r F V_r^{z_r}. \quad (7)$$

These expressions capture the fitness consequences of a marginal increase in cooperation by helpers and reproductives, respectively. The first term of each captures the fecundity cost to own type of producing more public good, whereas the second term and third term are the viability benefits that accrue to both types from this increased cooperation. If directional selection in both traits is zero ($\frac{\partial W(z_h, z_r)}{\partial z_h} \Big|_{z_h=z_r=z^*} = 0$ and $\frac{\partial W(z_h, z_r)}{\partial z_r} \Big|_{z_h=z_r=z^*} = 0$), then $z_h = z_r = z^*$ is a critical point, and **Equations 6** and **7** imply that

$$\frac{F'}{F} \Big|_{z_h=z_r=z^*} = - \frac{n_h V_h^{\tilde{h}} + n_r V_r^{\tilde{h}}}{n_h V_h} \Big|_{z_h=z_r=z^*}. \quad (8)$$

$$\frac{F'}{F} \Big|_{z_h=z_r=z^*} = - \frac{n_h V_h^{\tilde{r}} + n_r V_r^{\tilde{r}}}{n_h V_h} \Big|_{z_h=z_r=z^*}. \quad (9)$$

These equations mean that, if $z_h = z_r = z^*$ is a critical point, then any marginal viability benefit to the group of increased cooperation by one subgroup is cancelled by the fecundity cost to that same subgroup. Moreover, **Equations 8 and 9** together imply that

$$\frac{n_h V_h^{\tilde{h}} + n_r V_r^{\tilde{h}}}{n_h V_h} \Big|_{z_h=z_r=z^*} = \frac{n_h V_h^{\tilde{r}} + n_r V_r^{\tilde{r}}}{n_h V_h} \Big|_{z_h=z_r=z^*} \quad (10)$$

If this equation does not hold, then $z_h = z_r = z^*$ is not a critical point, that is, there is a difference in the viability-fecundity tradeoffs between subgroups such that some individuals (without loss of generality, helpers) can secure larger benefits for the group at the same fecundity cost as others (reproductives). This gives our first condition for division of labour being able to evolve:

The between-individual differences condition for division of labour

$$\frac{n_h V_h^{\tilde{h}} + n_r V_r^{\tilde{h}}}{n_h V_h} \Big|_{z_h=z_r=z^*} > \frac{n_h V_h^{\tilde{r}} + n_r V_r^{\tilde{r}}}{n_h V_h} \Big|_{z_h=z_r=z^*}. \quad (11)$$

If individuals are indistinguishable when both types invest equally in cooperation ($z_h = z_r = z$), then the viability functions satisfy $V_h(z, z) = V_r(z, z)$. In this case, Condition 11 can be restated as:

$$V_h^{\tilde{h}} + \frac{n_r}{n_h} V_r^{\tilde{h}} > V_r^{\tilde{r}} + \frac{n_h}{n_r} V_h^{\tilde{r}}. \quad (12)$$

This says that the contribution to total viability from the increased specialisation of helper individuals is strictly larger than the contribution to total viability from the increased specialisation of reproductives. As a result, helpers are predisposed to become more helper-like as they can gain larger viability gains for the group than the other type of individual.

Division of labour by an accelerating return from individual specialisation

Division of labour by an accelerating return from individual specialisation can occur if either of the third or fourth terms in the Taylor expansion (**Equation 2**) are positive in value ($W^{\tilde{h}\tilde{h}} > 0$ or $W^{\tilde{r}\tilde{r}} > 0$). Taking the second derivative of fitness (**Equation 24**) with respect to each trait and evaluating at the critical point of uniform cooperation z^* gives:

$$\frac{\partial^2 W}{\partial z_h^2} \Big|_{z_h=z_r=z^*} = 2n_h F' V_h^{\tilde{h}} + n_h F'' V_h + n_h F V_h^{\tilde{h}\tilde{h}} + n_r F V_r^{\tilde{h}\tilde{h}}. \quad (13)$$

$$\frac{\partial^2 W}{\partial z_r^2} \Big|_{z_h=z_r=z^*} = 2n_r F' V_r^{\tilde{r}} + n_r F'' V_r + n_h F V_h^{\tilde{r}\tilde{r}} + n_r F V_r^{\tilde{r}\tilde{r}}. \quad (14)$$

The terms of **Equations 13 and 14** capture the second-order effects of increased investment in cooperation. The first term of each captures the decline in the fitness benefit of increased cooperation due to the cross-interaction between fecundity and viability. For instance, as a helper invests more in cooperation (higher x), it increases its own viability (higher V_h), but its fecundity declines as well (lower F) and so the relative benefit of this increased viability is lessened (the cross term $F' V_h^{\tilde{h}}$ is negative). This represents a kind of decelerating return from cooperation. The second term of each captures the second-order effect of decreased investment in fecundity. If this term is positive, then this means that there is a diminishing fecundity cost to increased investment in cooperation, which can favour division of labour. The third and fourth terms capture the second-order effect of increased investment in viability, that is, does each successive investment in the public good lead to a larger or smaller increase in viability than the previous investment of the same size? The return on investment (ROI) in viability is accelerating if $V_h^{\tilde{h}\tilde{h}} > 0$, $V_r^{\tilde{h}\tilde{h}} > 0$, $V_h^{\tilde{r}\tilde{r}} > 0$, and $V_r^{\tilde{r}\tilde{r}} > 0$. The ROI is diminishing if these second derivatives are negative: $V_h^{\tilde{h}\tilde{h}} < 0$, $V_r^{\tilde{h}\tilde{h}} < 0$, $V_h^{\tilde{r}\tilde{r}} < 0$, and $V_r^{\tilde{r}\tilde{r}} < 0$.

Thus if either **Equation 13** or **Equation 14** is positive, then division of labour is favoured to evolve. This gives the second condition for division of labour.

The accelerating returns from individual specialisation condition for division of labour

$$\frac{n_h V_h^{z_h z_h} + n_r V_r^{z_h z_h}}{n_h V_h} |_{z_h=z_r=z^*} + \frac{F''}{F'} |_{z_h=z_r=z^*} + 2 \frac{F'}{F} \frac{V_h^{z_h}}{V_h} |_{z_h=z_r=z^*} > 0, \quad (15)$$

$$\frac{n_h V_h^{z_r z_r} + n_r V_r^{z_r z_r}}{n_r V_r} |_{z_h=z_r=z^*} + \frac{F''}{F'} |_{z_h=z_r=z^*} + 2 \frac{F'}{F} \frac{V_r^{z_r}}{V_r} |_{z_h=z_r=z^*} > 0. \quad (16)$$

Fixing our attention on just **Equation 15** (or equivalently on 16), this condition states that the sum of the second-order viability effect from increased cooperation (first term on left-hand side) and the second-order fecundity effect of increased cooperation (second term on left-hand side) must be larger than the marginal fecundity cost of increased investment in viability (third term on left-hand side). Note that the third term on left-hand side is always negative because increased investment in viability decreases the value of increased investment in fecundity. Therefore, division of labour by a single-trait mutation can only happen if there is an accelerating ROI in at least one of fecundity, $F(z)$; helper viability, $V_h(z_h, z_r)$; or reproductive viability, $V_r(z_h, z_r)$.

Division of labour by reciprocal specialisation

The last remaining scenario for division of labour is that the resident strategy of uniform cooperation is unstable to mutations in both traits, which can occur depending on the value of the last term of the Taylor expansion (**Equation 2**; $W^{z_h z_r} \Delta z_h \Delta z_r$). This kind of instability can arise if there is a joint mutation that affects the level of cooperation of both helpers and reproductives at the same time ($\Delta z_h \neq 0$ and $\Delta z_r \neq 0$). However, it could also occur if a slightly deleterious mutation in one trait invades by drift and destabilises the other trait so much that the population evolves away from the critical point. In either case, it will be found that same condition must be satisfied in order for division of labour to evolve. In the rest of this section, we give the general analysis of whether $(z_h, z_r) = (z^*, z^*)$ is unstable to two-trait mutations, and then consider a simplifying special case to clarify the biological interpretation of this analysis.

Suppose that the resident strategy of uniform cooperation ($(z_h, z_r) = (z^*, z^*)$) is stable against single-trait mutations (i.e. Conditions 15 and 16 not satisfied). Then the resident strategy is unstable to two-trait mutations if and only if the determinant of the Hessian is negative:

$$\frac{\partial^2 W}{\partial z_h^2} \frac{\partial^2 W}{\partial z_r^2} < \left(\frac{\partial^2 W}{\partial z_h \partial z_r} \right)^2. \quad (17)$$

This condition is satisfied if the strength of directional selection pushing the population back to the critical point along either of the trait-value directions is less than the strength of directional selection on a trait when moved off of the critical point along the other trait direction. To evaluate the Hessian condition, we first compute the second-order cross derivatives:

$$\frac{\partial^2 W}{\partial z_h \partial z_r} |_{z_h=z_r=z^*} = \frac{\partial^2 W}{\partial z_r \partial z_h} |_{z_h=z_r=z^*} = F' (n_h V_h^{z_r} + n_r V_r^{z_h}) + F (n_h V_h^{z_r z_h} + n_r V_r^{z_r z_h}). \quad (18)$$

Here, we have used that $V_i^{z_h z_r} = V_i^{z_r z_h}$. Substituting this, and **Equations 13 and 14**, into the Hessian condition gives:

The reciprocal specialisation condition for division of labour

$$n_h V_h n_r V_r \left(\frac{n_h V_h^{z_h z_h} + n_r V_r^{z_h z_h}}{n_h V_h} + \frac{F''}{F'} + 2 \frac{F'}{F} \frac{V_h^{z_h}}{V_h} \right) \left(\frac{n_h V_h^{z_r z_r} + n_r V_r^{z_r z_r}}{n_r V_r} + \frac{F''}{F'} + 2 \frac{F'}{F} \frac{V_r^{z_r}}{V_r} \right) |_{z_h=z_r=z^*} < \left((n_h V_h^{z_r z_h} + n_r V_r^{z_r z_h}) + \frac{F'}{F} (n_h V_h^{z_r} + n_r V_r^{z_h}) \right)^2 |_{z_h=z_r=z^*} \quad (19)$$

Assume that neither Condition 15 nor 16 is satisfied, that is, the individual ROI is non-accelerating. Then the left-hand side of the inequality is strictly positive, which means that Condition 19 is nontrivial. We will see in examples that Condition 19 can be satisfied, which means that division of labour can evolve by reciprocal specialisation even when the individual ROI is diminishing.

To clarify further the biological meaning of Condition 19, consider a simple family of models in which viability and fecundity are linear functions. In this case, all second derivatives are zero, and so we get the simplified condition:

Simplified reciprocal specialisation condition for division of labour

$$4 n_h V_h^{z_h} n_r V_r^{z_r} |_{z_h=z_r=z^*} < (n_h V_h^{z_r} + n_r V_r^{z_h})^2 |_{z_h=z_r=z^*}. \quad (20)$$

In the case that the viability functions are (**Equation 5**) this condition further simplifies to:

$$V_h^{c,r}|_{z_h=z_r=z^*} < V_h^{c,r}|_{z_h=z_r=z^*}. \quad (21)$$

This inequality is satisfied if the viability of reproductives increases faster with increased cooperation from helpers, than it does from increased cooperation from reproductives. This makes clear that reciprocal specialisation can evolve if reproductives stand to gain more from help from helpers than they would gain by helping themselves.

Acknowledgements

We thank Thomas Scott and Zheren Zhang for their helpful comments and suggestions; St John's College, Oxford (GAC), and the ERC (Horizon 2020 Advanced Grant 834164; SAW) for funding.

Additional information

Funding

Funder	Grant reference number	Author
H2020 European Research Council	834164	Stuart Andrew West

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Author contributions

Guy Alexander Cooper, Hadleigh Frost, Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review and editing; Ming Liu, Stuart Andrew West, Conceptualization, Formal analysis, Writing – original draft, Writing – review and editing

Author ORCIDs

Guy Alexander Cooper  <http://orcid.org/0000-0002-1748-8183>

Ming Liu  <http://orcid.org/0000-0002-5170-8688>

Stuart Andrew West  <http://orcid.org/0000-0003-2152-3153>

Decision letter and Author response

Decision letter <https://doi.org/10.7554/eLife.71968.sa1>

Author response <https://doi.org/10.7554/eLife.71968.sa2>

Additional files

Supplementary files

- Transparent reporting form

Data availability

The Matlab (R2020b) source code used to generate Figures 4D-F is available at <https://osf.io/nw8gz/>.

The following dataset was generated:

Author(s)	Year	Dataset title	Dataset URL	Database and Identifier
Cooper GA	2021	Does the evolution of division of labour require accelerating returns from individual specialisation?	https://doi.org/10.17605/OSF.IO/NW8GZ	Open Science Framework, 10.17605/OSF.IO/NW8GZ

References

- Ackermann M, Stecher B, Freed NE, Songhet P, Hardt WD, Doebeli M. 2008. Self-destructive cooperation mediated by phenotypic noise. *Nature* **454**: 987–990. DOI: <https://doi.org/10.1038/nature07067>, PMID: 18719588
- Biggart NW. 1776. Readings in Economic Sociology. Oxford, UK: Methuen. DOI: <https://doi.org/10.1002/9780470755679>
- Bourke AFG. 2011. Principles of Social Evolution. Oxford: Oxford University Press. DOI: <https://doi.org/10.1093/acprof:oso/9780199231157.001.0001>
- Cooper GA, West SA. 2018. Division of labour and the evolution of extreme specialization. *Nature Ecology & Evolution* **2**: 1161–1167. DOI: <https://doi.org/10.1038/s41559-018-0564-9>, PMID: 29807994
- Cooper GA, Liu M, Peña J, West SA. 2022. The evolution of mechanisms to produce phenotypic heterogeneity in microorganisms. *Nature Communications*. In press.
- Diard M, García V, Maier L, Remus-Emsermann MNP, Regoes RR, Ackermann M, Hardt WD. 2013. Stabilization of cooperative virulence by the expression of an avirulent phenotype. *Nature* **494**: 353–356. DOI: <https://doi.org/10.1038/nature11913>, PMID: 23426324
- Dragoš A, Kieseewalter H, Martin M, Hartmann R, Wechsler T, Eriksen C, Brix S, Drescher K, Stanley-Wall N, Kümmerli R, Kovács ÁT. 2018a. Division of Labor during Biofilm Matrix Production. *Current Biology* **28**: 1903–1913. DOI: <https://doi.org/10.1016/j.cub.2018.04.046>, PMID: 29887307
- Dragoš Anna, Martin M, Falcón García C, Kricks L, Pausch P, Heimerl T, Bálint B, Maróti G, Bange G, López D, Lieleg O, Kovács ÁT. 2018b. Collapse of genetic division of labour and evolution of autonomy in pellicle biofilms. *Nature Microbiology* **3**: 1451–1460. DOI: <https://doi.org/10.1038/s41564-018-0263-y>, PMID: 30297741
- Duarte A, Weissing FJ, Pen I, Keller L. 2011. An Evolutionary Perspective on Self-Organized Division of Labor in Social Insects. *Annual Review of Ecology, Evolution, and Systematics* **42**: 91–110. DOI: <https://doi.org/10.1146/annurev-ecolsys-102710-145017>
- Duarte A, Pen I, Keller L, Weissing FJ. 2012. Evolution of self-organized division of labor in a response threshold model. *Behavioral Ecology and Sociobiology* **66**: 947–957. DOI: <https://doi.org/10.1007/s00265-012-1343-2>, PMID: 22661824
- Flores E, Herrero A. 2010. Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nature Reviews. Microbiology* **8**: 39–50. DOI: <https://doi.org/10.1038/nrmicro2242>, PMID: 19966815
- Herrero A, Stavans J, Flores E. 2016. The multicellular nature of filamentous heterocyst-forming cyanobacteria. *FEMS Microbiology Reviews* **40**: 831–854. DOI: <https://doi.org/10.1093/femsre/fuw029>, PMID: 28204529
- Hölldobler B, Wilson EO. 1990. The Ants. United States: Harvard University Press.
- Ispolatov I, Ackermann M, Doebeli M. 2012. Division of labour and the evolution of multicellularity. *Proceedings. Biological Sciences* **279**: 1768–1776. DOI: <https://doi.org/10.1098/rspb.2011.1999>, PMID: 22158952
- Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuysen P, Jansa J, Bücking H. 2011. Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. *Science* **333**: 880–882. DOI: <https://doi.org/10.1126/science.1208473>, PMID: 21836016
- Koufopanou V. 1994. The Evolution of Soma in the Volvocales. *The American Naturalist* **143**: 907–931. DOI: <https://doi.org/10.1086/285639>
- Levin SR, West SA. 2017. The evolution of cooperation in simple molecular replicators. *Proceedings of the Royal Society B* **284**: 20171967. DOI: <https://doi.org/10.1098/rspb.2017.1967>
- Liu M, West SA, Cooper GA. 2021. The evolution of mechanisms to divide labour in non-clonal populations. *Ecology and Evolution* **11**: 14475–14489. DOI: <https://doi.org/10.1002/ece3.8067>
- Mavridou DAI, Gonzalez D, Kim W, West SA, Foster KR. 2018. Bacteria Use Collective Behavior to Generate Diverse Combat Strategies. *Current Biology* **28**: 345–355. DOI: <https://doi.org/10.1016/j.cub.2017.12.030>
- Maynard Smith J. 1982. Evolution and the Theory of Games. Cambridge: Cambridge University Press. DOI: <https://doi.org/10.1017/CBO9780511806292>
- Maynard Smith J, Szathmáry E. 1995. The Major Transitions in Evolution. Oxford University Press.
- Meeks JC, Elhai J. 2002. Regulation of Cellular Differentiation in Filamentous Cyanobacteria in Free-Living and Plant-Associated Symbiotic Growth States. *Microbiology and Molecular Biology Reviews* **66**: 94–121; . DOI: <https://doi.org/10.1128/MMBR.66.1.94-121.2002>, PMID: 11875129
- Michod RE. 2006. The group covariance effect and fitness trade-offs during evolutionary transitions in individuality. *PNAS* **103**: 9113–9117. DOI: <https://doi.org/10.1073/pnas.0601080103>, PMID: 16751277
- Mridha S, Kummerli R. 2021. Enforced Specialization Fosters Mutual Cheating and Not Division of Labour in the Bacterium *Pseudomonas aeruginosa*. *bioRxiv*. DOI: <https://doi.org/10.1101/2021.06.26.450018>
- Oster GF, Wilson EO. 1978. Caste and Ecology in the Social Insects. United States: Princeton University Press.
- Pepper JW. 2000. Relatedness in trait group models of social evolution. *Journal of Theoretical Biology* **206**: 355–368. DOI: <https://doi.org/10.1006/jtbi.2000.2132>, PMID: 10988021
- Queller DC. 1985. Kinship, reciprocity and synergism in the evolution of social behaviour. *Nature* **318**: 366–367. DOI: <https://doi.org/10.1038/318366a0>
- Queller DC. 1997. Cooperators since life began. *The Quarterly Review of Biology* **72**: 184–188. DOI: <https://doi.org/10.1086/419766>
- Queller DC. 2011. Expanded social fitness and Hamilton's rule for kin, kith, and kind. *PNAS* **108 Suppl 2**: 10792–10799. DOI: <https://doi.org/10.1073/pnas.1100298108>, PMID: 21690389

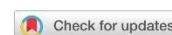
- Rueffler C, Hermisson J, Wagner GP. 2012. Evolution of functional specialization and division of labor. *PNAS* **109**: 326–335. DOI: <https://doi.org/10.1073/pnas.1110521109>, PMID: 22308336
- Schiessl KT, Ross-Gillespie A, Cornforth DM, Weigert M, Bigosch C, Brown SP, Ackermann M, Kümmerli R. 2019. Individual- versus group-optimality in the production of secreted bacterial compounds. *Evolution; International Journal of Organic Evolution* **73**:675–688. DOI: <https://doi.org/10.1111/evo.13701>, PMID: 30793292
- Solari CA, Kessler JO, Goldstein RE. 2013. A General Allometric and Life-History Model for Cellular Differentiation in the Transition to Multicellularity. *The American Naturalist* **181**: 369–380. DOI: <https://doi.org/10.1086/669151>, PMID: 23448886
- Staps M, Tarnita C. 2020. Evolution: How geometry shapes division of labor. *eLife* **9**: e63328. DOI: <https://doi.org/10.7554/eLife.63328>
- Strassmann JE, Zhu Y, Queller DC. 2000. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* **408**: 965–967. DOI: <https://doi.org/10.1038/35050087>
- Strmecki L, Greene DM, Pears CJ. 2005. Developmental decisions in *Dictyostelium discoideum*. *Developmental Biology* **284**: 25–36. DOI: <https://doi.org/10.1016/j.ydbio.2005.05.011>, PMID: 15964562
- Tverskoi D, Makarenkov V, Aleskerov F. 2018. Modeling functional specialization of a cell colony under different fecundity and viability rates and resource constraint. *PLOS ONE* **13**:0201446. DOI: <https://doi.org/10.1371/journal.pone.0201446>, PMID: 30089142
- Tverskoi D, Gavrilits S. 2021. The Evolution of Germ-Soma Specialization under Different Genetic and Environmental Effects. [bioRxiv]. DOI: <https://doi.org/10.1101/2021.04.02.438224>
- van Gestel J, Vlamakis H, Kolter R. 2015. Division of Labor in Biofilms: the Ecology of Cell Differentiation. *Microbiology Spectrum* **3**: 2014. DOI: <https://doi.org/10.1128/microbiolspec.MB-0002-2014>, PMID: 26104716
- Veening JW, Igoshin OA, Eijlander RT, Nijland R, Hamoen LW, Kuipers OP. 2008. Transient heterogeneity in extracellular protease production by *Bacillus subtilis*. *Molecular Systems Biology* **4**: 184. DOI: <https://doi.org/10.1038/msb.2008.18>
- West SA, Fisher RM, Gardner A, Kiers ET. 2015. Major evolutionary transitions in individuality. *PNAS* **112**: 10112–10119. DOI: <https://doi.org/10.1073/pnas.1421402112>
- West SA, Cooper GA. 2016. Division of labour in microorganisms: An evolutionary perspective. *Nature Reviews. Microbiology* **14**: 716–723. DOI: <https://doi.org/10.1038/nrmicro.2016.111>, PMID: 27640757
- Wyatt GAK, Kiers ET, Gardner A, West SA. 2014. A biological market analysis of the plant-mycorrhizal symbiosis. *Evolution; International Journal of Organic Evolution* **68**: 2603–2618. DOI: <https://doi.org/10.1111/evo.12466>, PMID: 24909843
- Yanni D, Jacobsen S, Márquez-Zacarias P, Weitz JS, Ratcliff WC, Yunker PJ. 2020. Topological constraints in early multicellularity favor reproductive division of labor. *eLife* **9**: e54348. DOI: <https://doi.org/10.7554/eLife.54348>, PMID: 32940598
- Zhang Z, Du C, de Barse F, Liem M, Liakopoulos A, van Wezel GP, Choi YH, Claessen D, Rozen DE. 2020. Antibiotic production in *Streptomyces* is organized by a division of labor through terminal genomic differentiation. *Science Advances* **6**:eaay5781. DOI: <https://doi.org/10.1126/sciadv.aay5781>, PMID: 31998842

A7

The evolution of mechanisms to
produce phenotypic
heterogeneity in microorganisms



ARTICLE



<https://doi.org/10.1038/s41467-021-27902-4>

OPEN

The evolution of mechanisms to produce phenotypic heterogeneity in microorganisms

Guy Alexander Cooper^{1,2✉}, Ming Liu², Jorge Peña³ & Stuart Andrew West²

In bacteria and other microorganisms, the cells within a population often show extreme phenotypic variation. Different species use different mechanisms to determine how distinct phenotypes are allocated between individuals, including coordinated, random, and genetic determination. However, it is not clear if this diversity in mechanisms is adaptive—arising because different mechanisms are favoured in different environments—or is merely the result of non-adaptive artifacts of evolution. We use theoretical models to analyse the relative advantages of the two dominant mechanisms to divide labour between reproductives and helpers in microorganisms. We show that coordinated specialisation is more likely to evolve over random specialisation in well-mixed groups when: (i) social groups are small; (ii) helping is more “essential”; and (iii) there is a low metabolic cost to coordination. We find analogous results when we allow for spatial structure with a more detailed model of cellular filaments. More generally, this work shows how diversity in the mechanisms to produce phenotypic heterogeneity could have arisen as adaptations to different environments.

¹St. John's College, Oxford OX1 3JP, UK. ²Department of Zoology, University of Oxford, Oxford OX1 3SZ, UK. ³Institute for Advanced Study in Toulouse, University of Toulouse Capitole, 31080 Toulouse, Cedex 6, France. ✉email: guy.cooper@zoo.ox.ac.uk

Different species use different mechanisms to produce adaptive phenotypic heterogeneity (Fig. 1)^{1–5}. In some cases, there is coordination across individuals to determine which individual will perform which role (*coordinated specialisation*)^{1,6}. This coordination could use signals, cues, or a developmental programme to provide information about the phenotypes adopted by other individuals in the group⁷. For example, when honey bee workers feed royal jelly to larvae to produce reproductive queens (Fig. 1a), or when the local density of a signalling molecule determines whether cyanobacteria cells develop into sterile nitrogen-fixing heterocysts (Fig. 1b)^{8–10}. In other cases, each individual adopts a helper phenotype with a certain probability, independently and without knowledge of the phenotypes adopted by other individuals (*random specialisation*)^{2,5,11,12}. For example, in *Salmonella enterica* co-infections, random biochemical fluctuations within each cell's cytoplasm are used to determine whether the cell sacrifices itself to trigger an inflammatory response that eliminates competitor species (Fig. 1d)^{12,13}. In yet further cases, the phenotype is influenced by the individual's genotype (genetic control). For instance, in some ant societies, whether individuals develop into queens, major or minor workers can be determined, in part, by their genes (Fig. 1c)^{3,14–16}. Across the tree of life, some species employ one mechanism to produce phenotypic heterogeneity whereas in other species mixed forms exist with a combination of coordinated specialisation, random specialisation, or genetic control^{3,15,17–22}.

We lack general evolutionary explanations for why different species use different mechanisms to produce phenotypic heterogeneity^{2,3,23,24}. Previous work has focused on the

non-reproductive division of labour in the social insects, and the proximate mechanisms that lead to different worker castes^{6,16,25–29}. However, the focus in that literature is on a different question—how different proximate mechanisms can produce coordinated specialisation—rather than the broader question of whether coordinated specialisation should be favoured over random specialisation or genetic control in the first place. It is with the reproductive division of labour that these three very different mechanisms have been observed in different species and for which there is an absence of evolutionary explanations^{2,3,23,24,30}.

Reproductive division of labour in bacteria and other microbes offers an excellent opportunity for studying why different mechanisms to produce phenotypic heterogeneity are favoured in different species^{1,2}. Reproductive division of labour occurs when social groups are composed of more cooperative ‘helpers’ who gain indirect fitness benefits by the aid they provide to less cooperative ‘reproductives’. Across microbes, the two primary mechanisms used to produce reproductive division of labour are coordinated and random specialisation (Fig. 2). Furthermore, while the form of cooperation and life histories of microbes share many similarities, they also vary in factors that could influence the evolution of division of labour, such as social group size^{31,32}.

We develop theoretical models to examine whether the relative advantages of random and coordinated specialisation can depend upon social or environmental conditions. Our aim is to use the reproductive division of labour in microbes as a ‘test system’ to address the broader question of whether evolutionary models can explain the diversity in the mechanisms that produce phenotypic heterogeneity more broadly. We show that coordinated specialisation is more likely to evolve over random specialisation in well-mixed groups when: (i) social groups are small; (ii) helping is more “essential”; and (iii) there is a low metabolic cost to coordination. We find the same qualitative results with deliberately simple models that are designed to capture the essence of the problem and with more detailed models that allow for spatial structure.

Results

We compare the relative fitness advantages of reproductive division of labour with either coordinated or random specialisation. Our first aim is to capture the problem in a deliberately

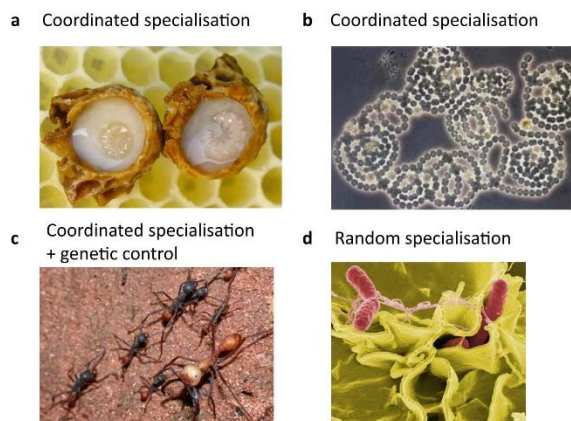


Fig. 1 Different mechanisms to produce phenotypic heterogeneity in nature. **a** In honey bee hives (*Apis mellifera*), larvae develop as sterile workers unless they are fed large amounts of royal jelly by adult workers (coordinated specialisation)⁸ (Photo by Waugsberg via the Wikimedia Commons). **b** In cyanobacteria filaments (*A. circinalis*), some individuals develop into sterile nitrogen fixers (lighter/yellow, round cells) if the amount of nitrogen fixed by their neighbours is insufficient (coordinated specialisation). This leads to a precise allocation of labour, with nitrogen-fixing cells distributed at fixed intervals along the filament⁹ (Picture by Dr. Imre Oldal via the Wikimedia Commons, cropped). **c** In the army ant (*Eciton Burchelli*), whether individual ants become a major or minor worker has a genetic component (genetic control)¹⁶ (Photo by Alex Wild via the Wikimedia Commons, cropped). **d** In *S. enterica* infections (serovar Typhimurium), each cell amplifies intracellular noise to determine whether it will self-sacrifice and trigger an inflammatory response that eliminates competing strains (random specialisation)¹³ (Photo by Rocky Mountain Laboratories, NIAID NIH via Wikimedia Commons).

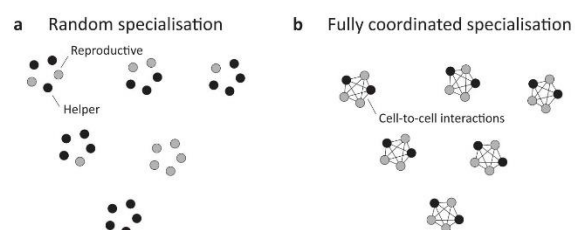


Fig. 2 Mechanisms to produce reproductive division of labour in clonal groups. We examine the relative advantages and disadvantages of the two key mechanisms to produce reproductive division of labour in social microorganisms^{1,5,11,46}. **a** Random specialisation occurs when cells randomly specialise into helpers or reproductives independently of one another. This can occur when a genetic feedback circuit is used to amplify small molecular fluctuations in the cytoplasm of each cell (phenotypic noise)^{4,11,12,78–80}. **b** Coordinated specialisation occurs when cells interact with one another, and share (or gain) phenotypic information while they are differentiating. This could occur through the secretion and detection of extracellular molecules (signals or cues), or with a shared developmental programme (epigenetics)^{1,2,25}.

simple model, which is easy to interpret, and can be applied across diverse microbe species^{33,34}.

We begin by assuming that coordinated specialisation always produces the optimal proportion of helpers and reproductives (fully coordinated specialisation) and that there is no within-group spatial structure (well-mixed groups; Methods: Well-mixed groups). We then test the robustness of our results by examining several alternate models for different biological scenarios (Supplementary Methods A–C) and by developing a more detailed model of growing cyanobacteria filaments that include the effects of within-group spatial structure (Methods: Cyanobacteria filaments). Throughout, we assume a form of cooperation that is common in microbes, where some individuals produce a ‘public good’ that benefits other cells^{1,2,9,12,21,35–40}.

Random specialisation vs fully coordinated specialisation. We assume that a single cell arrives on an empty patch and, through a fixed series of replications, produces a clonal group of n individuals that consists of k sterile helpers and $n - k$ pure reproductives ($k \in \{0, 1, 2, \dots, n\}$). We define the average group fecundity, $g_{k,n}$, as the reproductive success of a particular group in the absence of mechanism costs. This is measured as the per cell number of offspring that would disperse at the end of the group life cycle, given by

$$g_{k,n} = \frac{1}{n}(n - k)f_{k,n}, \quad (1)$$

where $n - k$ is the number of reproductives in the group, and $f_{k,n}$ is the fecundity of each reproductive in the absence of mechanism costs (Methods: Labour dividers and their fitness). We assume that $f_{k,n}$ increases with the number of helpers in the whole group (k).

Expression (1) highlights the trade-off between the number of reproductives in the group ($n - k$), which is higher when there are fewer helpers (lower k), and the amount of help that those reproductives obtain ($f_{k,n}$), which is higher when there are more helpers (higher k). If the division of labour is favoured, the balance of this trade-off leads to an optimal number of helpers, k^* , that is intermediate (i.e., $0 < k^* < n$), giving $g_{k^*,n}$ as the maximal reproductive success of the group (Methods: Fully coordinated specialisation).

In species that divide labour by coordination, the outcome of individual specialisation depends on the phenotypes of social group neighbours. Our first model is deliberately agnostic to the details of how phenotype information is shared between group members in order to facilitate predictions across different systems. For instance, individuals may share phenotype information via signalling between cells or with a common developmental programme (Fig. 2b)^{1,2,41}. We make the simplifying assumption that individuals coordinate fully so that coordinated groups always form with precisely the optimal number of helpers, k^* . The disadvantage of coordinated specialisation is that the mechanism could incur metabolic costs, such as the production of extracellular signalling molecules. The fitness of a group of coordinated specialisers is given by:

$$w_C = (1 - c_C)g_{k^*,n}, \quad (2)$$

where $g_{k^*,n}$ is the average group fecundity with the optimal number of helpers, k^* , and $0 \leq c_C \leq 1$ is the metabolic cost of coordination, whose form we leave unspecified but could in principle depend on further factors such as group size (Methods: Fully coordinated specialisation). We use ‘metabolic costs’ as a shorthand for all fixed costs at the time of differentiation, which could include other factors such as delaying reproduction. A number of different models have examined how different

proximate mechanisms can produce coordinated division of labour in specific systems^{6,25,28,29}.

In species that divide labour by random specialisation, each individual in the group independently becomes a helper with a given probability and a reproductive otherwise (Fig. 2a). Hence, the final number of helpers in the group is a binomial random variable. We assume here that the probability of adopting a helper role is equal to the optimal proportion of helpers ($p^* = k^*/n$). In principle, differences between the optimal probability of adopting a helper role and the optimal proportion of helpers could arise if there are different costs on average from producing groups with more or fewer helpers than is optimal. In Supplementary Methods A.2, we show that the same qualitative results arise if the probability of adopting a helper role maximises the fitness of randomly specialising cells. Thus, the expected fitness of a group of random specialisers is given by:

$$w_R = (1 - c_R) \sum_{k=0}^n \binom{n}{k} p^{*k} (1 - p^*)^{n-k} g_{k,n}, \quad (3)$$

where $0 \leq c_R \leq 1$ is the metabolic cost of random specialisation, which we assume is independent of the number of helpers in the group, k (Methods: Random specialisation). The potential advantage of random specialisation is that there may be fewer upfront metabolic costs from, for example, between cell signalling (i.e., if $c_R < c_C$ holds). The downside of random specialisation is that it can incur a stochastic cost: groups will often form with fewer or more helpers than is optimal (developmental stochasticity). Stochastic costs occur whenever groups arise with a sub-optimal composition, which is captured in our model by how the fitness of the group depends upon the number of helpers (Eq. 3).

The optimal proportion of helpers could depend upon the environment, and the probability of becoming a helper could be conditionally regulated in response to environmental cues. However, for simplicity, all our analyses assume a stable environment and ignore such regulation.

We need to specify how reproductive fecundity depends on the number of helpers in the group. This relationship will determine the functional cost of having a sub-optimal proportion of helpers. We focus on one of the most common forms of cooperation in microbes, where individuals secrete factors that provide a benefit to the local population of cells (‘public goods’)³⁸. We assume that the amount of public good in the social group depends linearly on the number of helpers in the group and is ‘consumed’ by all group members equally^{42,43}. An example of such public good is found in *Bacillus subtilis* populations, where only a subset of cells (helpers) produce and secrete proteases that degrade proteins into smaller peptides, but where these are then re-absorbed as a nutrient source by all cells^{44,45}. Further experimental evidence is needed to show that the non-helper cells in *B. subtilis* populations are more reproductive.

We allow the relative importance of producing public goods to vary between species. Each reproductive has a baseline fecundity, $b \geq 0$, that is independent of the amount of public good in the group. The fecundity benefit of helpers scales according to $h \geq 0$ as the amount of public good in the group increases. When reproductives have no baseline fecundity ($b = 0$) we say that cooperation is essential. When baseline fecundity is non-zero ($b > 0$), cooperation is non-essential and the ratio h/b provides a useful metric for the relative importance of cooperation.

Our assumptions give the following expression for the fecundity of a reproductive (Methods: Linear public goods):

$$f_{k,n} = b + h \frac{k}{n}, \quad (4)$$

By substituting Eq. 4 into Eqs. 1–3, we can determine when the fitness of coordinated specialisation is greater than the fitness of

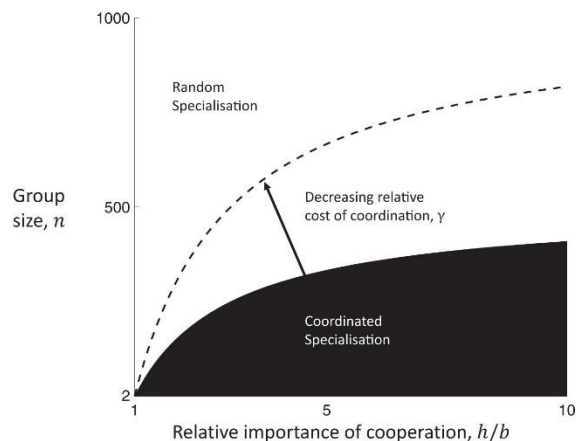


Fig. 3 Random versus coordinated specialisation. Small group sizes (lower n), relatively more important cooperation (higher h/b), and lower relative metabolic costs to coordination (lower γ) favour division of labour by coordinated specialisation (black) over division of labour by random specialisation (white). This is a visual depiction of Condition 5. We have used $\gamma = 2 \times 10^{-3}$ (solid boundary) and $\gamma = 1 \times 10^{-3}$ (dashed boundary). We note that the limit as the relative importance of cooperation goes to infinity (very large h/b) converges to the outcome for when cooperation is essential ($b = 0$).

random specialisation (i.e., $w_C > w_R$), which gives the simplified condition:

$$\gamma < \frac{h-b}{n(h+b)}, \quad (5)$$

where $\gamma = (c_C - c_R)/(1 - c_R)$ captures the relative change in metabolic costs paid when switching to coordinated specialisation from random specialisation (Methods: Linear public goods). If $h < b$, then sterile helpers are disadvantageous and the group is composed of all reproducers ($k^* = 0$). Thus, division of labour with sterile helpers is favoured to evolve only when $h > b$, which we will assume henceforth. In order for coordination to be favoured, the relative metabolic cost of coordination, γ , must be less than the relative fitness advantage of coordination over random specialisation in the absence of metabolic costs. Consequently, we term the right-hand side of Eq. 5 as the stochastic cost of random specialisation (Supplementary Discussion). Thus, Condition (5) specifies that coordinated specialisation is favoured when the relative metabolic cost of coordination (γ), is less than the stochastic cost of random specialisation (right-hand side). The condition can be used to predict how key environmental and ecological factors will influence which labour-dividing mechanism is more likely to evolve (Fig. 3).

Prediction 1. Smaller relative metabolic costs of coordination favour coordinated specialisation. When the metabolic cost of coordination is smaller (lower c_C) and the metabolic cost of random specialisation is larger (higher c_R), then the relative cost of switching from random specialisation to coordinated specialisation is lessened (smaller γ), which favours the evolution of coordinated specialisation (smaller left-hand side of Condition 5). If the metabolic costs of random specialisation are equal to or larger than the metabolic costs of coordination ($c_R \geq c_C \Rightarrow \gamma \leq 0$), then coordinated specialisation is always the favoured mechanism (Condition 5 always satisfied). Conversely, random specialisation can only ever be the favoured strategy ($w_R > w_C$; Condition 5 not satisfied) if the metabolic costs of random specialisation are less

than the metabolic costs of coordination ($c_C > c_R \Rightarrow \gamma > 0$; a necessary but not sufficient condition). This arises directly from our starting assumption that coordinated specialisation always produces groups with the optimal proportion of helpers whereas random specialisation may often produce groups that are sub-optimal.

Larger metabolic costs of coordinated specialisation ($c_C < c_R \Rightarrow \gamma > 0$) may be a reasonable assumption for many biological systems. The metabolic costs of random specialisation are determined by the production costs of the regulatory proteins employed in the genetic feedback circuit that amplifies intracellular noise^{4,5,46,47}. In contrast, coordinated specialisation requires both an intracellular genetic feedback circuit and some mechanism by which phenotype is communicated between cells, such as the costly production and secretion of extracellular signalling molecules^{1,2,9,41,48,49}. Coordinated specialisation could also take more time, leading to delayed reproduction.

If coordination is more metabolically costly ($c_C > c_R$), the optimal mechanism to divide labour depends on how the relative metabolic cost of coordination ($\gamma > 0$) balances against the benefit of avoiding the stochastic cost of random specialisation (right-hand side of Condition 5). The stochastic cost of random specialisation is determined entirely by: (i) the relative likelihood that random groups deviate from the optimal proportion of helpers, and (ii) the degree to which those deviations from the optimal proportion of helpers leads to a reduced average fecundity for the group (Methods: Linear public goods). Equation (5) shows how the importance of these two factors depends upon the size of the group (n) and on the relative importance of cooperation (h/b).

Prediction 2. Smaller social groups favour coordinated specialisation. The number of cells in the group has a large impact on the relative likelihood that random groups deviate from the optimal proportion of helpers (Fig. 3). In smaller groups, random specialisation can lead more easily to the formation of groups with a realised proportion of helpers that deviates significantly from the optimum. In contrast, in larger groups, the realised proportion of helpers will be more closely clustered about the optimal proportion with the highest fitness. This effect of group size on the stochastic cost of random specialisation is a consequence of the law of large numbers. For example, outcomes close to 50% heads are much more likely when tossing 100 coins in a row compared to only tossing 4 coins in a row where no heads or all heads may frequently occur.

Our prediction is related to a previous result from sex allocation theory. When mating occurs in small groups, small brood sizes select for more precise and less female-biased sex ratios, as there would otherwise be a high probability of producing a group containing no males at all^{50–52}. In another analogue, Wahl showed a mechanistically different effect when the division of labour is determined genetically and the number of group founders is small: groups may sometimes form that do not contain all of the genotypes required to produce all of the necessary phenotypes in the division of labour²⁴.

Prediction 3. The higher the relative importance of cooperation, the more coordinated specialisation is favoured. When the relative importance of cooperation is larger (higher h/b), the fitness costs incurred from producing too few helpers increases. In addition, as the relative importance of cooperation increases (higher h/b), the optimal proportion of helpers increases to 50% helpers ($p^* \approx \frac{1}{2}$). This increases the variance in the proportion of helpers produced by random specialisers, and so sub-optimal groups may arise even more frequently (Methods: Linear public goods). Thus, higher

relative importance of cooperation increases both: (i) the likelihood that groups deviate from the optimal proportion of helpers; and (ii) the scale of the fitness cost when they do. Both of these effects increase the stochastic cost of random specialisation (larger right-hand side of Condition 5), and thus favour the evolution of coordinated specialisation (Fig. 3).

Alternative forms of cooperation. The above analysis employs a deliberately simple public goods model, focusing on factors that are expected to be relevant across many microbial systems. This facilitates the interpretation of our results and generates broadly applicable predictions that are less reliant on the details of particular species.

In order to test the robustness of our results (predictions 1–3) we also developed a series of alternative simplified models corresponding to different biological scenarios (Supplementary Methods A and B; Supplementary Figs. 1–3). We examined the possibility that the public good provided by helpers: (i) is not consumed by its beneficiaries, as may occur when self-sacrificing *S. enterica* cells enter the gut to trigger an immune response that eliminates competitors (non-rivalrous or non-congestible collective good; Supplementary Methods B.2); or (ii) is only consumed by the reproductives in the group, as may preferentially occur for the fixed nitrogen secreted by heterocyst cells in *A. cylindrica* filaments (excludible or club good; Supplementary Methods B.3)^{9,12,53,54}. We allowed for randomly specialising cells to maximise their own probability of becoming helpers (Eq. 3; Supplementary Methods A.2), for reproductive fecundity to depend non-linearly on the proportion of helpers in the group (Supplementary Methods A.3), for helpers to have some fecundity (non-sterile helpers; Supplementary Methods B.4), and for division of labour to occur in each generation of group growth (Supplementary Methods B.5). In all of these alternative scenarios, we found qualitative agreement across the three predictions of the linear public goods model.

We found that less specialised helpers (with some fecundity) favour random specialisation over coordinated specialisation (Supplementary Methods B.4). In contrast to prediction 3, more fecund helpers can lead to a scenario where the larger relative importance of cooperation (higher h/b) disfavors coordinated specialisation. This occurs because high relative importance of cooperation (higher h/b) can produce groups composed predominantly of non-sterile helpers ($p^* \approx 1$), where the likelihood that random groups deviate from the optimal proportion of helpers is significantly diminished.

In Supplementary Methods C, we develop an individual-based simulation that also supports predictions 1–3. In addition, this simulation shows that costly coordination can evolve incrementally from random specialisation, and that intermediate levels of coordination can be favoured (Supplementary Fig. 4)⁵⁵.

Division of labour in a cyanobacteria filament. We then developed a more mechanistically detailed model of a growing cyanobacteria filament to investigate the impact of within-group spatial structure (Methods: Cyanobacteria filaments)⁵⁶. When there is insufficient fixed nitrogen (N_2) in the environment, some cyanobacteria species will facultatively divide labour between reproductive cells (autotrophs) that photosynthesise light and sterile helper cells (heterocysts) that fix and secrete environmental N_2 (Fig. 1b)^{9,57,58}. The fixed N_2 diffuses along the filament where it is used by reproductives to grow and produce new cells. Division of labour in cyanobacteria is a canonical example of coordinated specialisation as helpers produce a variety of signalling molecules that diffuse along the filament to ensure that a regular pattern of

phenotypes develops (Fig. 1b)^{9,57,58}. Previous models of cyanobacteria focused on determining the signalling and regulatory network required to recreate the exact pattern of heterocysts along the filament^{57,59–63}.

Cyanobacteria spores (hormogonium) tend to contain multiple cells^{9,57}. In order to consider the case where cooperation is essential, we assume that each filament begins as a clonal sequence of two reproductives (R) and two helpers (H) in the arrangement H-R-R-H (Methods: Life cycle). In Methods: Simulation results and Supplementary Fig. 6, we show that the same qualitative results hold for the alternative assumption where all spore cells are reproductive (R-R-R-R). Over time, the number of cells in the filament increases as reproductives grow and divide by binary fission to produce within-filament offspring cells, which become either helpers or reproductives (Fig. 4a). The group life cycle ends when the filament has reached a maximum size of L cells. At this time, the reproductives in the filament produce dispersing spores that found filaments in the next generation of the group life cycle and all remaining cells die (non-overlapping generations)⁹.

Reproductives grow over time by absorbing fixed N_2 , until they reach a critical size for cellular replication (Methods: Size of reproductives and Replication of reproductives). Each reproductive receives fixed N_2 from the abiotic environment at a rate of $\phi \geq 0$ units of fixed N_2 per unit time (uniform background density of fixed N_2)⁶³. In addition, each helper in the filament produces fixed N_2 , at a maximum rate of $\phi > 0$ units of fixed N_2 per unit time. We assume that the fixed N_2 produced by a helper disperses across the filament with a diffusion factor, $0 < \eta \leq 1$, where limited diffusivity (small η) means that only reproductives near the helper benefit from the fixed N_2 it produces and high diffusivity (large η) means that even distant reproductives along with the filament benefit (Methods: The local density of the public good across the filament). For the purposes of a focused analysis on the reproductive division of labour, we ignore other forms of phenotypic heterogeneity that cyanobacteria filaments may engage in, such as the production of ATP for the group by autotrophs (non-reproductive division of labour) and the formation of persister cells in some environments (bet-hedging)^{1,58,64–66}.

Upon replication, whether a new cell becomes a helper or a reproductive depends on four evolutionary traits that jointly determine the extent of division of labour and coordination in the filament (q, s, d , and v ; Fig. 4b; Methods: How cells specialise). The baseline probability ($0 \leq q \leq 1$) is the underlying probability that a cell becomes a helper in the absence of coordination. The level of signalling ($0 \leq s \leq 1$) is the fraction of resources that a helper commits to the production and secretion of signalling molecules. The signalling molecules produced by a helper disperses along the filament with a diffusivity that we assume is distinct from the N_2 diffusivity (Fig. 4a). The local density of signalling molecules allows new cells to estimate how close they are to a helper, or how many helpers there may be nearby.

Whether and how the new cell responds to the signal depends on the response sensitivity ($v \geq 0$) and the response threshold ($d \geq 0$; Fig. 4b; Methods: How cells specialise). If $v = 0$, then a new cell is insensitive to the signal and adopts the helper phenotype with the baseline probability q (random specialisation). If the new cell is sensitive to the signal ($v > 0$) then a local signal density that is greater than the response threshold, d , will lead the cell to be less likely to adopt the helper phenotype (Fig. 4b). A higher signal density than the threshold produces the opposite effect. As sensitivity increases (higher v), the response to the signal becomes more deterministic (Fig. 4b).

Increasing levels of coordination (higher v and s), allows for more precise patterning of helpers and reproductives in the

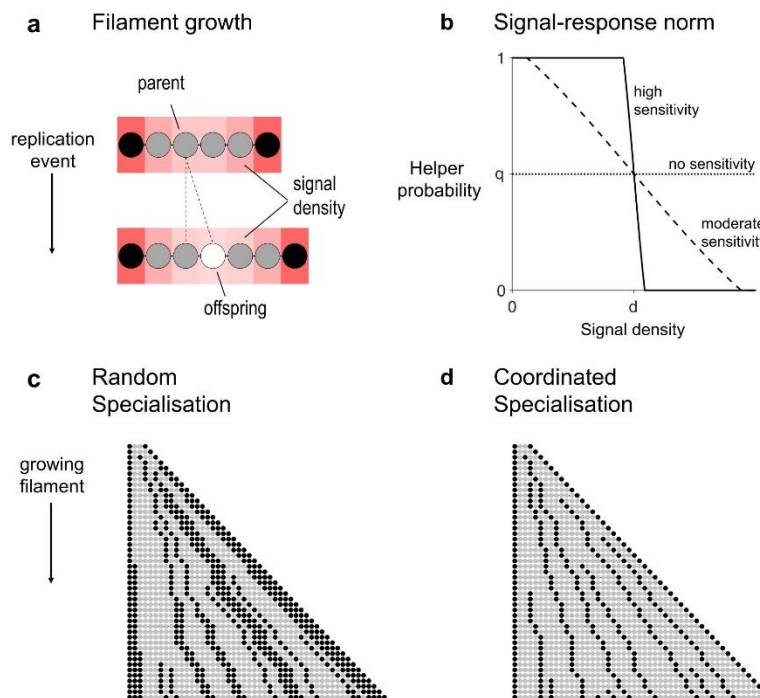


Fig. 4 Division of labour in a cyanobacteria filament. **a** Black cells represent helpers, and grey cells reproductives. When a reproductive replicates, the parent cell produces an offspring cell (white cell) to one side of itself along the filament. The blue shading shows the density of the signal molecule produced by the helpers as it diffuses along the filament. **b** When an offspring cell is sensitive to the signal ($v > 0$), a greater (lesser) signal density will decrease (increase) the probability that it becomes a helper ($q = 0.5, d = 5, v = 0, 0.2, 1$). **c** A simulated example of a filament growing that employs random specialisation ($q = 0.33, s = 0, d = 0$, and $v = 0$). **d** A simulated example of a filament growing that employs coordinated specialisation ($q = 0.33, s = 0.1, d = 1$ and $v = 1.5$) (Methods: Cyanobacteria filaments). The helper cells (black) are more evenly spaced out (less clumped) with coordinated specialisation compared to with random specialisation.

filament (compare Fig. 4c, d). However, we assume that increased coordination is metabolically costly. First, as helpers produce more signalling molecules (higher s), they can produce proportionally less fixed N_2 . Second, new cells that are more sensitive to the local density of the signalling molecule (higher v) incur a more severe time delay before they can specialise, such that reproductives ultimately take longer to reach the critical size of replication.

Cyanobacteria filaments employ such a signalling system and do not simply use the local density of fixed N_2 as a cue. A possible reason for this is that signalling molecules could be fast to produce and secrete and thus coordination can occur even before helpers begin to fix N_2 ⁶². Furthermore, using a dedicated signal could be more reliable than one based on fixed N_2 density alone, which might be biased by transient fluctuations in the background level of fixed N_2 (ϕ).

Simulations. We simulated an evolving population to estimate the strategy that is favoured by natural selection in different scenarios (q^*, s^*, d^*, v^*) (Methods: Evolution of coordination and Simulation results, and Supplementary Table). We started with a uniform population that specialises randomly ($s = d = v = 0$), and allowed the helper probability (q) to mutate and evolve for 500 generations, until an approximate equilibrium was reached. We then held the baseline helper probability (q) fixed and allowed the coordination traits (s, d and v) to mutate and evolve for 3500 generations. Each generation, the mutant strategy successfully replaces the resident strategy if it has a higher estimated average fitness. We calculate the fitness of individual filaments as

the summed fecundity of reproductives in the last generation of the group life cycle, divided by the amount of time that it took the filament to grow to L cells. The separate phases of the evolutionary simulation facilitate cleaner convergence of trait values, with an equilibrium generally being reached within 100–200 generations (Supplementary Fig. 5).

We found that the degree to which specialising cells evolve to coordinate can depend on social and environmental factors. In particular, both a lower background density of fixed N_2 (small ϕ) and more limited diffusion of fixed N_2 along the filament (smaller η) lead to the evolution of higher signalling levels (larger s^* ; Fig. 5a) and higher response sensitivities (larger v^* ; Fig. 5b). This produced filaments with a more precise allocation of labour across the filament (Fig. 5e). We quantify the extent of coordination by dividing the variance among the number of helpers in a contiguous sub-block of 10 cells by the variance that would be expected for a binomial random variable of the same mean (Methods: Simulation results). Higher values of the reciprocal of this ratio indicate the more precise division of labour.

The predictions of our cyanobacteria model agree broadly with those of our simpler analytical model. When there is limited diffusion of helper-fixed N_2 (low η), reproductives must depend primarily on helpers that are nearer along the filament, producing a smaller effective social group size (analogous to lower n). With random specialisation, a smaller social group can lead to proportions of helpers that deviate more from the optimum, increasing the benefit that can be obtained by coordination (Fig. 3). When the background density of fixed N_2 is small (low ϕ), this increases the relative benefit

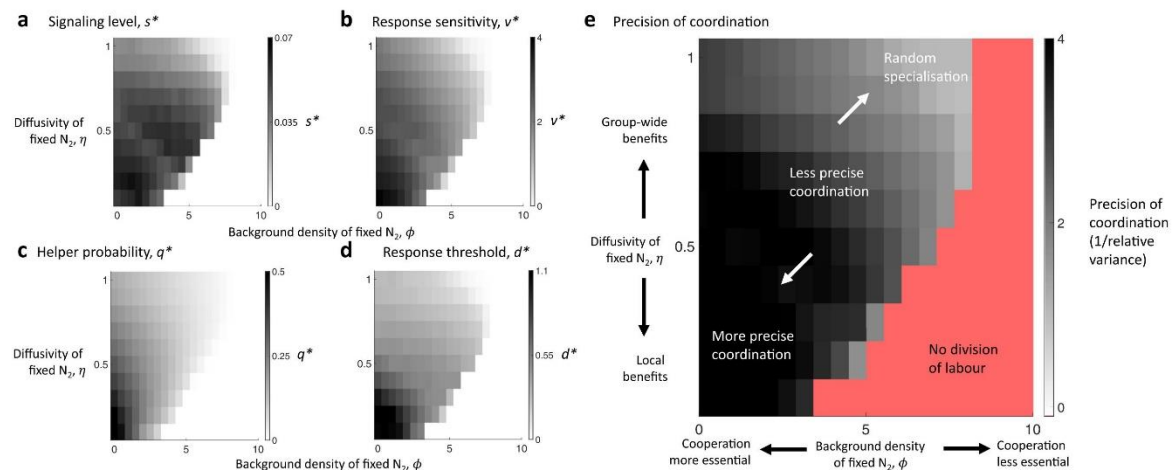


Fig. 5 The optimal level of coordination. We present simulation results for two key factors that affect the optimal level of coordination (Methods: Simulation results). A lower background density of fixed N_2 (smaller ϕ) and more limited diffusion of helper-fixed N_2 (smaller η) favours: **a** the evolution of a higher level of signalling (larger s^*); **b** a higher response sensitivity to the signal (larger v^*); **c** a higher baseline helper probability (larger q^*); and **d** a higher response threshold (larger d^*); **e** The effect of higher levels of both signalling (larger s^* in **a**) and response sensitivity (larger v^* in **b**) is that groups form with a more precisely coordinated helper distribution. The precision of coordination is calculated by dividing the variance in the number of helpers in a contiguous sub-block of 10 cells relative to the variance that would be expected for a binomial random variable of the same mean (Methods: Simulation results). Higher values of the reciprocal of this ratio suggest more precisely coordinated division of labour (darker shades).

of cooperation (analogous to higher h/b). With an increased benefit from cooperation, there is a greater advantage from coordinating to produce the optimum proportion of helpers (Fig. 3). In addition, our cyanobacteria model shows how intermediate coordination can be favoured in certain scenarios (Fig. 5).

However, care is required when examining factors in mechanistic models that can have additional effects unaccounted for by their analogues in simpler models. For instance, an increase in the background density of fixed N_2 (higher ϕ) means that cooperation is relatively less important (lower h/b), which we have found favours less coordination (Fig. 5). Relatively less important cooperation (lower h/b) in the mechanistic model also means that helpers may be willing to dedicate more effort to signal production (higher s) as there is then a relatively lower fitness cost to producing less of the public good. Another example is how helpers that produce more fixed N_2 (larger ϕ) not only lead to cooperation that is relatively more important (higher h/b) but can also lead to larger effective social groups sizes (larger n) as the increased good that helpers produce can then diffuse further along with the filament and benefit reproductives that are farther away.

Spatial structure and helper clumping. Our simulations show that coordination ($s^* > 0, v^* > 0$) is often favoured over random specialisation ($s^* \approx 0, v^* \approx 0$; Fig. 5a, b). In social groups with rigid spatial structure and local cooperation (lower η), an effective division of labour requires a regular distribution of helpers across the group. We hypothesised that random specialisation is particularly disadvantageous in such groups because it can lead to contiguous groups of helpers (clumps) that expand as the whole group grows, incurring a high functional or stochastic cost (compare Fig. 4c, d; Supplementary Fig. 7). The helpers within these clumps can neither reproduce to break up the clump nor are they close enough to reproductives to provide fixed N_2 . We performed additional simulations to investigate the likelihood and impact of helper clumping in growing filaments (Methods: The effect of helper clumping).

We found that a lower background density of fixed N_2 (smaller ϕ) and more limited diffusion of fixed N_2 (smaller η), leads to randomly specialising filaments with a larger average clump size (measured in the number of helpers per clump; Fig. 6a), and a higher cost of clumping (measured as the slope of the best-fit line of average clump size on filament fitness; Fig. 6b). A higher propensity to form clumps arises because a lower background density of fixed N_2 (smaller ϕ) and more limited diffusion of fixed N_2 (smaller η) means new cells are more likely to become helpers (larger q^* ; Fig. 5c). A higher cost to clumping arises in this case (smaller ϕ and η) because reproductives that are far from helpers have much lower fecundity, which increases the pressure for an even distribution of helpers (high functional costs). Combined, these patterns help to explain why random specialisation is disfavoured in this extreme (lower left corner of Fig. 5a, b, e).

Focusing on the extreme case of essential cooperation ($\phi = 0$) and very low diffusion of fixed N_2 ($\eta = 0.1$), we found that coordination has two effects on clumping. First, the fitness cost of clumping is more severe in coordinated filaments than in randomly specialising filaments (Fig. 6d). This occurs because coordinated helpers also invest in signalling molecules and so produce less of the public good than randomly specialised helpers, which amplifies the costs of clumping. Second, coordination leads to a large reduction in the average size of clumps, and so the cost associated with larger clumps is almost never paid (Fig. 6c, d). Consequently, coordination ($s^* > 0, v^* > 0$) can produce a substantial fitness advantage in spatial groups by decreasing the chance that costly helper clumps can form and grow.

Discussion

Our analyses provide a theoretical framework to help explain why different species of microorganisms use different mechanisms to divide labour². Coordinated division of labour is more likely to be favoured when: (i) social groups are small; (ii) helping is more “essential”; and (iii) there is a low metabolic cost to coordination. While testing our predictions with a formal comparative analysis would require data from more species, our predictions can help to

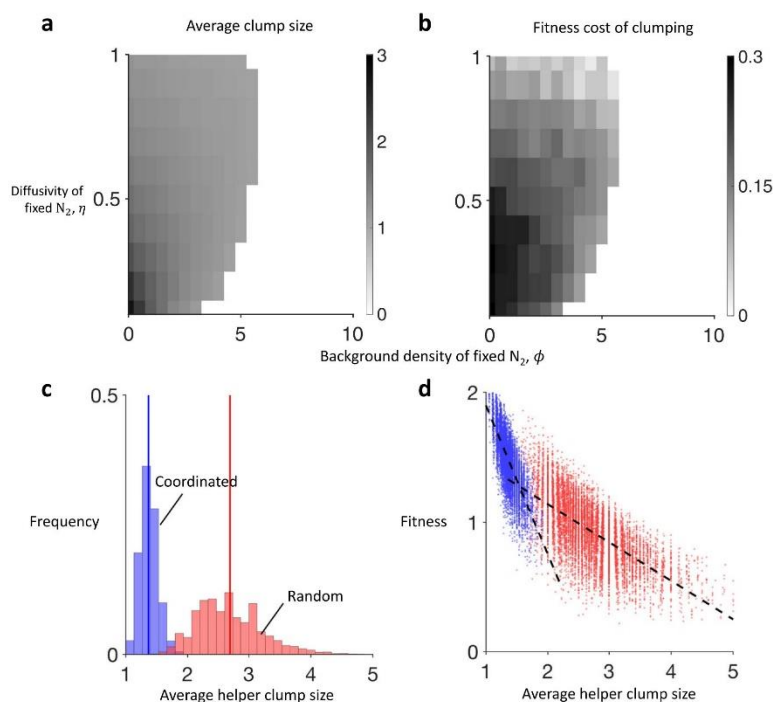


Fig. 6 Spatial structure and helper clumping. In randomly specialising filaments, a smaller background density of fixed N_2 (smaller ϕ) and more limited diffusion of helper-fixed N_2 (smaller η), lead to filaments with: **a** a larger average clump size, measured as the average number of helpers per clump; and **b** a higher fitness cost of clumping, measured as the slope of the least-squares linear regression of relative fitness on average clump size. We constructed **a**, **b** by performing 1000 independent simulations of growing filaments for each parameter combination, where the trait values are set to the associated optima for random specialisation determined in the previous analysis ($q = q^*$, $s = 0$, $d = 0$, and $v = 0$). We then performed 5000 independent simulations of both coordinated (blue) and random (red) filament growth at the extreme case of essential cooperation ($\phi = 0$) and very limited diffusion of fixed N_2 ($\eta = 0.1$). **c** Coordination leads to a dramatic reduction in average clump sizes across filaments (average clump size for random (red): 1.4 helpers and coordinated (blue): 2.7 helpers). **d** The absolute fitness cost of larger clumps is greater for coordination specialisation (blue) than for random specialisation (red) but filaments that pay the higher cost of coordination are rare. Slope of least-squares linear regression for random: -0.30 and coordinated: -1.1 . Mean squared error of fit for random: 0.033 and coordinated: 0.019. Source data for panels **c** and **d** are provided as a Source Data file. Further details are given in Methods: The effect of helper clumping.

understand the mechanisms that have evolved in well-studied examples.

There are many reasons why coordinated specialisation was favoured to evolve in cyanobacteria filaments. First, cyanobacteria only divide labour when fixed N_2 is growth-limiting and so the relative importance of cooperation is high (low ϕ and high h/b)^{9,58,63}. Second, the fixed nitrogen produced by helpers diffuses along the filament, preferentially aiding nearby reproductive and so the effective social group size is small (low η and small n)^{9,49,67}. Third, the initial costs of coordination may have been quite small as new cells could use the local level of fixed N_2 as a cue (low η)⁶⁸. Finally, cyanobacteria filaments have a rigid spatial structure with local benefits from cooperation and thus random specialisation could have led to the accumulation of large sterile clumps, which is a very inefficient distribution of phenotypes (high functional or stochastic cost; Fig. 6).

Colonies of *Volvox carteri* and *Dictyostelium discoideum* use coordination to divide labour, despite the fact that these groups are composed of large numbers of cells (high n ; on the order of 1000s of cells or more)^{20,69–71}. This highlights that no single factor can fully explain empirical patterns, and that further factors not captured by simple models might be relevant in specific cases. For instance, colonies of *Volvox carteri* require a specific spatial distribution of flagella beaters across the group, which may create a strong selection pressure for coordination, analogous to the avoidance of clumps in

cyanobacteria filaments. Furthermore, in some cases, details of the mechanism of division of labour are still not well understood. For instance, it is possible that there is also an initially random component to pre-stalk specialisation in *Dictyostelium*⁷⁰.

There are multiple reasons why random specialisation would have been favoured to evolve in other well-studied species. In *Salmonella enterica*, the self-sacrificing helper cells provide a competitive advantage that eliminates other microbes but is not “essential” to the replication of *Salmonella* cells (lower h/b)^{12,13}. Further, the benefits of cooperation are provided to all cells in the co-infection ($\eta = 1$) and so the effective social group size is reasonably large (higher n). Finally, *Salmonella* pathogens do not have a rigid spatial structure and so there is no scope for the accumulation of growing helper clumps as for cyanobacteria filaments. In *Bacillus subtilis*, a subset of cells become helpers that produce and secrete protein degrading proteases⁴⁴. However, these helper cells are not sterile and so the consequence of deviating from the optimal caste ratios is reduced (Supplementary Methods B.4).

To conclude, most previous work on phenotypic heterogeneity has tended to be either mechanistic, focusing on how different phenotypes are produced (caste determination), or evolutionary, focusing on why heterogeneity is favoured in the first place^{1–6,8,11,15,23–28,30,49,70,72–77}. We have used evolutionary models to address the broader question of why different mechanisms are used in different species^{2,3,12,23–25}. Focusing on

the reproductive division of labour in microorganisms, we have shown that coordinated specialisation is more likely to be favoured over random specialisation in small groups, when relative coordination costs are low, and when there are larger fitness costs to deviating from optimal caste ratios. We have also shown how these patterns can hold in groups with spatial structure, where there can be a large pressure for an even distribution of phenotypes. These results identify social and environmental factors that could help to explain the distribution of mechanisms to produce phenotypic heterogeneity that has been observed in bacteria, other microbes, and beyond. Aside from microorganisms, our results also suggest a hypothesis for why random caste determination has not been widely observed in animal societies. During the initial evolution of complex animal societies, group sizes were likely to be small and the relative costs of coordination might have been minor compared to each individual's day-to-day organismal metabolic expenditure.

Methods

Well-mixed groups

Labour dividers and their fitness. We assume that a single individual arrives on an empty patch and, through a fixed series of replications, forms a clonal group of n individuals, k of which are sterile helpers and $n - k$ of which are pure reproductives, where $k \in \mathcal{N} = \{0, 1, 2, \dots, n\}$. The average fecundity (fitness) of the group in the absence of mechanism costs, $g_{k,n}$, is measured by the per cell number of offspring that would disperse at the end of the life cycle. Denoting by $f_{k,n}$ the fecundity of each reproductive in the group in the absence of fecundity costs, the fecundity of the group is given by Eq. 1. We assume that $f_{k,n}$ depends only on the proportion of helpers in the group, $p = k/n$, with $p \in \mathcal{P} = \{0, \frac{1}{n}, \frac{2}{n}, \dots, \frac{n-1}{n}, 1\}$, so that we can write

$$f_{k,n} = F(p), \quad (6)$$

where F is a real function. We further assume that F is increasing on the interval $[0, 1]$ (i.e., the fecundity of each reproductive is increasing in the proportion of helpers in the group). We can then rewrite Eq. 1 as

$$g_{k,n} = (1 - k/n)F(k/n) = G(k/n), \quad (7)$$

where we have defined

$$G(p) = (1 - p)F(p). \quad (8)$$

Fully coordinated specialisation. With fully coordinated specialisation (C), we assume that some mechanism, such as signalling between cells, ensures that groups always form with the optimal proportion of helpers, p^* , where

$$p^* = \frac{k^*}{n}, \text{ with } k^* = \arg \max_{k \in \mathcal{N}} g_{k,n}. \quad (9)$$

The fitness of a group of coordinated specialisers with an optimal proportion of helpers, p^* , is given by Eq. 2, where

$$g_{k^*,n} = G(p^*) \quad (10)$$

and $0 \leq c_C \leq 1$ is the metabolic cost of coordination. We make no further assumptions on the functional form of c_C . However, we note that it could in principle depend on other model parameters, such as group size n .

Random specialisation. With random specialisation (R), each individual in the group independently becomes a helper with probability q , and a reproductive otherwise. Hence, the number K of helpers in the group is a binomial random variable with parameters n and q (i.e., $K \sim \text{Binomial}(n, q)$). In the following, it will also be convenient to write $Q = K/n$ for the random variable giving the proportion of helpers in the group. Then, the expected fitness of a group of random specialisers is given by

$$w_R(q) = \sum_{k=0}^n \binom{n}{k} q^k (1-q)^{n-k} (1 - c_R) g_{k,n}, \quad (11)$$

where $0 \leq c_R \leq 1$ is the metabolic cost of random specialisation. We assume that this cost is independent of the number of helpers k . In contrast to Eq. 3, we have not yet specified here that $q = p^*$.

Linear public goods. Our main model assumes that the fecundity function F is given by

$$F(p) = b + hp, \quad (12)$$

where $b \geq 0$ is a parameter that quantifies the baseline fecundity of reproductives in the absence of cooperation, and $h > 0$ is the scale of the benefits from increased cooperation (higher proportion of helpers). If there is no baseline fecundity

($b = 0$), cooperation by helpers is essential (i.e., the fecundity of reproductives is positive if and only if there are helpers around). If $b > 0$, cooperation is non-essential, with a lower value of b or a higher value of h leading to a larger relative importance of cooperation for the fecundity of reproductives. When cooperation is non-essential ($b > 0$), the ratio h/b is a useful metric for the relative importance of cooperation. Substituting Eq. 12 into Eq. 6 gives Eq. 4.

Replacing (12) into (8) we obtain

$$G(p) = (1 - p)(b + hp) \quad (13)$$

To find the fitness of coordinated specialisers, we first approximate p by a continuous variable, and calculate the derivative

$$G'(p) = h - b - 2hp.$$

This derivative is decreasing in p (i.e., $G(p)$ is concave), and has a single root given by

$$\hat{p} = \frac{h - b}{2h} = \frac{1}{2} \left(1 - \frac{b}{h} \right). \quad (14)$$

Such a root lies in the interval $(0, 1)$ if and only if $h > b$. Otherwise, the maximiser of $G(p)$ (and hence the optimal allocation of helpers) is given by $\hat{p} = 0$ (i.e., it is optimal to have no helpers). To avoid this trivial scenario without division of labour, henceforth we assume that $h > b$ holds. Further, to make progress we approximate the optimal allocation of helpers, p^* , by $\hat{p}$. The actual optimal value p^* will be a value near $\hat{p}$ but constrained by the permissible group compositions, since $p^* \in \mathcal{P}$ (cf. Supplementary Methods A.1, where we relax the assumption that $p^* \approx \hat{p}$). When cooperation is essential ($b = 0$), $\hat{p} = 1/2$. When cooperation is non-essential ($b > 0$), the approximate optimal proportion (Eq. 14) is an increasing function of h/b with $\lim_{b \rightarrow 0} \hat{p} = 1/2$. An approximation to the fitness of fully coordinated specialisers can be obtained by substituting Eq. 10 into Eq. 2 and letting $p^* \approx \hat{p}$:

$$w_C(p^*) = (1 - c_C)G(p^*) \approx (1 - c_C)G(\hat{p}). \quad (15)$$

To find the fitness of random specialisers, we substitute Eq. 13 into Eq. 7, Eq. 7 into Eq. 11, and simplify to obtain

$$\begin{aligned} w_R(q) &= (1 - c_R) \sum_{k=0}^n \binom{n}{k} q^k (1 - q)^{n-k} \left(1 - \frac{k}{n} \right) \left(b + h \frac{k}{n} \right) \\ &= (1 - c_R) \left(bE \left[1 - \frac{k}{n} \right] + hE \left[\left(1 - \frac{k}{n} \right) \frac{k}{n} \right] \right) \end{aligned} \quad (16)$$

$$\begin{aligned} &= (1 - c_R) \left(b(1 - q) + hq(1 - q) - h \frac{q(1 - q)}{n} \right) \\ &= (1 - c_R)G(q) - h\text{Var}(Q) \end{aligned} \quad (17)$$

where we have made use of the first two moments of the binomial distribution, $E[K] = nq$, $E[K^2] = nq(1 - q) + (nq)^2$ and of the fact that $\text{Var}(Q) = q(1 - q)/n$.

In order to determine the condition under which coordinated specialisation is favoured over random specialisation, we assume in a first step that random specialisers play the strategy, q^* , so that their fitness is given by

$$w_R(p^*) = (1 - c_R)(G(p^*) - h\text{Var}(P^*)), \quad (18)$$

where $P^* = K^*/n$ and $K^* \sim \text{Binomial}(n, p^*)$ (and hence $\text{Var}(P^*) = p^*(1 - p^*)/n$). This assumption simplifies our calculations and leads to results that are qualitatively similar to those that arise from the more parsimonious assumption that random specialisers play the strategy that maximises their fitness (cf. Supplementary Methods A.2, where we assume that random specialisers play optimally).

We can evaluate the condition for coordinated specialisation to be favoured over random specialisation (i.e., when $w_C(p^*) > w_R(p^*)$ holds) by comparing Eq. 15 and Eq. 18. The condition is given by

$$\text{Var}(P^*) \frac{h}{G(p^*)} > \frac{c_C - c_R}{1 - c_R} \equiv \gamma. \quad (19)$$

The left-hand side of this inequality is the normalised fecundity benefit of switching from random specialisation to coordinated specialisation, and the right-hand side of the inequality (γ) is the normalised relative change in metabolic costs paid from doing so. Inequality 19 shows that the fecundity benefit of coordination over random specialisation can be decomposed into a measure of the deviation from the optimal allocation of labour, $\text{Var}(P^*)$, and a quantity that captures the relative cost of deviating from the optimal proportion of helpers, $h/G(p^*)$.

To obtain a simple expression of Condition 19 in terms of our parameters (n , b , and h) we approximate p^* by $\hat{p}$ as given in Eq. 15 to obtain

$$\text{Var}(P^*) \approx \frac{(h - b)(h + b)}{4nh^2}, \quad (20)$$

$$\frac{h}{G(p^*)} \approx \frac{4h^2}{(h + b)^2}. \quad (21)$$

With these approximations, Condition 19 becomes Condition 5. Note that the left-hand side of Condition 5 is increasing in the benefits of cooperation h and decreasing in group size n and the baseline fecundity b . Since $G(p^*)$ is

(approximately) independent of n , we can say that the effect of increasing the group size acts primarily on the deviation of groups from the optimal proportion of helpers, $\text{Var}(P^*)$. In contrast, a smaller baseline fecundity (lower b) or more benefits from cooperation (larger h) both (i) push p^* closer to $1/2$ (which in turns increases the variance $\text{Var}(P^*)$) and (ii) increases the cost of deviation (larger $h/G(P^*)$) and thus acts via both factors.

Cyanobacteria filaments

Life cycle. Here, we give details on the processes that govern filament growth in our model. A filament begins as an array of four connected cells and increases in number until it reaches a maximum of L cells, at which point all reproductive cells produce a large number of offspring that disperse to found filaments in the next generation of the group life cycle. The remaining cells then die (i.e., generations are non-overlapping).

We let I_t be the number of cells in the filament at time $t \geq 0$, and $I_t = \{1, \dots, L_t\}$ be the set of (indexes to) individuals in the filament. Each individual cell has the fixed phenotype of a helper or a reproductive. Further, let $H_t \subset I_t$ and $R_t \subset I_t$ be, respectively, the set of helpers and reproductives in the filament at time t . We assume that the two interior cells are reproductives and the two exterior cells are helpers at the start of the filament growth ($t = 0$). That is, we have $I_0 = \{1, 2, 3, 4\}$, $H_0 = \{1, 4\}$, and $R_0 = \{2, 3\}$.

The local density of the public good across the filament. The rate at which a reproductive absorbs the public good depends on the local density of the public good at its location in the filament. We assume that the density of the public good at location $i \in R_t$ at time t is equal to

$$\Phi_i^t = \phi + \sum_{j \in H_t} \phi_{ij}^t, \quad (22)$$

where $\phi \geq 0$ is the uniform background density of the public good due to the environment, and $\phi_{ij}^t \geq 0$ is the increase in the local density of the public good that is due to helper j at time t , which is assumed to be given by

$$\phi_{ij}^t = \bar{\phi}(1-s)^{\xi} \frac{\eta^{i-j}}{\sum_{k \in I_t} \eta^{k-j}}, \quad (23)$$

where $\bar{\phi}$ is the maximum rate of public good production by a helper, $(1-s)^{\xi}$ is the degree to which this production decreases due to a trade-off with the production of signalling molecules (as described below), and the last factor ensures that ϕ_{ij}^t declines by a factor of $0 < \eta \leq 1$ for every cell position that separates the reproductive cell i from the helper j (diffusion factor of fixed nitrogen). When η is small, helpers only provide substantial public good benefits to their nearest neighbours. When η is large, even reproductives at a considerable distance along the filament receive public good benefits. The denominator of this last factor enforces a conservation principle such that an increase in diffusivity, η , does not artificially increase the amount of the public good produced by helpers. In the limit as $\eta \rightarrow 1$, all reproductives benefit equally from the efforts of each helper.

Size of reproductives. Reproductive cells grow over time as they absorb the public good from the environment. We could alternatively conceptualise this process as an increase in energy or resource reserve over time. Let π_i^t be the size of reproductive cell $i \in R_t$ at time t . We assume that each reproductive starts with base size $\pi_i^0 = 0$. For any time interval Δt during which no cell divides anywhere in the filament, the increase in size of a reproductive cell $i \in R_t$ is calculated as

$$\pi_i^{t+\Delta t} = \pi_i^t + \Psi_i^t \Delta t,$$

where Ψ_i^t is the instantaneous growth rate of reproductive $i \in R_t$. We assume that Ψ_i^t is an increasing but diminishing function of the rate of public good that is absorbed at its location, Φ_i^t , according to the functional form

$$\Psi_i^t = \psi \left(1 - e^{-\mu \Phi_i^t} \right), \quad (24)$$

where ψ is the maximum growth rate, and larger μ leads to a more diminishing curve.

Replication of reproductives. Reproductives grow until they reach a critical size $\bar{\pi}$, at which point they divide by budding off a daughter cell to one side of the parent cell along the filament. At any time, t , we calculate the time until the next replication, τ_i^t , using the following procedure. For each reproductive cell $i \in R_t$, we calculate its expected time until replication as

$$\tau_i^t = \frac{\bar{\pi} - \pi_i^t}{\Psi_i^t}, \quad (25)$$

i.e., the amount it has left to grow divided by its growth rate. Here, we have held fixed the growth/replication of all other reproductives. Thus, the next reproductive to divide, in this case, is simply the reproductive with the smallest expected time to replication, $\tau_i^t = \min_{j \in R_t} \tau_j^t$. When a reproductive cell $i \in R_t$ divides, it buds off a daughter cell either before or after the parent cell along the filament with equal probability. The positions, phenotypes, and sizes of all other cells in the filament are reindexed to account for the new cell (all cells to the right of the daughter cell

move one space further along the indexing array). Whether the daughter cell becomes a helper or a reproductive depends on the mechanism of specialisation (see below). The parent reproductive cell size is reset to zero and, if the daughter is reproductive, its size is set to zero as well. The group life cycle ends when the filament has reached a size of L cells, at which point all reproductives produce a large number of offspring that disperse to found filaments in the next generation of the group life cycle. The remaining cells then die (i.e., generations are non-overlapping).

How cells specialise. When a new cell is produced, whether the cell becomes a helper or a reproductive depends on the mechanism of specialisation. There are four co-evolving traits in our model that combined determine the mechanism of specialisation: (i) the baseline probability, $0 \leq q \leq 1$, (ii) the level of signalling, $0 \leq s \leq 1$, (iii) the response sensitivity, $v \geq 0$, and (iv) the response threshold, $d \geq 0$. The baseline probability, q , is the probability that the new cell adopts a helper role in the absence of coordination (i.e., if either $s = 0$ or $v = 0$). If $s > 0$, helper cells produce signalling molecules that diffuse along the length of the filament. Let i be the position of the new cell at time t in the filament and where the indices of all other cells have been updated to account for the new cell. The level of the signal detected by the cell is

$$\chi_i^t = \max \left(\lambda s \sum_{j \in H_t} \xi^{i-j} + \varepsilon, 0 \right), \quad (26)$$

where λs is the maximum rate of signal produced by each helper, and ξ is the factor by which the signal declines for each position that separates the helpers from the receiver. The term ε is a normal random variable with mean 0 and variance σ_ε^2 that accounts for the fact that new cells do not perfectly detect the level of the signal.

The degree to which the new cell responds to the signal when specialising depends on the interaction between the detected level of the signal, χ_i^t , and the trait values q , d , and v . Specifically, we model the probability, p , that the cell adopts a helper phenotype via the response norm

$$p = \min \left(1, \max \left(0, q + 1 - \frac{2}{1 + e^{-v(\chi_i^t - d)}} \right) \right). \quad (27)$$

The form of this function is depicted in Fig. 4b. We assume that if helpers emit no signal, then the response threshold d is also held at zero (i.e., $d = 0$ if $s = 0$). Random specialisation occurs if cells are insensitive to the signal (i.e., $v = 0$) or if cells send no signal at all (i.e., $s = 0$). In this case, Eq. 27 becomes $p = q$, and new cells adopt a helper phenotype with the baseline probability q . Coordination occurs when $v > 0$ and $s > 0$, in which case the probability of adopting a helper phenotype is affected by the detected level of the signal, χ_i^t . The larger the response sensitivity, v , and the larger the difference between the response threshold, d , and the detected level of the signal, χ_i^t , the more the probability of adopting a helper phenotype p is perturbed from the baseline probability q . If the detected signal level is greater than the response threshold (i.e., if $\chi_i^t > d$), then sensitive cells (with $v > 0$) increase their probability of adopting a helper phenotype (and thus $p > q$). In the limit, as cells become infinitely sensitive ($v \rightarrow \infty$), the response norm leads to a deterministic response where if the detected signal level is smaller than the threshold, the new cell always becomes a helper ($p = 1$), and if the detected signal level is greater than the threshold, the new cell always becomes a reproductive ($p = 0$).

In our model, higher coordination incurs larger metabolic costs. First, the more helpers invest in producing the signal (higher s) the fewer resources they have to produce the public good (Eq. 25). Second, we assume that filaments with more sensitive cells (higher v) grow more slowly, which we model as an increase in the growth-cap of reproductives, via

$$\bar{\pi} = \bar{\pi}_0 + e^{\beta v} - 1, \quad (28)$$

where $\bar{\pi}_0$ is the baseline growth-cap. More sensitive cells (higher v) lead to an exponentially increasing growth-cap with shape parameter β .

Consequently, in different scenarios, the optimal trait values q , s , d and v , will depend on the trade-offs as the filament grows between producing more helpers and producing more reproductives, and between the growth costs and the advantages of coordination.

Evolution of coordination. For a given set of model parameters, we estimate the evolutionarily optimal trait values q^* , s^* , d^* and v^* by simulating an evolving population of cyanobacteria filaments over 4000 generations. We initialise the population with the resident trait values all equal to zero ($q = s = d = v = 0$). Each generation, we consider an invading mutant strategy, which we draw from a multivariable random distribution, $\text{Normal}(\mu, \Sigma)$, with location μ equal to a vector

containing the resident trait values $(q, s, d, v)^T$ and with a covariance matrix

$$\Sigma = \begin{pmatrix} \sigma_q^2 & 0 & 0 & 0 \\ 0 & \sigma_s^2 & 0 & 0 \\ 0 & 0 & \sigma_d^2 & 0 \\ 0 & 0 & 0 & \sigma_v^2 \end{pmatrix}. \quad (29)$$

We do not allow all traits to mutate at the same time (but see Supplementary Fig. 6). For the first 500 generations, we consider only mutations in the baseline helper probability q , by setting $\sigma_s^2 = \sigma_d^2 = \sigma_v^2 = 0$. This allows the population to evolve to the optimal strategy for random specialisation. For the rest of the generations, we allow only the coordination traits s , d , and v to mutate by removing the constraint on σ_s^2 , σ_d^2 and σ_v^2 and by instead constraining $\sigma_q = 0$. We set the resident trait value of q to be its average trait value over generations 250 to 500. In addition, we set $d = 0$ whenever $s = 0$ to avoid neutral drift of d .

For each generation of the simulation, we estimate the fitness of the resident strategy and the mutant strategy by simulating the growth of 200 independent filaments using each strategy and averaging fitness across simulations. For a given simulation, let τ_L be the time at which the L th cell in the filament is produced. We calculate fitness as

$$w_{\text{filament}} = \frac{\sum_{i \in R_{\tau_L}} \Psi_i^{\tau_L}}{\tau_L}, \quad (30)$$

that is, as the sum of the fecundities of the reproductives in the last generation of the group life cycle (estimated as their growth rates), divided by the time it took the filament to grow to that size. If the estimated fitness of the mutant strategy is greater than the estimated fitness of the resident strategy, we replace the resident strategy with the mutant strategy before proceeding to the next generation. If the estimated fitness of the mutant strategy is less than the estimated fitness of the resident strategy, we keep the resident strategy when proceeding to the next generation. We approximate the evolved trait values (q^* , s^* , d^* , and v^*) for each evolutionary simulation as the average of each trait value over the last 2000 generations of the simulation.

Simulation results. There are 15 parameters in our model (see Supplementary Table). We focused our investigation on the particular patterns produced by two of these parameters, namely ϕ and η . The other parameters and simulation parameters were fixed to values given in the Supplementary Table. In Supplementary Fig. 5, we provide some sample plots to illustrate the evolutionary convergence of our simulations for the specific case of essential cooperation ($\phi = 0$) and very local cooperation ($\eta = 0.1$). We see that all trait values converge to the approximate final rolling average within 100–200 generations of being allowed to evolve. The results for the evolved level of signalling, s^* , response sensitivity, v^* , baseline helper probability, q^* , and response threshold, d^* are shown in Fig. 5. In these results, the evolved trait values (q^* , s^* , d^* and v^*) are averages across 5 independent evolutionary simulations for each parameter combination.

For each parameter combination examined, we performed further simulations to estimate the extent that the evolved level of coordination lead to a more or less precise division of labour. For each case, we ran $T = 10,000$ independent simulations of cyanobacteria growth using the evolved strategies (q^* , s^* , d^* and v^* ; Fig. 5a–d). For each of the T simulated filaments, indexed by $i \in \{1, \dots, T\}$, we recorded both the total number of helpers in the last generation of the filament, H_i , and the number of helpers in the leftmost non-terminal 10 cells in the last generation of the filament, $\tilde{H}_i$ (excluding the outside helper). We calculated the relative variance in the number of helpers in the leftmost non-terminal 10 cells as

$$\text{Relative variance} = \frac{\text{Var}_i(\tilde{H}_i)}{10h(1-h)}, \quad (31)$$

where the average proportion of helpers in the last generation across all T simulations is given by $h = \frac{1}{T} \sum_{i=1}^T \frac{H_i}{L}$. The numerator of Eq. 31 is the observed variance in the number of helpers in the leftmost non-terminal 10 cells. The denominator of Eq. 31 is the variance of a binomial distribution with 10 trials and probability of success equal to h , which is what we would expect if cells specialise randomly and independently from one another. If the level of coordination produces an allocation of labour that is indistinguishable from random specialisation, then the relative variance should be approximately one. As the level of coordination produces more and more precise allocations of labour, the relative variance is expected to decline. The precision of coordination as shown in Fig. 5 and Supplementary Fig. 6 corresponds to the reciprocal of the relative variance.

We also considered the possibility that filaments begin with no helpers. We repeated the above analyses, while ignoring the parameter combinations in which cooperation is essential ($\phi = 0$). Results for the evolved trait values and relative precision are given in Supplementary Fig. 6 and show similar qualitative patterns.

The effect of helper clumping. We ran additional simulations to quantify the propensity and cost of helper clumps.

For a given set of parameter values (Supplementary Table) we extracted the evolved trait values from the previous set of simulations and ran T independent simulations of filament growth with the associated evolved strategy (q^* , s^* , d^*

and v^*). When examining random specialisation, we set $s^* = d^* = v^* = 0$. For each simulation, we define a clump as any contiguous grouping of helpers in the last generation of the group growth. Thus, clump sizes can range from 1 (a single helper) to L (the entire filament). Within each individual simulation, indexed as $i \in \{1, \dots, T\}$, we calculated the average clump size, m_i , over all clumps in the filament. The average clump size across all T simulations is then the across-simulation average, $\bar{m} = \frac{1}{T} \sum_{i=1}^T m_i$. To determine the cost of clumping, we also recorded the fitness of each filament, w_i , for all T simulations (Equation 33). The cost of clumping is then approximated as the slope of the linear least-squares regression of filament fitness on average clump size, given by:

$$\text{Cost of clumping} = \frac{\text{Cov}(w_i, m_i)}{\text{Var}_i(m_i)}, \quad (32)$$

In Fig. 6a, b, we used the above approach to map the propensity and cost of clumping from random specialisation as a function of the background density of fixed nitrogen (ϕ) and the diffusivity of fixed nitrogen (η). In Fig. 6c, d, we used the above approach to determine the differences between random specialisation and coordinated specialisation, focusing on the extreme case of essential cooperation ($\phi = 0$) and very low diffusivity of fixed nitrogen ($\eta = 0.1$).

We then determined the effect of group growth on the formation of helper clumps in randomly specialising filaments. To do this, we ran T independent simulations where the filament does not grow and is composed of L cells. An individual cell becomes a helper with a probability equal to the optimal strategy for the growing group, q^* , and otherwise, it becomes a reproductive. We then quantified the average clump size in each simulation in the same way as for the previous analysis. Supplementary Fig. 7 shows the distribution of average clump sizes across $T = 10,000$ simulations of non-growing filaments (Supplementary Fig. 7A) and growing filaments (Supplementary Fig. 7B). This was calculated for the extreme case of essential cooperation $\phi = 0$ and very low diffusivity of fixed nitrogen ($\eta = 0.1$). We find that non-growing groups still form helper clumps but that the distribution has a smaller average value and has a smaller upper tail than for growing filaments. Consequently, helper clumping is more severe when cellular division and differentiation are “coupled”.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Source data for Fig. 6 and Supplementary Fig. 7 are provided as a Source Data file. Further data generated in this study are available on Github (<https://doi.org/10.5281/zenodo.5747159>). Source data are provided with this paper.

Code availability

All simulated data were generated using C and Matlab. The codes used for this study are available on Github (<https://doi.org/10.5281/zenodo.5747159>).

Received: 28 May 2020; Accepted: 16 December 2021;

Published online: 25 January 2022

References

- Ackermann, M. A functional perspective on phenotypic heterogeneity in microorganisms. *Nat. Rev. Microbiol.* **13**, 497–508 (2015).
- West, S. A. & Cooper, G. A. Division of labour in microorganisms: an evolutionary perspective. *Nat. Rev. Microbiol.* **14**, 716–723 (2016).
- Schwander, T., Lo, N., Beekman, M., Oldroyd, B. P. & Keller, L. Nature versus nurture in social insect caste differentiation. *Trends Ecol. Evol.* **25**, 275–282 (2010).
- Smits, W. K., Kuipers, O. P. & Veening, J. W. Phenotypic variation in bacteria: the role of feedback regulation. *Nat. Rev. Microbiol.* **4**, 259–271 (2006).
- Veening, J.-W., Smits, W. K. & Kuipers, O. P. Bistability, epigenetics, and Bet-Hedging in bacteria. *Annu. Rev. Microbiol.* **62**, 193–210 (2008).
- Duarte, A., Pen, I., Keller, L. & Weissing, F. J. Evolution of self-organized division of labor in a response threshold model. *Behav. Ecol. Sociobiol.* **66**, 947–957 (2012).
- Maynard Smith, J. & Harper, D. *Animal Signals*. (Oxford University Press, 2003).
- Linksvayer, T. A. et al. Larval and nurse worker control of developmental plasticity and the evolution of honey bee queen-worker dimorphism. *J. Evol. Biol.* **24**, 1939–1948 (2011).
- Flores, E. & Herrero, A. Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nat. Rev. Microbiol.* **8**, 39–50 (2010).
- Weaver, N. Rearing of honeybee larvae on royal jelly in the laboratory. *Science* **121**, 509–510 (1955).

11. Dubnau, D. & Losick, R. Bistability in bacteria. *Mol. Microbiol.* **61**, 564–572 (2006).
12. Ackermann, M. et al. Self-destructive cooperation mediated by phenotypic noise. *Nature* **454**, 987–990 (2008).
13. Diard, M. et al. Stabilization of cooperative virulence by the expression of an avirulent phenotype. *Nature* **494**, 353–356 (2013).
14. Hughes, W. O. H., Sumner, S., Van Borm, S. & Boomsma, J. J. Worker caste polymorphism has a genetic basis in *Acromyrmex* leaf-cutting ants. *Proc. Natl Acad. Sci. USA* **100**, 9394–9397 (2003).
15. Anderson, K. E., Linksvayer, T. A. & Smith, C. R. The causes and consequences of genetic caste determination in ants (Hymenoptera: Formicidae). *Myrmecol. N.* **11**, 119–132 (2008).
16. Jaffe, R., Kronauer, D. J. C., Kraus, F. B., Boomsma, J. J. & Moritz, R. F. A. Worker caste determination in the army ant *Eciton burchellii*. *Biol. Lett.* **3**, 513–516 (2007).
17. Zhang, Z. et al. Antibiotic production in *Streptomyces* is organized by a division of labor through terminal genomic differentiation. *Sci. Adv.* **6**, eaay5781 (2020).
18. Shelton, D. E., Desnitskiy, A. & Michod, R. E. Distributions of reproductive and somatic cell numbers in diverse *Volvox* (Chlorophyta) species. *Evol. Ecol. Res.* **14**, 707–727 (2012).
19. Griesemer, J. The units of evolutionary transition. *Selection* **1**, 67–80 (2001).
20. Smith, G. M. A comparative study of the species of *Volvox*. *Trans. Am. Microsc. Soc.* **63**, 265–310 (1944).
21. Dragoš, A. et al. Division of labor during biofilm matrix production. *Curr. Biol.* <https://doi.org/10.1016/j.cub.2018.04.046> (2018).
22. Dragoš, A. et al. Collapse of genetic division of labour and evolution of autonomy in pellicle biofilms. *Nat. Microbiol.* <https://doi.org/10.1038/s41564-018-0263-y> (2018).
23. Wahl, L. M. Evolving the division of labour: generalists, specialists and task allocation. *J. Theor. Biol.* **219**, 371–388 (2002).
24. Wahl, L. M. The division of labor: genotypic versus phenotypic specialization. *Am. Nat.* **160**, 135–145 (2002).
25. Duarte, A., Weissing, F. J., Pen, I. & Keller, L. An evolutionary perspective on self-organized division of labor in social insects. *Annu. Rev. Ecol. Syst.* **42**, 91–110 (2011).
26. Oster, G. F. & Wilson, E. O. *Caste and Ecology in The Social Insects*. (Princeton University Press, 1978).
27. Wilson, E. O. The ergonomics of caste in the social insects. *Am. Econ. Rev.* **102**, 41–66 (1978).
28. Duarte, A., Scholtens, E. & Weissing, F. J. Implications of behavioral architecture for the evolution of self-organized division of labor. *PLoS Comput. Biol.* **8**, e1002430 (2012).
29. Waibel, M., Floreano, D., Magnenat, S. & Keller, L. Division of labour and colony efficiency in social insects: Effects of interactions between genetic architecture, colony kin structure and rate of perturbations. *Proc. R. Soc. B Biol. Sci.* **273**, 1815–1823 (2006).
30. Bourke, A. F. G. *Principles of Social Evolution*. (Oxford University Press, 2011).
31. Powers, S. T. & Lehmann, L. When is bigger better? The effects of group size on the evolution of helping behaviours. *Biol. Rev.* <https://doi.org/10.1111/brv.12260> (2017).
32. Peña, J. & Nöldeke, G. Group size effects in social evolution. *J. Theor. Biol.* <https://doi.org/10.1016/j.jtbi.2018.08.004> (2018).
33. Parker, G. A. & Maynard Smith, J. Optimality theory in evolutionary biology. *Nature* **348**, 27–33 (1990).
34. May, R. M. Uses and abuses of mathematics in biology. *Science* **303**, 790–793 (2004).
35. Strassmann, J. E., Zhu, Y. & Queller, D. C. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* **408**, 965 (2000).
36. Diggle, S. P., Griffin, A. S., Campbell, G. S. & West, S. A. Cooperation and conflict in quorum-sensing bacterial populations. *Nature* **450**, 411 (2007).
37. Griffin, A. S., West, S. A. & Buckling, A. Cooperation and competition in pathogenic bacteria. *Nature* **430**, 1024–1027 (2004).
38. West, S. A., Diggle, S. P., Buckling, A., Gardner, A. & Griffin, A. S. The social lives of microbes. *Annu. Rev. Ecol. Syst.* **38**, 53–77 (2007).
39. Frost, I. et al. Cooperation, competition and antibiotic resistance in bacterial colonies. *ISME J.* <https://doi.org/10.1038/s41396-018-0090-4> (2018).
40. Gardner, A., West, S. A. & Buckling, A. Bacteriocins, spite and virulence. *Proc. R. Soc. B Biol. Sci.* **271**, 1529–1535 (2004).
41. van Gestel, J., Vlamakis, H. & Kolter, R. Division of labor in bio films: the ecology of cell differentiation. *Microb. Biofilms* **3**, 1–24 (2015).
42. Frank, S. A. A general model of the public goods dilemma. *J. Evol. Biol.* **23**, 1245–1250 (2010).
43. West, S. A. & Buckling, A. Cooperation, virulence and siderophore production in bacterial parasites. *Proc. R. Soc. B Biol. Sci.* **270**, 37–44 (2003).
44. Veening, J.-W. et al. Transient heterogeneity in extracellular protease production by *Bacillus subtilis*. *Mol. Syst. Biol.* **4**, 184 (2008).
45. Marlow, V. L. et al. The prevalence and origin of exoprotease-producing cells in the *Bacillus subtilis* biofilm. *Microbiology* **160**, 56–66 (2014).
46. Davidson, C. J. & Surette, M. G. Individuality in bacteria. *Annu. Rev. Genet.* **42**, 253–268 (2008).
47. McAdams, H. H. & Arkin, A. Stochastic mechanisms in gene expression. *Proc. Natl Acad. Sci. USA* **94**, 814–819 (1997).
48. Keller, L. & Surette, M. G. Communication in bacteria: an ecological and evolutionary perspective. *Nat. Rev. Microbiol.* **4**, 249–258 (2006).
49. Rossetti, V., Schirrmeyer, B. E., Bernasconi, M. V. & Bagheri, H. C. The evolutionary path to terminal differentiation and division of labor in cyanobacteria. *J. Theor. Biol.* **262**, 23–24 (2010).
50. West, S. A. & Herre, E. A. Stabilizing selection and variance in Fig Wasp Sex ratios. *Evolution* **52**, 475–485 (1998).
51. Nagelkerke, C. J. & Sabelis, M. W. Precise control of sex allocation in pseudo-arrhenotokous phytoseiid mites. *J. Evol. Biol.* **11**, 649–684 (1998).
52. Green, R. F., Gordh, G. & Hawkins, B. A. Precise sex ratios in highly inbred parasitic. *Am. Nat.* **120**, 653–665 (1982).
53. Dionisio, F. & Gordo, I. The tragedy of the commons, the public good dilemma and the meaning of rivalry and excludability in evolutionary biology. *Evol. Ecol. Res.* **8**, 321–332 (2006).
54. Peña, J., Nöldeke, G. & Lehmann, L. Evolutionary dynamics of collective action in spatially structured populations. *J. Theor. Biol.* <https://doi.org/10.1016/j.jtbi.2015.06.039> (2015).
55. Liu, M., West, S. A. & Cooper, G. A. Relatedness and the evolution of mechanisms to divide labor in microorganisms. *Ecol. Evol.* <https://doi.org/10.1002/ece3.8067> (2021).
56. Cooper, G. A., Ming, L., Peña, J. & West, S. A. The evolution of mechanisms to produce phenotypic heterogeneity in microorganisms. *GitHub* <https://doi.org/10.5281/zenodo.5747159> (2021).
57. Meeks, J. C. & Elhai, J. Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. *Microbiol. Mol. Biol. Rev.* **66**, 94–121 (2002).
58. Herrero, A., Stavans, J. & Flores, E. The multicellular nature of filamentous heterocyst-forming cyanobacteria. *FEMS Microbiol. Rev.* **40**, 831–854 (2016).
59. Wolk, C. P. Formation of one-dimensional patterns by stochastic processes and by filamentous blue-green algae. *Dev. Biol.* **46**, 370–382 (1975).
60. Gerdtsen, Z. P. et al. Modeling heterocyst pattern formation in cyanobacteria. *BMC Bioinformatics* **10**, 1–13 (2009).
61. Torres-Sánchez, A., Gómez-Gardeñes, J. & Falo, F. An integrative approach for modeling and simulation of heterocyst pattern formation in cyanobacteria filaments. *PLoS Comput. Biol.* **11**, 1–18 (2015).
62. Muñoz-García, J. & Ares, S. Formation and maintenance of nitrogen-fixing cell patterns in filamentous cyanobacteria. *Proc. Natl Acad. Sci. USA* **113**, 6218–6223 (2016).
63. Brown, A. I. & Rutenberg, A. D. Heterocyst placement strategies to maximize the growth of cyanobacterial filaments. *Phys. Biol.* **9**, 046002 (2012).
64. Fuch, D. & Chen, F. Presence of toxin-antitoxin systems in picocyanobacteria and their ecological implications. *ISME J.* **14**, 2843–2850 (2020).
65. Samuilov, V. D. et al. Tolerance to antimicrobial agents and persistence of *Escherichia coli* and cyanobacteria. *Biochem* **73**, 833–838 (2008).
66. Gardner, A., West, S. A. & Griffin, A. S. Is bacterial persistence a social trait? *PLoS ONE* **2**, e752 (2007).
67. Co, A. D., van Vliet, S., Kiviet, D. J., Schlegel, S. & Ackermann, M. Short-range interactions govern the dynamics and functions of microbial communities. *Nat. Ecol. Evol.* **4**, 366–375 (2020).
68. Brown, J., Sanderson, J. & Michod, E. Evolution of social behavior by reciprocation. *J. Theor. Biol.* **99**, 319–339 (1982).
69. Kirk, D. L. Germ-soma differentiation in *Volvox*. *Dev. Biol.* **238**, 213–223 (2001).
70. Strmecki, L., Greene, D. M. & Pears, C. J. Developmental decisions in *Dictyostelium discoideum*. *Dev. Biol.* **284**, 25–36 (2005).
71. Maree, A. F. M. & Hogeweg, P. How amoeboids self-organize into a fruiting body: multicellular coordination in *Dictyostelium discoideum*. *Proc. Natl Acad. Sci. USA* **98**, 3879–3883 (2002).
72. Kamakura, M. Royalactin induces queen differentiation in honeybees. *Nature* **473**, 478 (2011).
73. Gavrilits, S. Rapid transition towards the division of labor via evolution of developmental plasticity. *PLoS Comput. Biol.* **6**, e1000805 (2010).
74. Ispolatov, I., Ackermann, M. & Doebeli, M. Division of labour and the evolution of multicellularity. *Proc. R. Soc. B Biol. Sci.* **279**, 1768–1776 (2012).
75. Cooper, G. A. & West, S. A. Division of labour and the evolution of extreme specialization. *Nat. Ecol. Evol.* **2**, 1161–1167 (2018).
76. Linksvayer, T. A. Ant species differences determined by epistasis between brood and worker genomes. *PLoS ONE* **2**, e994 (2007).

77. Walsh, J. T., Warner, M. R., Kase, A., Cushing, B. J. & Linksvayer, T. A. Ant nurse workers exhibit behavioural and transcriptomic signatures of specialization on larval stage. *Anim. Behav.* **141**, 161–169 (2018).
78. Süel, G. M., Garcia-Ojalvo, J., Liberman, L. M. & Elowitz, M. B. An excitable gene regulatory circuit induces transient cellular differentiation. *Nature* **440**, 545–550 (2006).
79. Balázs, G., Van Oudenaarden, A. & Collins, J. J. Cellular decision making and biological noise: from microbes to mammals. *Cell* **144**, 910–925 (2011).
80. DeLong, E. F. et al. Noise in gene expression determines cell fate in *Bacillus subtilis*. *Science* **27**, 526–530 (2007).

Acknowledgements

We thank: Anna Dewar, Kevin Foster, Andy Gardner, Ashleigh Griffin, Asher Leeks, Matishalin Patel, Tom Scott, and Daniel Unterwegger for their helpful comments and suggestions; St. John's College, Oxford (GAC), the French National Research Agency (ANR) under the Investments for the Future (Investissements d'Avenir) program (grant ANR-17-EURE-0010 to the IAST; J.P.) and the ERC (Horizon 2020 Advanced Grant 834164; S.A.W.) for funding. We would like to acknowledge the use of the University of Oxford Advanced Research Computing (ARC) facility in carrying out this work. (<https://doi.org/10.5281/zenodo.22558>).

Author contributions

G.A.C., S.A.W., and J.P. conceived the study. G.A.C. and J.P. designed and analysed the analytical models, G.A.C. and M.L. designed and analysed the simulation models. G.A.C. and S.A.W. wrote the first draft. All authors contributed toward writing the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41467-021-27902-4>.

Correspondence and requests for materials should be addressed to Guy Alexander Cooper.

Peer review information *Nature Communications* thanks the anonymous reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2022

A8

Environmental quality mediates
the ecological dominance of
cooperatively breeding birds

Received: 16 November 2022 | Revised: 8 March 2023 | Accepted: 30 March 2023

DOI: 10.1111/ele.14226

LETTER

ECOLOGY LETTERS  WILEY

Environmental quality mediates the ecological dominance of cooperatively breeding birds

Yu-Heng Lin¹  | Ying-Yu Chen¹ | Dustin R. Rubenstein^{2,3}  | Ming Liu⁴  | Mark Liu¹ | Sheng-Feng Shen¹ ¹Biodiversity Research Center, Academia Sinica, Taipei, Taiwan²Department of Ecology, Evolution and Environmental Biology, Columbia University, New York City, New York, USA³Center for Integrative Animal Behavior, Columbia University, New York City, New York, USA⁴Department of Biology, University of Oxford, Oxford, UK

Correspondence

Sheng-Feng Shen, Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan.

Email: shensf@sinica.edu.tw

Funding information

Academia Sinica, Grant/Award Number: AS-IA-106-L01 and AS-SS-106-05; Minister of Science and Technology of Taiwan, Grant/Award Number: 100-2621-B-001-004-MY3, 104-2311-B-001-028-MY3, 108-2314-B-001-009-MY3 and 110-2628-B-001-022; National Science Foundation, Grant/Award Number: IOS-1656098

Editor: Noa Pinter-Wollman

Abstract

Although social species as diverse as humans and ants are among the most abundant organisms on Earth, animals cooperate and form groups for many reasons. How these different reasons for grouping affect a species' ecological dominance remains unknown. Here we use a theoretical model to demonstrate that the different fitness benefits that animals receive by forming groups depend on the quality of their environment, which in turn impacts their ecological dominance and resilience to global change. We then test the model's key predictions using phylogenetic comparative analysis of >6500 bird species. As predicted, we find that cooperative breeders occurring in harsh and fluctuating environments have larger ranges and greater abundances than non-cooperative breeders, but cooperative breeders occurring in benign and stable environments do not. Using our model, we further show that social species living in harsh and fluctuating environments will be less vulnerable to climate change than non-social species.

KEYWORDS

cooperation, cooperative breeding, ecological consequences, range size, sociality, species distribution

INTRODUCTION

Over 150 years ago, Charles Darwin proposed in *On the Origin of Species* that explaining interspecific variation in geographic range size is critical for understanding both the “present welfare” and the “future success” of species (Darwin, 1859). Linking species distributions and organismal success takes on greater meaning today in an era of anthropogenic climate change where environments are becoming increasingly unpredictable (IPCC, 2021) and extreme weather events are occurring with greater frequency (Mora et al., 2017; Prein et al., 2017). One of the primary ways that organisms cope with these and other forms of global change is to adjust their distributions, moving, for example, up mountain ranges

as temperatures increase (Chen et al., 2011; Lenoir et al., 2020) or into new habitats as precipitation patterns shift (Pearce-Higgins et al., 2015). Environmental change most often negatively impacts the demography of populations or species, including both reductions in range size (Erasmus et al., 2002) and abundance (Both et al., 2010; Pearce-Higgins et al., 2015), yet animals that cooperate and form societies may be able to compensate and outcompete their non-social neighbours or relatives under periods of global change (Cornwallis et al., 2017; Sun et al., 2014). Although the ability to form cooperative societies has been argued to be one of the primary reasons for the dominance of social organisms on Earth, there are not only many fewer social species than non-social ones (Brooks et al., 2017; Wilson, 1990, 2012), but

Yu-Heng Lin and Ying-Yu Chen contributed equally to this work.

there is also no formal framework explaining why some social species have become ecologically dominant while others have not. To understand how cooperation influences ecological dominance, we must determine not only how ecology impacts the evolution of social living, but also how sociality influences ecological success and population demography for species living in environments of varying quality (Lin et al., 2019; Shen et al., 2017).

Several comparative studies in a variety of taxa suggest that both the mean and variation in climatic conditions have shaped the evolution of animal societies (Fisher et al., 2021). For example, harsh mean environmental conditions and/or fluctuating environments have favoured the evolution of cooperative breeding behaviour in ants (La Richelière et al., 2022), wasps (Sheehan et al., 2015), bees (Kocher et al., 2014), birds (Jetz & Rubenstein, 2011; Rubenstein & Lovette, 2007), and mammals (Firman et al., 2020; Lukas & Clutton-Brock, 2017), including humans (Martin et al., 2020). Yet, high population density and habitat saturation, both characteristics of species living in benign and/or stable environments, have also been suggested to favour the evolution of cooperative breeding behaviour in insects (Choe & Crespi, 1997; Costa, 2006), arachnids (Choe & Crespi, 1997), birds (Gonzalez et al., 2013), and mammals (Solomon & French, 1997). This phenomenon of cooperative breeding behaviour being favoured in both harsh and fluctuating environments as well as under benign and stable conditions constitutes the paradox of environmental quality and sociality (Lin et al., 2019; Shen et al., 2017). To help resolve this paradox, researchers proposed that cooperative breeding behaviour may evolve for different reasons in different environments (Shen et al., 2017). According to this “dual benefits framework”, animals form social groups to gain resource defence (RD) benefits against intraspecific competitors in stable and/or benign environments, but they form groups to gain collective action (CA) benefits in the face of interspecific competition or environmental challenges in harsh and/or fluctuating environments (Lin et al., 2019; Shen et al., 2017). Despite contrasting and testable predictions from the dual benefits framework (Shen et al., 2017; Shen & Rubenstein, 2019), we still lack a mathematical formulation of these ideas, as well as broad-scale comparative and biogeographic studies investigating how the relationships among cooperative breeding behaviour and ecology influence the demography and ecological success of social organisms.

Here, we integrate socioecological and macroecological perspectives on animal behaviour, demography, and global change by combining theory and phylogenetic comparative approaches to test the paradox of environmental quality and sociality. First, we construct a spatially explicit, individual-based model to explore how the effects of both mean environmental conditions and environmental fluctuation (variation in environmental conditions) influence the evolution of cooperative investment in

different types of grouping benefits, and how these combined environmental effects impact the ecological and demographic consequences (range size and abundance) of social species that form groups for different reasons (RD or CA benefits). Using phylogenetic comparative methods, we subsequently test the model's key predictions by first examining how environmental and social factors affect the evolution of cooperative breeding behaviour in birds, and then determining whether cooperative breeding has different ecological and demographic consequences (range size and abundance) for species that exhibit cooperative breeding behaviour under different environmental conditions. Because birds are known to show variation among clades in the relationship between cooperative breeding and environmental quality (Lin et al., 2019), we begin by conducting a clade-level analysis within avian lineages that have evolved cooperative breeding behaviour to investigate how different environmental factors influence cooperative breeding. To examine the alternative ecological and demographic consequences of cooperative breeding, we then separate these avian clades into two categories, those in which cooperative breeding behaviour is either positively associated with environmental harshness/stability or with environmental benignness/fluctuation, and compare species range sizes and abundances in cooperative and non-cooperative species in these two categories. To explore further, we combine all clades and species into a single comparative analysis examining range size and abundance in cooperative and non-cooperative species. Finally, we use our model to further investigate the vulnerability to climate change of species that form cooperatively breeding groups for different reasons in different environments. Ultimately, we show not only that the relationships between cooperative breeding behaviour and demography (range size and abundance) depend on the relationship between cooperative breeding and environmental quality, but that social species living in harsh or fluctuating environments will be less vulnerable to climate change than non-social species.

METHODS

Modelling social evolution and its ecological consequences

To examine the ecological and demographic consequences of cooperation, we began by building a spatially explicit, individual-based model that assumes a population evolves in a habitat matrix of 23×23 patches. Each patch can be occupied by more than one individual, and individuals in the same patch interact, or cooperate, to generate either CA or RD benefits. Individuals are classified into (1) those that invest in generating CA benefits by cooperating to outcompete interspecific competitors or to cope with

environmental challenges (CA cooperators), thereby increasing the amount of group resources that are shared equally among group members, (2) those that invest in creating RD benefits through the cooperative defence of group resources within the patch against intraspecific competitors (RD cooperators), or (3) those that neither cooperate nor produce anything, but that share group resources (free-loaders) (Shen et al., 2017). Cooperators contribute to the grouping benefits at a cost to themselves, whereas non-cooperators bear no cost. Within each simulation time step, the reproductive rate is determined by the amount of resources that individuals consume. After individuals produce offspring, those offspring either stay or disperse to other patches, and then some of the reproductive individuals die.

To account for the relationship between the mean and variation in environmental conditions, and to introduce temporal variation into our model by simulating a fluctuating environment, we sampled the resource availability of the whole grid every time step from a uniform distribution. To tease out the ecological and demographic consequences of cooperation, we recorded the proportion of cooperators in the population and the average degree of cooperation that each individual exhibits over time, as well as the total abundance and range size (i.e., the number of occupied patches in the habitat matrix). We then compared the results of this model to those of a non-social population composed exclusively of non-cooperators. Finally, we simulated scenarios under which climate change results in lower mean available resources or higher fluctuation in resources. We investigated the impacts of climate change on CA and RD collaborators by adjusting the mean resource level of the environment and increasing the degree of fluctuation in environmental resources. More detailed model descriptions are provided in the Supplementary Methods.

Phylogenetic comparative analysis

Trait and environmental data

The avian species used in this study came from published data (Jetz & Rubenstein, 2011). Because we were interested in how climatic factors influence the evolution of cooperatively breeding behaviour, we excluded clades containing 100% non-cooperatively breeding species (2409 species belonging to 52 clades) and 100% cooperatively breeding species (58 species belonging to 5 clades). We also compared the species list with the most recent *Handbook of the Birds of the World* and BirdLife Taxonomic Checklist v5 (BirdLife International, 2020) to exclude subspecies from our analysis ($N=290$ species). The social system of each species was classified as “cooperative” or “non-cooperative”

and was determined through published accounts (Cockburn, 2006; Jetz & Rubenstein, 2011). Following Griesser and Suzuki (2016), we also reran our comparative analyses excluding 152 species that only occasionally breed cooperatively, finding nearly identical results (see Tables S1–S3). Since 47 of these occasional cooperative breeders were present in the clades containing all non-cooperative species and already removed, we ended up excluding 105 species in our re-analysis without occasional cooperative breeders. Body mass was also collected from published data (Dunning Jr, 2007; Tobias et al., 2022) and included in our analysis because of its potential effect on influencing social behaviour (Jetz & Rubenstein, 2011). Range sizes of every species in the dataset were extracted from digital bird species distribution maps of the world (BirdLife International, 2020), and global species abundances (i.e., the estimated number of individuals within a species) were taken from a previously published study (Callaghan et al., 2021). To ensure sufficient statistical power to assess the relationship between climatic factors and cooperative breeding behaviour, we restricted our analysis to clades containing more than 20 species (Blomberg et al., 2003). Thus, our clade-level analysis includes 6553 species belonging to 52 clades (Table S4). Finally, 100 phylogenies were generated from the open-access dataset of BirdTree.org (Jetz et al., 2012) using the Hackett backbone (Hackett et al., 2008). To account for phylogenetic uncertainty, we used posterior tree samples to create a maximum clade credibility (MCC) consensus tree, using 10% burn-in and common ancestor height in TreeAnnotator v2.6.6 (Bouckaert et al., 2019).

We extracted environmental data from the distributional ranges of each species using the CRU TS 4.05 database (Harris et al., 2020) and calculated the mean values of six climate variables for each grid cell within the geographic distribution range of each species: (1) mean annual temperature (°C); (2) diurnal temperature range (DTR, °C); (3) seasonal temperature range (STR, °C); (4) mean annual precipitation (MAP, mm); (5) within-year variation in precipitation (i.e., the mean among all years of the within-year standard deviations of the 3-month values); and (6) among-year variation in precipitation (i.e., the standard deviation within the same 3-month period across all years) (Chan et al., 2016; Ficetola et al., 2017; Gonzalez et al., 2013; Rubenstein & Lovette, 2007). We then calculated within- and among-year variation in precipitation as the mean of log10-transformed values across all 55 × 55 km grid cells occupied by a species over 120 years (Jetz & Rubenstein, 2011). Following similar comparative studies, harsh and fluctuating environments were defined as those with high mean annual temperature (Cornwallis et al., 2017), low mean annual precipitation (Botero et al., 2014; Firman et al., 2020), high variability in precipitation (Cornwallis et al., 2017; Rubenstein & Lovette, 2007), and high variability in

temperature (Botero et al., 2014; Firman et al., 2020), and vice versa for stable and predictable environments.

Phylogenetically-informed tests

To explore the ecological causes and consequences of cooperative breeding behaviour comparatively in birds, we used a series of phylogenetically-informed tests (see flow diagram in Figure S1). First, to assess the relationship between social system and climate for each of the 52 avian clades (selected according to the criteria described above), we examined whether the six environmental variables and body mass predicted the occurrence of cooperative breeding behaviour in each clade. We first tested a full model using the *phylglm* function with all of the environmental variables and life history traits in the R package *phylolm* (Ho et al., 2018), with the binary dependent variable (cooperative breeding, hereafter referred to as “cooperative” or non-cooperative breeding, hereafter “non-cooperative”). We then examined the variance inflation factor (VIF) of the full model for each climatic factor to assess potential multicollinearity. We identified and retained variables with robust effects after iteratively removing variables with $VIF > 5$ (O'Brien, 2007). Finally, we determined the best-fitting models with a corrected Akaike information criterion (AICc) (Hurvich & Tsai, 1989), using model averaging for models with $AICc < 2$ (Anderson & Burnham, 2004) in the R package *AICcmodavg* (Mazerolle, 2013). We then classified each clade into one of the three groups based on the relationship between environmental factors and social system from *phylglm*: (1) cooperative breeding behaviour is positively associated with environmental harshness/stability; (2) cooperative breeding behaviour is positively associated with environmental benignness/fluctuation; or (3) there is no association between cooperative breeding behaviour and environmental quality. To include as many clades as possible in our analysis, we used a liberal threshold of $p < 0.25$ for classifying clades into one of the three categories.

Next, we performed a clade-level analysis, testing the effects of both cooperative breeding behaviour and climate on demography (both species range size and abundance) using phylogenetic least squares (PGLS) models separately for species in clades that cooperate in benign and/or stable environments ($N = 1786$ species), those that cooperate in harsh and/or fluctuating environments ($N = 2426$ species) by implementing the *phylolm* function using lambda model (Pagel, 1999) in the R package *phylolm*. We also tested the VIF of the full model between each climatic factor to examine for potential multicollinearity. As before, we identified and retained variables with robust effects after iteratively removing variables with $VIF > 5$ (O'Brien, 2007) and used model averaging methods to average the models with $\Delta AICc < 2$. We also ran a PGLS analysis using the same approach but

combining all clades and species ($N = 6553$ species) into a single model. Finally, we explored the potential causal relationships among climate, body mass, social system, and demography in the two categories in which cooperative breeding was associated with environmental quality using phylogenetic confirmatory path analysis (PCPA) (Hardenberg & Gonzalez-Voyer, 2013) with the lambda model in the R package *phylopath* (van der Bijl, 2018). More detailed descriptions of PCPA are provided in the Supplementary Methods.

RESULTS

Model results

First, we explored which environmental conditions favour CA or RD cooperation strategies. Since high quality patches are only worth defending when environmental conditions are predictable, we found that RD benefits only evolve in a temporally stable environment with low variability in resource availability (Figure 1a). In addition, both stable and benign environments (i.e., those with high resource availability) will lead to higher intraspecific competition over resources and thus, favour cooperative investment in resource defence (Figure S2). In contrast, CA benefits are more likely to emerge when mean environmental conditions are harsh (i.e., low resource availability) and when resource availability fluctuates greatly (Figure 1b and Figure S2). This is because extra group resources produced by cooperators are essential for individuals to reproduce successfully in both harsh and fluctuating environments. As a result, we found that CA and RD benefits occur in environments with distinct properties. Finally, our model also shows that non-cooperators have a reproductive advantage over cooperators under a wide range of environmental conditions, including in both benign environments for CA benefits and fluctuating environments for RD benefits.

Next, we explored the effects of cooperation on demography by determining the consequences of CA and RD benefits on range size and abundance. We found that RD cooperators and non-cooperators have very similar range sizes (Figure 2d) and abundances (Figure 2e,f) in harsh environments with low resource availability. In contrast, the population and range sizes of CA cooperators are larger than those of non-cooperators (Figure 2d–f) because individuals are more cooperative in harsh environments with low resource availability (Figure 1b). Nevertheless, CA cooperators and non-cooperators have similar range sizes and abundances in benign environments with high resource availability (Figure 2a–c), but the range sizes (Figure 2a) and abundances (Figure 2b,c) of RD cooperators are smaller or similar to non-cooperators. We also found that CA cooperators always have larger

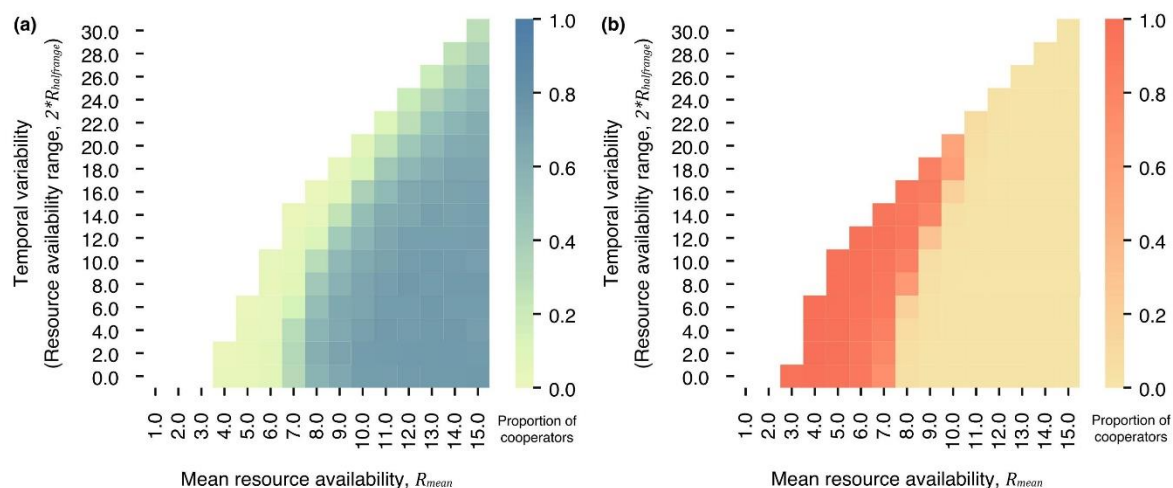


FIGURE 1 Model predictions of the relationships among grouping benefits, the mean and variability of resource availability, and the evolution of cooperation. Proportions of (a) resource defence (RD) cooperators and (b) collective action (CA) cooperators in each population in relation to mean resource availability. Each grid of the heat map is an average of over 100 repeated simulations, and each simulation lasts 10,000 time steps. We calculated the proportion of cooperators (i.e., individuals with non-zero degrees of cooperation) at the end of the simulations. Descriptions for resource availability in the simulation model are provided in the Methods.

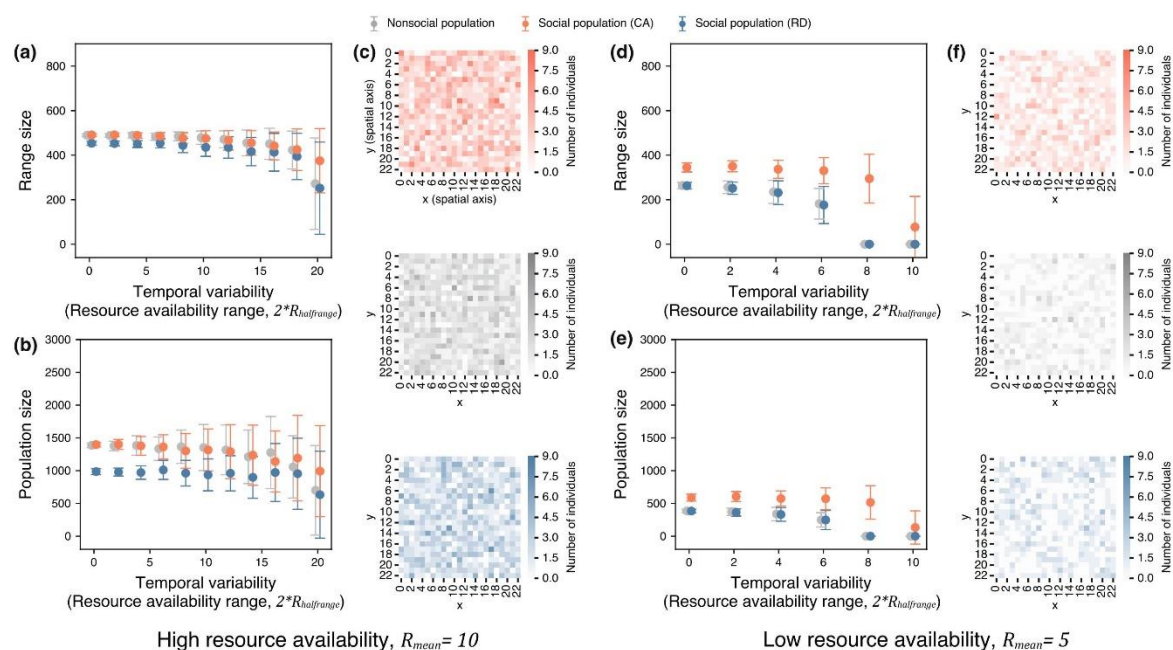


FIGURE 2 Model predictions about the relationships among grouping benefits, the mean and variability of resource availability, and the ecological consequences of cooperation. Comparisons of (a) range size, (b) species abundance, and (c) the spatial distribution of individuals of social populations that form because of collective action (CA) benefits (orange), social populations that form because of resource defence (RD) benefits (blue), and non-social populations (grey) in relation to the temporal variability in benign environments with high resource availability. (d) Range size, (e) species abundance, and (f) the spatial distribution of individuals of social populations in harsh environments with low resource availability. Points represent means, and error bars represent standard deviations. The colour darkness of heat maps indicates the number of individuals occupying a patch. All data are collected in the same way as in Figure 1.

range sizes and abundances than non-cooperators in harsh environments (Figure 2d–f). Fluctuating environmental conditions generally lead to a reduction in the range size (Figure 2d) and abundance (Figure 2e,f)

of both cooperators and non-cooperators in harsh environments with low resource availability. However, the reductions in range size (Figure 2d) and abundance (Figure 2e,f) of CA cooperators are relatively

small, except in extremely fluctuating environments. In contrast, the range sizes and abundances of CA cooperators are comparable to those of non-cooperators when mean resource availability is high (Figure 2a–c). Nevertheless, RD cooperators have smaller or similar range sizes (Figure 2a) and abundances (Figure 2b,c) to non-cooperators because most individuals defend high-quality patches (i.e., high resource availability; larger R_{mean} in Figure 1a). Interestingly, our model makes the same predictions for populations in both harsh and fluctuating environments, as well as similar predictions for those in both benign and stable environments, suggesting that the effect of highly fluctuating environments on the evolution of cooperative breeding behaviour is equivalent to the effect of harsh mean environmental conditions.

Comparative tests of ecological dominance in avian cooperative breeders

To comparatively test the set of predictions of our model (summarized in Table S5), together with previous work showing that the ecological drivers of cooperative breeding behaviour in birds may be different in different clades (Cockburn, 2020; Lin et al., 2019; Shen et al., 2017), we first examined how environmental factors affect the evolution of cooperatively and non-cooperatively breeding species separately in each avian clade (those showing either an association between cooperative breeding behaviour and environmental harshness/fluctuation, an association between cooperative breeding behaviour and environmental benignness/stability, or no association between cooperative breeding behaviour and environmental quality), and then compared species range sizes and abundances between cooperative and non-cooperative breeders in just the clades that showed an association between cooperative breeding behaviour and environmental quality. Although cooperative breeding behaviour is unrelated to environmental quality in 22 clades containing 2341 species (including 236 cooperative breeders), cooperatively breeding species tend to occur in harsh and fluctuating environments in 15 clades containing 2426 total species (including 306 cooperative breeders), but in benign and stable environments in 15 clades containing 1786 total species (including 169 cooperative breeders) (Table S4). Thus, as has been argued previously for birds (Lin et al., 2019), cooperative breeding behaviour varies greatly across the avian tree of life and appears to have evolved for different reasons in different groups of birds: 30% of clades show an association between cooperative breeding behaviour and environmental harshness/fluctuation (indicating that groups are likely to form because of CA benefits), 30% show an association between cooperative breeding behaviour and environmental benignness/stability (indicating that groups are likely to form because of RD benefits), and

40% show no association between cooperative breeding behaviour and environmental quality.

Next, we explicitly tested our simulation model predictions about the ecological and demographic consequences of sociality by comparing species range sizes and abundances in cooperative and non-cooperative breeders separately in clades that showed an association between cooperative breeding and environmental quality. In clades where cooperative breeding is associated with environmental benignness/stability (Table S6a), cooperative and non-cooperative breeders have similar range sizes (Figure 3a and Table S6b) and abundances (Table S6c). However, in clades where cooperation is associated with environmental harshness/fluctuation (Table S7a), cooperatively breeding species tend to have marginally larger range sizes than non-cooperative species (Figure 3b and Table S7b) and species with larger range sizes have greater abundances (Table S7c). Both of these results are consistent with the predictions of our simulation model. CA cooperators are more likely to evolve in harsh/fluctuating environments, and thus, those cooperatively breeding species occurring in such environments are more likely to have larger species range sizes and abundances than non-cooperative species. In contrast, RD cooperators are more likely to evolve in benign/stable environments, and thus, those cooperatively breeding species occurring in such environments are more likely to have similar species range sizes and abundances to non-cooperative species.

Although the results of our clade-level analysis were consistent with the key predictions of our simulation model, they excluded the 40% of avian species in clades that did not show an association between cooperative breeding behaviour and environmental quality. Therefore, to further test our model predictions using the complete dataset, we combined all of the avian clades into a single analysis (52 clades, including 6553 species). As has been shown previously for birds (Cornwallis et al., 2017; Jetz & Rubenstein, 2011; Rubenstein & Lovette, 2007), we found that both high environmental variability (high among-year variation in precipitation) and harsh mean environmental conditions (low mean annual precipitation) best explain the evolution of cooperative breeding behaviour (Table S8a). However, we went a step further than previous analyses and also show that cooperatively breeding species, which are more likely to occur in harsh and fluctuating environments, have significantly larger range sizes (Figure 3c and Table S8b) and marginally larger species abundances than non-cooperatively breeding species (Table S8c), as predicted by our model and consistent with the clade-level analysis.

Finally, to infer causal relationships among climate, cooperative breeding behaviour, and demography, we performed phylogenetic path analysis for both the clade-level and combined analyses. For clades in which cooperative breeders are more likely to occur in benign and stable environments, cooperation does not influence

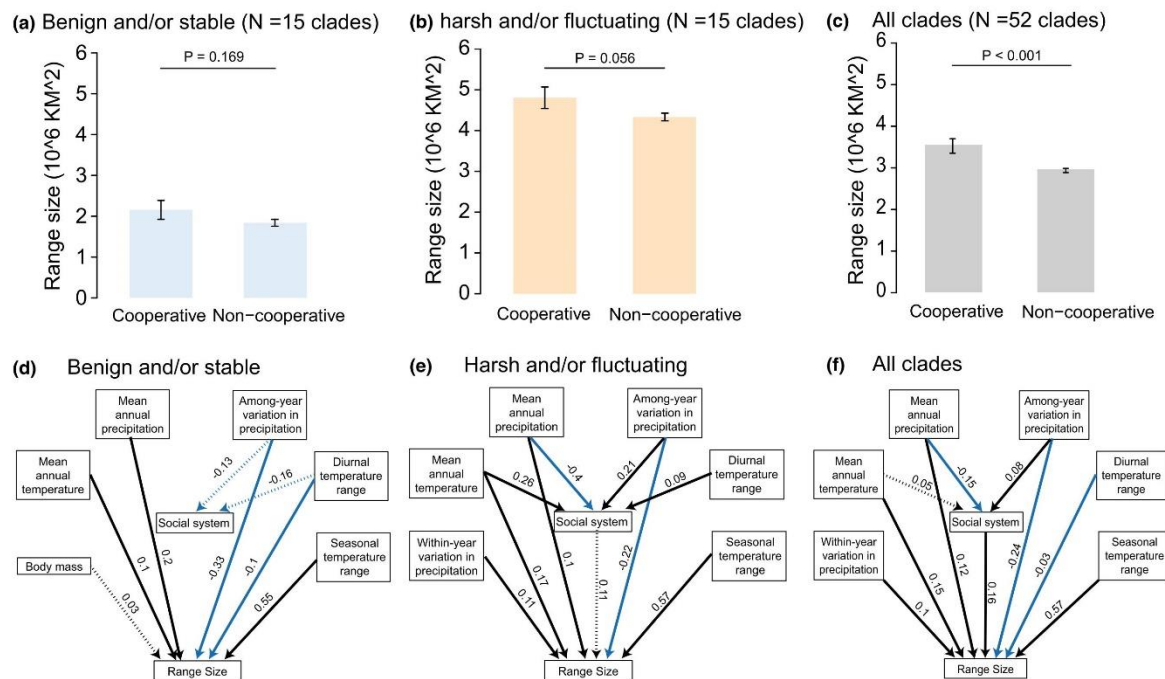


FIGURE 3 Relationships among environmental factors, social system, and range size. Comparison of geographic range sizes of cooperatively and non-cooperatively breeding species in (a) benign and/or stable environments ($N=15$ clades), (b) harsh and/or fluctuating environments ($N=15$ clades), and (c) in all clades in all environments, including those that did not show relationships between cooperative breeding behaviour and environmental quality ($N=52$ clades). Causal relationships among environmental factors, cooperative breeding, and range size in clades that cooperate in (d) benign and/or stable environments, (e) harsh and/or fluctuating environments, and (f) in all clades of cooperatively breeding birds in all environments. Arrows indicate direct effects, and colours represent direction (black: positive; blue: negative), with solid lines signifying significant causal relationships and dash lines signifying marginally significant causal relationships. Numbers represent standardized regression coefficients.

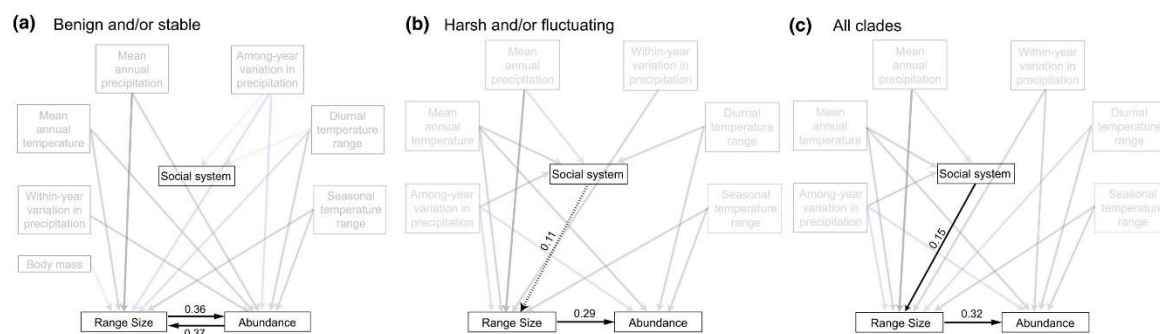


FIGURE 4 Causal relationships among social system, range size, and species abundance for (a) cooperatively breeding species that occur in benign and/or stable environments, (b) cooperatively breeding species that occur in harsh and/or fluctuating environments, and (c) all clades of cooperatively breeding birds. Arrows indicate direct effects, and colours represent direction (black: positive; blue: negative; grey: relationships that have been described in Figure 3), with solid lines signifying significant causal relationships and dash lines signifying marginally significant causal relationships. Numbers represent standardized regression coefficients. Since 424 species did not have species abundance information, we performed the same phylogenetic path analysis using this subset of the data ($N=6129$ species) that includes both species range size and abundance. The faded text and lines in the figure represent similar relationships to those presented in Figure 3, so we have faded them to improve the readability of this figure.

range size (Figure 3d and Table S9a), but species with larger ranges still have greater abundances (Figure 4a and Table S9b). However, for clades in which cooperative

breeders are more likely to occur in harsh and fluctuating environments, cooperative species have marginally larger range sizes (Figure 3e and Table S10a) and greater

abundances than non-cooperative species (Figure 4b and Table S10b). In the combined analysis of all clades, we similarly found that cooperative breeding behaviour is more likely to occur in harsh and fluctuating environments, and that cooperative species in these environments have larger range sizes (Figure 3f and Table S11a) and greater abundances than non-cooperative species (Figure 4c and Table S11b).

Considering all of these comparative analyses together indicates that cooperation appears to enable social species to outcompete non-social species to achieve greater abundances in environments where resource availability is low and variability high, a result consistent with the key prediction of our simulation model. In contrast, species that cooperate in benign and stable environments do not have greater abundances than non-cooperative species in those same environments, a result that is also consistent with the prediction from our theoretical model. In other words, cooperative breeding behaviour does not affect species range size or abundance in benign and/or stable environments, only in harsh and/or fluctuating environments. Thus, our comparative analyses suggest that cooperation appears to be a consequence—rather than a cause—of high intraspecific competition when species are abundant.

Vulnerability of social species to climate change

Given our finding that cooperation enables social species to outcompete non-social species and achieve greater abundance in environments where resource availability is low and fluctuates, we returned to our simulation model to investigate the possible effects of climate change (e.g., an increase in environmental fluctuation) on populations of CA cooperators, RD cooperators, and non-cooperators. Our model compares CA benefit species (populations containing both CA cooperators and non-cooperators), RD benefit species (populations containing both RD cooperators and non-cooperators), and non-social species (populations consisting entirely of non-cooperators). We found that in an originally harsh environment with low resource availability, CA cooperators are more resilient when climate change causes a decrease in the average availability of resources (Figure 5a–f). Therefore, the proportion of CA cooperators increases as the environment becomes increasingly harsh (Figure 5b,e), and CA cooperators maintain a relatively higher abundance compared to RD cooperators and non-cooperators (Figure 5a,d). In contrast, RD cooperators persist at a lower proportion in the population when the average environmental conditions deteriorate (Figure 5c,f,i,l), but environmental change has only a minimal or negative impact on the population abundance of RD cooperators compared to that of non-cooperators (Figure 5a,d,g,j). We also find that the effect of greater environmental fluctuations on both

the population abundance and the proportion of cooperators in the population is similar to that of reduced average resource availability (Figure S3). Thus, species that cooperate in harsh and/or fluctuating environments will be less vulnerable to climate change than both non-social species and those that cooperate in benign and/or stable environments.

DISCUSSION

To more directly test the paradox of environmental quality and sociality, we combined simulation modelling and phylogenetic comparative analysis to examine the ecological and demographic consequences of sociality (summarized in Table S5). Our model showed that CA cooperators living in harsh and/or fluctuating environments have larger range sizes and species abundances than non-cooperators in those environments, but that RD cooperators and non-cooperators living in stable and/or benign environments have similar range sizes and species abundances. Results from our comparative analyses were consistent with the model predictions, which help explain the paradox of why similar forms of social organization occur under such seemingly disparate ecological circumstances (Emlen, 1982; Shen et al., 2017). Yet, previous studies have argued that the influence of ecology on social evolution is not as easily quantified across species as kinship, making it difficult to develop a general understanding of how ecology influences on social evolution (Korb & Heinze, 2008; West et al., 2021). The obvious challenge is that a species often faces various types of environmental pressures (i.e., CA and RD benefits can occur in the same species). Thus, although environmental factors have been consistently recognized as key causes of social evolution, surprisingly, the specific environmental factors—agents of selection—influencing the evolution of cooperative breeding behaviour have only been well-studied in a few empirical studies (Shen et al., 2017; Wcislo & Tierney, 2009).

The dual-benefits framework provides a series of predictions that allow us to conduct broad-scale comparative studies to understand the ecological causes and consequences of cooperative breeding behaviour. Our analysis of the global distribution of avian species suggests that different taxa form cooperatively breeding groups for different reasons and in environments of contrasting quality. Among all birds, we found that species tend to cooperate in harsh environments with low mean annual precipitation and fluctuating environments with high among-year variation in precipitation, as has been shown previously for birds (Cornwallis et al., 2017; Jetz & Rubenstein, 2011; Rubenstein & Lovette, 2007). However, at the clade level, we found that similar numbers of clades containing roughly equal numbers of species cooperate in harsh and/or fluctuating environments and in benign and/or stable environments. We interpret these results to

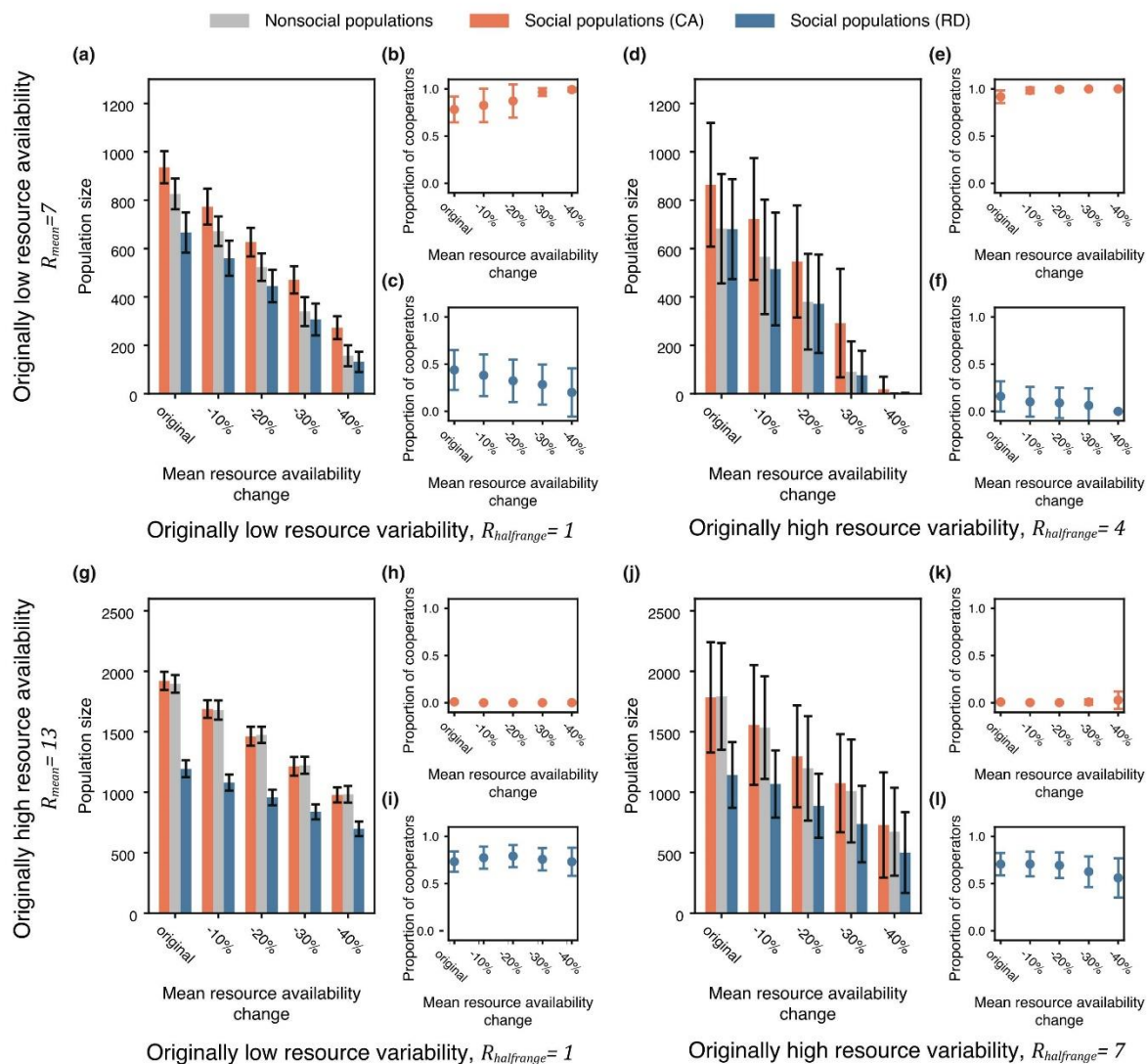


FIGURE 5 Model predictions of the effects of decreasing mean resource availability due to climate change. Comparison of (a) species abundance, (b) proportion of collective action (CA) cooperators, and (c) proportion of resource defence (RD) cooperators in an environment originally with low mean resource availability and small fluctuations in resources. Comparison of (d) species abundance, (e) proportion of CA cooperators, and (f) proportion of RD cooperators in an environment originally with low mean resource availability and large fluctuations in resources. Comparison of (g) species abundance, (h) proportion of CA cooperators, and (i) proportion of RD cooperators in an environment originally with high mean resource availability and small fluctuations in resources. Finally, a comparison of (j) species abundance, (k) proportion of CA cooperators, and (l) proportion of RD cooperators in an environment originally with high mean resource availability and large fluctuations in resources.

mean that although there is a general trend for cooperative breeding behaviour to evolve in harsh and/or unpredictable environments, birds appear to cooperate in these contrasting environments for different reasons, as argued by the paradox of environmental quality and sociality (Lin et al., 2019; Shen et al., 2017). Importantly, without performing clade-level analyses as we did here, researchers are likely to overlook the effects of benign/stable environments on the evolution of cooperative breeding behaviour.

Our results are consistent with the idea that cooperative breeding behaviour should be considered as an umbrella category, and care should be taken to differentiate between clades that form cooperatively breeding groups for different ecological reasons when conducting large-scale comparative studies (Shen et al., 2017). In addition, there are many clades of birds for which we were unable to find a relationship between environmental quality and cooperative breeding behaviour, either because CA and RD benefits

may both play a part in these lineages, or other factors may influence the propensity to cooperate. Therefore, we believe that future studies that examine the effects of environmental and social factors on cooperative breeding behaviour at the clade level—rather than just lumping all the data together—will be fruitful. Equally important, although CA and RD benefits are likely to act together in the same species, we believe that in most species one particular type of benefit will be more important for influencing the evolution of cooperative breeding behaviour. For example, while alloparents typically feed offspring and help increase the overall provisioning rate (a type of a CA benefit) (Hatchwell, 1999), a lack of outside breeding vacancies often favours offspring remaining on the natal territory (a type of RD benefit) and is therefore likely to be the primary ecological cause of cooperative breeding in these species (Emlen, 1982, 1991). According to the dual-benefits framework, we can understand which CA or RD benefits play the most important role in shaping the cooperatively breeding behaviour of species (or population) through observations (e.g., the relationship between group size and per capita productivity) or experimental manipulations (e.g., alteration of reproductive vacancies or population density; see table 1 in Shen et al., 2017) (Liu et al., 2020; Sun et al., 2014). Ultimately, determining the influence of CA or RD benefits on the evolution of cooperative breeding behaviour at the species level will be key to our understanding of environmental influences on the evolution of sociality (Shen & Rubenstein, 2019).

It is worth noting that our simulation model generates similar effects of high environmental fluctuation and poor mean environmental conditions on the evolution of cooperative behaviour, a result that is likely to occur because environments with high climatic fluctuations are, by definition, bound to include periods of both low and high environmental quality. Thus, in environments with greater environmental fluctuations, there is a stronger selection for social group formation during harsh periods with small population sizes than during benign periods with relatively large population sizes. Most studies examining the effects of population size fluctuations on the strength of selection have focused on the effects of genetic drift in small populations (Carson, 1990; Lande, 1976), or have examined how environmental fluctuations affect changes in gene frequency across genotypes at a fixed population size, thus focusing on reductions in fitness variance (Slatkin, 1974; Starrfelt & Kokko, 2012). However, based on our model that assumes population size varies with environmental fitness (a biologically realistic assumption for most species), strategies to cope with harsh environments may be key to understanding how organisms adapt to fluctuating environments.

Finally, although some of the most ecologically successful species on Earth form cooperative societies, it is often assumed that cooperation enables their ‘social conquest’ (Wilson, 1987, 2012). Here, we provide a formal mathematical framework for understanding precisely how cooperation could lead to ecological dominance in some taxa but not in others. Our model results also suggest that species that cooperate in harsh and/or fluctuating environments (Firman et al., 2020; Jetz & Rubenstein, 2011; Kocher et al., 2014; La Richelière et al., 2022; Lukas & Clutton-Brock, 2017; Martin et al., 2020; Rubenstein & Lovette, 2007; Sheehan et al., 2015) will be less vulnerable to climate change than both non-social species and those that cooperate against intraspecific competitors in benign and/or stable environments (Borger et al., 2023; Choe & Crespi, 1997; Cornwallis et al., 2017; Costa, 2006; Gonzalez et al., 2013; Solomon & French, 1997). This framework linking the ecological causes and consequences of cooperation can thus be used to explore a range of topics relevant to the changing world in which we now find ourselves, including the fluctuation and resilience of species abundances of social and non-social species over evolutionary and ecological timescales, as well as range size shifts in relation to changes in social behaviour under different climate change scenarios. Under intensifying anthropogenic climate change (Bonfils et al., 2020; Paik et al., 2020; Zhang et al., 2013), now more than ever, we urgently need to understand how social behaviours and environmental conditions synergistically influence the ecological success—or failure—of animals.

AUTHOR CONTRIBUTIONS

S.-F.S. conceived the idea of the paper. Y.-Y. C. and S.-F.S. constructed the simulation model. Y.-H. L., S.-F. S., and D.D.R. analysed the empirical data. S.-F. S., Y.-Y. C., Y.-H. L., M.L., M.L., and D.D.R. designed the study and wrote the manuscript.

ACKNOWLEDGEMENTS

S.-F.S. was supported by the Academia Sinica (AS-SS-106-05 and AS-IA-106-L01) and the Minister of Science and Technology of Taiwan (100-2621-B-001-004-MY3, 104-2311-B-001-028-MY3, 108-2314-B-001-009-MY3 and MOST 110-2628-B-001-022). D.D.R. was supported by the National Science Foundation (IOS-1656098).

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ele.14226>.

DATA AVAILABILITY STATEMENT

All code and data are available on GitHub (https://github.com/mingpapilio/Codes_Social_Conquest) and archived on Zenodo (doi.org/10.5281/zenodo.7820320).

ORCID

Yu-Heng Lin  <https://orcid.org/0000-0001-9316-8643>Dustin R. Rubenstein  <https://orcid.org/0000-0002-4999-3723>Ming Liu  <https://orcid.org/0000-0002-5170-8688>Sheng-Feng Shen  <https://orcid.org/0000-0002-0631-6343>

REFERENCES

- Anderson, D. & Burnham, K. (2004) *Model selection and multi-model inference*, 2nd edition. NY: Springer-Verlag.
- BirdLife International. (2020) Handbook of the Birds of the World and BirdLife International digital checklist of the birds of the world. Version.
- Blomberg, S.P., Garland, T., Jr. & Ives, A.R. (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, 57, 717–745.
- Bonfils, C.J.W., Santer, B.D., Fyfe, J.C., Marvel, K., Phillips, T.J. & Zimmerman, S.R.H. (2020) Human influence on joint changes in temperature, rainfall and continental aridity. *Nature Climate Change*, 10, 726–731.
- Borger, M.J., Richardson, D.S., Dugdale, H., Burke, T. & Komdeur, J. (2023) Testing the environmental buffering hypothesis of cooperative breeding in the Seychelles warbler. *Acta Ethologica*. <https://doi.org/10.1007/s10211-022-00408-y>
- Botero, C.A., Dor, R., McCain, C.M. & Safran, R.J. (2014) Environmental harshness is positively correlated with intraspecific divergence in mammals and birds. *Molecular Ecology*, 23, 259–268.
- Both, C., Van Turnhout, C.A.M., Bijlsma, R.G., Siepel, H., Van Strien, A.J. & Foppen, R.P.B. (2010) Avian population consequences of climate change are most severe for long-distance migrants in seasonal habitats. *Proceedings of the Royal Society B: Biological Sciences*, 277, 1259–1266.
- Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A. et al. (2019) BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, 15, e1006650.
- Brooks, K.C., Maia, R., Duffy, J.E., Hultgren, K.M. & Rubenstein, D.R. (2017) Ecological generalism facilitates the evolution of sociality in snapping shrimps. *Ecology Letters*, 20, 1516–1525.
- Callaghan, C.T., Nakagawa, S. & Cornwell, W.K. (2021) Global abundance estimates for 9,700 bird species. *Proceedings of the National Academy of Sciences*, 118, e2023170118.
- Carson, H.L. (1990) Increased genetic variance after a population bottleneck. *Trends in Ecology & Evolution*, 5, 228–230.
- Chan, W.-P., Chen, I.-C., Colwell, R.K., Liu, W.-C., Huang, C.-Y. & Shen, S.-F. (2016) Seasonal and daily climate variation have opposite effects on species elevational range size. *Science*, 351, 1437–1439.
- Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B. & Thomas, C.D. (2011) Rapid range shifts of species associated with high levels of climate warming. *Science*, 333, 1024–1026.
- Choe, J.C. & Crespi, B.J. (1997) *The evolution of social behavior in insects and arachnids*. Cambridge, UK: Cambridge University Press.
- Cockburn, A. (2006) Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society B: Biological Sciences*, 273, 1375–1383.
- Cockburn, A. (2020) Chapter six—Can't see the “hood” for the trees: can avian cooperative breeding currently be understood using the phylogenetic comparative method? *Advances in the Study of Behavior*, 52, 243–291.
- Cornwallis, C.K., Botero, C.A., Rubenstein, D.R., Downing, P.A., West, S.A. & Griffin, A.S. (2017) Cooperation facilitates the colonization of harsh environments. *Nature Ecology & Evolution*, 1, 0057.
- Costa, J.T. (2006) *The other insect societies*. Cambridge, MA: Belknap Press of Harvard University Press.
- Darwin, C. (1859) *On the origin of species by means of natural selection, or the preservation of Favoured races in the struggle for life*. London: John Murray.
- Dunning, J.B., Jr. (2007) *CRC handbook of avian body masses*. Florida, USA: CRC Press.
- Emlen, S.T. (1982) The evolution of helping I: an ecological constraints model. *The American Naturalist*, 119, 29–39.
- Emlen, S.T. (1991) Evolution of cooperative breeding in birds and mammals. In: Krebs, J.R. & Davies, N.B. (Eds.) *Behavioural ecology: an evolutionary approach*. Oxford, UK: Blackwell Science Oxford, pp. 301–337.
- Erasmus, B.F., Van Jaarsveld, A.S., Chown, S.L., Kshatriya, M. & Wessels, K.J. (2002) Vulnerability of south African animal taxa to climate change. *Global Change Biology*, 8, 679–693.
- Ficetola, G.F., Mazel, F. & Thuiller, W. (2017) Global determinants of zoogeographical boundaries. *Nature Ecology & Evolution*, 1, 0089.
- Firman, R.C., Rubenstein, D.R., Moran, J.M., Rowe, K.C. & Buzatto, B.A. (2020) Extreme and variable climatic conditions drive the evolution of sociality in Australian rodents. *Current Biology*, 30, 691–697.
- Fisher, D.N., Kilgour, R.J., Siracusa, E.R., Foote, J.R., Hobson, E.A., Montiglio, P.-O. et al. (2021) Anticipated effects of abiotic environmental change on intraspecific social interactions. *Biological Reviews*, 96, 2661–2693.
- Gonzalez, J.-C.T., Sheldon, B.C. & Tobias, J.A. (2013) Environmental stability and the evolution of cooperative breeding in hornbills. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20131297.
- Griesser, M. & Suzuki, T.N. (2016) Occasional cooperative breeding in birds and the robustness of comparative analyses concerning the evolution of cooperative breeding. *Zoological Letters*, 2, 1–11.
- Hackett, S.J., Kimball, R.T., Reddy, S., Bowie, R.C., Braun, E.L., Braun, M.J. et al. (2008) A phylogenomic study of birds reveals their evolutionary history. *Science*, 320, 1763–1768.
- Hardenberg, A.V. & Gonzalez-Voyer, A. (2013) Disentangling evolutionary cause-effect relationships with phylogenetic confirmatory path analysis. *Evolution: International Journal of Organic Evolution*, 67, 378–387.
- Harris, I., Osborn, T.J., Jones, P. & Lister, D. (2020) Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. *Scientific Data*, 7, 1–18.
- Hatchwell, B. (1999) Investment strategies of breeders in avian cooperative breeding systems. *American Naturalist*, 154, 205–219.
- Ho, L.S.T., Ane, C., Lachlan, R., Tarpinian, K., Feldman, R., Yu, Q. et al. (2018) Package ‘phylolm’.
- Hurvich, C.M. & Tsai, C.-L. (1989) Regression and time series model selection in small samples. *Biometrika*, 76, 297–307.
- IPCC. (2021) *Climate change 2021: the physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate Change*. Cambridge: Cambridge University Press.
- Jetz, W. & Rubenstein, D.R. (2011) Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Current Biology*, 21, 72–78.
- Jetz, W., Thomas, G., Joy, J., Hartmann, K. & Mooers, A. (2012) The global diversity of birds in space and time. *Nature*, 491, 444–448.
- Kocher, S.D., Pellissier, L., Veller, C., Purcell, J., Nowak, M.A., Chapuisat, M. et al. (2014) Transitions in social complexity along elevational gradients reveal a combined impact of season length and development time on social evolution. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20140627.

- Korb, J. & Heinze, J. (2008) *Ecology of social evolution*. New York: Springer.
- La Richelière, F., Muñoz, G., Guénard, B., Dunn, R.R., Economo, E.P., Powell, S. et al. (2022) Warm and arid regions of the world are hotspots of superorganism complexity. *Proceedings of the Royal Society B: Biological Sciences*, 289, 20211899.
- Lande, R. (1976) Natural selection and random genetic drift in phenotypic evolution. *Evolution*, 30, 314–334.
- Lenoir, J., Bertrand, R., Comte, L., Bourgeaud, L., Hattab, T., Murienne, J. et al. (2020) Species better track climate warming in the oceans than on land. *Nature Ecology & Evolution*, 4, 1044–1059.
- Lin, Y.-H., Chan, S.-F., Rubenstein, D.R., Liu, M. & Shen, S.-F. (2019) Resolving the paradox of environmental quality and sociality: the ecological causes and consequences of cooperative breeding in two lineages of birds. *The American Naturalist*, 194, 207–216.
- Liu, M., Chan, S.-F., Rubenstein, D.R., Sun, S.-J., Chen, B.-F. & Shen, S.-F. (2020) Ecological transitions in grouping benefits explain the paradox of environmental quality and sociality. *The American Naturalist*, 195, 818–832.
- Lukas, D. & Clutton-Brock, T. (2017) Climate and the distribution of cooperative breeding in mammals. *Royal Society Open Science*, 4, 160897.
- Martin, J., Ringen, E., Duda, P. & Jacggi, A. (2020) Harsh environments promote alloparental care across human societies. *Proceedings of the Royal Society B: Biological Sciences*, 287, 20200758.
- Mazerolle, M.J. (2013) AICcmodavg: model selection and multimodel inference based on (Q) AIC (c). R Package Version, 1, 35.
- Mora, C., Dousset, B., Caldwell, I.R., Powell, F.E., Geronimo, R.C., Bielecki, C.R. et al. (2017) Global risk of deadly heat. *Nature Climate Change*, 7, 501–506.
- O'Brien, R.M. (2007) A caution regarding rules of thumb for variance inflation factors. *Quality & Quantity*, 41, 673–690.
- Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature*, 401, 877–884.
- Paik, S., Min, S.-K., Zhang, X., Donat, M.G., King, A.D. & Sun, Q. (2020) Determining the anthropogenic greenhouse gas contribution to the observed intensification of extreme precipitation. *Geophysical Research Letters*, 47, e2019GL086875.
- Pearce-Higgins, J.W., Ockendon, N., Baker, D.J., Carr, J., White, E.C., Almond, R.E.A. et al. (2015) Geographical variation in species' population responses to changes in temperature and precipitation. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20151561.
- Prein, A.F., Rasmussen, R.M., Ikeda, K., Liu, C., Clark, M.P. & Holland, G.J. (2017) The future intensification of hourly precipitation extremes. *Nature Climate Change*, 7, 48–52.
- Rubenstein, D.R. & Lovette, I.J. (2007) Temporal environmental variability drives the evolution of cooperative breeding in birds. *Current Biology*, 17, 1414–1419.
- Sheehan, M.J., Botero, C.A., Hendry, T.A., Sedio, B.E., Jandt, J.M., Weiner, S. et al. (2015) Different axes of environmental variation explain the presence vs. extent of cooperative nest founding associations in *Polistes* paper wasps. *Ecology Letters*, 18, 1057–1067.
- Shen, S.-F., Emlen, S.T., Koenig, W.D. & Rubenstein, D.R. (2017) The ecology of cooperative breeding behaviour. *Ecology Letters*, 20, 708–720.
- Shen, S.-F. & Rubenstein, D.R. (2019) Environmental uncertainty and social behavior. In: Choe, J.C. (Ed.) *Encyclopedia of animal behavior*. Cambridge, MA: Elsevier, Academic Press, pp. 807–815.
- Slatkin, M. (1974) Hedging one's evolutionary bets. *Nature*, 250, 704–705.
- Solomon, N.G. & French, J.A. (1997) *Cooperative breeding in mammals*. Cambridge: Cambridge University Press.
- Starrfelt, J. & Kokko, H. (2012) Bet-hedging—a triple trade-off between means, variances and correlations. *Biological Reviews*, 87, 742–755.
- Sun, S.-J., Rubenstein, D.R., Chen, B.-F., Chan, S.-F., Liu, J.-N., Liu, M. et al. (2014) Climate-mediated cooperation promotes niche expansion in burying beetles. *eLife*, 3, e02440.
- Tobias, J.A., Sheard, C., Pigot, A.L., Devenish, A.J., Yang, J., Sayol, F. et al. (2022) AVONET: morphological, ecological and geographical data for all birds. *Ecology Letters*, 25, 581–597.
- van der Bijl, W. (2018) Phylopath: easy phylogenetic path analysis in R. *PeerJ*, 6, e4718.
- Weislo, W.T. & Tierney, S.M. (2009) The evolution of communal behavior in bees and wasps: an alternative to eusociality. In: Gadau, J. & Fewell, J. (Eds.) *Organization of Insect Societies from genome to Sociocomplexity*. MA: Harvard University Press Cambridge, pp. 148–169.
- West, S.A., Cooper, G.A., Ghoul, M.B. & Griffin, A.S. (2021) Ten recent insights for our understanding of cooperation. *Nature Ecology & Evolution*, 5, 419–430.
- Wilson, E.O. (1987) Causes of ecological success: the case of the ants. *Journal of Animal Ecology*, 56, 1–9.
- Wilson, E.O. (1990) *Success and dominance in ecosystems: the case of the social insects*. Oldendorf: International Ecology Institute.
- Wilson, E.O. (2012) *The social conquest of earth*. W. W. Norton, New York: Liveright.
- Zhang, X., Wan, H., Zwiers, F.W., Hegerl, G.C. & Min, S.-K. (2013) Attributing intensification of precipitation extremes to human influence. *Geophysical Research Letters*, 40, 5252–5257.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Lin, Y.-H., Chen, Y.-Y., Rubenstein, D.R., Liu, M., Liu, M. & Shen, S.-F. (2023) Environmental quality mediates the ecological dominance of cooperatively breeding birds. *Ecology Letters*, 26, 1145–1156. Available from: <https://doi.org/10.1111/ele.14226>

