Cooperation and conflict across the major transitions

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Statement of originality

I declare that I have composed this thesis and that the work contained herein is my own except where explicitly stated. This work has not been submitted for any degree, diploma, certificate, or other qualification, in this University or elsewhere.

Nicholas G. Davies

For my parents

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General abstract

The history of life is punctuated by major transitions in individuality, when previously-independent biological units assemble into new agents. The stability of these evolutionary alliances is governed by the extent of cooperation and conflict among their constituents. In this thesis, I explore how ecology influences these social interactions. *Part I* investigates cooperation in arthropod societies. W. D. Hamilton's haplodiploidy hypothesis, which holds that asymmetries in sibling relatedness explain the exclusively-female worker caste of the social Hymenoptera, has fallen out of favour; instead, the preadaptation of females for rearing young is thought to explain who helps in these taxa. Analysing the evolution of paternal care, I show that this pattern of preadaptation is not itself a consequence of haplodiploidy inhibiting brood care by males. I then show how five key factors of sexual ecology—sex-specific preadaptation, labile sex allocation, sib-mating, promiscuity, and reproductive autonomy—have a major impact upon the evolution of sex-biased helping in arthropod societies. Finally, inclusive-fitness theory predicts that monogamy should promote worker sterility in insect societies, but a recent population-genetics model has challenged this prediction. I show that relaxing this model's genetic, evolutionary, and ecological assumptions supports inclusive-fitness theory. *Part II* investigates conflict within genomes. I show that transposons are under selection to drive their host populations extinct, and explore the transposon–host and transposon–transposon interactions that might prevent this outcome. While neither selection against transposons' deleterious effects nor exploitation by parasites can plausibly prevent extinction, I show that host suppression of transposons can; however, this is only stable in the long term if suppression makes transposition costlier. I conclude that complex life could not exist without the active suppression of genetic conflict. Overall, I argue that ecology explains patterns of cooperation and conflict across the major transitions.

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Chapter 1

Introduction

The great achievement of the major transitions paradigm (Maynard Smith & Szathmáry 1995; Queller 1997a) has been to provide an attainable viewpoint from which, taking in the landscape of natural history from primordial beginnings to late elaborations, the social bedrock upon which all biological complexity rests can clearly be discerned (Table 1). In the two decades that have passed since the publication of *The Major Transitions in Evolution* (Maynard Smith & Szathmáry 1995), the major transitions view has itself become sufficiently foundational that the list of life's successful synergies—genes to genomes, prokaryotes to eukaryotes, cells to bodies, individuals to societies—is a familiar refrain among acolytes of certain sociobiological denominations. Indeed, as the basis for a research programme, the basic conceptual tool—that many of the most significant developments in evolution have involved the conjunction of lower-level building blocks, formerly independent entities, into higher-level, interdependent and co-replicating units, in effect a new level of individuality—is sufficiently elegant and powerful that it is difficult to shake loose once grasped.

While Maynard Smith and Szathmáry's book (1995) synthesized the major transitions view from its precursors (*e.g.* Wheeler 1911; Bonner 1974; Buss 1987; Maynard Smith 1988), it was a subsequent book review and précis (Queller 1997a) that was largely responsible for systematizing them. In Queller's schema, major transitions in individuality can be understood as the culmination of one of two types of evolutionary alliance. In the first type—fraternal alliances—the units that coalesce are alike, such that a member of such an alliance, in promoting the survival and replication of

Transition	Example	Type
Independent replicators → populations of replicators	Genome	Egalitarian
Separate unicells → symbiotic unicell	Eukaryotic cell	Egalitarian
Unicells → multicellular organism	Animals, plants, fungi	Fraternal
Multicellular organisms → eusocial society	Eusocial insects	Fraternal
Heterospecific organisms → obligate interspecific mutualism	*Buchnera* and pea aphids	Egalitarian

Table 1: The major transitions in individuality (adapted from West *et al.* 2015). The history of life on earth has been punctuated by major transitions in individuality, which are characterized by lower-level individuals coalescing into a new, higher-level individual through an evolutionary alliance. The two requirements for a major transition in individuality are complementary totipotency and negligible within-group conflict: complementary totipotency means that the transition partners, whose ancestors were able to replicate independently, now cannot replicate themselves in the absence of their partners; and negligible within-group conflict means that there must be sufficiently low scope for conflict between partners that adaptations can manifest at the level of the new individual. Transitions in individuality come in two types—while a fraternal transition is driven by indirect fitness gains through kinship, an egalitarian transition is driven by direct fitness gains through mutual benefit.

another, is promoting the survival of its own type. Such alliances obtain in multicellular bodies, where somatic cells help propagate the germline. To the soma, this is as good as promoting self-propagation, because the genetic bottleneck of passing through a single-cell stage ensures that germ and soma are genetically identical (Buss 1987); indeed, no transition to obligate multicellularity has occurred via the formation of non-clonal groups (Fisher *et al.* 2013). Fraternal alliances also underpin the organization of eusocial insect colonies, where workers sacrifice their reproductive potential to help raise their siblings. Under strict lifetime monogamy, individuals have equal relatedness to their siblings as to their offspring (Boomsma 2007); here, too, no transition to obligate eusociality has occurred in clades without strict lifetime monogamy (Hughes *et al.* 2008). In such fraternal alliances, enhancing the fitness of an ally is interchangeable with enhancing one's own fitness.

In contrast, in an egalitarian alliance, the units that coalesce are not alike. Rather, they cooperate because sticking together leads to more lasting evolutionary success than staying apart; the viability of this strategy necessarily depends on the suppression of conflict between units, since there is no kinship to provide indirect fitness benefits to allies. An egalitarian alliance obtains between genes in a genome: a gene lives and dies with its carrier, and so in order to promote its own survival a gene must promote the survival of its carrier (Williams 1966; Dawkins 1976). This strategy is equally effective for genes residing in sexual organisms as it is for their counterparts within asexuals on account of the fairness of meiosis (Leigh 1971): in a sexual organism, a given gene has only a 50% chance of being passed on per offspring, but that is the same chance afforded to any other gene. The subversion of fair meiosis—for example, by transposons, meiotic drivers, or other selfish genetic elements—introduces the potential for intragenomic conflict (Burt & Trivers 2006).

Overall, then, a fraternal alliance is built on relatedness and is fundamentally altruistic: individuals promote the survival of their allies as an alternative to personal reproduction. An egalitarian alliance, on the other hand, is built on codependence and is fundamentally mutualistic: individuals promote the survival of their allies as a means of securing their personal reproduction. Each reflects alternative strategies for propagating one's type. That evolutionary alliances are at the core of major transitions in

individuality serves as a sort of organizing principle for principles of organization, in which the principals at one level of individuality become organs at another.

But if this view sets out necessary conditions for major transitions, questions remain over what constitutes the sufficient conditions. Major transitions are rare occurrences, despite the necessary generating materials being commonplace. What accounts for the occurrence of major transitions in some qualifying clades, and not in others? And what explains the various patterns that obtain in the end products of major transitions? Answering these questions requires delving into details of ecology: the factors governing an individual's interactions with its physical and social environment. The aim of this thesis is to identify some of those factors, and to show how they influence patterns of cooperation and conflict within evolutionary alliances. Specifically, this thesis focuses on two systems: cooperation in arthropod societies, and conflict in transposon–host coevolution (Fig. 1).

Part I: Sexual ecology and cooperation in arthropod societies

Sociality, relatedness, and ecology

In terms of biological organization, eusocial insects provide some of the most complex examples of life on earth. The social insects exhibit an extreme form of reproductive altruism matched by few other multicellular species, in which specialized workers forfeit their reproductive potential in order to invest in the welfare of their siblings. Darwin (1859) saw the workers of social insects as posing a slight difficulty for his theory of natural selection (Ratnieks *et al.* 2011) which he dispatched by proposing that had it been "profitable to the community that a number should have been annually born capable of work, but incapable of procreation, I can see no very great difficulty in this being effected by natural selection", *i.e.*, "by acting on the fertile parents" (Darwin 1859, pp. 236 & 241).

A more precise treatment of selection "applied to the family" (Darwin 1859, p. 237) was taken up by Hamilton (1964). By kin selection theory, genes for altruistic

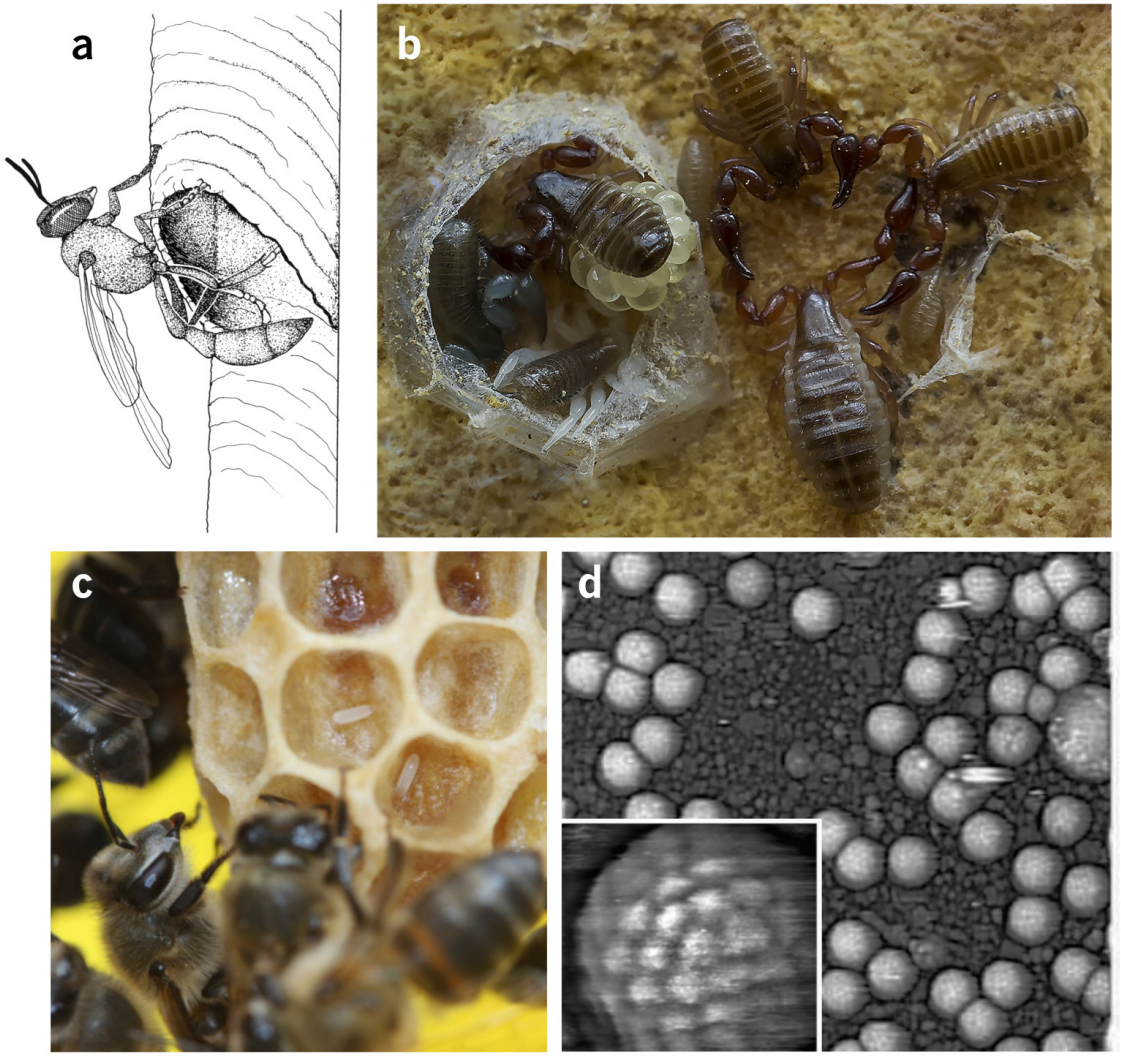


Figure 1: Topics studied in this thesis. **(a)** The evolution of paternal care. Paternal care is rare in the solitary Hymenoptera. An exception is furnished by *Trypoxylon* wasps, males of which engage in brood-guarding and nest-building behaviour. Here, a *Trypoxylon* (*Trypargilum*) *monteverdeae* male guards the entrance to his mate's nest. From Brockmann (1992). **(b)** Helping in arthropod societies. Altruistic sib-rearing in arthropods occurs not only in the eusocial Hymenoptera and termites, but also in some lesser-known primitively-social species, such as this social pseudoscorpion (*Paratemnoides nidificator*). Here, adults and nymphs are gathered around a silk chamber. Photo by José Roberto Peruca. **(c)** Worker sterility in insect colonies. Workers in most hymenopteran societies, although unmated, retain the ability to produce sons through arrhenotokous parthenogenesis. Here, worker-laid eggs are found on the exterior of a queen cell. From Goudie and Oldroyd (2014). **(d)** Transposon–host coevolution. Transposons are genetic parasites that make copies of themselves within their host genome. Here, the Ty3 retrotransposon virus-like particle in yeast is imaged using atomic force microscopy. Inside the spherical capsid structure, an RNA fragment is reverse transcribed into DNA, ready to be inserted into the genome. The larger picture is 1 μm wide, while the inset is 80 nm wide; from Kuznetsov *et al.* (2005).

behaviour can spread in a population when the marginal cost to the altruist, C, is less than the marginal benefit to the beneficiary, B, discounted by the genetic relatedness between altruist and beneficiary, R; that is, $RB - C > 0$. In Hamilton's (1964, 1972) "haplodiploidy hypothesis", relatedness asymmetries stemming from haplodiploidy had a special role in explaining patterns of sociality among insects. Hamilton proposed that because a haplodiploid female's relatedness to her sisters (life-for-life $R = 3/4$) exceeds her relatedness to offspring ($R = 1/2$), haplodiploid females should be more willing to invest in sib-rearing than in rearing offspring. This hypothesis purported to explain not only the apparent predisposition for eusociality among haplodiploids, but also the exclusively-female worker caste of the social Hymenoptera (Hamilton 1964, 1972).

This theory fell out of the favour with the realization that, because a haplodiploid female's low relatedness to brothers ($R = 1/4$) exactly cancels her high relatedness to sisters, her overall relatedness to siblings is equal to her relatedness to offspring ($R = 1/2$ in both cases), and therefore that haplodiploidy should neither promote sociality in general, nor encourage female helping in particular (Fig. 2; Trivers & Hare 1976; Gardner *et al.* 2012). Instead, a more recent "monogamy hypothesis" (Boomsma 2007, 2009, 2013) holds that the most important relatedness-based factor in the evolution of eusociality is strict lifetime monogamy between colony founders, which ensures that relatedness between siblings is no lower than parent-offspring relatedness, permitting the interchangeability required for a fraternal major transition.

However, although strict lifetime monogamy is rare in the animal kingdom, it is not the only requirement for the evolution of eusociality. Prominent critics of inclusive fitness theory (*e.g.* Wilson 2005; Nowak *et al.* 2010; Wilson & Nowak 2014) have seemingly suggested that relatedness has had no effect on the taxonomic distribution of eusociality, and that other ecological factors are exclusively responsible for promoting sociality among arthropods. But inclusive fitness theory does not deny the importance of ecological factors beyond genetic relatedness; rather, it provides a framework for translating ecological costs and benefits—and specificities of the genetic system—into a common currency that explains features of organismal design (Frank 1998; Foster *et al.* 2006; West & Gardner 2013). Therefore, an investigation into the ecological corre-

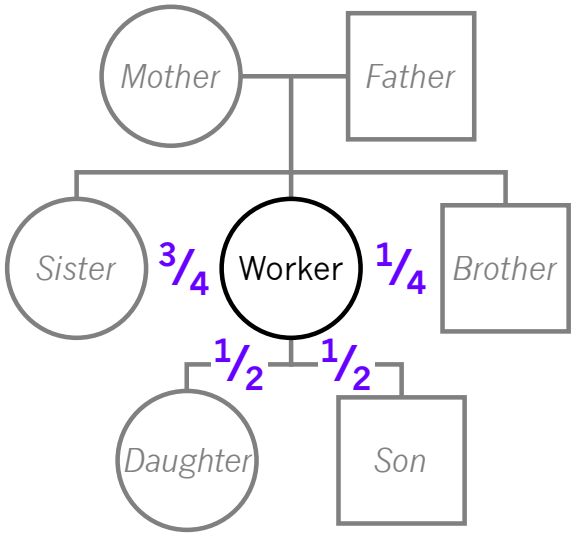


Figure 2: Pedigree illustrating the life-for-life relatedness between a female worker of a haplodiploid society and her family members. A worker is more related to her full sisters ($R = 3/4$) than to her offspring ($R = 1/2$), but less related to her brothers ($R = 1/4$), such that under strict lifetime monogamy, her average relatedness to siblings ($R = 1/2$) is equal to her average relatedness to offspring.

lates of eusociality does not comprise an alternative to inclusive fitness theory, but identifies factors modulating the cost, benefit, and relatedness terms in Hamilton's rule.

In Part 1 of this thesis, some of the ecological factors underlying the origins and elaboration of arthropod sociality are explored. I develop a conceptual framework focusing on "the ecology of sex"—that is, the structure of the social environment with respect to sexual reproduction—and explore its importance in shaping patterns of helping in arthropod societies.

The evolution of paternal care (chapter 2)

Relatedness asymmetries between the sexes stemming from haplodiploidy should not render females more willing to help their siblings of both sexes than males, and hence the genetics of sex determination *per se* does not explain sex-biased helping in social insects (Trivers & Hare 1976, Gardner *et al.* 2012; Ross *et al.* 2013). In particular, it appears instead that workers in the social Hymenoptera are female because females, not males, are preadapted for sib-rearing, on account of having been the exclusive providers of parental care in the solitary ancestors of these social species (Lin & Mich-

ener 1972; Alexander 1974; West-Eberhard 1975; Evans 1977; Charlesworth 1978; Eickwort 1981; Craig 1982; Andersson 1984; Starr 1985; Bourke & Franks 1995; Queller & Strassmann 1998; Ross *et al.* 2013). However, haplodiploidy could still be responsible for female-biased helping in this clade if it facilitated the evolution of maternal and not paternal care. Gardner (2012) showed that, under the most realistic assumptions, haplodiploidy either inhibits or has no effect on the evolution of maternal care. However, the same pattern of maternal-only care could have resulted if haplodiploidy inhibited paternal care relative to diploidy. In this chapter, I show that haplodiploidy either promotes (under inbreeding) or does not affect (under outbreeding) the evolution of paternal care relative to diploidy, further demonstrating that if haplodiploidy does have an impact upon the evolution of parental care, it has the opposite effect to that required to explain female-biased helping in the social Hymenoptera.

The ecology of sex and who helps in arthropod societies (chapter 3)

Haplodiploidy *per se* does not seem to have had a major impact upon patterns of helping in eusocial insects (Gardner *et al.* 2012; Gardner 2012; Ross *et al.* 2013; Davies & Gardner 2014). However, the validity of Hamilton's "haplodiploidy hypothesis" has not been evaluated in the context of sib-mating and local mate competition—a mating system that is common at the origins of arthropod societies—and so its applicability beyond the outbred social insects remains unclear. Moreover, if haplodiploidy promotes neither sex biases in helping nor the evolution of sociality, what does explain patterns of helping in arthropods? Addressing these questions, I construct a model to formally test the "preadaptation hypothesis" (Ross *et al.* 2013) that sex-biased helping reflects ancestral preadaptations for helping, under both diploidy and haplodiploidy. Additionally, I examine the impact of four other key factors of sexual ecology—sib-mating, promiscuity, labile sex allocation, and reproductive autonomy—showing that these also modulate the sex bias and extent of helping. These predictions are tested using a comparative survey, demonstrating that the ecology of sex, not ploidy *per se*, strongly shapes patterns of helping in arthropod societies.

Worker sterility, population genetics, and inclusive fitness (chapter 4)

A later elaboration in the evolution of haplodiploid insect societies—and an especially conspicuous example of sibling-directed altruism—is the evolution of sterility among female workers. In most social hymenopteran colonies, workers, despite remaining unmated, continue to produce sons through arrhenotokous parthenogenesis; but in some colonies, workers have partially or entirely stopped producing sons in order to redirect their resources towards colony productivity. Like other examples of sibling-directed altruism, such helpful worker sterility should be promoted by monogamy, since monogamy ensures high relatedness between a worker and the beneficiaries of her altruistic sterility, her siblings. However, a recent paper by Olejarz *et al.* (2015) has challenged this view, arguing that an explicit population-genetics model supports no clear effect of monogamy on worker sterility. In this chapter, I show that the conclusions of Olejarz *et al*. depend on restrictive assumptions concerning the genetics, evolution, and ecology of worker sterility. Performing a more comprehensive analysis of their model, I show that—in keeping with the predictions of inclusive fitness theory—monogamy has a clear promoting effect on helpful worker sterility, and demonstrate that a less restrictive formulation of Olejarz *et al*.'s population-genetics model corroborates inclusive-fitness theory more generally.

Part II: Cellular ecology and conflict within genomes

Selfish genes in parliament

The "gene's-eye view" of evolution (Dawkins 1976) locates adaptation at the level of the gene, suggesting that individual genetic elements within a genome—apparently cooperating harmoniously to generate organismal design—are really pursuing their own naked self-interest. Although attributing organismal adaptation to exclusively-"selfish" genes breeds certain conceptual difficulties (Gardner & Welch 2011), there are clear links between allelic success and organismal adaptation. In the face of natural selection, alleles normally spread in a population insofar as they increase the inclusive fitness of their host—and, by extension, the transmission of all other genes in the

genome. However, the conventional link between host fitness and allelic transmission is not automatic; it depends on the suppression of conflict among genes (Leigh 1971; Buss 1987). These enforcement mechanisms can be circumvented, allowing a selfish genetic element which promotes its own transmission at the expense of lowering host fitness to spread nonetheless within a population. Such misalignment of evolutionary interests between constituents of the same genome is termed intragenomic conflict (Burt & Trivers 2006).

Selective pressures on the rest of the genome operate in opposition to genetic selfishness; in the terminology of Leigh (1977), the conflicting evolutionary interest of the "parliament of genes" promotes the suppression of selfish genetic elements. Yet in many instances of intragenomic conflict, the coevolution of genetic selfishness and host suppression remains poorly understood. In the final chapter of this thesis, I explore genetic conflict caused by transposons and the mechanisms of host suppression deployed to prevent it.

The tragedy of the transposons (chapter 5)

Transposons are genes that duplicate themselves within host genomes, increasing their rate of transmission in the process. They have had a major impact upon eukaryotic evolution as sources of genetic variation, drivers of genome size evolution, rewirers of gene regulatory networks, and promoters of species diversity. Transposons are also of clinical importance, having been implicated in human diseases including cancers, schizophrenia, and multiple hereditary disorders. Eukaryotic hosts deploy various counter-adaptations to suppress the proliferation of transposons, from the highly-conserved, highly-adaptable Piwi-piRNA pathway to more ad-hoc, taxon-specific suppressors of particular transposon families. But our understanding of the co-evolution of transposons and their hosts is limited. In particular, we still rely heavily on population genetics papers from the 1980s that do not account for adaptation by transposons and counter-adaptation by their hosts.

In this chapter, drawing from ecology and social evolution theory, I show that transposons are under continual selection to increase their replication rate, even to the point of driving the host population extinct, analogously to the classic "tragedy of

the commons". I demonstrate that host suppression plays a crucial role in preventing host extinction, and that particular assumptions about host strategies of suppression are needed to explain how extinction is averted in natural populations. I explore how aspects of cellular ecology play into the coevolution of transposons and their hosts, finding that these considerations lead to fundamentally different predictions for the long-term evolutionary outcome. Reclassifying transposons according to their mode of duplication—public, private, or parasitic—I show that predicted patterns of host suppression hold across three multicellular eukaryotes with differing genomic proportions of each type of transposon.

Part I

Sexual ecology and cooperation in arthropod societies

Chapter 2

The evolution of paternal care

Abstract

W. D. Hamilton famously suggested that the inflated relatedness of full sisters under haplodiploidy explains why all workers in the social Hymenoptera are female. This suggestion has not stood up to further theoretical scrutiny, and is not empirically supported. Rather, it appears that altruistic sib-rearing in the social Hymenoptera is performed exclusively by females because this behaviour has its origins in parental care, which was performed exclusively by females in the ancestors of this insect group. However, haplodiploidy might still explain the sex of workers if this mode of inheritance has itself been responsible for the rarity of paternal care in this group. Here, we perform a theoretical kin-selection analysis to investigate the evolution of paternal care in diploid and haplodiploid populations. We find that haplodiploidy may either inhibit or promote paternal care depending on model assumptions, but that under the most plausible scenarios it promotes—rather than inhibits—paternal care. Our analysis casts further doubt upon there being a causal link between haplodiploidy and eusociality.

Introduction

W. D. Hamilton (1964, 1972) famously suggested that the inflated genetic relatedness of full sisters under haplodiploidy (life-for-life $R = 3/4$) explains why females, and not

males, engage in altruistic sib-rearing in hymenopteran societies. This haplodiploidy hypothesis has fallen out of favour with the realization that the average value of siblings of both sexes is exactly the same ($R = 1/2$) for females and males, irrespective of the sex ratio (Charlesworth 1978; Charnov 1978; Craig 1979; Bourke & Franks 1995; Ross *et al.* 2013). Rather, it appears that the sex bias in altruistic sib-rearing within this group of insects owes to a sex bias in the provision of parental care among their solitary ancestors, a pattern which holds across other eusocial species in which the original function of workers was nursing (Ross *et al.* 2013). That is, although their altruism is clearly driven by kin selection, hymenopteran workers are female because females were already equipped with the behaviours and morphologies conducive to successful sib-rearing—a possibility that Hamilton (1964) acknowledged.

Nevertheless, haplodiploidy could still explain the sex of workers if it was itself responsible for the ancestral pattern of parental care in this group. One possibility is that haplodiploidy promoted the evolution of maternal care among the ancestors of the social Hymenoptera (Wade 2001; Linksvayer & Wade 2005), but theoretical analysis suggests that haplodiploidy is more likely to inhibit than promote maternal care, and in many instances will have no effect at all (Gardner 2012). Alternatively, haplodiploidy might have inhibited the evolution of paternal care, yielding a similar outcome. This possibility has not yet been explored.

Here, we investigate whether haplodiploidy inhibits the evolution of paternal care. Specifically, we consider the evolutionary fate of paternal care genes that incur costs for their male and/or female carriers but provide benefits to their male carriers' offspring, under diploidy versus haplodiploidy, outbreeding versus inbreeding, and adding versus averaging of gene effects. We derive analytical results using kin selection methodology (Hamilton 1964; Taylor & Frank 1996), and we confirm these results with numerical simulation of an explicit population genetics model. In doing so, we evaluate the possibility that haplodiploidy is responsible for social hymenopteran workers being female because it inhibited the evolution of paternal care among their ancestors.

Model and results

Kin selection model

Gardner (2012) considered a kin selection model of the evolution of maternal care, in order to evaluate the possibility that haplodiploidy has facilitated eusociality by promoting the evolution of maternal care. We adapt that model to investigate the evolution of paternal care, in order to evaluate the possibility that haplodiploidy has led to an exclusively female worker caste by inhibiting the evolution of paternal care. We assume an infinite population of females and males with diploid or haplodiploid inheritance. We consider the evolution of a paternal care gene which benefits the offspring of its male carriers while carrying a cost for its female and/or male carriers[1]. Accordingly, the fitness of a female is $w_{\mathrm{f}}(G, G')$ and the fitness of a male is $w_{\mathrm{m}}(G, G')$, where G is the individual's breeding value for the paternal care gene and G' is the individual's father's breeding value. We denote by $C_{\mathrm{f}} = -\partial w_{\mathrm{f}}/\partial G$ and $C_{\mathrm{m}} = -\partial w_{\mathrm{m}}/\partial G$ the direct cost of the paternal care gene for females and males, respectively, and we denote by $B_{\mathrm{f}} = \partial w_{\mathrm{f}}/\partial G'$ and $B_{\mathrm{m}} = \partial w_{\mathrm{m}}/\partial G'$ the indirect benefit for female and male offspring of male carriers of the paternal care gene, respectively. We assume that breeding values are determined either through adding or averaging of gene effects. For diploids, $G = g_1 + g_2$ under adding, where g_1 and g_2 are the individual's two genic values, and $G = (g_1 + g_2)/2$ under averaging. For haploids, $G = g$, irrespective of whether gene effects add or average together (see Gardner 2012 for more discussion of adding versus averaging of gene effects).

Adding gene effects

Under the assumption that gene effects add together, we find that natural selection favours paternal care under diploidy when

$$-(2+2f)C_{\mathrm{f}} + (1+3f)B_{\mathrm{f}} - (2+2f)C_{\mathrm{m}} + (1+3f)B_{\mathrm{m}} > 0\,, \tag{1}$$

[1] These costs could potentially owe to pleiotropic effects of the paternal care gene, rather than simply the cost of the behaviour itself. Behavioural genes with pleiotropic effects have been described in nature; for example, genetic variation in the vertebrate melanocortin system has pleiotropic effects on coloration and behaviour in both sexes (Ducrest *et al.* 2008), and parental care behaviours in burying beetles show positive genetic correlations between sexes (Walling *et al.* 2008).

where f is the coefficient of inbreeding (see *Methods* for derivation). The corresponding condition for haplodiploidy is

$$-(2+2f)C_{\mathrm{f}} + (1+f)B_{\mathrm{f}} - C_{\mathrm{m}} + fB_{\mathrm{m}} > 0\,. \tag{2}$$

Adopting some simplifying assumptions, conditions 1 and 2 can be rearranged into the form $C/B < A$, where A describes the 'potential for paternal care' (*cf.* Gardner 2012). That is, a relatively high potential means that paternal care is favoured by natural selection even if there is a relatively high cost/benefit ratio, whereas a relatively low potential means that paternal care is disfavoured by natural selection unless the cost/benefit ratio is correspondingly low. In particular, we assume that: (i) the benefit of paternal care accrues equally to a caring father's sons and daughters ($B_{\mathrm{f}} = B_{\mathrm{m}} = B$); (ii) the benefit is positive ($B > 0$); and (iii) one of three scenarios applies regarding the cost of paternal care, namely, that females and males bear the cost of paternal care equally ($C_{\mathrm{f}} = C_{\mathrm{m}} = C$), only females bear the cost of paternal care ($C_{\mathrm{f}} = C, C_{\mathrm{m}} = 0$), or only males bear the cost of paternal care ($C_{\mathrm{f}} = 0, C_{\mathrm{m}} = C$). The corresponding potentials for paternal care under the adding model of gene effects are given in Table 1 and illustrated in Fig. 1a–c. These analytical results are confirmed by numerical simulation of an explicit population genetics model (Fig. 2; see *Methods* for details).

Under full outbreeding ($f = 0$), haplodiploidy either inhibits the evolution of paternal care or has no effect. Specifically: if both sexes bear the cost of paternal care, then the potential for paternal care is $A = 1/2$ under diploidy and $A = 1/3$ under haplodiploidy (Fig. 1a); if only females bear the cost of paternal care, then the potential for paternal care is $A = 1$ under diploidy and $A = 1/2$ under haplodiploidy (Fig. 1b); and if only males bear the cost of paternal care, then the potential for paternal care is $A = 1$ under both diploidy and haplodiploidy (Fig. 1c). Conversely, under full inbreeding ($f = 1$), haplodiploidy may promote or inhibit the evolution of paternal care. Specifically: if both sexes bear the cost of paternal care, then the potential for paternal care is $A = 1$ under diploidy and $A = 3/5$ under haplodiploidy (Fig. 1a); if only females bear the cost of paternal care, then the potential for paternal care is $A = 2$ under diploidy and $A = 3/4$ under haplodiploidy (Fig. 1b); and if only males bear the

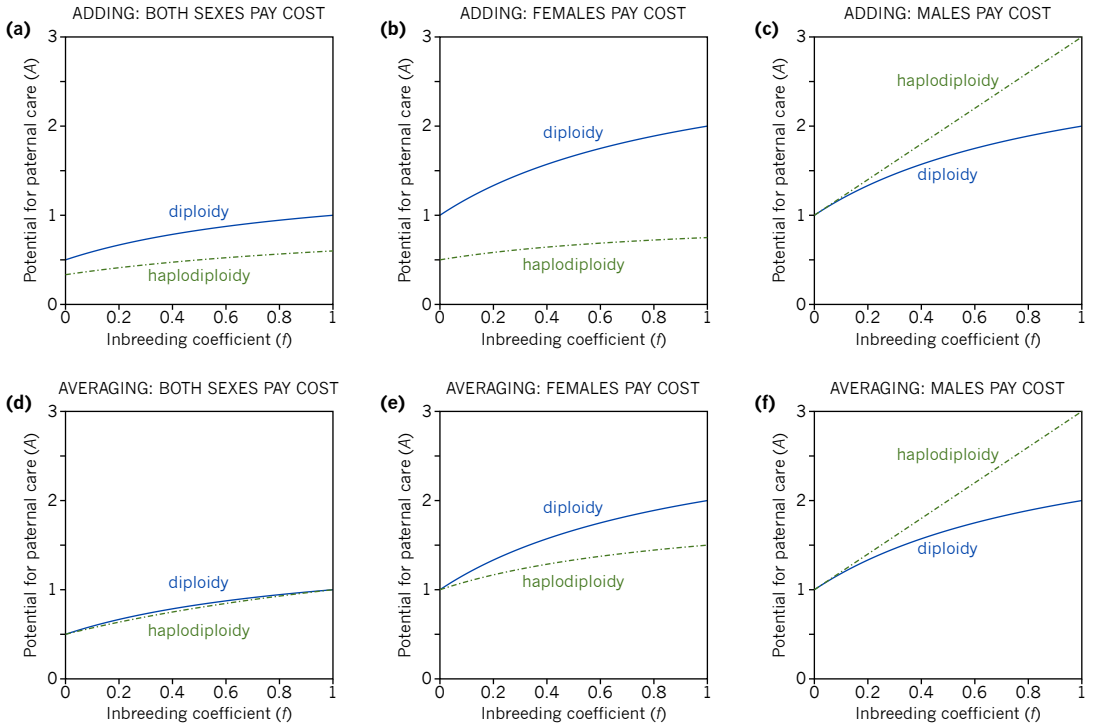


Figure 1: Potential for paternal care under diploidy versus haplodiploidy. Analytical solutions for the potential for paternal care (A, such that the condition for natural selection to favour paternal care is $C/B < A$) under diploidy (solid blue lines) versus haplodiploidy (dashed green lines). Results are shown for: adding of gene effects, with costs experienced by **(a)** both sexes equally ($C_f = C_m = C$), **(b)** females only ($C_f = C, C_m = 0$), or **(c)** males only ($C_f = 0, C_m = C$); and averaging of gene effects, with costs experienced by **(d)** both sexes equally ($C_f = C_m = C$), **(e)** females only ($C_f = C, C_m = 0$), or **(f)** males only ($C_f = 0, C_m = C$).

	Potential for paternal care		
Genetic system	Direct cost accrues to both sexes	Direct cost accrues to females only	Direct cost accrues to males only
Gene effects add			
Diploidy	$\frac{1+3f}{2+2f}$	$\frac{1+3f}{1+f}$	$\frac{1+3f}{1+f}$
Haplodiploidy	$\frac{1+2f}{3+2f}$	$\frac{1+2f}{2+2f}$	$1+2f$
Gene effects average			
Diploidy	$\frac{1+3f}{2+2f}$	$\frac{1+3f}{1+f}$	$\frac{1+3f}{1+f}$
Haplodiploidy	$\frac{1+2f}{2+f}$	$\frac{1+2f}{1+f}$	$1+2f$

Table 1: Potential for paternal care (A, such that the condition for natural selection to favour paternal care is $C/B < A$) as a function of genetic architecture, ploidy, sex-specific effects, and the coefficient of inbreeding (f).

cost of paternal care, then the potential for paternal care is $A = 2$ under diploidy and $A = 3$ under haplodiploidy (Fig. 1c). Intermediate rates of inbreeding ($0 < f < 1$) yield intermediate results (Fig. 1a–c).

Averaging gene effects

Under the assumption that gene effects average together, the condition for natural selection to favour paternal care under diploidy is

$$-(2+2f)C_{\mathrm{f}} + (1+3f)B_{\mathrm{f}} - (2+2f)C_{\mathrm{m}} + (1+3f)B_{\mathrm{m}} > 0\,. \tag{3}$$

Note that condition 3 is identical to condition 1. The corresponding condition for haplodiploidy is

$$-(1+f)C_{\mathrm{f}} + (1+f)B_{\mathrm{f}} - C_{\mathrm{m}} + fB_{\mathrm{m}} > 0\,. \tag{4}$$

Following the procedure outlined in the previous section, conditions 3 and 4 can

also be rearranged into the form $C/B < A$, where A describes the potential for paternal care. The corresponding potentials for paternal care under the averaging model of gene effects are given in Table 1 and illustrated in Fig. 1d–f; these analytical results are confirmed by numerical simulation (Fig. 2).

Under full outbreeding ($f = 0$), haplodiploidy has no effect upon the evolution of paternal care. Specifically: if both sexes bear the cost of paternal care, then the potential for paternal care is $A = 1/2$ under both diploidy and haplodiploidy (Fig. 1d); if only females bear the cost of paternal care, then the potential for paternal care is $A = 1$ under both diploidy and haplodiploidy (Fig. 1e); and if only males bear the cost of paternal care, then the potential for paternal care is $A = 1$ under both diploidy and haplodiploidy (Fig. 1f). Conversely, under full inbreeding ($f = 1$), haplodiploidy may promote or inhibit or have no effect upon the evolution of paternal care. Specifically: if both sexes bear the cost of paternal care, then the potential for paternal care is $A = 1$ under both diploidy and haplodiploidy (Fig. 1d); if only females bear the cost of paternal care, then the potential for paternal care is $A = 2$ under diploidy and $A = 3/2$ under haplodiploidy (Fig. 1e); and if only males bear the cost of paternal care, then the potential for paternal care is $A = 2$ under diploidy and $A = 3$ under haplodiploidy (Fig. 1f). Intermediate rates of inbreeding ($0 < f < 1$) yield intermediate results (Fig. 1d–f); note that when both sexes bear the cost of paternal care, haplodiploidy inhibits the evolution of paternal care relative to diploidy for intermediate rates of inbreeding (Fig. 1d).

Discussion

It appears that workers are female in the social Hymenoptera because their altruistic sib-rearing derives from the maternal care practiced by their solitary ancestors (Lin & Michener 1972; Alexander 1974; West-Eberhard 1975; Evans 1977; Charlesworth 1978; Eickwort 1981; Craig 1982a; Andersson 1984; Starr 1985; Bourke & Franks 1995; Queller & Strassmann 1998; Ross *et al.* 2013). Paternal care is rare among solitary Hymenoptera, having only been reported in the genus *Trypoxylon* (Hamilton 1964; West-Eberhard 1975; Brockmann 1980), while maternal care is widespread throughout

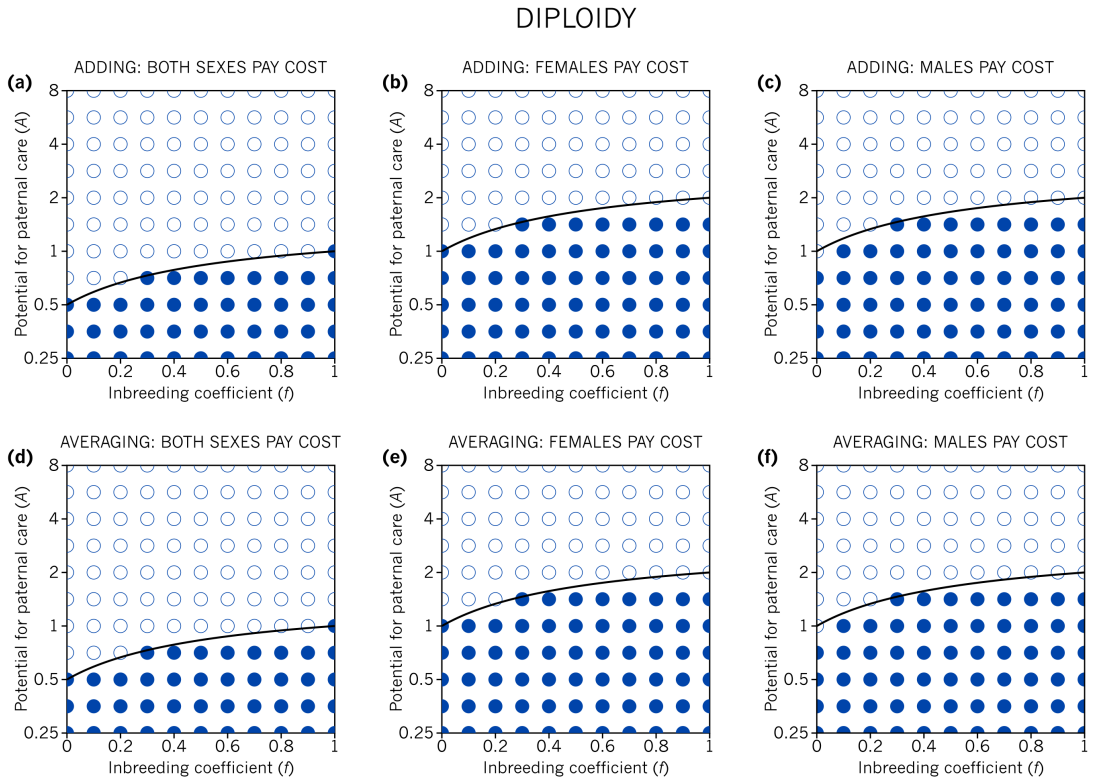


Figure 2: Invasion of a paternal-care allele under diploidy and haplodiploidy. Black lines present analytical solutions for the potential for paternal care (A, such that the condition for natural selection to favour paternal care is $C/B < A$), while overlaid circles present numerical results confirming the analytical solutions, indicating invasion (filled circles) or non-invasion (open circles) of a paternal care allele from rarity. Results for diploidy are presented in **(a)**-**(f)** for: adding of gene effects, with costs experienced by **(a)** both sexes equally ($C_f = C_m = C$), **(b)** females only ($C_f = C, C_m = 0$), and **(c)** males only ($C_f = 0, C_m = C$); and averaging of gene effects, with costs experienced by **(d)** both sexes equally ($C_f = C_m = C$), **(e)** females only ($C_f = C, C_m = 0$), and **(f)** males only ($C_f = 0, C_m = C$). *(Continued on next page.)*

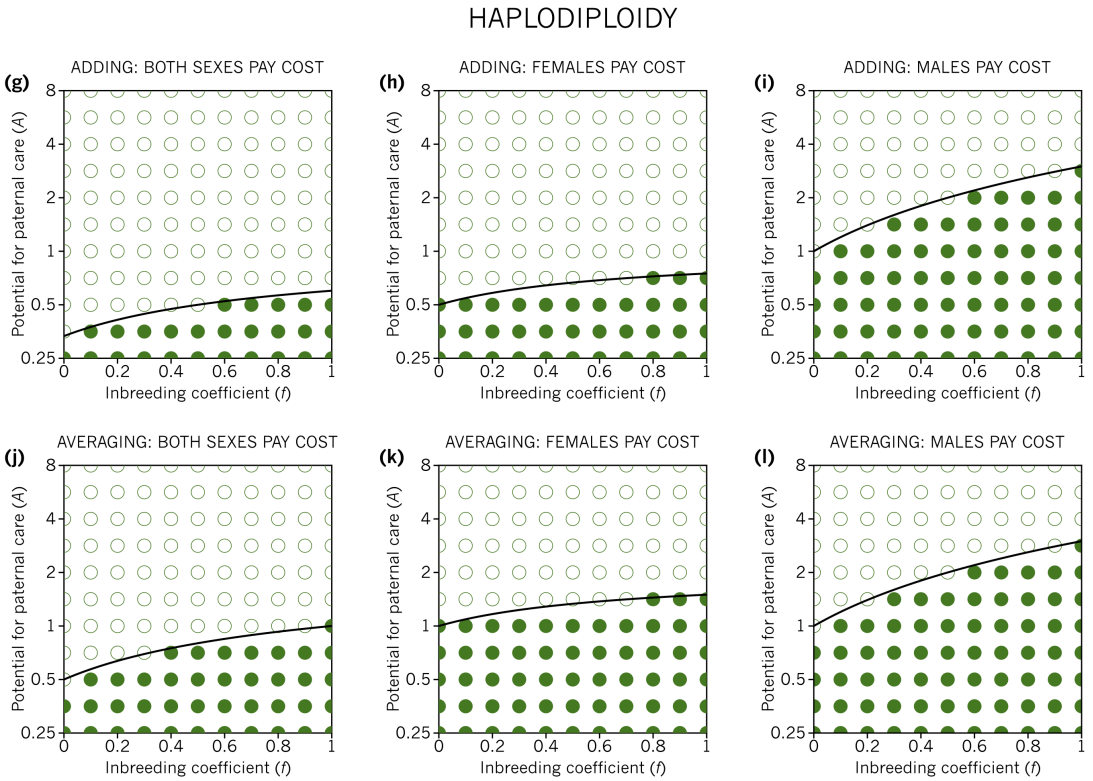


Figure 2: *(Continued from previous page.)* Invasion of a paternal-care allele under diploidy and haplodiploidy. Black lines present analytical solutions for the potential for paternal care (A, such that the condition for natural selection to favour paternal care is $C/B < A$), while overlaid circles present numerical results confirming the analytical solutions, indicating invasion (filled circles) or non-invasion (open circles) of a paternal care allele from rarity. Results for haplodiploidy are presented in **(g)-(l)** for: adding of gene effects, with costs experienced by **(g)** both sexes equally ($C_\mathrm{f} = C_\mathrm{m} = C$), **(h)** females only ($C_\mathrm{f} = C, C_\mathrm{m} = 0$), and **(i)** males only ($C_\mathrm{f} = 0, C_\mathrm{m} = C$); and averaging of gene effects, with costs experienced by **(j)** both sexes equally ($C_\mathrm{f} = C_\mathrm{m} = C$), **(k)** females only ($C_\mathrm{f} = C, C_\mathrm{m} = 0$), and **(l)** males only ($C_\mathrm{f} = 0, C_\mathrm{m} = C$).

this group (Hamilton 1964; Hölldobler & Wilson 1990; Alexander *et al.* 1991). Furthermore, both theory and empirical evidence suggest that the origin of hymenopteran worker behaviour lies in parental care redirected towards siblings (Wheeler 1928; Kennedy 1966; Michener 1969; Wilson 1971; Hamilton 1972; Alexander 1974; West-Eberhard 1987; Alexander *et al.* 1991; Bourke & Franks 1995; Linksvayer & Wade 2005; Amdam *et al.* 2006; Boomsma 2007; Toth *et al.* 2007; Boomsma *et al.* 2011). Hence, the absence of male workers among the social Hymenoptera reflects an ancestral dearth of paternal care.

Here, we have investigated the possibility that haplodiploidy inhibits the evolution of paternal care, which, if correct, would potentially explain the pattern of parental care among the ancestors of the social Hymenoptera, and hence the contemporary sex bias in worker castes. We have found that haplodiploidy may mediate the evolution of paternal care, but that it may have a positive or negative effect or no effect at all, depending upon which individuals experience the costs of paternal care, the degree of inbreeding, and how gene effects combine to determine phenotypes.

Of most biological relevance is the scenario in which the father pays the cost of paternal care. Here, we have found that for outbred populations ($f = 0$) haplodiploidy neither promotes nor inhibits the evolution of paternal care, irrespective of whether gene effects add or average together, because the value that a male places upon his offspring relative to the value that he places upon himself is the same under diploidy and haplodiploidy. Specifically, although a male places less value upon his 'sons' under haplodiploidy (*i.e.*, zero value, because they develop from his partner's unfertilized eggs, and hence are unrelated to him; Hamilton 1972), he places more value upon his daughters (because the reproductive value of females is greater under haplodiploidy; Hamilton 1972), and these two effects exactly cancel.

However, for inbred populations ($f > 0$), haplodiploidy promotes the evolution of paternal care, irrespective of whether gene effects add or average together. This is because inbreeding leads a male to be more related to his offspring under haplodiploidy than under diploidy. Specifically, inbreeding increases the consanguinity of father and offspring under both diploidy and haplodiploidy, increasing the absolute value of offspring under both modes of inheritance. But while inbreeding

increases the consanguinity of a male to himself under diploidy (because he is inbred), it does not increase the consanguinity of a male to himself under haplodiploidy (because a haploid male cannot be inbred), such that the absolute value a male places upon himself is increased by inbreeding under diploidy but not under haplodiploidy. The net effect is that, in the context of inbreeding, a male places relatively more value upon his offspring under haplodiploidy than under diploidy, and hence, under inbreeding, haplodiploidy promotes the evolution of paternal care.

Although we have treated the degree of inbreeding as a fixed parameter when assessing the impact of diploidy versus haplodiploidy upon the evolution of paternal care, it is possible that rates of inbreeding are systematically different for diploid versus haplodiploid taxa. One possibility is that haplodiploidy promotes inbreeding by purging the recessive deleterious alleles that make inbreeding costly (White 1945; Suomalainen 1962; Crozier 1970; Brückner 1978; Henter 2003). As we have found that inbreeding ($f > 0$) tends to accentuate the impact of haplodiploidy, this effect would tend to magnify the differences between diploidy and haplodiploidy in our analysis. Alternatively, for hymenopterans in particular, inbreeding could be relatively inhibited because of costs associated with the production of sterile diploid males in the context of complementary sex determination (Whiting 1925; Whiting 1933; Cook & Crozier 1995; van Wilgenburg *et al.* 2006; for an exception, see Cowan & Stahlhut 2004). A negative association between haplodiploidy and inbreeding would tend to reduce the differences observed between diploidy and haplodiploidy in our analysis. The relative importance of these two factors has not been systematically studied, but although inbreeding rates vary among both diploids and haplodiploids, haplodiploidy does appear to be overrepresented among chronic inbreeders (Hamilton 1967; Werren 1993).

We have also considered two further scenarios, in which both females and males, or only females, pay the cost of paternal care. These particular cost scenarios are included primarily for synthesis with the analyses of Wade (2001) and Gardner (2012). We have found that haplodiploidy may either inhibit or have no impact upon the evolution of paternal care in these scenarios, depending upon the degree of inbreeding and whether gene effects add or average together to determine the phenotype. Conceptualization

of these results is complicated because there are two actor classes: males who actually enact the paternal care behaviour, and females who pay (at least part of) the cost. Broadly speaking, haplodiploidy inhibits the evolution of paternal care when females pay a cost because females have greater reproductive value under haplodiploidy and hence the evolutionary impact of this cost is magnified (*cf.* Gardner 2012).

These results could illuminate the evolutionary trajectories of particular paternal care genes that incur known costs for their female carriers. This might be relevant in a case where morphologies or behaviours enhancing parental care in males are deleterious when expressed in females, and where the genes underlying these traits are not differentially regulated in the two sexes. Such genes may be sexually antagonistic—that is, experiencing opposing selective pressures in females and in males (Lande 1980; Rice 1984). Sexually-antagonistic genes have been identified in insect populations (*e.g.* Innocenti & Morrow 2010). However, the absence of a clear and necessary ecological trade-off underlying sexual antagonism in paternal care traits means that these results may be of limited relevance to the broader question of whether and how haplodiploidy impacts upon the evolution of paternal care.

Thus, if haplodiploidy has a robust impact upon the evolution of paternal care, our analysis suggests that it is in the opposite direction from that required to explain the female bias in parental care among the ancestors of the social Hymenoptera. This provides further support for the idea that haplodiploid inheritance is not responsible for the exclusively female worker caste of the social Hymenoptera: either directly, as a result of the inflated genetic relatedness of haplodiploid sisters (Charlesworth 1978; Charnov 1978; Craig 1979); or indirectly, by pre-adapting females and not males for sib-rearing (Gardner 2012 and the present analysis). Instead, although ancestral female bias in parental care provides the best explanation for the female bias in altruistic sib-rearing among the social Hymenoptera (Ross *et al.* 2013), the relative absence of paternal care in this group mirrors the more general absence of paternal care across the terrestrial animal kingdom, which owes to other fundamental asymmetries between the sexes (Queller 1997b; Kokko & Jennions 2012).

More generally, the issue of whether haplodiploidy has been an important determinant of the evolution of helping in the social Hymenoptera (the evidence suggests

that it has not) is distinct from the issue of whether kin selection has played a key role (the evidence overwhelmingly suggests that it has). Ecological factors, such as those concerning which sex or sexes exhibited parental care in the ancestral species, do not provide alternatives to kin selection, but rather modulate the cost and benefit terms appearing in Hamilton's (1963, 1964, 1970) rule.

Methods

1. Kin selection analysis

Here, we perform a kin selection analysis of the model presented above, analogously to the model of maternal care analysed by Gardner (2012). Having defined w_f and w_m above, we write $\bar{w}_\mathrm{f}$ and $\bar{w}_\mathrm{m}$ for the average fitness of females and males, respectively, and $W_\mathrm{f} = w_\mathrm{f}/\bar{w}_\mathrm{f}$ and $W_\mathrm{m} = w_\mathrm{m}/\bar{w}_\mathrm{m}$ for the relative fitness of a female and a male, respectively. Since we assume the paternal care gene is vanishingly rare, $\bar{w}_\mathrm{f} = \bar{w}_\mathrm{m}$. The condition for natural selection to favour any trait is $c_\mathrm{f}(\mathrm{d}W_\mathrm{f}/\mathrm{d}g_\mathrm{f}) + c_\mathrm{m}(\mathrm{d}W_\mathrm{m}/\mathrm{d}g_\mathrm{m}) > 0$ (Taylor 1996; Taylor & Frank 1996; Frank 1997, 1998; Taylor *et al.* 2007); here, c_f and c_m are the class reproductive values of females and males, respectively, and g_f and g_m denote the genic values of random genes picked from a female and a male, respectively.

A gene's value has both direct and indirect impacts on its carrier's fitness. The direct effect follows from the genic value (g_f or g_m) modifying its carrier's breeding value (G_f or G_m), which in turn may be associated with a fitness cost to the gene's carrier. The indirect effect follows from the statistical association, due to heredity, between the gene's value (g_f or g_m) and its carrier's father's breeding value (G'_f or G'_m), which in turn is associated with the father's investment in paternal care, which may yield a fitness benefit for the gene's carrier. Thus, the two actors who may modify a given recipient's fitness are the recipient itself and the recipient's father. From the chain rule, we have $\mathrm{d}W_\mathrm{f}/\mathrm{d}g_\mathrm{f} = (\partial W_\mathrm{f}/\partial G_\mathrm{f}) \times (\mathrm{d}G_\mathrm{f}/\mathrm{d}g_\mathrm{f}) + (\partial W_\mathrm{f}/\partial G'_\mathrm{f}) \times (\mathrm{d}G'_\mathrm{f}/\mathrm{d}g_\mathrm{f})$. Here: $\partial W_\mathrm{f}/\partial G_\mathrm{f} = -C_\mathrm{f}/\bar{w}_\mathrm{f}$; $\mathrm{d}G_\mathrm{f}/\mathrm{d}g_\mathrm{f}$ is equal to $2p_\mathrm{f}$ for adding of gene effects and p_f for averaging of gene effects, where p_f is the consanguinity (Bulmer 1994) of a female to herself; $\partial W_\mathrm{f}/\partial G'_\mathrm{f} = B_\mathrm{f}/\bar{w}_\mathrm{f}$; and $\mathrm{d}G'_\mathrm{f}/\mathrm{d}g_\mathrm{f}$ is equal to $2p_\mathrm{d}$ for adding of gene effects under diploidy and p_d for adding of gene effects under haplodiploidy and averaging of

gene effects under diploidy or haplodiploidy, where p_{d} is the consanguinity of father and daughter. Similarly, $\mathrm{d}W_{\mathrm{m}}/\mathrm{d}g_{\mathrm{m}} = (\partial W_{\mathrm{m}}/\partial G_{\mathrm{m}}) \times (\mathrm{d}G_{\mathrm{m}}/\mathrm{d}g_{\mathrm{m}}) + (\partial W_{\mathrm{m}}/\partial G'_{\mathrm{m}}) \times (\mathrm{d}G'_{\mathrm{m}}/\mathrm{d}g_{\mathrm{m}})$. Here: $\partial W_{\mathrm{f}}/\partial G_{\mathrm{m}} = -C_{\mathrm{m}}/\bar{w}_{\mathrm{m}}$; $\mathrm{d}G_{\mathrm{m}}/\mathrm{d}g_{\mathrm{m}}$ is equal to $2p_{\mathrm{m}}$ for adding of gene effects under diploidy and p_{m} for adding of gene effects under haplodiploidy and averaging of gene effects under diploidy or haplodiploidy, where p_{m} is the consanguinity of a male to himself; $\partial W_{\mathrm{m}}/\partial G'_{\mathrm{m}} = B_{\mathrm{m}}/\bar{w}_{\mathrm{m}}$; and $\mathrm{d}G'_{\mathrm{m}}/\mathrm{d}g_{\mathrm{m}}$ is equal to $2p_{\mathrm{s}}$ for adding of gene effects under diploidy and p_{s} for adding of gene effects under haplodiploidy and averaging of gene effects under diploidy or haplodiploidy, where p_{s} is the consanguinity of father and son.

Here, we have framed our kin selection analysis in terms of coefficients of consanguinity and class reproductive values. In the Introduction, we framed the haplodiploidy hypothesis in terms of life-for-life relatedness, which combines both genetic similarity and individual reproductive value in a single quantity. Gardner *et al.* (2012, Appendix A) provides an overview of the connections between consanguinity, life-for-life and regression relatedness and individual and class reproductive value.

Note that: $c_{\mathrm{f}} = c_{\mathrm{m}} = 1/2$ under diploidy and $c_{\mathrm{f}} = 2/3$ and $c_{\mathrm{m}} = 1/3$ under haplodiploidy, regardless of the sex ratio (Price 1970; Taylor 1996); $p_{\mathrm{f}} = (1+f)/2$, where $f = \mathrm{d}g_1/\mathrm{d}g_2 = \mathrm{d}g_2/\mathrm{d}g_1$ is the coefficient of inbreeding; $p_{\mathrm{d}} = (1+3f)/4$ under diploidy and $p_{\mathrm{d}} = (1+f)/2$ under haplodiploidy; $p_{\mathrm{m}} = (1+f)/2$ under diploidy and $p_{\mathrm{m}} = 1$ under haplodiploidy; and $p_{\mathrm{s}} = (1+3f)/4$ under diploidy and $p_{\mathrm{s}} = f$ under haplodiploidy. Making these substitutions into the condition $c_{\mathrm{f}}(\mathrm{d}W_{\mathrm{f}}/\mathrm{d}g_{\mathrm{f}}) + c_{\mathrm{m}}(\mathrm{d}W_{\mathrm{m}}/\mathrm{d}g_{\mathrm{m}}) > 0$ recovers inequalities 1–4 under *Model and results*.

2. Population genetics analysis

Here, we develop an explicit population genetics model of the evolution of paternal care, analogous to the maternal care model analysed by Gardner (2012).

2.1 Diploidy

We assume an infinite population of females and males with genetic variation at a single locus with two alleles, the paternal care allele A and the null allele a. The

proportion of females carrying $x \in \{0,1,2\}$ copies of A is ϕ_x, and the proportion of males carrying $u \in \{0,1,2\}$ copies of A is μ_u. Similarly, we index maternal genotypes by $y \in \{0,1,2\}$ and paternal genotypes by $v \in \{0,1,2\}$. In the construction of mating pairs, we use the parameter α to control inbreeding. First, females and males are paired preferentially with 'matching' genotypes—AA females with AA males, Aa females with Aa males, and aa females with aa males—with any unpaired individuals paired randomly; then, a proportion $1-\alpha$ of these pairs is broken up and repaired randomly. Therefore, the frequency of x-female / u-male pairs is

$$\gamma_{xu} = \begin{cases} \alpha \min(\phi_x, \mu_u) + (1-\alpha)\phi_x\mu_u & \text{if } x = u \\ \alpha \dfrac{(\phi_x - \min(\phi_x, \mu_x))\,(\mu_u - \min(\phi_u, \mu_u))}{\sum_{z\in\{0,1,2\}}(\mu_z - \min(\phi_z, \mu_z))} + (1-\alpha)\phi_x\mu_u & \text{if } x \neq u \end{cases}.$$

Note that when genotype frequencies are equal for males and females, the $x \neq u$ case gives $\gamma_{xu} = (1-\alpha)\phi_x\mu_u$. Each pair produces a large number of offspring with an even sex ratio; each offspring's genotype comprises a random allele from the mother and a random allele from the father. Thus, the probability that a daughter born to a y-mother and a v-father is of genotype x is $\pi_{xyv=}\sum_{X\in\{0,1\}}\mathscr{B}(X; y/2)\mathscr{B}(x-X; v/2)$, where $\mathscr{B}(q;r)$ is equal to r if $q = 1$, $1-r$ if $q = 0$, and 0 otherwise; and the probability that a son born to a y-mother and a v-father is of genotype u is $\rho_{uyv} = \sum_{U\in\{0,1\}}\mathscr{B}(U; y/2)\mathscr{B}(u-U; v/2)$. The total proportion of x-daughters with a v-father is $\phi_{xv} = \sum_y \pi_{xyv}\gamma_{yv}$, and the total proportion of u-sons with a v-father is $\mu_{uv} = \sum_y \rho_{uyv}\gamma_{yv}$. Offspring undergo viability selection; the proportion of x-daughters with v-fathers who survive to adulthood is $s_{xv} = \beta - C_\mathrm{f}\varepsilon_\mathrm{f}x + B_\mathrm{f}\varepsilon_\mathrm{m}v$, and the proportion of u-sons with v-fathers who survive to adulthood is $t_{uv} = \beta - C_\mathrm{m}\varepsilon_\mathrm{m}u + B_\mathrm{m}\varepsilon_\mathrm{m}v$, where: β is baseline fitness; C_f, B_f, C_m, and B_m are as defined under *Model and results*; and $\varepsilon_\mathrm{f} = \varepsilon_\mathrm{m} = 1$ under adding of gene effects and $\varepsilon_\mathrm{f} = \varepsilon_\mathrm{m} = 1/2$ under averaging of gene effects. The average viability for females is $S = \sum_{x,v} s_{xv}\phi_{xv}$, and the average viability for males is $T = \sum_{u,v} t_{uv}\mu_{uv}$. Finally, an equal number of surviving females and males become the breeders for the next generation, returning the population to the beginning of the cycle with genotype frequencies $\phi'_x = \sum_v s_{xv}\phi_{xv}/S$ for females and $\mu'_u = \sum_v t_{uv}\mu_{uv}/T$ for males.

2.2 Haplodiploidy

We follow the same procedure as above, except for the following changes: male genotypes range over $u, v \in \{0,1\}$; AA females preferentially pair with A males, aa females preferentially pair with a males, and Aa females have no preference, making the frequency of x-female / u-male breeding pairs

$$\gamma_{xu} = \begin{cases} \alpha \min(\phi_x, \mu_u) + (1-\alpha)\phi_x\mu_u & \text{if } x = 2u \\ \alpha\left(\mu_u - \min(\phi_{2u}, \mu_u) - (\phi_{2-2u} - \min(\phi_{2-2u}, \mu_u))\right) + (1-\alpha)\phi_x\mu_u & \text{if } x = 1 \\ \alpha\left(\phi_{2-2u} - \min(\phi_{2-2u}, \mu_u)\right) + (1-\alpha)\phi_x\mu_u & \text{otherwise} \end{cases};$$

the probability that a daughter born to a genotype-y mother and a genotype-v father is of genotype x is $\pi_{xyv} = \sum_{X\in\{0,1\}} \mathscr{B}(X; y/2)\mathscr{B}(X; y/2)$ and the probability that a son born to a genotype-y mother and a genotype-v father is of genotype u is $\rho_{uyv} = \mathscr{B}(u; y/2)$; and $\varepsilon_m = 1$ under both adding and averaging of gene effects.

2.3 Inbreeding

To compute the coefficient of inbreeding f in our simulated population, we define a random variable κ according to the presence ($\kappa = 1$) or absence ($\kappa = 0$) of allele A in a random gamete produced by a random female, and a random variable λ according to the presence ($\lambda = 1$) or absence ($\lambda = 0$) of allele A in a random gamete produced by the same female's mate. Then, $f = \mathrm{cov}(\kappa,\lambda)/\mathrm{cov}(\kappa,\kappa)$, where $\mathrm{cov}(\kappa,\lambda) = \mathrm{E}(\kappa\lambda) - \mathrm{E}(\kappa)\mathrm{E}(\lambda) = (\sum_{x,u} \frac{xu}{4}\gamma_{xu}) - (\sum_{x,u} \frac{x}{2}\gamma_{xu})(\sum_{x,u} \frac{u}{2}\gamma_{xu})$ under diploidy and $\mathrm{cov}(\kappa,\lambda) = (\sum_{x,u} \frac{xu}{2}\gamma_{xu}) - (\sum_{x,u} \frac{x}{2}\gamma_{xu})(\sum_{x,u} u\gamma_{xu})$ under haplodiploidy, and $\mathrm{cov}(\kappa,\kappa) = \mathrm{E}(\kappa^2) - \mathrm{E}(\kappa)^2 = (\sum_{x,u} \frac{x}{2}\gamma_{xu}) - (\sum_{x,u} \frac{x}{2}\gamma_{xu})^2$ under both diploidy and haplodiploidy (Gardner 2012). We use the parameter α to control the amount of inbreeding during the construction of mating pairs; for an inbreeding population at equilibrium under neutrality, $f = \alpha/(2-\alpha)$. This follows because under neutrality, allele frequencies are the same in both sexes, and so $\mathrm{E}(\kappa\lambda) - \mathrm{E}(\kappa)\mathrm{E}(\lambda) = \left(\alpha(\frac{\phi_1}{4} + \phi_2) + (1-\alpha)p^2\right) - p^2$ and $\mathrm{E}(\kappa^2) - \mathrm{E}(\kappa)^2 = p - p^2$, where $\phi_2 = p^2 + fpq$ and $\phi_1 = 2pq(1-f)$ (Wright 1921; Li 1955), p is the frequency of allele A, and $q = 1 - p$.

2.4 Numerical simulations

We initialize genotype frequencies to $\phi_0 = \mu_0 = 0.999$, $\phi_1 = \mu_1 = 0.001$ and $\phi_2 = \mu_2 = 0.000$ under diploidy and $\phi_0 = 0.999$, $\phi_1 = 0.001$, $\phi_2 = 0.000$, $\mu_0 = 0.9995$ and $\mu_1 = 0.0005$ under haplodiploidy. For viability selection, we assume that $\beta = 1$ and $C = 0.001$. We then perform the above numerical recursions for ϕ_x and μ_u, allowing 100 generations to pass with the paternal care gene 'switched off' (*i.e.*, setting $C_\mathrm{f} = C_\mathrm{m} = B_\mathrm{f} = B_\mathrm{m} = 0$). Then we record the coefficient of inbreeding f and the frequency of the paternal care gene (*i.e.* $\frac{1}{2}\left[\frac{\phi_1}{2} + \phi_2\right] + \frac{1}{2}\left[\frac{\mu_1}{2} + \mu_2\right]$ for diploidy and $\frac{2}{3}\left[\frac{\phi_1}{2} + \phi_2\right] + \frac{\mu_1}{3}$ for haplodiploidy). We then 'switch on' the paternal care gene, setting C_f, C_m, B_f, and B_m to their desired values, and allow another 900 generations to pass. We consider the paternal care gene to have invaded if its frequency after the 900 generations of selection is greater than it was before those 900 generations.

Acknowledgments

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Chapter 3

The ecology of sex and who helps in arthropod societies

Abstract

Across arthropod societies, sib-rearing (*e.g.* nursing or nest defence) may be provided by females, by males, or by both sexes. According to Hamilton's "haplodiploidy hypothesis", this diversity reflects the relatedness consequences of diploid versus haplodiploid inheritance. However, an alternative "preadaptation hypothesis" instead emphasizes an interplay of ecology and the co-option of ancestral, sexually-dimorphic traits for sib-rearing. The preadaptation hypothesis has recently received empirical support, but remains to be formalized. Here, we mathematically model the coevolution of sex-specific helping and sex allocation, contrasting these hypotheses. We find that ploidy *per se* has little effect. Rather, the ecology of sex shapes patterns of helping: sex-specific preadaptation strongly influences who helps; a freely-adjustable sex ratio magnifies sex biases and promotes helping; and sib-mating, promiscuity, and reproductive autonomy also modulate the sex and abundance of helpers. An empirical survey reveals that patterns of sex-specific helping in arthropod taxa are consistent with the preadaptation hypothesis.

Introduction

Arthropod societies are characterized by individuals who, voluntarily or under manipulation, sacrifice their reproductive potential for their siblings' welfare. This "helping" behaviour—whether it involves nursing juveniles, habitat construction, or home defence—is often sex-biased, meaning that either females or males systematically invest more into sib-rearing. A familiar example of sex-biased helping is furnished by the social Hymenoptera (wasps, bees, and ants), whose exclusively-female workforce is often contrasted with the mixed-sex workforce of the social cockroaches (termites).

W.D. Hamilton's (1964; 1972) "haplodiploidy hypothesis" invoked the genetics of sex determination to explain this diversity. Hamilton argued that haplodiploid inheritance, as exhibited by the Hymenoptera, promotes female-biased helping because haplodiploid females are more related to sisters (life-for-life $R = 3/4$) than offspring ($R = 1/2$). In contrast, the termites' diploid inheritance does not promote a sex bias, because diploid individuals are equally related to siblings and offspring of either sex ($R = 1/2$). Under this view, inflated sororal relatedness also explains haplodiploids' apparent predisposition for sociality.

But in spite of its elegance, the haplodiploidy hypothesis has not stood up to further theoretical or empirical scrutiny. Crucially, haplodiploid females have comparatively low relatedness to brothers ($R = 1/4$), which exactly balances their high relatedness to sisters. Hence, haplodiploidy's relatedness asymmetries should neither promote helping overall, nor encourage female helping in particular, compared to diploidy (Trivers & Hare 1976; Craig 1979; Bourke & Franks 1995; Crozier & Pamilo 1996; Boomsma 2007; Gardner *et al.* 2012; Ross *et al.* 2013). Moreover, a broader view of arthropod sociality, beyond the eusocial insects, reveals extensive diversity in the sex of helpers—from all-female to mixed-sex to all-male—that is not readily explained by ploidy (Fig. 1).

Accordingly, an alternative "preadaptation hypothesis" has been suggested to explain patterns of helping in arthropods. This hypothesis holds that sex-biased helping reflects ancestral sexual dimorphism for traits subsequently co-opted for

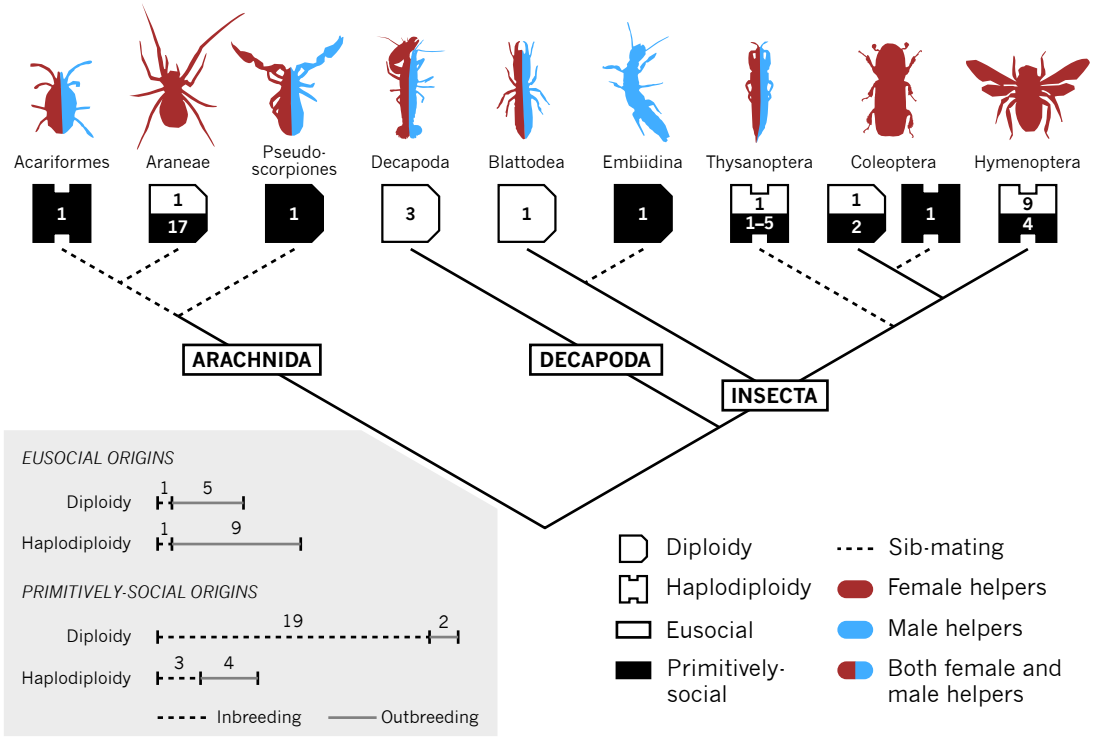


Figure 1: The 44 known independent origins of sib-rearing among eusocial and primitively-social arthropods, broken down by ploidy, type of social organization, presence or absence of sib-mating, and sex of helpers. Numbers within tree tips give the number of independent origins of sib-rearing within each taxon. **Inset, lower left:** distribution of origins of eusociality and primitive sociality, for diploidy versus haplodiploidy and inbreeding (sib-mating) versus outbreeding taxa. Phylogeny is based on Misof *et al.* (2014).

sib-rearing (Ross *et al.* 2013). For example, female-biased alloparental care is expected where ancestors provided maternal, but not paternal, care (Wheeler 1928; Lin & Michener 1972; Alexander 1974; West-Eberhard 1975; Evans 1977; Charlesworth 1978; Eickwort 1981; Craig 1982a; Andersson 1984; Starr 1985; Bourke & Franks 1995; Crozier & Pamilo 1996; Queller & Strassmann 1998; Gardner & Ross 2013). Indeed, this logic explains more variation in helper sex across animal societies than the classic ploidy-centric view (Ross *et al.* 2013).

However, the preadaptation hypothesis has only been verbally articulated, not theoretically formalized. Moreover, the ecology of sex more generally—that is, the structure of the social environment with respect to sexual reproduction—remains little-explored as an evolutionary driver of patterns of arthropod sociality, perhaps owing to the traditional emphasis on ploidy in this clade (Boomsma 2007). For instance, while sex allocation in cooperative-breeding vertebrates is comparatively well-understood (Emlen *et al.* 1986, Pen & Weissing 2000), more recent theory has highlighted a potential for positive feedback between sex-ratio bias and sex-biased helping, particularly in haplodiploid taxa that can readily adjust the sex ratio (Gardner & Ross 2013); yet the implications of such dynamics under sex-specific preadaptation to helping remain obscure. And although inbreeding is prevalent among social arthropods (Fig. 1), and impacts upon relatedness in sex-specific and ploidy-specific ways (Frank 1985; Herre 1985), inbreeding-associated mating ecologies have been largely neglected in relation to the haplodiploidy and preadaptation hypotheses (Bourke & Franks 1995; Crozier & Pamilo 1996).

Here, we develop a mathematical kin-selection model exploring the coevolution of sex allocation and sex-biased helping, under diploidy versus haplodiploidy and over a range of sexual ecologies: varying sex differences in preadaptation for sib-rearing; fixed versus evolutionarily-labile sex allocation; mating systems spanning full outbreeding to full sib-mating and strict monogamy to extreme promiscuity; and voluntary versus maternally-manipulated helping. This analysis yields quantitative predictions for when we expect helping by females, males, or both sexes, and we evaluate these predictions qualitatively with an empirical comparative survey of all known origins of sib-rearing among arthropods.

Mathematical analysis

Life cycle

A mated female produces many offspring on a patch, spending a proportion $1 - z$ of her resources on daughters and z on sons. On average, daughters invest a proportion x, and sons invest a proportion y of their reproductive resources into helping. To simplify interpretation, we assume females and males are equally costly, making z the sex ratio, and assume x and y are propensities for joining a sterile helper caste, although they could represent any costly investment into sib-rearing. Each female helper raises b_f, and each male helper b_m extra siblings; thus, $b_\mathrm{f} \neq b_\mathrm{m}$ indicates sex-specific preadaptation to sib-rearing. A proportion $1 - s$ of reproductive males disperse, and males from other patches immigrate. Then, mating occurs randomly within patches; consequently, sib-mating occurs with probability s. By default we assume strict monogamy, as this is most empirically-relevant (Boomsma 2007, 2009, 2013; Hughes *et al.* 2008), but we also provide results for any degree of promiscuity. After mating, gravid females disperse to found new patches, restarting the cycle (Fig. 2).

Dynamics and statics

Our analysis comprises three methodologies. First, we employ Taylor & Frank's (1996) neighbour-modulated fitness method, a recipient-centred approach to kin selection (Hamilton 1964). This yields analytical conditions for natural selection's effect on sex allocation, female helping, and male helping, allowing us to identify stable states for each. Second, we determine these states' reachability by numerically integrating the selection gradients starting from no helping ($x = y = 0$). Third, we assess our analytical results' robustness against a more realistic interplay of selection, mutation, drift, and standing genetic variation using stochastic individual-based simulations. (See *Methods* for full methodology and simulation results.)

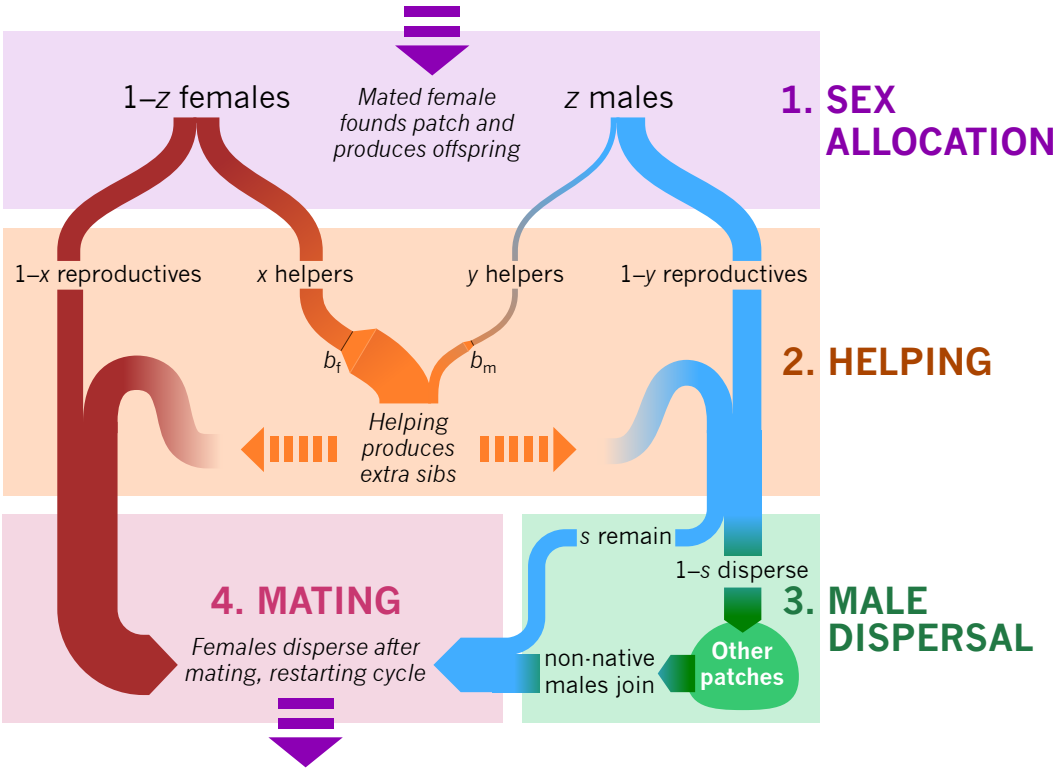


Figure 2: Life cycle used in the model. (1) Sex allocation: the foundress allocates a proportion z of her resources to sons and 1–z to daughters. (2) Helping: daughters invest a proportion x of their resources into sib-rearing, and sons invest a proportion y. The benefit of helping by females and males (b_f and b_m, respectively) determine the number of additional siblings surviving to maturity. (3) Male dispersal: reproductive males stay at home to mate with probability s, otherwise dispersing to other patches, and males from other patches immigrate to the focal patch. (4) Mating: reproductives mate within patches, such that s is the expected probability of sib-mating. After mating, gravid females disperse, founding new patches.

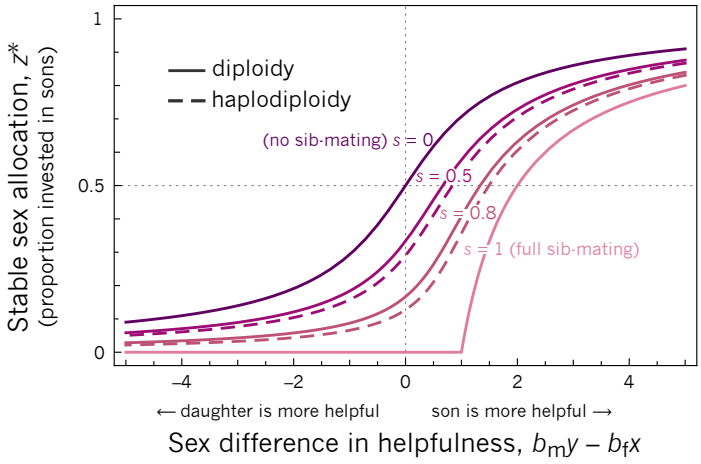


Figure 3: The stable strategy for sex allocation. Mothers are favoured to overproduce individuals of the more helpful sex, according to the sex difference in helpfulness $b_m y - b_f x$ (whether a daughter or a son helps raise more offspring, on average; ordinate axis) and to produce more daughters as the rate of sib-mating, s, increases (darker to lighter lines), a tendency which is exaggerated under haplodiploidy (dashed lines) when sib-mating is intermediate ($0 < s < 1$).

Sex allocation

Natural selection favours mothers to increase their investment into sons, z, when

$$\underbrace{-p_{\mathrm{dau}}\frac{c_{\mathrm{f}}}{N(1-z)}+p_{\mathrm{son}}\frac{c_{\mathrm{m}}}{Nz}}_{\text{rarer–sex effect}}+\underbrace{(b_{\mathrm{m}}y-b_{\mathrm{f}}x)\left[(1-z)\,p_{\mathrm{dau}}\frac{c_{\mathrm{f}}}{N(1-z)}+zp_{\mathrm{son}}\frac{c_{\mathrm{m}}}{Nz}\right]}_{\text{local resource enhancement effect}}$$
$$\underbrace{-sp_{\mathrm{son}}\frac{c_{\mathrm{m}}}{N(1-z)}-s^2p_{\mathrm{son}}\frac{c_{\mathrm{m}}}{Nz}}_{\text{local mate competition effect}}+\underbrace{(b_{\mathrm{m}}y-b_{\mathrm{f}}x)\left[(1-z)\,sp_{\mathrm{son}}\frac{c_{\mathrm{m}}}{N(1-z)}-zs^2p_{\mathrm{son}}\frac{c_{\mathrm{m}}}{Nz}\right]}_{\text{local mate enhancement effect}}>0\,. \tag{1}$$

To interpret this condition, read the left-hand side as gathering the inclusive-fitness effects experienced by a mother who sacrifices a daughter to produce an extra son. If the sum of these effects is greater than zero, this trade improves the mother's inclusive fitness, so natural selection will favour an increase in z (Fig. 3). Each term in condition 1 is constructed from three measures of value (Frank 1998): a marginal fitness cost or benefit for a recipient; the foundress's consanguinity with the recipient (the probability that randomly-picked genes from each are identical by descent; Bulmer 1994); and the recipient's reproductive value (RV), measuring their long-term genetic contribution to the population.

Accordingly, the rarer-sex effect captures the face value of trading away a daughter

for a son, ignoring knock-on effects for other relatives. The daughter has consanguinity p_{dau} with her mother, and her RV is $\frac{c_{\text{f}}}{N(1-z)}$, while the son has consanguinity p_{son} with his mother, and his RV is $\frac{c_{\text{m}}}{Nz}$. Here: $p_{\text{dau}} = p_{\text{son}} = \frac{1}{4-3s}$ under diploidy, and $p_{\text{dau}} = \frac{1}{4-3s}$, $p_{\text{son}} = \frac{2-s}{4-3s}$ under haplodiploidy (see *Methods*); c_{f} and c_{m} are the total RV of females and males, respectively ($c_{\text{f}} = c_{\text{m}} = 1/2$ under diploidy, while $c_{\text{f}} = 2/3$, $c_{\text{m}} = 1/3$ under haplodiploidy); and N is the total population size. For diploids and outbred haplodiploids, $p_{\text{dau}}c_{\text{f}} = p_{\text{son}}c_{\text{m}}$, so the rarer-sex effect favours increased investment into sons when $z < 1/2$, but decreased investment into sons when $z > 1/2$, and hence an even sex allocation, $z = 1/2$, overall (Darwin 1871; Düsing 1883; Fisher 1930). But for inbred haplodiploids, $p_{\text{dau}}c_{\text{f}} > p_{\text{son}}c_{\text{m}}$, and hence inbreeding *per se* increases a mother's relative valuation of her daughters under haplodiploidy, favouring a relatively female-biased sex ratio (Hamilton 1972; Frank 1985; Herre 1985).

The local resource enhancement (LRE) effect obtains when the sexes differ in helpfulness ($b_{\text{f}}x \neq b_{\text{m}}y$). In trading away a potential female helper for a potential male helper, the mother gains, on average, $b_{\text{m}}y - b_{\text{f}}x$ successfully-raised offspring: a proportion $1 - z$ of these are daughters, each worth $p_{\text{dau}}\frac{c_{\text{f}}}{N(1-z)}$, and a proportion z are sons, each worth $p_{\text{son}}\frac{c_{\text{m}}}{Nz}$. The LRE effect favours a relative sex-ratio bias towards the more-helpful sex, increasing the overall investment in helping (Trivers & Willard 1973; Pen & Weissing 2000; Gardner & Ross 2013).

The local mate competition (LMC) effect obtains in the context of sib-mating ($s > 0$). Trading a daughter for a son means one fewer potential mating partner (the daughter) and one more potential mate competitor (the son) for males mating on the patch (Hamilton 1967; Taylor 1981). As male RV comes from mating with females, the daughter's forfeiture incurs—on average—the loss of a fraction $\frac{1}{N(1-z)}$ of total male RV for a locally-mating male, whose expected consanguinity to the foundress is sp_{son}. Simultaneously, the RV gained by the additional son, $\frac{c_{\text{m}}}{Nz}$, incurs an equal loss from his mate competitors, who are his brothers to the extent s^2 that neither he nor his brothers disperse before mating, and hence have consanguinity s^2p_{son} to the foundress. These costs of LMC favour a relatively female-biased sex ratio (Hamilton 1967).

Finally, the "local mate enhancement" (LME) effect stems from an interaction between LRE and LMC. Specifically, in trading a daughter for a son, the mother gains

$(b_{\mathrm{m}}y - b_{\mathrm{f}}x)(1-z)$ daughters and $(b_{\mathrm{m}}y - b_{\mathrm{f}}x)z$ sons through sib-rearing. As above, each daughter yields an inclusive-fitness benefit $sp_{\mathrm{son}}\frac{c_{\mathrm{m}}}{N(1-z)}$ by alleviating LMC, while each son yields an inclusive-fitness cost $-s^2p_{\mathrm{son}}\frac{c_{\mathrm{m}}}{Nz}$ by intensifying LMC. The net effect of LME is zero when sib-mating is absent ($s = 0$) or complete ($s = 1$), but otherwise ($0 < s < 1$) it slightly favours investment in the more-helpful sex (Fig. M1, *Methods*).

Sex-specific helping

Voluntary helping

Natural selection favours an increase in voluntary helping by females, x, when

$$\underbrace{-p_{\mathrm{self|f}}\frac{c_{\mathrm{f}}}{N(1-z)(1-x)}}_{\text{sacrifice effect}} + \underbrace{b_{\mathrm{f}}\left[(1-z)\,p_{\mathrm{sis|f}}\frac{c_{\mathrm{f}}}{N(1-z)} + zp_{\mathrm{bro|f}}\frac{c_{\mathrm{m}}}{Nz}\right]}_{\text{sib-rearing effect}}$$
$$\underbrace{-sp_{\mathrm{bro|f}}\frac{c_{\mathrm{m}}}{N(1-z)(1-x)}}_{\text{local mate competition effect}} + \underbrace{b_{\mathrm{f}}\left[(1-z)\,sp_{\mathrm{bro|f}}\frac{c_{\mathrm{m}}}{N(1-z)} - zs^2p_{\mathrm{bro|f}}\frac{c_{\mathrm{m}}}{Nz}\right]}_{\text{local mate enhancement effect}} > 0\,. \tag{2}$$

The left-hand side gathers the inclusive-fitness effects experienced by a reproductive female who joins a sterile helper caste. Analogously to condition 1, the four labelled effects reflect distinct selection pressures which, together, shape female helping (Fig. 4a) . Above, $p_{\mathrm{self|f}} = \frac{2-s}{4-3s}$, $p_{\mathrm{sis|f}} = p_{\mathrm{bro|f}} = \frac{1}{4-3s}$ under diploidy, and $p_{\mathrm{self|f}} = \frac{2-s}{4-3s}$, $p_{\mathrm{sis|f}} = \frac{3-s}{8-6s}$, $p_{\mathrm{bro|f}} = \frac{1}{4-3s}$ under haplodiploidy.

The sacrifice effect captures the female's lost RV—the total female RV divided by the number of reproductive females in the population—weighted by her consanguinity to herself, $p_{\mathrm{self|f}}$. All else being equal, as the sex ratio becomes more female-biased, the direct cost of helping decreases, promoting female helping (*cf.* Gardner & Ross 2013). This mirrors the rarer-sex effect (condition 1) in that female reproduction is devalued when females are more abundant.

The sib-rearing effect describes the inclusive-fitness benefit of rearing extra siblings, in the absence of any knock-on effects on mating ecology. Specifically, the female raises $b_{\mathrm{f}}(1-z)$ extra sisters, each worth $p_{\mathrm{sis|f}}\frac{c_{\mathrm{f}}}{N(1-z)}$, and $b_{\mathrm{f}}z$ extra brothers, each worth

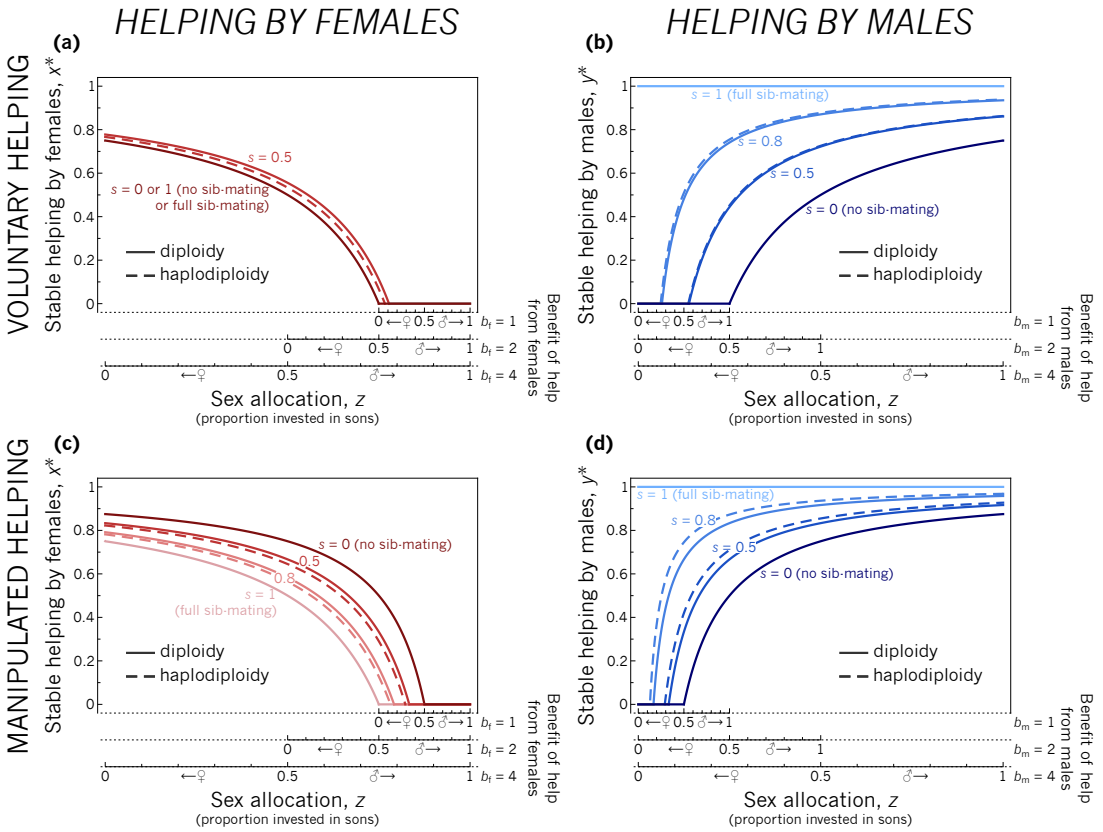


Figure 4: The stable strategy for helping by females and males. Individuals are favoured to help more as their sex becomes relatively more abundant (along ordinate axes) and as the benefits of helping increase (between ordinate axes); under haplodiploidy (dashed lines), females help slightly less, and males help slightly more, than under diploidy (solid lines), provided there is an intermediate rate of sib-mating ($0 < s < 1$). The effect of sib-mating, s, depends on the sex in question and on whether helping is voluntary or maternally-manipulated. **(a)** Voluntary helping by females: helping is promoted under intermediate sib-mating. **(b)** Voluntary helping by males: sib-mating promotes helping. **(c)** Maternally-manipulated helping by females: sib-mating inhibits helping. **(d)** Maternally-manipulated helping by males: sib-mating promotes helping.

$p_{\mathrm{bro|f}}\frac{c_\mathrm{m}}{Nz}$. By increasing relatedness, inbreeding ($s > 0$) increases a female's relative valuation of her siblings, which promotes helping, and this effect is stronger under diploidy than haplodiploidy.

The LMC effect obtains in the context of sib-mating ($s > 0$), such that a female joining the sterile helper caste means one fewer potential mate for her brothers. Specifically, a reproductive female represents, on average, $\frac{c_\mathrm{m}}{N(1-z)(1-x)}$ units of male RV (*cf. Sex allocation*, above), so the female helper incurs this loss for locally-mating males, who have expected consanguinity $sp_{\mathrm{bro|f}}$ to the female. This cost of LMC inhibits female helping and, indeed, exactly cancels the promoting effect of inbreeding (previous paragraph), under both diploidy and haplodiploidy.

Finally, the LME effect captures an interaction between sib-rearing and LMC. By helping, the female rears $b_\mathrm{f}(1-z)$ extra sisters and $b_\mathrm{f}z$ extra brothers. Each sister, by alleviating LMC, yields an inclusive-fitness benefit $sp_{\mathrm{bro|f}}\frac{c_\mathrm{m}}{N(1-z)}$, while each brother, by intensifying LMC, yields an inclusive-fitness cost $-s^2p_{\mathrm{bro|f}}\frac{c_\mathrm{m}}{Nz}$ (*cf. Sex allocation*, above). LME has no effect when sib-mating is absent ($s = 0$) or complete ($s = 1$), but otherwise ($0 < s < 1$) it moderately promotes female helping (Fig. M2a, *Methods*).

Natural selection favours an increase in voluntary helping by males, y, when

$$\underbrace{-p_{\mathrm{self|m}}\frac{c_\mathrm{m}}{Nz\,(1-y)}}_{\text{sacrifice effect}}+\underbrace{b_\mathrm{m}\left[(1-z)\,p_{\mathrm{sis|m}}\frac{c_\mathrm{f}}{N\,(1-z)}+zp_{\mathrm{bro|m}}\frac{c_\mathrm{m}}{Nz}\right]}_{\text{sib-rearing effect}}$$
$$+\underbrace{s^2p_{\mathrm{bro|m}}\frac{c_\mathrm{m}}{Nz\,(1-y)}}_{\text{local mate competition effect}}+\underbrace{b_\mathrm{m}\left[(1-z)\,sp_{\mathrm{bro|m}}\frac{c_\mathrm{m}}{N\,(1-z)}-zs^2p_{\mathrm{bro|m}}\frac{c_\mathrm{m}}{Nz}\right]}_{\text{local mate enhancement effect}}\;>\;0\,. \tag{3}$$

The left-hand side gathers the inclusive-fitness effects experienced by a reproductive male who joins a sterile helper caste, which together shape male helping (Fig. 4b). Above, $p_{\mathrm{self|m}}=\frac{2-s}{4-3s}$, $p_{\mathrm{sis|m}}=p_{\mathrm{bro|m}}=\frac{1}{4-3s}$ under diploidy, and $p_{\mathrm{self|m}}=1$, $p_{\mathrm{sis|m}}=\frac{1}{4-3s}$, $p_{\mathrm{bro|m}}=\frac{2-s}{4-3s}$ under haplodiploidy.

The sacrifice, sib-rearing and LME effects in condition 3 exactly mirror the corresponding effects in condition 2. Hence, as the sex ratio becomes relatively male-biased, male helping is promoted because its direct cost is reduced. However, the symmetry

breaks down with respect to LMC: while the LMC effect in condition 2 captures an indirect cost for a female abstaining from mating, the LMC effect in condition 3 captures an indirect benefit for a male abstaining from mating. Specifically, the male's forfeited RV, $\frac{c_m}{Nz(1-y)}$, yields a corresponding increase in RV for his mate competitors, who are his brothers to the extent s^2 that neither he nor his brothers would disperse before mating, and hence have expected consanguinity $s^2 p_{\mathrm{bro|m}}$ with the male.

Accordingly, sib-mating—by increasing sibling relatedness, incentivising abstention from LMC, and returning benefits through LME (Fig. M2b, *Methods*)—promotes a male's willingness to help. Indeed, under sib-mating ($s > 0$), males are generally more willing to help than females, for an even sex ratio ($z = 1/2$) and equal helping ability ($b_f = b_m$; Fig. 4a & b). And, in contrast with Hamilton's (1964, 1972) haplodiploidy hypothesis, this relative predisposition for male helping may be magnified under haplodiploidy. This is because, under haplodiploidy: (i) inbreeding leads a female to be more consanguineous with herself, increasing her cost of self-sacrifice, but this is not true for males, as a haploid individual cannot be inbred (*cf.* Davies & Gardner 2014); and (ii) inbreeding leads males to value brothers more than females do, increasing their willingness to abstain from LMC and their indirect benefits through LME.

Maternally-manipulated helping

If mothers control their offspring's investment into helping, then natural selection favours an increase in helping by females when

$$\underbrace{-p_{\mathrm{dau}} \frac{c_f}{N(1-z)(1-x)}}_{\text{sacrifice effect}} + \underbrace{b_f \left[(1-z)\, p_{\mathrm{dau}} \frac{c_f}{N(1-z)} + z p_{\mathrm{son}} \frac{c_m}{Nz} \right]}_{\text{sib-rearing effect}}$$
$$\underbrace{-s p_{\mathrm{son}} \frac{c_m}{N(1-z)(1-x)}}_{\text{local mate competition effect}} + \underbrace{b_f \left[(1-z)\, s p_{\mathrm{son}} \frac{c_m}{N(1-z)} - z s^2 p_{\mathrm{son}} \frac{c_m}{Nz} \right]}_{\text{local mate enhancement effect}} > 0 . \tag{4}$$

and an increase in helping by males when

$$\underbrace{-p_{\text{son}}\frac{c_{\text{m}}}{Nz\left(1-y\right)}}_{\text{sacrifice effect}}+\underbrace{b_{\text{m}}\left[\left(1-z\right)p_{\text{dau}}\frac{c_{\text{f}}}{N\left(1-z\right)}+zp_{\text{son}}\frac{c_{\text{m}}}{Nz}\right]}_{\text{sib}-\text{rearing effect}}$$
$$+\underbrace{s^{2}p_{\text{son}}\frac{c_{\text{m}}}{Nz\left(1-y\right)}}_{\text{local mate competition effect}}+\underbrace{b_{\text{m}}\left[\left(1-z\right)sp_{\text{son}}\frac{c_{\text{m}}}{N\left(1-z\right)}-zs^{2}p_{\text{son}}\frac{c_{\text{m}}}{Nz}\right]}_{\text{local mate enhancement effect}}\;>\;0\,. \tag{5}$$

Conditions 4 and 5 may be obtained by substituting into conditions 2 and 3 the consanguinities p_{dau} instead of $p_{\text{self}|\text{f}}$ and $p_{\text{sis}|\text{f}}$, and p_{son} instead of $p_{\text{self}|\text{m}}$ and $p_{\text{bro}|\text{f}}$, reflecting that, for manipulated helping, natural selection acts on the mother's inclusive fitness rather than that of the helpers. The resulting effect on helping is illustrated in Fig. 4c & d. All else being equal, mothers prefer more helping than potential helpers themselves do when $s < 1$—therefore, there is potential for parent–offspring conflict over the extent of sib-rearing (Alexander 1974; Trivers 1974; González-Forero 2014). Analogously to voluntary helping, mothers tend to recruit more male helpers in the context of inbreeding ($s > 0$), because recruiting males alleviates LMC, while recruiting females exacerbates LMC; this effect is magnified under haplodiploidy, because inbreeding increases a mother's valuation of her daughters relative to her sons (see *Sex allocation*, above).

Promiscuity and helping

Although we focus on monogamy above, our model allows us to consider promiscuity's effect on sex-specific helping. Under diploidy, promiscuity tends to inhibit voluntary helping by decreasing sibling relatedness. Under haplodiploidy, promiscuity tends to inhibit voluntary helping by females, but has no effect on helping by males, because haploid males are only related to their siblings through their mothers (Fig. M4, *Methods*). Note that promiscuity does not affect sex allocation or maternally-manipulated helping, because it does not alter a mother's comparative relatedness to her daughters versus sons (see *Methods* for details).

Coevolution of sex allocation and sex-biased helping

Because sex allocation depends on the helpfulness of females versus males (see condition 1), while helping depends on sex allocation (see conditions 2–5), there is potential for coevolution between sex allocation and sex-biased helping. Indeed, whilst both sexes may help when the sex ratio is fixed at a particular value, we find that only one sex helps when we permit these traits to coevolve (Fig. 5) .

When sex allocation is fixed at $z = 1/2$, individuals of both sexes may help. For voluntary helping, females and males each help according to their ability (Fig. 5a); for maternally-manipulated helping, the mother recruits female and male helpers according to her need (Fig. 5c). In either case, sib-mating tends to promote male helping, an effect which is slightly magnified under haplodiploidy (see Sex-specific helping, above).

In contrast, when the sex ratio is evolutionarily-labile, typically only one sex helps. For voluntary helping, this is because any sex difference in helpfulness ($b_f x \neq b_m y$) attracts a sex-allocation bias favouring the more-helpful sex (due to the LRE effect, condition 1), while any change in sex allocation makes the favoured sex more willing to help, and the disfavoured sex less willing to help (due to the sacrifice effect, conditions 2 & 3). This positive feedback between sex-biased helping and sex-ratio bias ultimately promotes helping by one sex only. Which sex helps may be influenced by several factors but, over some regions of parameter space, both female-only and male-only helping represent possible alternative stable states (see *Methods*). In particular, in the context of outbreeding ($s = 0$) and no sex bias in ability ($b_f = b_m$), all-female helping and all-male helping are equally-likely alternatives. A bias in ability ($b_f \neq b_m$) tends to promote exclusive helping by the more-able sex, while sib-mating ($s > 0$)—by favouring a female-biased sex allocation—tends to promote exclusive helping by females (Fig. 5b).

For maternally-manipulated helping, only one sex helps when sex allocation is labile because full control of both sex allocation and helping allows the mother to recruit helpers solely from the more-able sex, adjusting her sex allocation as needed to produce the desired adult sex ratio. Hence, only the more-able sex is expected to help,

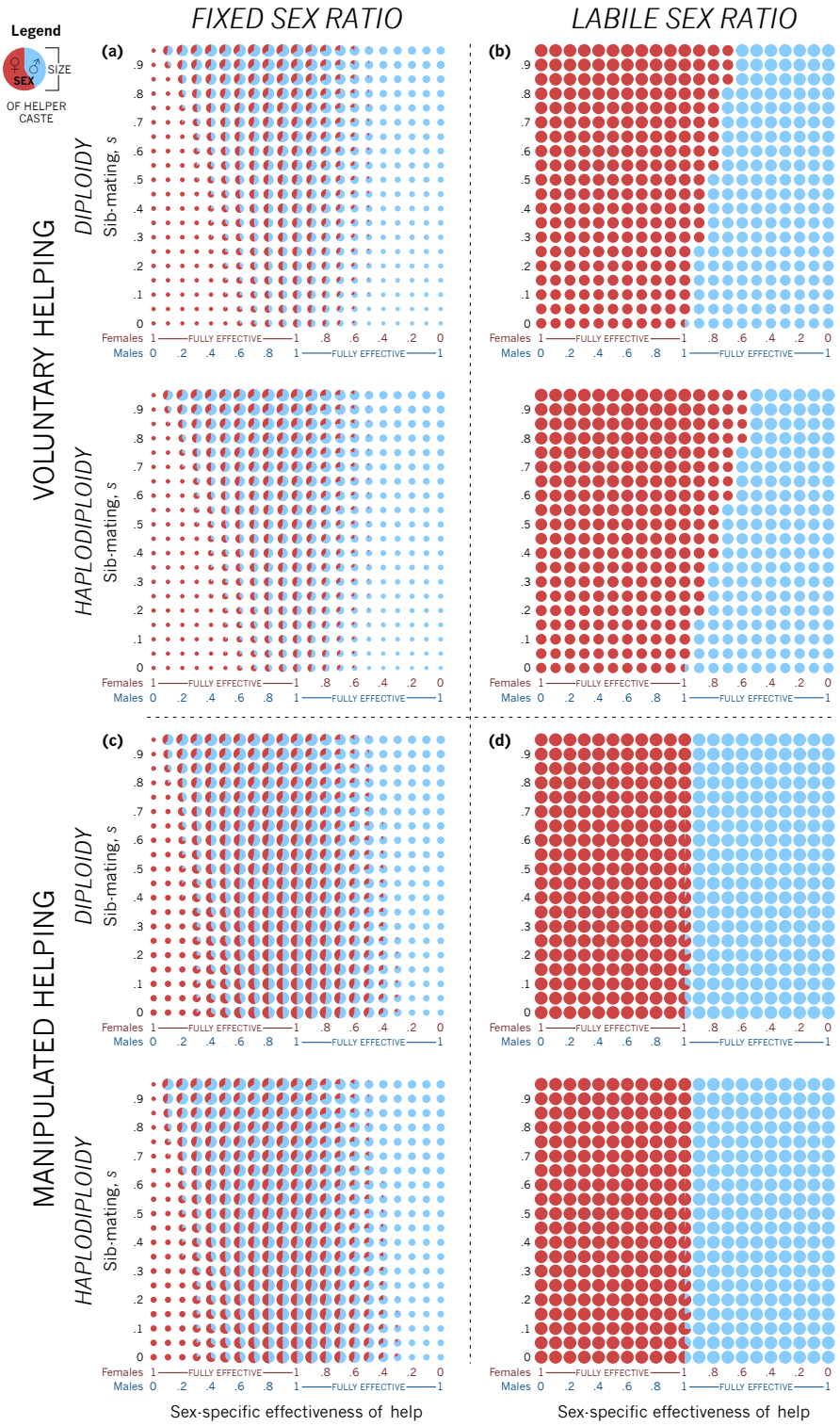


Figure 5: Patterns of helping as a function of sex differences in helping ability, sib-mating, diploidy versus haplodiploidy, voluntary versus maternally-manipulated helping, and fixed versus labile sex allocation. Each pie chart shows the equilibrium state following the evolution of helping, giving the percentage of helpers who are female (red) versus male (blue), as well as the size of the helper caste (diameter of pie chart), for a given set of parameters. Each chart is obtained by numerically integrating selection gradients (conditions 1–5) starting from no helping ($x = y = 0$). Sex allocation is either fixed at $z = 1/2$ **(a, c)**, or set to its equilibrium value in the absence of helping, then permitted to freely coevolve with helping **(b, d)**. Here, we assume a maximum sib-rearing benefit of $b_{\max} = 4$, *i.e.* $b_{\rm f} = 4e_{\rm f}$ and $b_{\rm m} = 4e_{\rm m}$, where $e_{\rm f}$ and $e_{\rm m}$ are the "effectiveness of help" for females and males, respectively (bottom of each plot). Note that differences between diploidy and haplodiploidy *per se* are minor, while sex differences in ability have a major influence on the sex of helpers.

regardless of ploidy or sib-mating (Fig. 5d). In the absence of sex-specific preadaptation ($b_f = b_m$), the helper sex ratio is selectively neutral from the mother's perspective, and any sex bias may depend on other factors.

Finally, we find that evolutionarily-labile sex allocation promotes helping overall, because it allows mothers to invest more in helpers. This result holds regardless of the relative helping ability of females versus males, except for manipulated helping when females and males are exactly equally-preadapted, in which case labile sex allocation has no effect (Fig. 5).

Empirical survey

Focusing on the origins of sociality, we collected published data from across the social arthropods, including both primitively-social species, where individuals delay dispersal to conduct sib-rearing, and facultatively-eusocial species, with non-reproductive helper castes. However, we excluded eusocial species with complementary totipotency (Crespi & Yanega 1995; Boomsma 2013) as these are highly-derived and may display secondarily-evolved traits that hinder straightforward interpretation of helper sex (*e.g.* sex-specific task specialization). For each independent origin of social behaviour, we recorded: the sex of helpers; any behavioural sex differences in willingness to help; whether sociality evolved under inbreeding or outbreeding; and ploidy. Finally, we assessed possible sex-specific preadaptation for helping. Following Ross *et al.* (2013), where helping involves brood care, we assumed that the sex or sexes that ancestrally provided parental care are pre-adapted. Where helpers defend the colony, we assumed preadaptation if one sex is better-equipped for the task, *e.g.* through superior body size or weaponry. We included multiple species per origin of sociality if there was variation for any trait of interest. Data were analysed using a phylogenetically-controlled mixed-model approach using the R packages MCMCglmm (Hadfield & Nakagawa 2010) and MCMCglmmRAM (Hadfield 2015). We estimated a molecular phylogeny for 31 taxa, each covering a separate evolutionary origin, using BEAST (Drummond *et al.* 2012) with published sequences (5929 aligned sites across the following loci: *16S*, *18S*, *28S*, *coi*, *coii*, *cytb*, *ef1a*, *h3*, *wingless*; see

Methods for full methodology).

We identified 16 eusocial and 28 primitively-social origins of sib-rearing among arthropods (Fig. 1). Remarkably, and in contrast with assumptions of previous theory on the evolution of eusociality, over half of these origins appear in the context of inbreeding and LMC. Our survey also shows that even in species where both sexes help, there are generally subtle sex-biases, either numerical or behavioural, and completely-unbiased helping is rare. Of the 44 origins we identified, full data were available for 30 (Table M3 & Fig. M11, *Methods*). Undertaking taxonomically- and phylogenetically-controlled comparative analyses, we find that sex biases in helping are primarily determined by sex differences in helping ability (Fig. 6a; $p_{\mathrm{MCMC}} < 0.001$ for both analyses), while neither ploidy (phylogenetic $p_{\mathrm{MCMC}} = 0.50$, taxonomic $p_{\mathrm{MCMC}} = 0.996$) nor sib-mating (phylogenetic $p_{\mathrm{MCMC}} = 0.50$, taxonomic $p_{\mathrm{MCMC}} = 0.25$) are significant determinants of helper sex.

A key prediction of our model is that, while both sexes may help if sex allocation is constrained, helping should be restricted to one sex if sex allocation is evolutionarily-labile. As there is little direct information concerning which species can bias the sex ratio, we used indirect estimates where necessary, assuming that haplodiploids and any species with ancestral LMC have this ability. We find that, with one exception (the *Austroplatypus* ambrosia beetle), single-sex workers are restricted to taxa able to freely adjust sex allocation, and the association between helper sex bias and sex-ratio lability is significant (phylogenetic $p_{\mathrm{MCMC}} = 0.02$) or marginally non-significant (taxonomic $p_{\mathrm{MCMC}} = 0.07$) across independent origins of helping (Fig. 6b).

Discussion

We have analysed the coevolution of sex allocation and sib-rearing in a demographically-explicit mathematical model, contrasting the impact of ploidy versus preadaptation upon patterns of helping in arthropod societies. We find that ploidy has little effect, while five key aspects of sexual ecology—sex-specific preadaptation, sex-ratio constraints, sib-mating, promiscuity, and reproductive autonomy—influence the sex and abundance of helpers. We have assessed the empirical support for and

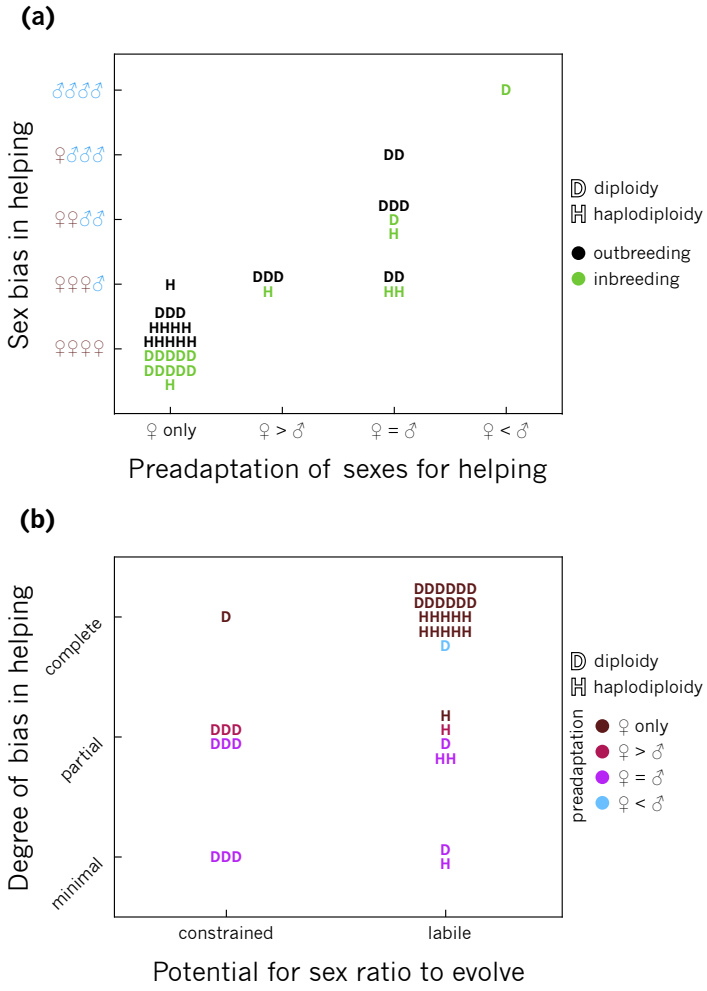


Figure 6: Data from the empirical survey providing a qualitative test of model predictions. **(a)** Across origins of helping in arthropods for which data are available, sex-specific preadaptation for helping tasks is significantly associated with the sex of helpers (phylogenetic $p_{\mathrm{MCMC}} < 0.001$, taxonomic $p_{\mathrm{MCMC}} < 0.001$). **(b)** The helper sex ratio tends to exhibit a greater bias towards single-sex helping in clades with an inferred ancestral ability to bias the sex ratio (phylogenetic $p_{\mathrm{MCMC}} = 0.02$, taxonomic $p_{\mathrm{MCMC}} = 0.07$).

relevance of these predictions by conducting a comparative survey of all known origins of sociality in arthropods.

Our analysis provides a formal foundation for the "preadaptation hypothesis" that sex biases in helping may reflect ancestral sexual dimorphism for traits co-opted for sib-rearing. In other words, sex-biased sib-rearing—whether by immature reproductives or sterile helpers—is modulated by intrinsic sex differences in helping ability, as established empirically by Ross *et al.* (2013). Innate differences may be expected if, for example, helpers originate as nurses in a taxon with ancestral maternal-only care—as in the aculeate Hymenoptera, where maternal care is widespread (Hamilton 1964; Hölldobler & Wilson 1990; Alexander *et al.* 1991) and sib-rearing derives from brood care redirected towards siblings (Wheeler 1928; Kennedy 1966; Michener 1969; Wilson 1971; Hamilton 1972; Alexander 1974; West-Eberhard 1987; Amdam *et al.* 2006; Boomsma 2007; Toth *et al.* 2007).

Crucially, we have shown that sex differences in helping ability need not be absolute to have a major influence on who helps. Any initial sex difference in helpfulness promotes a sex-ratio bias favouring the more-helpful sex via LRE (Fig. 3), which selects for further-biased helping by comparatively decreasing the more-helpful individuals' RV (Fig. 4), eventually leading to single-sex helping (Fig. 5). Here, sex allocation impacts upon sib-rearing because helping becomes comparatively more appealing as mating opportunities diminish in value; in contrast, Johnstone & Cant (2008)—who assumed that non-breeders cannot help—found no impact of sex allocation upon helping. In contradistinction to the haplodiploidy hypothesis, our analysis highlights that single-sex helping should emerge by default—and that it is mixed-sex workforces that demand a special explanation, such as relatively-constrained sex allocation.

Because sexually-dimorphic traits relevant for helping may stem from male-female asymmetries in sexual selection, the mating system, and gametic investment (Queller 1997b), sex-specific preadaptation itself reflects a species' ancestral sexual ecology. We have shown that contemporary sexual ecology also plays a crucial role, as it both drives sex-biased helping directly and generates sex-ratio biases that themselves promote biased helping. In particular, sib-mating, with concomitant inbreeding and LMC, may have a major impact upon sex-specific helping, depending on the species' ability to

adjust the sex ratio. Specifically, under a fixed sex ratio—for example, owing to an inflexible chromosomal mode of sex determination—LMC may drive relatively male-biased helping as an alternative means of diverting resources from male to female reproduction (Fig. 5a & c). In contrast, under an evolutionarily-labile sex ratio, LMC may drive female-biased sex allocation, decreasing females' RV and hence encouraging relatively female-biased helping (Fig. 5b & d). Hence, kin competition among helpers not only influences sex allocation directly (Wild 2006) but also indirectly, by promoting sex-biased helping. Surprising as it is, to our knowledge our analysis is the first to link LMC to the evolution of sex-biased helping, and we suspect this owes to an inordinate focus on the social Hymenoptera leading researchers to believe that inbreeding is of little empirical relevance to the origin of sociality—a misconception that we hope Fig. 1 helps to dispel.

Besides addressing sex biases in helping, our analysis also highlights aspects of sexual ecology that may promote the emergence of sociality. In addition to suggesting that inflated sororal relatedness explains the all-female workforce of the social Hymenoptera, the haplodiploidy hypothesis suggests that it is also responsible for this group's multiple independent origins of eusociality (Hamilton 1964, 1972). This view has received criticism on both theoretical and empirical grounds (Trivers & Hare 1976; Craig 1979; Gardner *et al.* 2012), and instead the current consensus is that the presence or absence of strict monogamy is the most important determinant of taxonomic patterns of sociality (Boomsma 2007, 2009, 2013; Hughes *et al.* 2008). This "monogamy hypothesis" suggests that, because full siblings are as related to each other as an individual is to her own offspring, there has been little barrier to the evolution of altruistic sib-rearing in taxa exhibiting strict monogamy, a mating system that is rare especially among iteroparous taxa in which sib-rearing can feasibly arise. This, too, supports the preadaptation view, as it highlights prerequisites for sociality that have not evolved to promote helping: for example, the sperm-storage abilities of the aculeate Hymenoptera enable females to produce multiple broods throughout a potentially long lifetime, despite mating only once. We have confirmed that monogamy dramatically increases selection for sib-rearing, particularly among outbred taxa (Fig. M5, *Methods*).

The ability to adjust the sex ratio, in addition to its role in magnifying sex biases in helping, also acts to promote helping, by enabling the mother to invest more in helpers. Frank & Crespi (1989) proposed an alternative feedback, with Trivers & Willard's (1973) effect driving split sex ratios, which—in conjunction with haplodiploidy—then drive female-biased helping. Our model predicts more investment into helping, exhibiting a greater sex bias, than existing models that do not consider coevolution between sex allocation and helping (Pen & Weissing 2000; Wild 2006; Johnstone & Cant 2008). Similar positive feedback was shown by Gardner & Ross (2013), although their model assumed female-only helping from the outset rather than allowing sex-biased helping to evolve from scratch. They noted that an evolutionarily-inflexible sex ratio poses a significant barrier to the evolution of eusociality when only females can help, and we have shown that this result extends to both sexes (potentially) helping, whether voluntarily or under maternal manipulation (Fig. 5). This may explain why, among 44 identified origins of sib-rearing in arthropods, 37 (84%) occurred in clades with an inferred ancestral ability to adjust the sex ratio.

We also propose that sib-mating may promote sociality in arthropods (Fig. 5; Figs. M5 & M6, *Methods*). The effect of inbreeding on the evolution of sib-rearing has been considered before: because it reduces relatedness asymmetries between a haplodiploid female and her siblings of either sex (Hamilton 1972; Crozier & Pamilo 1996); because it may influence the speed or favourability of the evolution of altruism under particular genetic architectures (Michod 1993); or because cyclical inbreeding could result in individuals being more related to (inbred) siblings than to their potential (outbred) offspring (Hamilton 1972; Bartz 1979; Chapman 2003; *cf.* Myles & Nutting 1988). Here we have outlined a novel mechanism by which inbreeding may promote altruistic sib-rearing, through the particular ecological context of LMC. In this context, LME may promote helping both by increasing an individual's willingness to help and by further promoting a sex-ratio bias favouring the more-helpful sex (Figs. M1 & M2, *Methods*). Furthermore, male helping in particular is promoted via the inclusive-fitness benefits of abstaining from LMC: a male who gives up breeding to help does not completely forfeit his genetic interest in a potential mate's offspring if she simply mates with his brother instead. It is suggestive that, among 44 identified origins of sociality, 24 (55%)

have occurred in clades ancestrally associated with sib-mating (Fig. 1).

A further aspect of sexual ecology is whether individuals make their own breeding decisions (Alexander 1974; Michener & Brothers 1974). Maternally-manipulated helping is suggested to be more readily-favoured than voluntary helping (Alexander 1974; Charlesworth 1978; Charnov 1978) and, indeed, we find that maternal manipulation promotes helping, particularly under outbreeding (Fig. 5). Nonetheless, maternally-manipulated helping—while theoretically plausible—may not readily explain eusociality's absence outwith ancestrally monogamous taxa (*cf.* González-Forero & Gavrilets 2013).

Although our comparative survey supports the prediction that sex-specific preadaptation explains sex biases in helping (Fig. 6), single-sex helping is not always empirically observed in taxa exhibiting a labile sex ratio. We suggest three possible reasons for this. First, helping may yield direct fitness benefits, incentivising individuals of both sexes to engage in nest defence, cooperative feeding, or (cryptic) parental care. Second, the cost of reproduction under local mating may be sufficiently low that there is a point at which further investment in helping by a particular sex is disfavoured, arresting the feedback between sex allocation and sex-biased helping. Third, if helping is manipulated and there is no significant sex bias in ability, mothers may not be selected to recruit helpers from one sex preferentially.

In our model we have taken sex-specific helping ability as given and not subject to evolution. However, helpers, once established, will be favoured to improve their helping ability. Accordingly, empirically-measured sex differences in helping ability may be partly a consequence, not a cause, of sex differences in willingness to help. Moreover, a constrained sex ratio not only promotes mixed-sex helping but, by inducing strong selection for improvement in the less-able sex, may also act to abolish sex differences in helping ability, further obscuring the direction of causality. However, it is likely that extant sex differences in ability reflect the direction, if not the magnitude, of ancestral sex differences that would have driven the initial establishment of sex-biased helping. In this vein, recent theoretical work has shown that voluntary helping can arise as an elaboration of manipulated helping when helping efficiency evolves over time (González-Forero 2014). In such a case, our model

would predict that contemporary sex biases in helping reflect ancestral biases, while contemporary investment in helping reflects current helping efficiency.

More generally, our analysis shows that haplodiploidy *per se* has little relevance to the evolution of sex-specific helping; and when it does impact the sex of helpers—*i.e.*, under sib-mating and/or promiscuity—it may have the opposite effect to that predicted by Hamilton, inhibiting helping by females and promoting helping by males. Rather, the main impact of haplodiploidy upon the evolution of eusociality appears to be (i) by its providing a ready means of sex-ratio adjustment (de la Filia *et al.* 2015), which magnifies both sex biases and investment in helping; and (ii) by its preadapting taxa for inbreeding ecologies—which may promote sib-rearing—by purging recessive alleles associated with inbreeding depression and by allowing a sex-ratio response to LMC.

Methods

1. Summary

Here, we present methodological details and some additional results. Section 2 gives the derivation of the kin-selection model that yields conditions 1–5 of the main text. Section 3 presents additional results stemming from this analysis, isolating the effect of local mate enhancement on sex allocation and helping, exploring the impact of promiscuous mating on the analysis, giving thresholds for the evolution of sociality, and presenting simplified versions of conditions 1–5. Section 4 relates methodology and notes relating to data collection and statistical analysis for the empirical survey.

In Section 5, we derive candidate convergence-stable points for helping and sex allocation using the conditions derived in Section 2, and we analyse the evolutionary dynamics of these traits in an infinite population. In Section 6, we present an individual-based model verifying our analytical results in a finite population subject to mutation and drift. In Section 7, we provide the R code used for our comparative analysis and give summary result tables for fixed effects.

2. Derivation of inclusive-fitness effects

Conditions 1–5 in the main text are derived using the method of Taylor & Frank (1996), which is a recipient-centred approach to kin selection (Hamilton 1964). In the following analysis, the recipient is a juvenile female or male, who: may or may not be born, according to their mother's sex-allocation phenotype; may or may not survive to reproduce, according to their helping phenotype and those of their siblings; and may or may not achieve reproductive success, according to competition for mates and breeding sites. The life cycle follows the model detailed above (see *Life cycle* section and Fig. 2).

We proceed according to the following programme. The condition for natural selection to favour an increase to a trait is $\frac{\mathrm{d}W}{\mathrm{d}g}|_{g=\bar{g}} > 0$, where: g is the genic value of a gene for that trait picked randomly from a juvenile recipient in the population; W is this individual's expected relative fitness; $\bar{g}$ is the population-average genic value for the trait; and recipients are weighted according to their reproductive value (Taylor & Frank 1996). The recipient's relative fitness W may be affected both by their own expression of the trait and by the expression of the same trait by their social partners. All individuals whose trait expression affects the fitness of the recipient (including the recipient themselves) are termed actors. Actor-recipient relatedness provides the link between changes to the recipient's genic value and changes to the actors' expression of the trait.

Note that throughout the *Methods*, we use lowercase letters for traits of individuals (*i.e.*, x, y, and z), uppercase letters for traits averaged over individuals within a patch (*i.e.*, X and Y), and lowercase letters with overbars for population-average traits (*i.e.*, $\bar{x}$, $\bar{y}$, and $\bar{z}$). However, in the main text and in all figures, references to the traits x, y, and z denote the population-average values of these traits; there, we have dropped the overbars to simplify presentation.

2.1 Neighbour-modulated fitness model

We begin by constructing expressions for the expected reproductive success of a focal female and of a focal male (expressions A1 and A2 below). These are functions of: (i) the mother's sex allocation strategy; (ii) the individual's expected investment in helping; (iii) the amount of help received from the individual's siblings; and (iv) the

probability of sib-mating.

For mathematical convenience in modelling the mother's sex allocation, we suppose that she produces a large number K of "female" eggs and an equal number of "male" eggs, but only hatches a fraction 1–z of the female eggs and a fraction z of the male eggs, such that she is left with K juvenile offspring overall, $(1–z)K$ females and zK males (*cf.* Taylor & Frank 1996). We make a similar simplifying assumption in modelling the benefit of helpers: we assume that in the absence of help, each juvenile has a probability $B < 1$ of surviving to reproductive age, but that this probability increases with the average investment in helping by the individual's siblings. These two assumptions allow us to proceed mathematically as though the total number of females and males produced on a patch were independent of sex allocation and helping, but the model can equally be interpreted in terms of the mother producing a variable proportion of female versus male offspring based on her sex ratio strategy, or of help increasing her brood size rather than improving the survival of her already-hatched offspring. We assume that any density regulation experienced by a dispersing mated female is uncorrelated with her genotype, from which it follows that the analysis is independent of any variation in population size between generations.

Following these assumptions, the expected fitness of a focal female offspring depends on three factors. First, her mother's sex allocation z determines the female's probability of hatching, 1–z. Second, the female's expected investment in help x determines her probability of reproducing, 1–x. Third, the amount of help the female receives from her siblings determines whether she survives to maturity. Hence, the expected fitness of a focal daughter is

$$w_{\mathrm{f}} = (1 - z)(1 - x)B \tag{A1}$$

where $B = B(h)$ is the survival on a patch with $h = (1–z)e_{\mathrm{f}}X + ze_{\mathrm{m}}Y$ help. Here: $e_{\mathrm{f}}, e_{\mathrm{m}} \in [0, 1]$ are the relative effectiveness of helping by females and males, respectively; X is the average of x over the focal female's sisters; and Y is the average of y over the focal female's brothers.

The expected fitness of a focal son, w_{m}, is similar, but must also be multiplied by

his expected share of mates, which varies depending on the probability s that he mates at home:

$$w_{\mathrm{m}} = z(1-y)B\left[s\frac{(1-z)(1-X)B}{sz(1-Y)B+(1-s)\bar{z}(1-\bar{y})\bar{B}}+(1-s)\frac{(1-\bar{z})(1-\bar{x})\bar{B}}{\bar{z}(1-\bar{y})\bar{B}}\right] \quad \text{(A2)}$$

where X is the average of x over the focal male's sisters; Y is the average of y over the focal male's brothers; and $\bar{x}$, $\bar{y}$, $\bar{z}$, and $\bar{B}$ are the expected values of X, Y, z, and B on a random patch. The term in square brackets is the focal son's expected share of mates: with probability 1–s he disperses before mating, and so his expected mating success is the population-average number of reproductive females per reproductive male; with probability s he mates locally, and so his expected mating success is the number of reproductive females on his native patch divided by the number of reproductive males on his native patch, whether they dispersed into the patch from elsewhere or were born there. Note that, because a large number of offspring are produced on each patch (K is large), we assume that X and Y in expressions A1 and A2 are equal.

The average female fitness $\bar{w}_{\mathrm{f}}$ and average male fitness $\bar{w}_{\mathrm{m}}$ may be obtained by substituting the average trait values $\bar{x}$, $\bar{y}$, $\bar{z}$, and $\bar{B}$ into expressions A1 and A2, which yields $\bar{w}_{\mathrm{f}} = \bar{w}_{\mathrm{m}} = (1-\bar{z})(1-\bar{x})\bar{B}$.

2.2 Kin-selection analysis

In a sex-structured population, natural selection favours an increase to a trait g when

$$c_{\mathrm{f}}\frac{\mathrm{d}W_{\mathrm{f}}}{\mathrm{d}g_{\mathrm{f}}}+c_{\mathrm{m}}\frac{\mathrm{d}W_{\mathrm{f}}}{\mathrm{d}g_{\mathrm{m}}}>0\,, \quad \text{(A3)}$$

where: c_{f} and c_{m} are the class reproductive value of females and of males, respectively (with $c_{\mathrm{f}} = c_{\mathrm{m}} = 1/2$ under diploidy and $c_{\mathrm{f}} = 2/3$, $c_{\mathrm{m}} = 1/3$ under haplodiploidy); $W_{\mathrm{f}} = w_{\mathrm{f}}/\bar{w}_{\mathrm{f}}$ and $W_{\mathrm{m}} = w_{\mathrm{m}}/\bar{w}_{\mathrm{m}}$ are the relative fitness of a focal female and of a focal male, respectively; g_{f} and g_{m} denote the genic value of a gene picked randomly from the locus for the trait g in a focal female or in a focal male, respectively (Taylor & Frank 1996); and the condition is evaluated in a population monomorphic for all traits (*i.e.*, for this analysis, at the point $x = X = \bar{x}$, $y = Y = \bar{y}$, $z = \bar{z}$).

Several classes of actor may affect the focal recipient's fitness through their expression of the trait g. (For example, a focal female recipient's fitness will be affected by the trait of "helping by females" through two actor classes: herself, because her own investment in helping x affects her reproductive potential; and her sisters, because their average investment in helping X affects the amount of help she receives and hence her survival to reproductive age.) So, in condition A3 above, $\frac{\mathrm{d}W_\mathrm{f}}{\mathrm{d}g_\mathrm{f}} = \sum_a \frac{\partial W_\mathrm{f}}{\partial g_a}\frac{\mathrm{d}g_a}{\mathrm{d}g_\mathrm{f}}$, where the summation is taken over all actor classes affecting the recipient's fitness through their expression of trait g; $\frac{\partial W_\mathrm{f}}{\partial g_a}$ is the effect of trait g expressed by actor class a on the focal female's relative fitness; and $\frac{\mathrm{d}g_a}{\mathrm{d}g_\mathrm{f}}$ is the regression of the actor's trait g_a on the genic value g_f of the focal female, which is proportional to the consanguinity (a measure of relatedness) $p_{a|\mathrm{f}}$ between actor and recipient. Similarly, $\frac{\mathrm{d}W_\mathrm{m}}{\mathrm{d}g_\mathrm{m}} = \sum_a \frac{\partial W_\mathrm{m}}{\partial g_a}\frac{\mathrm{d}g_a}{\mathrm{d}g_\mathrm{m}}$. All consanguinities are derived in section 2.3, below.

Evaluating condition A3 for sex allocation, z, gives that natural selection favours an increase in sex allocation (an increase in resources allocated to males) if

$$c_\mathrm{f}\frac{\partial W_\mathrm{f}}{\partial z}p_\mathrm{dau} + c_\mathrm{m}\frac{\partial W_\mathrm{m}}{\partial z}p_\mathrm{son} > 0\,, \tag{A4}$$

where $\frac{\partial W_\mathrm{f}}{\partial z} = -\frac{1}{1-\bar{z}} + \Delta h$ and $\frac{\partial W_\mathrm{m}}{\partial z} = \frac{1-s^2}{\bar{z}} - \frac{s}{1-\bar{z}} + \Delta h(1+s-s^2)$. The sex difference in helpfulness Δh is equal to $b_\mathrm{m}\bar{y} - b_\mathrm{f}\bar{x}$, where $b_\mathrm{f} = e_\mathrm{f}(\frac{\mathrm{d}B}{\mathrm{d}h}|_{h=\bar{h}})/\bar{B}$ and $b_\mathrm{m} = e_\mathrm{m}(\frac{\mathrm{d}B}{\mathrm{d}h}|_{h=\bar{h}})/\bar{B}$; thus b_f and b_m relate the marginal relative survival benefit of help from females and males, respectively. Making these substitutions in condition A4 and dividing both sides by N, the population size, recovers condition 1 of the main text. The division is done to facilitate interpreting condition 1 in terms of individual reproductive value, but has no effect on the results of the analysis.

Similarly, natural selection favours an increase in voluntary helping by females if

$$c_\mathrm{f}(\frac{\partial W_\mathrm{f}}{\partial x}p_{\mathrm{self}|\mathrm{f}} + \frac{\partial W_\mathrm{f}}{\partial X}p_{\mathrm{sis}|\mathrm{f}}) + c_\mathrm{m}\frac{\partial W_\mathrm{m}}{\partial X}p_{\mathrm{bro}|\mathrm{f}} > 0\,, \tag{A5}$$

and favours an increase in maternally-manipulated helping by females if

$$c_\mathrm{f}(\frac{\partial W_\mathrm{f}}{\partial x}p_\mathrm{dau} + \frac{\partial W_\mathrm{f}}{\partial X}p_\mathrm{dau}) + c_\mathrm{m}\frac{\partial W_\mathrm{m}}{\partial X}p_\mathrm{son} > 0\,, \tag{A6}$$

where: $\frac{\partial W_\mathrm{f}}{\partial x} = -\frac{1}{1-\bar{x}}$; $\frac{\partial W_\mathrm{f}}{\partial X} = b_\mathrm{f}(1-\bar{z})$; and $\frac{\partial W_\mathrm{m}}{\partial X} = -\frac{s}{1-\bar{x}} + b_\mathrm{f}(1-\bar{z})(1+s-s^2)$. Making these substitutions in conditions A5 and A6 and dividing both sides by $N(1-\bar{z})$ recovers conditions 2 and 4, respectively, of the main text. The division is done to facilitate interpreting conditions 2 and 4 in terms of individual reproductive value, but has no effect on the results of the analysis.

Finally, natural selection favours an increase in voluntary helping by males if

$$c_\mathrm{f}\frac{\partial W_\mathrm{f}}{\partial Y}p_{\mathrm{sis|m}} + c_\mathrm{m}\left(\frac{\partial W_\mathrm{m}}{\partial y}p_{\mathrm{self|m}} + \frac{\partial W_\mathrm{m}}{\partial Y}p_{\mathrm{bro|m}}\right) > 0\,, \tag{A7}$$

and favours an increase in maternally-manipulated helping by males if

$$c_\mathrm{f}\frac{\partial W_\mathrm{f}}{\partial Y}p_{\mathrm{dau}} + c_\mathrm{m}\left(\frac{\partial W_\mathrm{m}}{\partial y}p_{\mathrm{son}} + \frac{\partial W_\mathrm{m}}{\partial Y}p_{\mathrm{son}}\right) > 0\,, \tag{A8}$$

where: $\frac{\partial W_\mathrm{f}}{\partial Y} = b_\mathrm{m}\bar{z}$; $\frac{\partial W_\mathrm{m}}{\partial y} = -\frac{1}{1-\bar{y}}$; and $\frac{\partial W_\mathrm{m}}{\partial Y} = \frac{s^2}{1-\bar{y}} + b_\mathrm{m}\bar{z}(1+s-s^2)$. Making these substitutions in conditions A7 and A8 and dividing both sides by $N\bar{z}$ recovers conditions 3 and 5, respectively, of the main text. The division is done to facilitate interpreting conditions 3 and 5 in terms of individual reproductive value, but has no effect on the results of the analysis.

2.3 Relatedness calculations

The consanguinity of two individuals A and B is the probability that an allele drawn randomly with replacement from individual A is identical by descent to an allele drawn randomly with replacement from the corresponding locus of individual B, with individuals A and B permitted to be the same individual (Bulmer 1994). This probability increases with inbreeding, so consanguinities are typically given in terms of f, the inbreeding coefficient, which is also equal to the average consanguinity of mating partners (Table M1).

Under diploidy, the consanguinity of a full brother and sister is $\frac{1+3f}{4}$, and the consanguinity of a maternal half-brother and sister is $\frac{1+7f}{8}$. Therefore, the expected consanguinity of brothers and sisters is $p_{\mathrm{bro|f}} = p_{\mathrm{sis|m}} = m\frac{1+3f}{4} + (1-m)\frac{1+7f}{8} = \frac{1+7f+m(1-f)}{8}$, where $m \in [0,1]$ measures promiscuity, *i.e.* the probability that two

Notation	Relationship	Diploidy	Haplodiploidy
$p_{\text{self}\|\text{f}}$	Female to self	$\frac{1+f}{2}$	$\frac{1+f}{2}$
$p_{\text{fsis}\|\text{f}}$	Female to full sister	$\frac{1+3f}{4}$	$\frac{3+5f}{8}$
$p_{\text{fbro}\|\text{f}}$	Female to full brother	$\frac{1+3f}{4}$	$\frac{1+3f}{4}$
$p_{\text{hsis}\|\text{f}}$	Female to maternal half-sister	$\frac{1+7f}{8}$	$\frac{1+7f}{8}$
$p_{\text{hbro}\|\text{f}}$	Female to maternal half-brother	$\frac{1+7f}{8}$	$\frac{1+3f}{4}$
p_{dau}	Female to mother	$\frac{1+3f}{4}$	$\frac{1+3f}{4}$
$p_{\text{self}\|\text{m}}$	Male to self	$\frac{1+f}{2}$	1
$p_{\text{fsis}\|\text{m}}$	Male to full sister	$\frac{1+3f}{4}$	$\frac{1+3f}{4}$
$p_{\text{fbro}\|\text{m}}$	Male to full brother	$\frac{1+3f}{4}$	$\frac{1+f}{2}$
$p_{\text{hsis}\|\text{m}}$	Male to maternal half-sister	$\frac{1+7f}{8}$	$\frac{1+3f}{4}$
$p_{\text{hbro}\|\text{m}}$	Male to maternal half-brother	$\frac{1+7f}{8}$	$\frac{1+f}{2}$
p_{son}	Male to mother	$\frac{1+3f}{4}$	$\frac{1+f}{2}$

Table M1: Familial consanguinities in terms of f, the inbreeding coefficient, under diploidy and haplodiploidy.

siblings share the same father. Here, $m = 1$ represents strict lifetime monogamy, $m = 0$ represents extreme promiscuity, and intermediate values represent mating ecologies in between. Two random mating partners are brother and sister with probability s and are unrelated with probability $1 - s$, which makes the average consanguinity between mating partners under diploidy $f = sp_{\mathrm{bro|f}} = sp_{\mathrm{sis|m}} = s\frac{1+7f+m(1-f)}{8}$. This gives $f = \frac{s+ms}{8-7s+ms}$. Under haplodiploidy, males do not receive any genetic contribution from their "father", so the consanguinity of a full brother and sister is the same as the consanguinity of a maternal half-brother and half-sister, $\frac{1+3f}{4}$. Consequently, the average consanguinity between mating partners under haplodiploidy is $f = s\frac{1+3f}{4}$, which gives $f = \frac{s}{4-3s}$.

These values of f can be substituted into the consanguinities given in Table M1 to yield consanguinities in terms of s and m, with the average consanguinity of two random siblings being m times the consanguinity of full siblings plus $1 - m$ times the consanguinity of maternal half-siblings (Table M2).

2.4 Simplified conditions

In the main text, conditions 1–5 are presented in a form which favours conceptual clarity over concision. However, the conditions can be made mathematically simpler; we present simplified forms here.

Sex allocation Natural selection favours mothers to increase their investment into sons, $\bar{z}$, when (*cf.* condition 1)

$$\left(-\frac{1}{1-\bar{z}} + \Delta h\right) p_{\mathrm{dau}} c_{\mathrm{f}} + \left(-\frac{s}{1-\bar{z}} + \frac{1-s^2}{\bar{z}} + (1+s-s^2)\Delta h\right) p_{\mathrm{son}} c_{\mathrm{m}} > 0\,,$$

where $\Delta h = b_{\mathrm{m}}\bar{y} - b_{\mathrm{f}}\bar{x}$.

Voluntary helping Natural selection favours an increase in voluntary helping by females, $\bar{x}$, when (*cf.* condition 2)

$$\frac{-p_{\mathrm{self|f}} c_{\mathrm{f}} - s p_{\mathrm{bro|f}} c_{\mathrm{m}}}{1-\bar{x}} + b_{\mathrm{f}}(1-\bar{z})\left(p_{\mathrm{sis|f}} c_{\mathrm{f}} + (1+s-s^2) p_{\mathrm{bro|f}} c_{\mathrm{m}}\right) > 0\,.$$

Notation	Relationship	Diploidy	Haplodiploidy
$p_{\text{self}\|\text{f}}$	Female to self	$\frac{4-(3-m)s}{8-(7-m)s}$	$\frac{2-s}{4-3s}$
$p_{\text{sis}\|\text{f}}$	Female to sister	$\frac{1+m}{8-(7-m)s}$	$\frac{1+s+2m(1-s)}{8-(7-m)s}$
$p_{\text{bro}\|\text{f}}$	Female to brother	$\frac{1+m}{8-(7-m)s}$	$\frac{1}{4-3s}$
p_{dau}	Female to mother	$\frac{2-(1-m)s}{8-(7-m)s}$	$\frac{1}{4-3s}$
$p_{\text{self}\|\text{m}}$	Male to self	$\frac{4-(3-m)s}{8-(7-m)s}$	1
$p_{\text{sis}\|\text{m}}$	Male to sister	$\frac{1+m}{8-(7-m)s}$	$\frac{1}{4-3s}$
$p_{\text{bro}\|\text{m}}$	Male to brother	$\frac{1+m}{8-(7-m)s}$	$\frac{2-s}{4-3s}$
p_{son}	Male to mother	$\frac{2-(1-m)s}{8-(7-m)s}$	$\frac{2-s}{4-3s}$

Table M2: Familial consanguinities in terms of s, the probability of sib-mating, and m, the probability that two siblings share the same father, under diploidy and haplodiploidy.

Natural selection favours an increase in voluntary helping by males, $\bar{y}$, when (*cf.* condition 3)

$$\frac{(-p_{\mathrm{self|m}} + s^2 p_{\mathrm{bro|m}})c_{\mathrm{m}}}{1-\bar{y}} + b_m \bar{z} \left(p_{\mathrm{sis|m}} c_{\mathrm{f}} + (1 + s - s^2) p_{\mathrm{bro|m}} c_{\mathrm{m}}\right) > 0 \,.$$

Maternally-manipulated helping Natural selection favours an increase in manipulated helping by females, $\bar{x}$, when (*cf.* condition 4)

$$\frac{-p_{\mathrm{dau}} c_{\mathrm{f}} - s p_{\mathrm{son}} c_{\mathrm{m}}}{1-\bar{x}} + b_{\mathrm{f}} (1-\bar{z}) \left(p_{\mathrm{dau}} c_{\mathrm{f}} + (1 + s - s^2) p_{\mathrm{son}} c_{\mathrm{m}}\right) > 0 \,.$$

Natural selection favours an increase in manipulated helping by males, $\bar{y}$, when (*cf.* condition 5)

$$\frac{(-1 + s^2) p_{\mathrm{son}} c_{\mathrm{m}}}{1-\bar{y}} + b_m \bar{z} \left(p_{\mathrm{dau}} c_{\mathrm{f}} + (1 + s - s^2) p_{\mathrm{son}} c_{\mathrm{m}}\right) > 0 \,.$$

3. Additional results

3.1 Impact of local mate enhancement

In the main text, we identify the selection pressure of "local mate enhancement" (LME), which captures the knock-on effect of additional individuals raised by helpers on local mate competition. It is possible to quantify the impact of LME on sex allocation and helping by removing the LME effect from conditions 1–5, calculating convergence-stable solutions for $\bar{x}$, $\bar{y}$, and $\bar{z}$ in the absence of LME, and taking the difference between convergence-stable solutions with and without LME as the effect of LME *per se* on the evolution of sex allocation (Fig. M1) and of sex-biased helping (Fig. M2).

Overall, the LME effect increases the brood-level investment in helping for intermediate levels of sib-mating ($0 < s < 1$), because it both promotes helping by females and by males (see Fig. M2) and selects for an additional sex-allocation bias in favour of the more helpful sex (see Fig. M1).

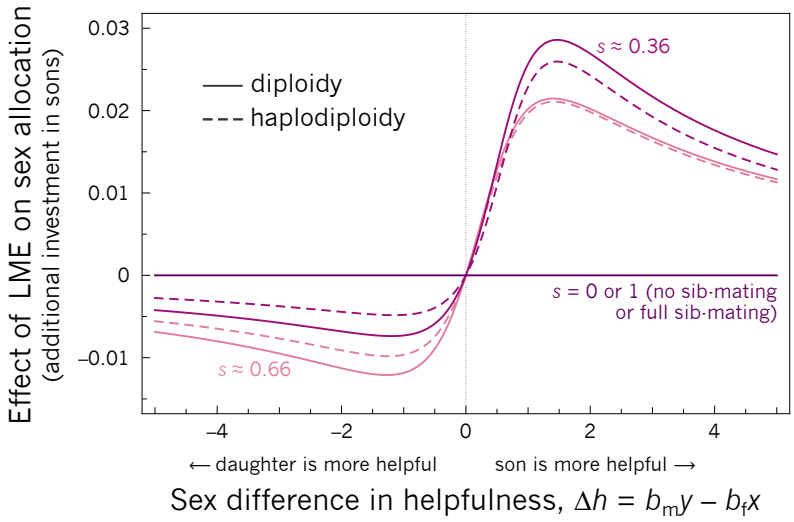


Figure M1: Change to stable sex allocation following from the "local mate enhancement" (LME) effect. In the context of intermediate sib-mating ($0 < s < 1$), the stable sex allocation tends to be biased more towards the more helpful sex, because some of the mother's sons are local competitors, which means they benefit from having more mates as a result of help. As s, the level of sib-mating, increases to 1, this effect is cancelled by the cost to those individuals of also having more competitors as a result of help. The particular curves shown here illustrate the values of s, under diploidy, for which the LME effect has its greatest male-biasing effect (increasing z by ~0.0286, for $s \approx 0.363$) and its greatest female-biasing effect (decreasing z by 0.0121, for $s \approx 0.656$).

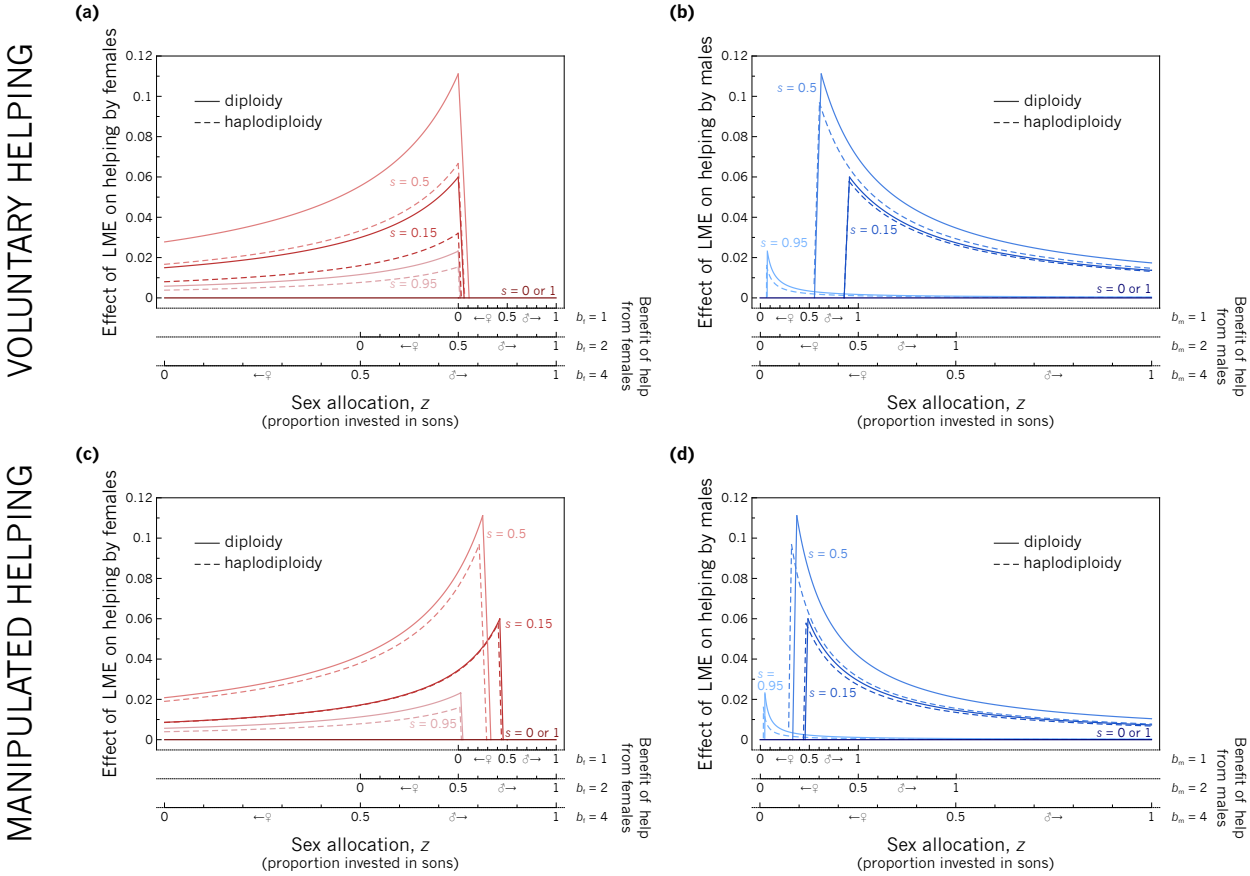


Figure M2: Change to stable voluntary helping following from the "local mate enhancement" (LME) effect. In the context of intermediate sib-mating ($0 < s < 1$), helping tends to be promoted because some of the helpers' brothers are local competitors, which means they benefit from having more mates as a result of help. As s, the level of sib-mating, increases to 1, this effect is cancelled by the cost to those individuals of also having more competitors as a result of help.

3.2 Effects of promiscuity

In this section, we consider the effects of promiscuity on the coevolution of sex allocation and sib-rearing. Because in our model, offspring live with their mother, only promiscuity by females affects relatedness between nestmates; therefore, we focus on the mating behaviour of females.

Importantly, the level of promiscuity (m) does not affect a mother's relative valuation of her daughters versus her sons, so it does not have an impact upon the convergence-stable sex ratio or maternally-manipulated helping, although promiscuity might affect the rate of evolution of these traits by decreasing the strength of selection. Promiscuity may, however, impact the stable level of *voluntary* helping by both females and males.

To explore the impact of promiscuity on voluntary helping by females, we can divide both sides of condition 2 in the main text by the "sacrifice effect" term, which allows us to evaluate how the magnitude of the three indirect effects of helping by females (the sib-rearing effect, the LMC effect, and the LME effect) changes with promiscuity, relative to the focal female's direct fitness cost of helping (Fig. M3a–c). Similarly, the impact of promiscuity on voluntary helping by males can be explored by dividing both sides of condition 3 in the main text by the "sacrifice effect" term within it (Fig. M3d–f).

Voluntary helping by females Under diploidy, a decrease in promiscuity increases the relatedness between a female and her siblings of both sexes. Accordingly, an increase in m yields the following inclusive-fitness effects for a focal female helper: (i) the relative benefit of rearing siblings increases, for $s \neq 1$ (Fig. M3a); (ii) the relative cost of abstaining from local mating increases, for $0 < s < 1$ (Fig. M3b); and (iii) the relative benefit of local mate enhancement increases, for $0 < s < 1$ (Fig. M3c). Overall, monogamy tends to increase a female's willingness to help: although increasing m can increase the indirect costs of helping (point ii, above), this is never sufficient to reduce a female's willingness to help because it increases the indirect benefits of helping more than enough to compensate (points i and iii, above).

Under haplodiploidy, a decrease in promiscuity increases the relatedness between

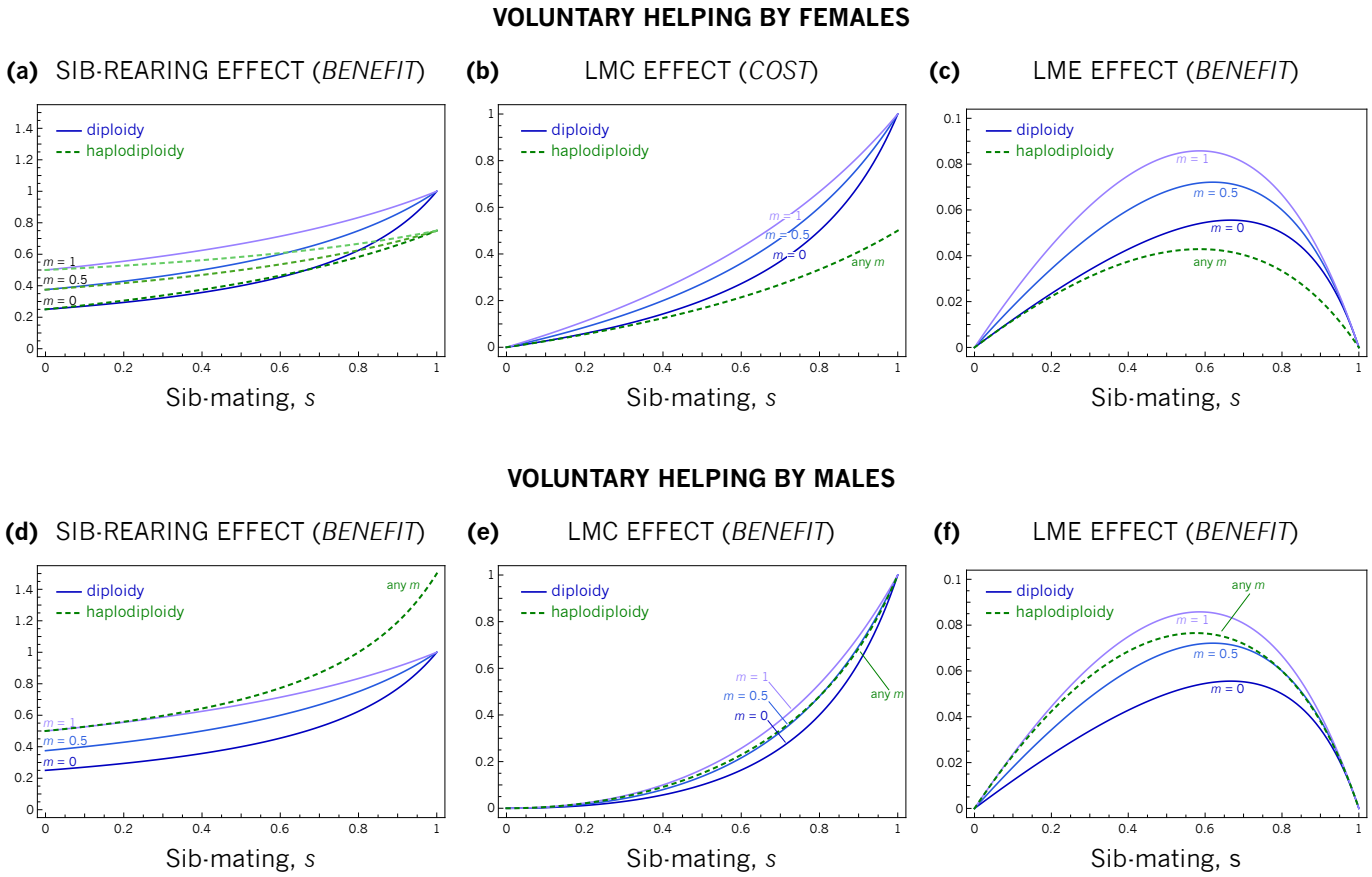


Figure M3: Impact of sib-mating, s, and monogamy, m, on the relative magnitude of the sib-rearing, LMC, and LME indirect inclusive-fitness effects on voluntary helping by **(a–c)** females, as given in condition 2 of the main text, and **(d–f)** males, as given in condition 3 of the main text. Vertical axes give the magnitude of each effect relative to the direct fitness cost of helping (the "sacrifice effect") paid by the focal individual. Blue solid lines illustrate magnitudes of effects under diploidy, while green dashed lines illustrate magnitudes of effects under haplodiploidy. Where the magnitude of a given effect varies with the level of monogamy (m), illustrative curves are shown with values of m labelled, while "any m" denotes that the curve is the same for any value of $m \in [0,1]$: this holds for panels **b–f** under haplodiploidy only. These plots assume $b_\mathrm{f} = b_\mathrm{m} = 1$, $x = y = 0$, and $z = 1/2$; for other values of these parameters, scale the vertical axis of panels **a** and **c** by $2b_\mathrm{f}(1-x)(1-z)$ and the vertical axis of panels **d** and **f** by $2b_\mathrm{m}yz$.

a female and her sisters, but does not affect the relatedness between a female and her brothers. Therefore, an increase in m increases the relative benefit of helping siblings when $s \neq 1$ (because the sib-rearing effect benefits siblings of both sexes, Fig. M3a), but does not affect the relative magnitude of either the LMC or the LME effects (because those effects target brothers only, Fig. M3b&c).

Voluntary helping by males Under diploidy, a decrease in promiscuity increases the relatedness between a male and his siblings of both sexes. Accordingly, an increase in m yields the following inclusive-fitness effects for a focal male helper: (i) the relative benefit of helping siblings increases, for $s \neq 1$ (Fig. M3d); (ii) the relative benefit of abstaining from local mating increases, for $0 < s < 1$ (Fig. M3e); and (iii) the relative benefit of local mate enhancement increases, for $0 < s < 1$ (Fig. M3f). Overall, monogamy promotes voluntary helping by males under diploidy when $s \neq 1$, because it increases the relative magnitude of the three indirect benefits associated with helping.

Under haplodiploidy, promiscuity has no effect on the relatedness between a focal male and his siblings of either sex. Therefore, monogamy does not affect the level of voluntary helping by males under haplodiploidy (Fig. M3d–f).

Overall, then, promiscuity tends to reduce the amount of voluntary helping, particularly in the context of outbreeding. However, under haplodiploidy, promiscuity does not reduce a male's willingness to help, so promiscuity could result in male-biased helping in haplodiploid taxa. The effect of promiscuity on which sex or sexes provide help is illustrated in Fig. M4 .

Under both diploidy and haplodiploidy, changes to promiscuity can affect who helps when the sex ratio is labile through another, more subtle mechanism. By reducing the relative importance of the LRE and LME effects (as compared to the rarer-sex and LMC effects) in determining a mother's sex allocation, an increase in promiscuity can alter the coevolutionary dynamics between $\bar{x}$, $\bar{y}$, and $\bar{z}$, leading to an alternative solution being reached from a given starting point. However, this effect does not typically have a decisive impact on which sex provides help.

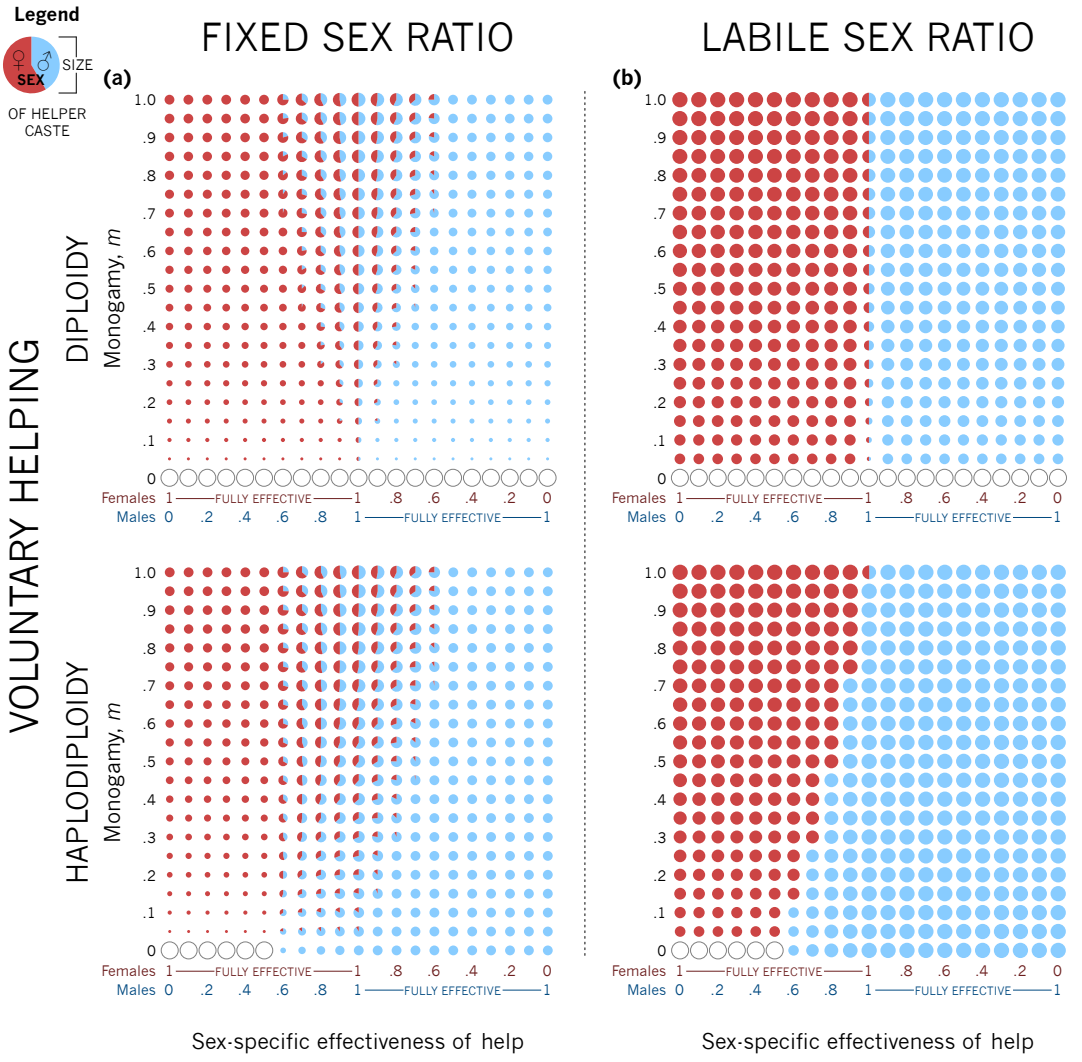


Figure M4: Patterns of voluntary helping as a function of sex differences in helping ability, monogamy, diploidy versus haplodiploidy, and fixed versus labile sex allocation. Each pie chart shows the equilibrium state following the evolution of helping, giving the percentage of helpers who are female (red) versus male (blue), as well as the size of the helper caste (diameter of pie chart), for a given set of parameters. Empty circles indicate parameter values for which helping did not evolve. These results assume $b_{\max} = 4$, as an illustrative example. Note that in the upper-right panel, the comparatively small helper caste when females and males are equally preadapted is a consequence of numerical integration settling on an unstable equilibrium at which both females and males help. Here, any small perturbation would result in single-sex helping at a greater level.

3.3 Thresholds for sociality

In Section 5, we derive analytical conditions (A15, A17, A19, A20, A22, and A24) which can be rearranged to give the minimum values of b_f and b_m required for at least some helping by either males or females to be potentially maintained by natural selection. In doing so, we can compare the propensity for social behaviour among different sexual ecologies. Our approach is to solve for the minimum value of b which satisfies the conditions referred to above for stable helping by either females or males, where we compare three regimes of sex-specific preadaptation, in which: (i) only females are preadapted for helping ($b_f = b$, $b_m = 0$); (ii) both females and males are preadapted for helping ($b_f = b_m = b$); or (iii) only males are preadapted for helping ($b_f = 0$, $b_m = b$). Lower threshold values of b then correspond to helping behaviour evolving more easily. Results are provided in Fig. M5 for voluntary helping and Fig. M6 for maternally-manipulated helping.

Voluntary helping When sex allocation is fixed at $\bar{z} = 1/2$, helping becomes easier with increasing s and with increasing m for both females and males under diploidy and for females under haplodiploidy; while helping becomes easier with increasing s, but does not depend on m, for males under haplodiploidy. We also see that when there is a high rate of sib-mating, maternal promiscuity becomes less of a barrier to the evolution of helping for females and males under diploidy and for females under haplodiploidy. When both sexes are equally preadapted, males have a higher propensity to start helping than females (when $s > 0$; Fig. M5).

When sex allocation is evolutionarily labile, helping becomes easier with increasing s and with increasing m for both females and males under diploidy and for females under haplodiploidy; while helping becomes easier with intermediate s (as s approaches 0.5) but does not depend on m for males under haplodiploidy. For a high rate of sib-mating, maternal promiscuity becomes less of a barrier to the evolution of helping especially for females under diploidy and haplodiploidy and somewhat for males under diploidy. Under diploidy, when both sexes are equally preadapted, males have a higher propensity to start helping than females (when $s > 0$). Under haplodiploidy, when both sexes are equally preadapted, males have a

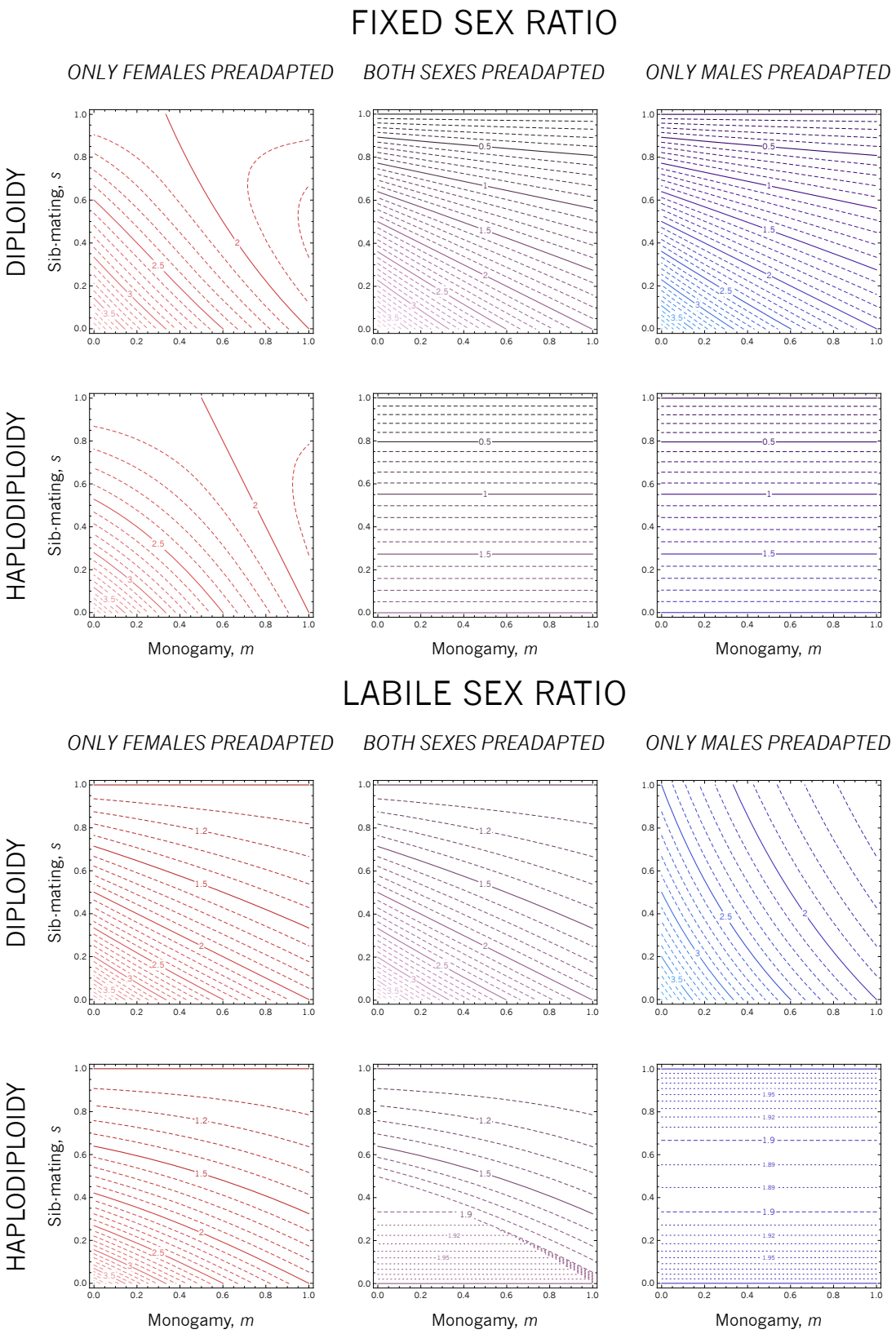


Figure M5: Thresholds for the evolution of voluntary helping as a function of promiscuity and degree of sib-mating. These give the minimum value of b for which at least some helping can stably persist, where $b_{\mathrm{f}} = b$, $b_{\mathrm{m}} = 0$ if only females are preadapted, $b_{\mathrm{f}} = b_{\mathrm{m}} = b$ if both sexes are preadapted, and $b_{\mathrm{f}} = 0$, $b_{\mathrm{m}} = b$ if only males are preadapted for helping.

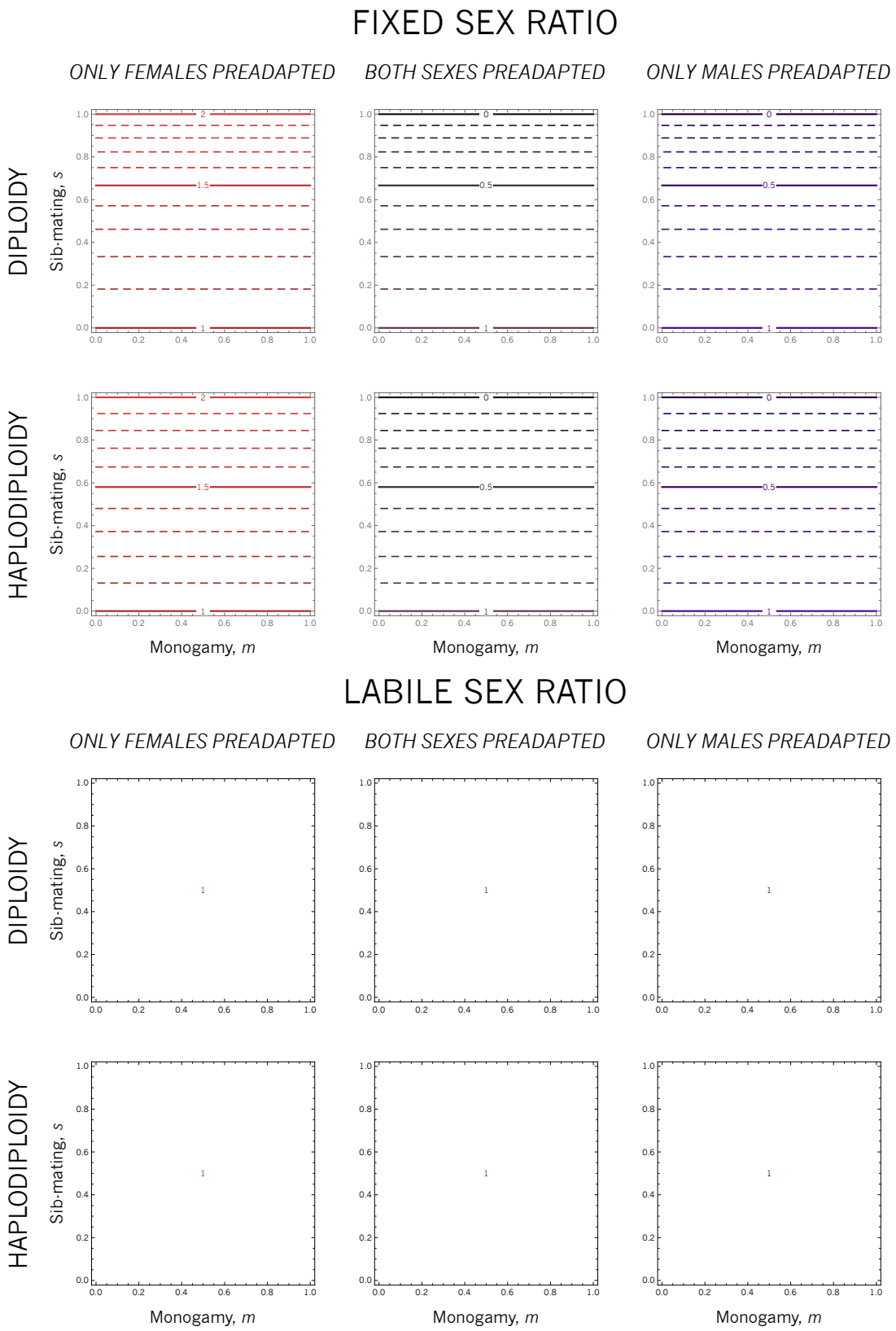


Figure M6: Thresholds for the evolution of maternally-manipulated helping as a function of promiscuity and degree of sib-mating. These give the minimum value of b for which at least some helping can stably persist, where $b_{\mathrm{f}} = b$, $b_{\mathrm{m}} = 0$ if only females are preadapted, $b_{\mathrm{f}} = b_{\mathrm{m}} = b$ if both sexes are preadapted, and $b_{\mathrm{f}} = 0$, $b_{\mathrm{m}} = b$ if only males are preadapted for helping.

higher propensity to start helping than females for low to intermediate m and low s, and females have a higher propensity to start helping otherwise (Fig. M5).

Maternally-manipulated helping In this case, maternal promiscuity has no effect on thresholds for helping by females or by males. For a fixed sex ratio, the threshold for helping by females increases from $b_{\mathrm{f}} = 1$ when $s = 0$ to $b_{\mathrm{f}} = 2$ when $s = 1$, for both diploidy and haplodiploidy. This is because in the context of sib-mating, females become increasingly valuable to the mother insofar as they are mates for her sons; therefore, she is less willing to recruit females as helpers. In contrast, the threshold for helping by males decreases from $b_{\mathrm{m}} = 1$ when $s = 0$ to $b_{\mathrm{m}} = 0$ when $s = 1$, for both diploidy and haplodiploidy. This is because insofar as there is sib-mating, sons compete with each other for mates, so recruiting sons as helpers relieves local mate competition. When females and males are equally preadapted, helping by males evolves more easily when $s > 0$ (Fig. M6). For a labile sex ratio, the threshold for helping is $b > 1$ regardless of the rate of sib-mating and maternal promiscuity (Fig. M6).

4. Notes on data collection and comparative analysis

4.1 Data used for empirical survey

Data used for the empirical survey are reproduced in Table M3; sequences used for the phylogenetic analysis are presented in Table M4.

4.2 General assumptions for data collection

Helper sex ratio Here we aim to capture both the absolute sex ratio of helpers as well as any difference between the sexes in tendency to help. The factor we analyse has five categories: 0 = female only, 0.25 = female-biased (helper sex ratio 0.05–0.45, or unbiased but with higher tendency to help by females), 0.5 = unbiased, 0.75 = male-biased (helper sex ratio 0.55–0.95, or unbiased but with higher tendency to help by males), 1 = male only.

order	family	genus	species	type[a]	ploidy[b]	helper sex ratio	preadaptation	sib-mating/LMC	biased colony sex ratio	labile sex ratio	references	in phylogeny[c]
Acari	Tetranychidae	*Stigmaeopsis*	*celarius*	PS	HD	0.25	0.25	yes	yes	yes	1, 2	yes
Araneae	Agelenidae	*Agelena*	*consociata*	PS	D	0	0	yes	yes	yes	3	no
Araneae	Agelenidae	*Agelena*	*republicana*	PS	D	0	0	yes	yes	yes	3	no
Araneae	Dictynidae	*Aebutina*	*binotata*	PS	D	0	0	yes	yes	yes	3	no
Araneae	Dictynidae	*Mallos*	*gregalis*	PS	D	0	0	yes	yes	yes	3	(g)
Araneae	Eresidae	*Stegodyphus*	*dumicola*	PS	D	0	0	yes	yes	yes	3	yes
Araneae	Eresidae	*Stegodyphus*	*mimosarum*	PS	D	0	0	yes	yes	yes	3	yes
Araneae	Eresidae	*Stegodyphus*	*sarasinorum*	PS	D	0	0	yes	yes	yes	3	no
Araneae	Oxyopidae	*Tapinillus*	sp.	PS	D	0	0	no	yes	yes	3	no
Araneae	Sparassidae	*Delena*	*cancerides*	PS	D	0	0	no	yes	yes	3	yes
Araneae	Theredíidae	*Achaeranenea*	*wau*	PS	D	0	0	yes	yes	yes	3	no
Araneae	Theridiidae	*Anelosimus*	*eximius*	eusocial	D	0	0	yes	yes	yes	3	yes
Araneae	Theridiidae	*Theridion*	*nigroannulatum*	PS	D	0	0	yes	yes	yes	3	yes
Araneae	Thomisidae	*Diaea*	sp.	PS	D	0.25	0.5	no	yes	yes	3, 4	(g)
Coleoptera	Curculionidae	*Austroplatypus*	*incompertus*	eusocial	D	0	0	no	yes	no	5, 6, 7	yes
Coleoptera	Curculionidae	*Xyleborinus*	*saxeseni*	PS	HD	0	0	yes	yes	yes	8	yes
Decapoda	Alpheidae	*Synalpheus*	*chacei*	eusocial	D	0.25	0.25	no	no	no	9, 10, 11, 12	yes
Decapoda	Alpheidae	*Synalpheus*	*filidigitus*	eusocial	D	0.25	0.25	no	no	no	9, 10, 11, 12	yes
Decapoda	Alpheidae	*Synalpheus*	*regalis*	eusocial	D	0.25	0.25	no	no	no	9, 10, 11, 12	yes
Embiidina	Australembiidae	*Australembia*	*nodosa*	PS	D	1	0.75	yes	yes	yes	13	(g)
Hymenoptera	Apidae	*Allodape*	*punctata*	eusocial	HD	0	0	no	yes	yes	14	yes
Hymenoptera	Halictidae	*Augochlorella*	*striata*	eusocial	HD	0	0	no	yes	yes	14	yes
Hymenoptera	Halictidae	*Halictus*	*marginatus*	eusocial	HD	0	0	no	yes	yes	14	(g)
Hymenoptera	Halictidae	*Lasioglossum*	*rowheri*	eusocial	HD	0	0	no	yes	yes	14	(g)
Hymenoptera	Vespidae	*Liostenogaster*	*flavolineata*	eusocial	HD	0	0	no	yes	yes	14	yes
Hymenoptera	Vespidae	*Ropalidia*	*marginata*	eusocial	HD	0.25	0	no	yes	yes	15	no
Hymenoptera	Vespidae	*Stenogastrinae*	sp.	PS	HD	0	0	no	yes	yes	14, 16	no
Hymenoptera	Vespidae	*Polistinae*	sp.	PS	HD	0	0	no	yes	yes	14	no
Hymenoptera	Apidae	*Eulaema*	*nigrita*	PS	HD	0	0	no	yes	yes	14	yes
Hymenoptera	Formicidae	*Pristomyrmex*	*pungens*	PS	HD	0	0	no	yes	yes	14	yes
Blattodea	Kalotermitidae	*Cryptotermes*	*domesticus*	eusocial	D	0.5	0.5	no	no	no	17, 18	yes
Blattodea	Kalotermitidae	*Cryptotermes*	*secundus*	eusocial	D	0.75	0.5	no	no	no	17, 18	yes
Blattodea	Kalotermitidae	*Neotermes*	*connexus*	eusocial	D	0.75	0.5	no	no	no	18	no
Blattodea	Rhinotermitidae	*Prorhinotermes*	*inopinatus*	eusocial	D	0.5	0.5	no	no	no	18	(g)
Blattodea	Rhinotermitidae	*Termitogeton*	*planus*	eusocial	D	0.25	0.5	no	no	no	18	yes
Blattodea	Termopsidae	*Hodotermopsis*	*sjostedti*	eusocial	D	0.5	0.5	no	no	no	18	yes
Pseudoscorpiones	Atemnidae	*Paratemnoides*	*nidificator*	PS	D	0.5	0.5	yes	no	yes	13, 19	(g)
Thysanoptera	Phlaeothripidae	*Oncothrips*	*habrus*	eusocial	HD	0.25	0.5	yes	yes	yes	20, 21	yes
Thysanoptera	Phlaeothripidae	*Oncothrips*	*waterhousei*	eusocial	HD	0.25	0.5	yes	yes	yes	20, 21	yes
Thysanoptera	Phlaeothripidae	*Kladothrips*	*hamiltoni*	eusocial	HD	0.5	0.5	yes	yes	yes	20, 21, 22	yes

Table M3: Data used in comparative survey. See section 4.2 for details on data collection and coding of categories. [a] PS = primitively social. [b] D = diploidy, HD = haplodiploidy. [c] (g) = genus match. References: 1, Mori & Saito 2005; 2, Saito *et al.* 2004; 3, Avilés 1997; 4, Evans 2000; 5, Smith *et al.* 2009; 6, Kent & Simpson 1992; 7, Kent 2001; 8, Biedermann & Taborsky 2011; 9, Duffy 1996; 10, Duffy *et al.* 2000; 11, Chak *et al.* 2015a; 12, Chak *et al.* 2015b; 13, Costa 2006; 14, Ross *et al.* 2013; 15, Sen & Gadagkar 2006; 16, Strassman *et al.* 1994; 17, Muller & Korb 2008; 18, Bourguignon & Miura 2012; 19, Zeh & Zeh 1990; 20, Chapman *et al.* 2002; 21, Crespi & Mound 1997; 22, Kranz *et al.* 2001.

taxon	Ef1a	16S	Cytb	COI	COII	H3	28S	18S
Allodape punctata			JN564695.1	JN564689.1				
Anelosimus eximius		AY230956.1		JX977216.1		AY230991.1	AY231089.1	EF050191.1
Augochlorella striata				DQ645731.1				
Australembia rileyi				EU157063.1		EU157029.1	AY521771.1	AY521847.1
Austroplatypus incompertus				FJ867832.1				
Cryptotermes domesticus			AF189115.1		AF189087.1			
Cryptotermes secundus			AF189120.1	EU253840.1	AF189093.1		DQ441901.1	DQ882635.1
Delena cancerides		KF372686.1		KF442799.1		KF442871.1	KF372787.1	
Diaea subdola		EU168118.1		EU168174.1		AX281976.1		JN816823.1
Eulaema nigrita			AY916095.1	AJ581116.1				
Halictus rubicundus				AF438481.1			AY654510.1	AY995651.1
Hodotermes sjostedti			EU253928.1	JN615371.1	EU253893.1		DQ441931.1	EU253796.1
Kladothrips hamiltoni	AF386706.1	AF386659.1		AF386683.1				
Lasioglossum leucozonium				JX831587.1				
Liostenogaster flavolineata		AF066939.1		GU596839.1			GU596730.1	EF190726.1
Mallos pallidus				FJ949008.1				FJ948884.1
Oncothrips habrus	AF386716.1	DQ246465.1		AY920992.1				
Oncothrips waterhousei	AF386720.1	AF386673.1		AF386696.1				
Paratemnoides elongatus				U70217.1				
Pristomyrmex pungens	EU204444.1		EU602313.1				AB126783.1	AB126782.1
Prorhinotermes canalifrons			EU253925.1	EU253849.1	EU253888.1		EU253672.1	EU253791.1
Stegodyphus mimosarum				DQ973161.1		FJ949054.1		FJ948893.1
Stigmaeopsis celarius				AB531823.1				
Stigmaeopsis takahashii				AB531836.1				
Synalpheus chacei				AF230792.1				KJ595265.1
Synalpheus filidigitus				HQ435386.1				KJ595275.1
Synalpheus regalis				AF230794.1				
Termitogeton planus					AB109539.1	EF050369.1	DQ442039.1	
Theridion nigroannulatum				EF050324.1				EF458147.1
Xyleborinus saxeseni	AF259876.1			KC845519.1			HM099727.1	AJ850038.1

Table M4: Sequences used for the phylogenetic analysis. Codes are Genbank accession numbers. In those cases where there were no sequence data available for a given species in the database, we randomly selected a species from the same genus and substituted this in the phylogeny.

Ability to bias the sex ratio In order to assess the ability of mothers to bias the sex ratio of their brood, we assume that haplodiploid taxa have the ability by default. For diploid taxa, we assess either direct experimental evidence for this ability, or indirect evidence in the form of either significantly-biased colony/reproductive sex ratios or the presence of local mate competition. Although our mathematical analysis focuses on freely-adjustable versus fixed sex allocation, many species may fall somewhere in between (*e.g.*, sex ratio adjustment may be possible, but relatively costly). However, data on the relative strength of any constraints on sex ratio adjustment are not generally available, so we simply code a species' sex allocation as either constrained or labile.

Sex-specific preadaptation for helping Direct estimates of a sex difference in ability to help are very difficult to obtain. We therefore obtained an indirect estimate based on a number of factors. First of all, we assumed that if brood care is an important component of help, then the sex that ancestrally preformed parental care in the non-social ancestor is better equipped to do so (see Ross *et al.* 2013). In those cases where help involves defence, we assumed that the larger sex, or the sex with a morphological defence adaptation (*e.g.* Decapoda, Embiidina and Pseudoscorpiones, see below) is better equipped. As with helper sex ratio, we coded preadaptation for helping using five categories: 0 = only females preadapted, 0.25 = females better-preadapted, 0.5 = both sexes equally preadapted, 0.75 = males better-preadapted, 1 = only males preadapted.

4.3 Data collection notes for each taxonomic group

Acari Primitive sociality has been described for seven species across two genera. Helper tasks include defence and nest building; however, although the former is shared by both sexes, the latter is performed exclusively by females (Mori & Saito 2005; Saito *et al.* 2004; Mori & Saito 2006). This sex difference in helping might be because of the strong size dimorphism, where females on average are 50% larger than males (Saito *et al.* 2004). LMC has been experimentally confirmed for two species and is assumed for the other species based on their population structure and biased sex ratios.

Araneae Advanced sociality is common among spiders and has evolved repeatedly. Generally only females help. With a single exception, colony and disperser sex ratios are extremely female-biased. Evidence for LMC comes from population structure and mating behaviour (females mate within natal colonies) for all but one species where allozyme data suggests that within-colony relatedness is consistent with outbreeding (Evans & Goodisman 2002). Finally, there is empirical evidence that *Anelosimus domingo* females are able to bias the sex ratio of their broods (Aviles *et al.* 2006).

Coleoptera True (facultative) eusociality is found in a single species of ambrosia beetle, while primitive sociality is more widespread. Sociality in beetles is found in both haplodiploid and diploid species. For those species where the helper sex ratio is known, only females appear to help, with tasks including brood care and nest building/maintenance. Sociality appears to have evolved from maternal care, which might suggest that females are better equipped to help, especially with brood care (Ross *et al.* 2013). *Xyleborinus* ambrosia beetles are strongly inbred and matings take place within the natal nest. *Austroplatypus incompertus*, on the other hand, appears to be outbred (Kent 2001). *A. incompertus* disperser sex ratios are 1:1, but colony sex ratios overall appear to be slightly female-biased (Kent 2001). This could suggest that mothers might have some ability to bias the sex ratio of their offspring, but the evidence is currently not strong enough to classify them as having labile sex ratios. We were only able to include data on *A. incompertus* and two species of *Xyleborinus*, as data on helper sex were not available for *Trachyostus* or Passalidae beetles (Costa 2006).

Decapoda We were able to include data on three eusocial species of snapping shrimp from the genus *Synalpheus*. In all species, the colony sex ratio, as well as the sex ratio of helpers, is even. However, the gonadal development of females, but not males, is suppressed by the presence of the queen, suggesting female-biased investment in helping. In addition, females tend to have larger competitive weapons compared to males and might therefore be better equipped to help with nest defence (Chak 2015a, b).

Embiidina Primitive sociality or possibly even true eusociality has been found in a single species of webspinner. Although this species is poorly studied, it seems that they occur as single family groups, founded by a single female and her (adult) offspring. Intriguingly, the male offspring are dimorphic, with some displaying enlarged mouthparts, suggesting a male-limited soldier morph. LMC is assumed based on the fact that females appear to mate within the nest and that colony sex ratios are female-biased (Costa 2006). There is no data on the ploidy of this species, yet all other webspinners studied to date are diploid, with male heterogametic genetic sex determination (Beukeboom & Perrin 2014).

Hymenoptera Eusociality has evolved nine times among the Hymenoptera (Hughes *et al.* 2008). In addition, there are a number of origins of primitive sociality within the order. There is little variability in the traits of interest within the order. With a single exception (see table M3), only females help in hymenopteran societies. All hymenopterans are haplodiploid (Beukeboom & Perrin 2014). Sib-mating is rare among social Hymenoptera, possibly because their mechanism of sex determination causes strong inbreeding depression (Beukeboom & Perrin 2014). Due to the lack of variability and the large number of social species, we chose to include a single species per origin. We excluded obligate eusocial species as here the sex ratio is often under worker control, and as such is not covered by our model.

Blattodea Eusociality has evolved once and is present in all members of the Isoptera, yet there is a lot of variability in the sex of helpers. Termites can roughly be divided into two groups: (i) those with a single irreversibly sterile caste (soldiers only) and (ii) those with both soldiers and sterile workers. It is generally assumed that soldier-only colonies represent the ancestral state (Korb *et al.* 2012). Both soldier and working castes can vary from unbiased to single-sex, either male-only or female-only (Muller & Korb 2008; Bourguignon & Miura 2012). A major complication is that often this leads to sex-specific task specialization, a type of behaviour not covered by our model. In addition there is limited information on the relative numbers of soldiers versus workers, making it difficult to get an accurate estimate of overall helper sex ratio. We have there-

fore decided to restrict our analysis to soldier-only colonies. However, even in soldier-only colonies with a single soldier sex, helping is not strictly sex-limited, as immature non-soldiers can delay their maturation in order to help. We therefore score colonies with sex-biased or sex-limited soldier castes as having a partly biased helper sex ratio. Termites are generally outbred, and the reproductive castes tend to have unbiased sex ratios. There are a few interesting exceptions, though, such as some members of the *Reticulitermes*, where parthenogenetically-produced replacement queens mate with their brothers (Matsuura 2009). These systems might prove an excellent empirical system to test the predictions of our model, as inbreeding varies over time within a single colony. However, there is insufficient data to include them in the current analysis.

Pseudoscorpiones Primitive sociality is present in two related species of pseudoscorpion. Both sexes engage in helping behaviour, including cooperative prey capture and food sharing, cooperative nest building, and cooperative parental care. The sexes have similar body size and ability to produce poison, suggesting no sex bias in their ability to help (Zeh & Zeh 1990; Tizo Pedroso & Del Claro 2011). There is task specialization based on both sex and age, though, with males and females specializing on different tasks according to their abilities (Tizo Pedroso & Del Claro 2011). LMC is assumed based on mating behaviour (mating within natal nest) and slightly biased colony sex ratios (Zeh & Zeh 1990). The latter also suggests that mothers might be able to bias the sex ratio of their brood.

Thysanoptera Both facultative eusociality and primitive sociality are found among Australian gall thrips. The eusocial species have a distinct soldier caste that is responsible for colony defence, and although not completely sterile, has a reduced reproductive output compared to the founding female (Chapman *et al.* 2002). The sex ratio of the soldier caste varies from even to female-biased depending on the species (Crespi & Mound 1997; Kranz *et al.* 2001; Chapman *et al.* 2002). In those species with biased helper sex ratios there is also some evidence for behavioural differences in the tendency to help, with females appearing more eager to help (Crespi & Mound 1997).

Evidence for LMC comes from mating behaviour, population genetics analyses and biased disperser sex ratios (Chapman *et al.* 2000). There is little sexual dimorphism and both sexes can use their enlarged forelimbs for defence and to secrete a substance to control fungal pathogens (Crespi & Mound 1997).

4.4 Comparative analysis

We analysed the data on helper sex ratio using taxonomic and phylogenetic mixed models (Hadfield & Nakagawa 2010) with the R packages MCMCglmm (for the taxonomic models; Hadfield 2010) and MCMCglmmRAM (for the phylogenetic models; Hadfield 2015). We corrected for phylogenetic non-independence between origins by using either nested taxonomic levels (Order / Family / Genus) or the reconstructed molecular phylogeny. We used threshold models with helper sex ratio (0 = female only, 0.25 = female-biased, 0.5 = unbiased, 0.75 = male-biased, 1 = male only) or type of bias (0 = minimal bias, 0.5 = partial bias, 1 = complete bias) as ordinal response variables. As predictors for helper sex ratio we included sex-specific preadaptation, ploidy (haplodiploidy vs. diploidy) and LMC (present or absent), while as predictors for type of bias we included sex-specific preadaptation and evolutionary lability of the sex ratio (0 = constrained, 1 = labile).

We used a weakly informative Gelman prior (Gelman *et al.* 2008, Hadfield *et al.* 2013) for the fixed effects and an inverse Wishart prior for random effects, and fixed the residual variance to 1. Due to the small size of our phylogeny, there is very little information to estimate the phylogenetic heritability of helper sex. We therefore present results from two different analyses, for which we either: (i) assume a phylogenetic heritability $\lambda = 1$, as suggested by Felsenstein (2005), and use a reduced animal model approach (Hadfield 2015; models m1a and m2a, Section 7); or (ii) estimate λ from the tree using a χ^2 prior, as suggested by de Villemereuil (2012; models m1b and m2b, Section 7). This prior prohibits very large values of $\lambda > 0.9$, thereby preventing numerical overflow. All models were run for 1.3 million iterations with a burn-in of 300,000 iterations. We report the significance of our fixed effects in terms of p_{MCMC}, which is twice the posterior probability that the estimate is negative or positive (whichever probability is smallest). This value can be interpreted as a Bayesian equivalent to the

traditional p-value (Hadfield & Nakagawa 2010, Hadfield *et al.* 2013).

5. Stable states and evolutionary dynamics

By analysing conditions 1–5 from the main text, we can identify locally-stable solutions for the traits $\bar{x}$, $\bar{y}$, and $\bar{z}$—that is, values of each that will resist invasion by mutants of small effect. This is partly captured by the concept of "convergence stability"; however, classic mathematical treatments of convergence stability (Christiansen 1991; Taylor 1996) are strictly only appropriate for trait values not lying on the boundaries of trait space. Therefore, we expand upon classic definitions of convergence stability to handle trait boundaries. We consider a trait to have attained a convergence-stable point (CSP) when: (i) natural selection favours neither a small increase nor a small decrease in the trait, insofar as an increase or decrease in the trait is possible; and (ii) small perturbations to the trait lead natural selection to favour returning the trait to its original value. To illustrate these conditions, consider a trait g with a population-average value of $\bar{g}$ and a potential CSP g^*, and suppose that the condition for natural selection to favour an increase in $\bar{g}$ is $D(\bar{g}) > 0$. Furthermore, suppose that the trait g is bounded by α and ω, such that $\alpha \leq g \leq \omega$. If $\alpha < g^* < \omega$, condition i is met when $D(\bar{g})|_{\bar{g}=g^*} = 0$, and condition ii is met when $\frac{\partial D(\bar{g})}{\partial \bar{g}}|_{\bar{g}=g^*} < 0$. If $g^* = \alpha$, conditions i and ii are met when $D(\bar{g})|_{\bar{g}=g^*} < 0$. And if $g^* = \omega$, conditions i and ii are met when $D(\bar{g})|_{\bar{g}=g^*} > 0$.

CSPs for $\bar{x}$, $\bar{y}$, and $\bar{z}$ vary depending on whether helping is voluntary or maternally-manipulated and on ploidy. Below, we begin by giving CSPs for sex allocation given a fixed investment in helping by females and males, and then give CSPs for helping given a fixed sex allocation. Then, we give candidate CSPs for both sex allocation and helping simultaneously. Finally, we analyse the evolutionary dynamics which may allow a candidate CSP to be reached.

5.1 Solutions for sex allocation, given helping

By analysis of condition 1 in the main text, we find that the CSP for sex allocation is

$$\begin{aligned} z^* &= \frac{-2C}{B+\sqrt{B^2-4AC}}, \\ \text{with} \quad & A = b_{\mathrm{m}}\bar{y} - b_{\mathrm{f}}\bar{x} \\ & B = 1 + b_{\mathrm{f}}\bar{x} - b_{\mathrm{m}}\bar{y} \\ & C = \frac{-(1-s^2)c_{\mathrm{m}}p_{\mathrm{son}}}{c_{\mathrm{f}}p_{\mathrm{dau}} + (1+s-s^2)c_{\mathrm{m}}p_{\mathrm{son}}}. \end{aligned} \tag{A9}$$

Under diploidy, C in A13 reduces to

$$C = -\frac{1-s}{2-s}, \tag{A9a}$$

while under haplodiploidy,

$$C = -\frac{(2-s)(1-s^2)}{4+s-3s^2+s^3}. \tag{A9b}$$

If $\bar{x}$ and $\bar{y}$ take fixed values, these can be substituted into A9 to find the corresponding convergence-stable sex allocation.

5.2 Solutions for helping, given sex allocation

Voluntary helping By analysis of condition 2 in the main text, we find that the convergence-stable level of voluntary helping by females is

$$x^* = \begin{cases} 0 & \text{if } F^* < 0 \\ F^* & \text{otherwise} \end{cases},$$

where

$$F^* = 1 - \frac{c_{\mathrm{f}}p_{\mathrm{self|f}} + sc_{\mathrm{m}}p_{\mathrm{sis|m}}}{b_{\mathrm{f}}\left(c_{\mathrm{f}}p_{\mathrm{sis|f}} + (1+s-s^2)c_{\mathrm{m}}p_{\mathrm{sis|m}}\right)(1-\bar{z})}. \tag{A10}$$

Under diploidy, F^* reduces to

$$F^* = 1 - \frac{2\,(2+(1-m)s)}{b_{\mathrm{f}}(1+m)(2-s)(1+s)(1-\bar{z})}, \tag{A10a}$$

while under haplodiploidy,

$$F^* = 1 - \frac{4 - s}{b_f \left(2 + 2m(1 - s) + (2 - s)s\right)\left(1 - \bar{z}\right)}. \tag{A10b}$$

These solutions are illustrated in Fig. 3a of the main text.

By analysis of condition 3 in the main text, we find that the convergence-stable level of voluntary helping by males is

$$y^* = \begin{cases} 0 & \text{if } M^* < 0 \\ M^* & \text{otherwise} \end{cases},$$

where

$$M^* = 1 - \frac{c_m p_{\text{self}|m} - s^2 c_m p_{\text{bro}|m}}{b_m \left(c_f p_{\text{bro}|f} + (1 + s - s^2) c_m p_{\text{bro}|m}\right) \bar{z}}. \tag{A11}$$

Under diploidy, M^* reduces to

$$M^* = 1 - \frac{(1 - s)(4 + s + ms)}{b_m (1 + m)(2 - s)(1 + s)\bar{z}}, \tag{A11a}$$

while under haplodiploidy,

$$M^* = 1 - \frac{(1 - s)\left(4 + (1 - s)s\right)}{b_m \left(4 + s - 3s^2 + s^3\right)\bar{z}}. \tag{A11b}$$

These solutions are illustrated in Fig. 3b of the main text.

Maternally-manipulated helping By analysis of condition 4 in the main text, we find that the convergence-stable level of maternally-manipulated helping by females is

$$x^* = \begin{cases} 0 & \text{if } F^* < 0 \\ F^* & \text{otherwise} \end{cases},$$

where

$$F^* = 1 - \frac{c_f p_{\text{dau}} + s c_m p_{\text{son}}}{b_f \left(c_f p_{\text{dau}} + (1 + s - s^2) c_m p_{\text{son}}\right)\left(1 - \bar{z}\right)}. \tag{A12}$$

Under diploidy, F^* reduces to

$$F^* = 1 - \frac{1}{b_f(2-s)(1-\bar{z})}, \tag{A12a}$$

while under haplodiploidy,

$$F^* = 1 - \frac{2+(2-s)s}{b_f\left(4+s-3s^2+s^3\right)(1-\bar{z})}. \tag{A12b}$$

These solutions are illustrated in Fig. 3c of the main text.

By analysis of condition 5 in the main text, we find that the convergence-stable level of maternally-manipulated helping by males is

$$y^* = \begin{cases} 0 & \text{if } M^* < 0 \\ M^* & \text{otherwise} \end{cases},$$

where

$$M^* = 1 - \frac{(1-s^2)c_m p_{son}}{b_m\left(c_f p_{dau} + (1+s-s^2)c_m p_{son}\right)\bar{z}}. \tag{A13}$$

Under diploidy, M^* reduces to

$$M^* = 1 - \frac{1-s}{b_m(2-s)\bar{z}}, \tag{A13a}$$

while under haplodiploidy,

$$M^* = 1 - \frac{(2-s)(1-s)(1+s)}{b_m\left(4+s-3s^2+s^3\right)\bar{z}}. \tag{A13b}$$

These solutions are illustrated in Fig. 3d of the main text.

When sex allocation is fixed to take a particular value, that value can be substituted into equations A10–A13 above to determine the convergence-stable level of female and male helping under a particular sex allocation with voluntary helping (A10 & A11) or maternally-manipulated helping (A12 & A13).

5.3 Simultaneous solutions for sex allocation and helping

In sections 3.1 and 3.2 above, we saw that the CSP for $\bar{z}$ depends on $\bar{x}$ and $\bar{y}$, while the CSPs for $\bar{x}$ and $\bar{y}$ depend on $\bar{z}$. If both helping and sex allocation are evolutionarily labile, we can identify candidate multidimensional CSPs by solving for all three one-dimensional CSPs simultaneously. These candidate CSPs will not necessarily remain stable to perturbation of more than one trait simultaneously (Leimar 2009); hence, we use numerical integration, or stochastic individual-based simulations, to verify both the stability and reachability of these states.

We find that there are three possible simultaneous candidate CSPs for $\bar{x}$, $\bar{y}$, and $\bar{z}$. One corresponds to no helping by females or males; one corresponds to helping by females only; and one corresponds to helping by males only. Focusing on sex allocation, we denote these solutions by z_0^*, z_f^*, and z_m^*, respectively; these values can then be substituted into equations A10–A13 above to find the corresponding candidate CSPs for helping.

We give z_0^*, z_f^*, and z_m^* below; the form of each solution and the conditions under which each is a valid candidate CSP depend on whether helping is voluntary or maternally-manipulated and on ploidy.

Voluntary helping When helping is voluntary, the three possible candidate CSPs for sex allocation are as follows.

Corresponding to no helping, the first solution is

$$z_0^* = \frac{(1-s^2)c_\mathrm{m}p_\mathrm{son}}{c_\mathrm{f}p_\mathrm{dau} + (1+s-s^2)c_\mathrm{m}p_\mathrm{son}}, \tag{A14}$$

which is equal to

$$z_0^* = \frac{1-s}{2-s} \tag{A14a}$$

under diploidy and

$$z_0^* = \frac{(2-s)(1-s^2)}{4+s-3s^2+s^3} \tag{A14b}$$

under haplodiploidy. When $\bar{z}$ takes this value, the indirect benefits of helping are not sufficient for voluntary helping by females or males to increase from zero. Specifically,

z_0^* is a valid candidate CSP for sex allocation when

$$\begin{aligned} b_\mathrm{f} &< \frac{\left(c_\mathrm{f}p_{\mathrm{self|f}} + sc_\mathrm{m}p_{\mathrm{sis|m}}\right)\left(c_\mathrm{f}p_\mathrm{dau} + (1+s-s^2)c_\mathrm{m}p_\mathrm{son}\right)}{\left(c_\mathrm{f}p_\mathrm{dau} + sc_\mathrm{m}p_\mathrm{son}\right)\left(c_\mathrm{f}p_{\mathrm{sis|f}} + (1+s-s^2)c_\mathrm{m}p_{\mathrm{sis|m}}\right)}, \\ b_\mathrm{m} &< \frac{\left(c_m p_{\mathrm{self|m}} - s^2 c_\mathrm{m}p_{\mathrm{bro|m}}\right)\left(c_\mathrm{f}p_\mathrm{dau} + (1+s-s^2)c_\mathrm{m}p_\mathrm{son}\right)}{(1-s^2)\,c_\mathrm{m}p_\mathrm{son}\left(c_\mathrm{f}p_{\mathrm{bro|f}} + (1+s-s^2)c_\mathrm{m}p_{\mathrm{bro|m}}\right)}. \end{aligned} \tag{A15}$$

This reduces to

$$\begin{aligned} b_\mathrm{f} &< \frac{4-2s+2ms}{1+m+s+ms}, \\ b_\mathrm{m} &< \frac{4+s+ms}{1+m+s+ms} \end{aligned} \tag{A15a}$$

under diploidy, and

$$\begin{aligned} b_\mathrm{f} &< \frac{(4-s)(4+s-3s^2+s^3)}{(2+s(2-s))^2 + 2m\,(2-s^2(3-s))}, \\ b_\mathrm{m} &< \frac{4+s-s^2}{2+s-s^2} \end{aligned} \tag{A15b}$$

under haplodiploidy. There is no voluntary helping by females or by males ($x^* = y^* = 0$) when these conditions are met, which can be verified by substituting z_0^* into equations A10 & A11 above.

Corresponding to voluntary helping by females only, the second candidate CSP for sex allocation is

$$\begin{aligned} z_\mathrm{f}^* &= \frac{-2C}{B - \sqrt{B^2 - 4AC}}, \\ \text{with} \quad & A = b_\mathrm{f}\left(c_\mathrm{f}p_{\mathrm{sis|f}} + (1+s-s^2)c_\mathrm{m}p_{\mathrm{sis|m}}\right) \\ & B = c_\mathrm{f}p_{\mathrm{self|f}} - c_\mathrm{f}p_{\mathrm{sis|f}} - c_\mathrm{m}p_{\mathrm{sis|m}} - b_\mathrm{f}\left(c_\mathrm{f}p_{\mathrm{sis|f}} + (1+s-s^2)c_\mathrm{m}p_{\mathrm{sis|m}}\right) \\ & C = \frac{(1-s^2)c_\mathrm{m}p_\mathrm{son}\left(c_\mathrm{f}p_{\mathrm{sis|f}} + (1+s-s^2)c_\mathrm{m}p_{\mathrm{sis|m}}\right)}{c_\mathrm{f}p_\mathrm{dau} + (1+s-s^2)c_\mathrm{m}p_\mathrm{son}}. \end{aligned} \tag{A16}$$

Under diploidy, the coefficients

$$\begin{aligned} A &= b_\mathrm{f}(1+m)(2-s)(1+s) \\ B &= (1-s)\,(2-s-m(2+s)) - b_\mathrm{f}(1+m)(2-s)(1+s) \end{aligned}$$

$$C = (1+m)(1-s^2) \tag{A16a}$$

yield z_f^* when substituted into equation A16. Under haplodiploidy, the coefficients

$$\begin{aligned} A &= b_f\,(2+2m(1-s)+s(2-s)) \\ B &= (1-s)(2-2m-s) - b_f\,(2+2m(1-s)+s(2-s)) \\ C &= \frac{(1-s^2)(2-s)\,(2+2m(1-s)+s(2-s))}{4+s-3s^2+s^3} \end{aligned} \tag{A16b}$$

yield z_f^* when substituted into equation A16. When $\bar{z}$ takes this value, the convergence-stable level of voluntary helping is nonzero for females and zero for males, which can be verified by substituting z_f^* into equations A10 and A11 above. In particular, z_f^* is a valid candidate CSP for sex allocation under diploidy when

$$\begin{aligned} b_f &> \begin{cases} 2\sqrt{\frac{(3-m)(1-s)^2}{(1+m)(2-s)^2(1+s)}} + \frac{(1-s)(4+s+ms)}{(1+m)(2-s)(1+s)} & \text{if } m \le \frac{2-7s+3s^2}{2-s+s^2} \\ \frac{4-2s+2ms}{1+m+s+ms} & \text{otherwise} \end{cases}, \\ b_m &< \frac{4-2s+2ms}{(1+m)(2-s)(1+s)z_f^*}, \end{aligned} \tag{A17a}$$

and under haplodiploidy when

$$\begin{aligned} b_f &> \begin{cases} 2\sqrt{\frac{(2-s)(1-s)^2(1+s)(4(3+s)-s^2(9-2s)-2m(2+(2-s)s))}{(2+2m(1-s)+(2-s)s)(4+s-3s^2+s^3)^2}} & \\ \quad +1+\frac{4-s}{2+2m(1-s)+(2-s)s} - \frac{2(2+(2-s)s)}{4+s-3s^2+s^3} & \text{if } m \le \frac{4-14s-7s^2+10s^3-2s^4}{2(1-s)(2+(2-s)s)} \\ \frac{(4-s)(4+s-3s^2+s^3)}{(2+(2-s)s)^2+2m(2-(3-s)s^2)} & \text{otherwise} \end{cases}, \\ b_m &< \frac{(1-s)\,(4+(1-s)s)}{(4+s-3s^2+s^3)z_f^*}. \end{aligned} \tag{A17b}$$

General forms of condition A17a and A17b—*i.e.*, in terms of consanguinities p and class reproductive values c as placeholders, rather than with their particular values for diploidy and haplodiploidy substituted in and the resulting conditions simplified—do exist, but we omit them here because they are very lengthy and not particularly informative (for example, they take different forms depending on whether individuals

are more related to their siblings than they are to themselves, which may not represent results of general importance).

Finally, corresponding to voluntary helping by males only, the third candidate CSP for sex allocation is

$$\begin{aligned} z_{\mathrm{m}}^{*} &= \frac{-2C}{B+\sqrt{B^2-4AC}}, \\ \text{with} \quad & A = b_{\mathrm{m}}\left(c_{\mathrm{f}}p_{\mathrm{bro|f}} + (1+s-s^2)c_{\mathrm{m}}p_{\mathrm{bro|m}}\right) \\ & B = -c_{\mathrm{m}}p_{\mathrm{self|m}} + c_{\mathrm{f}}p_{\mathrm{bro|f}} + (1+s)c_{\mathrm{m}}p_{\mathrm{bro|m}} \\ & \quad -b_{\mathrm{m}}\left(c_{\mathrm{f}}p_{\mathrm{bro|f}} + (1+s-s^2)c_{\mathrm{m}}p_{\mathrm{bro|m}}\right) \\ & C = c_{\mathrm{m}}p_{\mathrm{self|m}} - c_{\mathrm{f}}p_{\mathrm{bro|f}} - (1+s)c_{\mathrm{m}}p_{\mathrm{bro|m}} \\ & \quad + \frac{\left(c_{\mathrm{f}}p_{\mathrm{dau}} + sc_{\mathrm{m}}p_{\mathrm{son}}\right)\left(c_{\mathrm{f}}p_{\mathrm{bro|f}} + (1+s-s^2)c_{\mathrm{m}}p_{\mathrm{bro|m}}\right)}{c_{\mathrm{f}}p_{\mathrm{dau}} + (1+s-s^2)c_{\mathrm{m}}p_{\mathrm{son}}}. \end{aligned} \tag{A18}$$

Note that the form of A17 and A18 differ by the sign of the radical in the denominator as well as by the coefficients A, B, and C. Under diploidy, the coefficients

$$\begin{aligned} A &= b_{\mathrm{m}}(1+m)(2-s)(1+s) \\ B &= 2(2s+m-1) - b_{\mathrm{m}}(1+m)(2-s)(1+s) \\ C &= (3-m)(1+s) \end{aligned} \tag{A18a}$$

yield z_{m}^{*} when substituted into equation A18. Under haplodiploidy, the coefficients

$$\begin{aligned} A &= b_{\mathrm{m}}(4+s-3s^2+s^3) \\ B &= (4-s)s - b_{\mathrm{m}}(4+s-3s^2+s^3) \\ C &= 2(1-s) \end{aligned} \tag{A18b}$$

yield z_{m}^{*} when substituted into equation A18. When $\bar{z}$ takes this value, the convergence-stable level of voluntary helping is zero for females and nonzero for males, which can be verified by substituting z_{m}^{*} into equations A10 and A11 above. In particular, z_{m}^{*} is

a valid candidate CSP for sex allocation under diploidy when

$$\begin{aligned} b_{\mathrm{f}} &< \frac{2-(1-m)s}{(1+m)^2(2-s)(1+s)^2}\Bigg\{-2+2m+4s+b_{\mathrm{m}}(1+m)(2-s)(1+s) \\ &\quad +\sqrt{\begin{array}{l}4(1-m-2s)^2-4b_{\mathrm{m}}(1+m)(2-s)\,(2-(1-m)s) \\ +\left(b_{\mathrm{m}}(1+m)(2-s)(1+s)\right)^2\end{array}}\Bigg\}, \\ b_{\mathrm{m}} &> 2\left(\sqrt{\frac{(3-m)(1-s)}{(1+m)(2-s)^2(1+s)}}+\frac{2-s+ms}{(1+m)(2-s)(1+s)}\right), \end{aligned} \tag{A19a}$$

and under haplodiploidy when

$$\begin{aligned} b_{\mathrm{f}} &< \frac{4-s}{2\left((2+(2-s)s)^2+2m\,(2-(3-s)s^2)\right)}\Bigg\{(4-s)s+b_{\mathrm{m}}(4+s-3s^2+s^3) \\ &\quad +\sqrt{\begin{array}{l}(4-s)^2s^2-2b_{\mathrm{m}}(2-s)(2+s)(4+s-3s^2+s^3) \\ +\left(b_{\mathrm{m}}(4+s-3s^2+s^3)\right)^2\end{array}}\Bigg\}, \\ b_{\mathrm{m}} &> \frac{\sqrt{8\,(2-(3-s)s^2)}+4-s^2}{4+s-3s^2+s^3}. \end{aligned} \tag{A19b}$$

General forms of these conditions exist, but we omit them here.

In Fig. M7 we illustrate conditions A15, A17 and A19, in order to provide examples of the parameter space over which solutions A14, A16, and A18 are valid in the context of strict monogamy ($m = 1$). Fig. M8 gives a single example of the same for promiscuity ($m = 0$), illustrating that in the absence of strict monogamy there are regions of parameter space in which all three possible solutions given above represent valid candidate CSPs.

Maternally-manipulated helping When helping is maternally manipulated, the three possible candidate CSPs for sex allocation are as follows.

The first solution, corresponding to no helping, is identical to the solution corresponding to no voluntary helping, given by A14 above; however, under maternally-manipulated helping, the conditions for the validity of this candidate CSP are

$$b_{\mathrm{f}} < 1, b_{\mathrm{m}} < 1, \tag{A20}$$

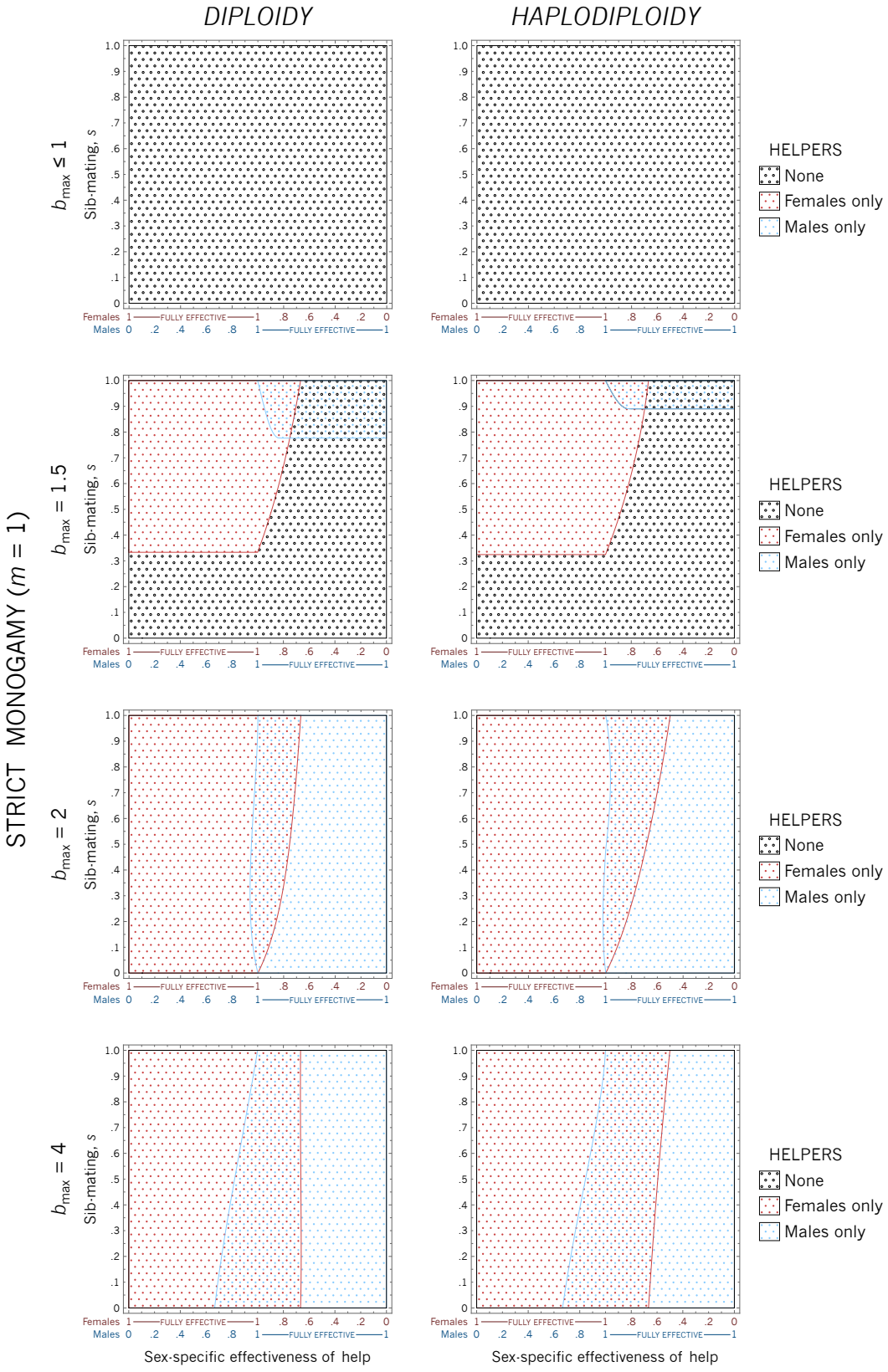


Figure M7: Conditions under which voluntary helping may stably persist, for helping by females, males, or neither, assuming strict monogamy ($m = 1$). Each row gives a different illustrative value of the maximum sib-rearing benefit of $b_{\max}$, *i.e.* such that $b_{\mathrm{f}} = b_{\max} e_{\mathrm{f}}$ and $b_{\mathrm{m}} = b_{\max} e_{\mathrm{m}}$, where e_{f} and e_{m} are the "effectiveness of help" for females and males, respectively (bottom of each plot).

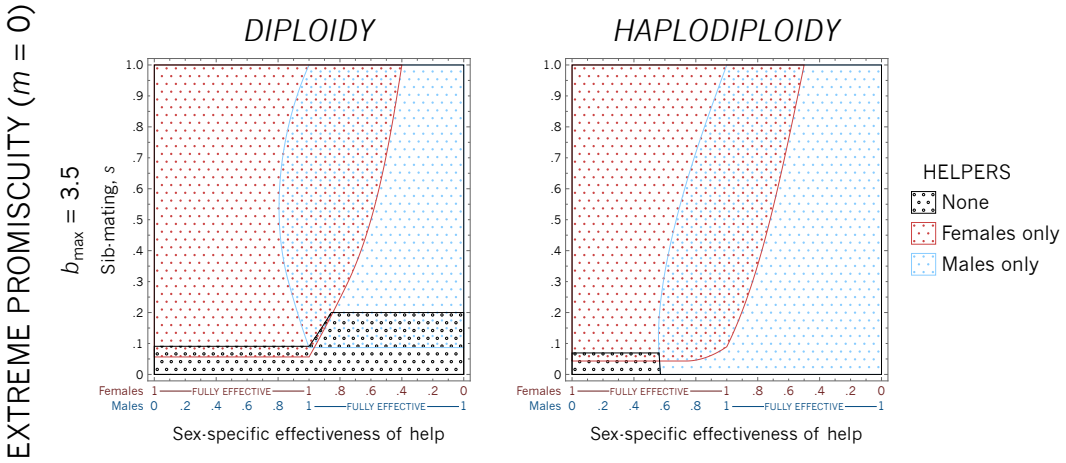


Figure M8: Conditions under which voluntary helping may stably persist, for helping by females, males, or neither, assuming extreme promiscuity ($m = 0$). Results are given for $b_{\max} = 3.5$ as an illustrative example.

regardless of ploidy. Under condition A20, recruiting helpers of either sex will not increase a mother's inclusive fitness (since those helpers would not sib-rear the additional offspring needed to replace them), so there is no maternally-manipulated helping by females or by males ($x^* = y^* = 0$) when these conditions are met, which can be verified by substituting z_0^* from equation A14 into equations A12 and A13 above.

Corresponding to manipulated helping by females only, the second candidate CSP for sex allocation is

$$z_{\mathrm{f}}^* = \frac{(1-s^2)c_{\mathrm{m}}p_{\mathrm{son}}}{b_{\mathrm{f}}\left(c_{\mathrm{f}}p_{\mathrm{dau}} + (1+s-s^2)c_{\mathrm{m}}p_{\mathrm{son}}\right)}, \tag{A21}$$

which is

$$z_{\mathrm{f}}^* = \frac{1-s}{b_{\mathrm{f}}(2-s)} \tag{A21a}$$

under diploidy and

$$z_{\mathrm{f}}^* = \frac{(2-s)(1-s^2)}{b_{\mathrm{f}}(4+s-3s^2+s^3)} \tag{A21b}$$

under haplodiploidy. When $\bar{z}$ takes this value, the convergence-stable level of maternally-manipulated helping is nonzero for females and zero for males, which can be verified by substituting z_{f}^* into equations A12 and A13 above. In particular, z_{f}^* is a

valid candidate CSP for sex allocation when

$$b_f > 1, b_f > b_m, \tag{A22}$$

regardless of ploidy.

Finally, corresponding to manipulated helping by males only, the third candidate CSP for sex allocation is

$$z_m^* = 1 - \frac{c_f p_{dau} + s c_m p_{son}}{b_m \left(c_f p_{dau} + (1 + s - s^2) c_m p_{son}\right)}, \tag{A23}$$

which is

$$z_m^* = 1 - \frac{1}{b_m(2 - s)} \tag{A23a}$$

under diploidy and

$$z_m^* = 1 - \frac{2 + s(2 - s)}{b_m(4 + s - 3s^2 + s^3)} \tag{A23b}$$

under haplodiploidy. When $\bar{z}$ takes this value, the convergence-stable level of maternally-manipulated helping is zero for females and nonzero for males, which can be verified by substituting z_m^* into equations A12 and A13 above. In particular, z_m^* is a valid candidate CSP for sex allocation when

$$b_m > 1, b_m > b_f, \tag{A24}$$

regardless of ploidy.

We illustrate conditions A20, A22, and A24 in Fig. M9 .

5.4 Evolutionary dynamics

Evolutionary dynamics were computed by interpreting the left-hand sides of conditions A4–A8 above as rates of change due to natural selection. (Note that the "non-normalized" versions of these conditions were used; that is, A4 was not divided by N, A5 and A6 were not divided by $N(1 - \bar{z})$, and A7 and A8 were not divided by $N\bar{z}$.) These were used to generate Figs. 5 and M4.

Rates of change were numerically integrated in C++ using the ODEINT library,

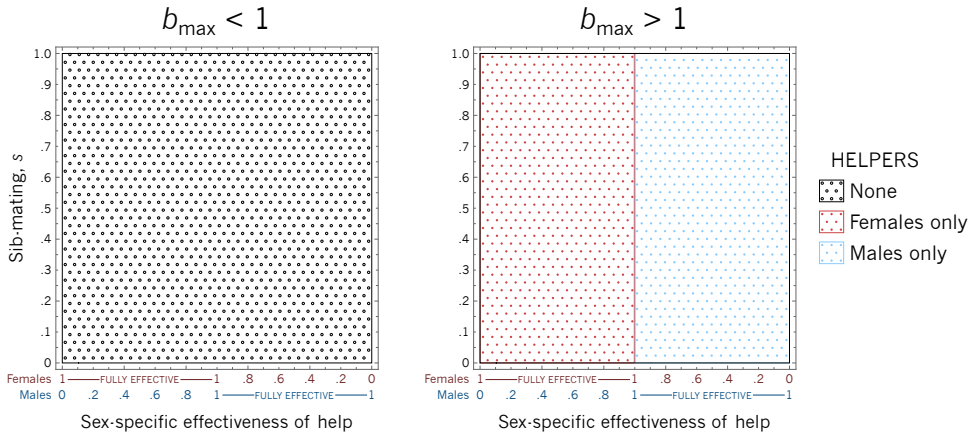


Figure M9: Conditions under which maternally-manipulated helping may stably persist, for helping by females, males, or neither. Each panel holds for both diploidy and haplodiploidy, regardless of the level of promiscuity.

version 2 (Ahnert & Mulanksy 2011), integrating with the default 5th-order Cash-Karp error-controlled stepper with adaptive step size. We start integration from an initial condition of no helping, with $\bar{x} = \bar{y} = 0$, and with $\bar{z}$ initially assigned its analytically-determined equilibrium value under no helping as identified in equation A14 above. Integrations were given sufficient time (50 units) to reach equilibrium before the final values of $\bar{x}$, $\bar{y}$, and $\bar{z}$ were reported.

6. Individual-based model

To verify the results of our kin selection analysis, we implemented a stochastic individual-based model in C++. Here, each individual comprises three loci, one for each trait. Each locus comprises one or two genes, depending on whether the individual is haploid or diploid, and each gene is represented by a real number $\gamma \in [0,1]$. Phenotypes are determined by averaging the genic values at the corresponding locus: hence, a haploid individual with genotype $\{\gamma_x, \gamma_y, \gamma_z\}$ has phenotype $x = \gamma_x, y = \gamma_y, z = \gamma_z$, while a diploid individual with genotype $\{\gamma_{x1}, \gamma_{x2}, \gamma_{y1}, \gamma_{y2}, \gamma_{z1}, \gamma_{z2}\}$ has phenotype $x = \frac{1}{2}(\gamma_{x1} + \gamma_{x2})$, $y = \frac{1}{2}(\gamma_{y1} + \gamma_{y2})$, $z = \frac{1}{2}(\gamma_{z1} + \gamma_{z2})$. (Whether a given trait is actually "expressed" by an individual depends on their sex and on model parameters.)

At the beginning of each generation, M mated females each found a patch by laying

K eggs, which develop into females or males depending on the foundress's sex allocation strategy z. Specifically, each egg independently develops into a male with probability z, otherwise developing into a female, such that the number of males on the patch is drawn from a binomial distribution of K trials with success probability z. Each female has a probability x of developing into a helper, and each male has a probability y of developing into a helper; otherwise, the individual develops into a reproductive. For voluntary help, x and y are the focal individual's phenotypic traits, while for maternally-manipulated help, x and y are the mother's traits. Each reproductive son or daughter is assigned a fitness $w = B(h)$, with $h = \frac{1}{K}(e_{\mathrm{f}}H_{\mathrm{f}} + e_{\mathrm{m}}H_{\mathrm{m}})$. Here: e_{f} and e_{m} are the effectiveness of female and male helpers, respectively, as defined in section 2.1 above; H_{f} is the number of female helpers on the patch; and H_{m} is the number of male helpers on the patch. We define the function relating the survival benefit of helping as $B(h) = \exp\left(b(h–1)\right)$, because this function satisfies $0 < B(h) \leq 1$ for $0 \leq h \leq 1$, and because $(dB/dh|_{h=\bar{h}})/\bar{B}$ is then equal to a constant, b; note that from the definitions of b_{f} and b_{m} in section 2.1 above, $b_{\mathrm{f}} = be_{\mathrm{f}}$ and $b_{\mathrm{m}} = be_{\mathrm{m}}$.

Then each reproductive male disperses with probability $1 - s$, with dispersing males randomly distributed over all patches. Specifically, from each patch, $k_{\mathrm{d}} \sim \mathrm{Binomial}(r_{\mathrm{m}}, 1 - s)$ males are gathered, where r_{m} is the number of reproductive males on the patch. The group of n_{d} dispersed males is randomly shuffled, then the dispersed males are evenly distributed over each patch, such that each of the M patches receives between $\mathrm{Floor}(\frac{n_{\mathrm{d}}}{M})$ and $\mathrm{Ceil}(\frac{n_{\mathrm{d}}}{M})$ males. This procedure is done rather than assigning each male independently to a random patch in order to minimize the probability of any patches being devoid of reproductive males, which would result in females going unmated. Finally, for each patch, a new foundress is selected randomly from among all females in the population (excluding mothers of the current generation), with probability proportional to the female's fitness; her mate is selected randomly from among the males on her patch, with probability proportional to the male's fitness; and all other individuals die. For our numerical simulations, we assume strict monogamy, and hence each foundress has at most one mate. Should a foundress be unmated because there are no reproductive males on her patch, under haplodiploidy she can still produce an all-male brood, but under diploidy she does not produce any

offspring. After mating, the remaining males die and the population is returned to the beginning of the cycle.

At birth, an individual's genotype may change due to mutation. Specifically, each gene in a newly-born individual has a probability μ of mutating, in which case its genic value changes from γ to $\gamma' = \max(0, \min(\gamma + \varepsilon, 1))$, where ε is drawn from a normal distribution with mean 0 and standard deviation σ.

We begin all simulations with a monomorphic population in which all $\gamma_x = \gamma_y = 0$ and all $\gamma_z = 0.5$, and hence $x = 0$, $y = 0$, $z = 0.5$ for each individual. We allow the sex ratio to evolve for 10,000 generations in the absence of helping. Then, helping is permitted to evolve. The individual-based model confirms the results of our kin selection analysis (Fig. M10).

7. Comparative analysis R code and result tables

Here, we provide excerpts from the R code used to perform our statistical analyses and the summary result tables for fixed effects generated by it. Throughout, the `help` dataframe corresponds to data from Table M3 above, according to the following scheme: `sex.ability` = *preadaptation*, `genetic.sys` = *ploidy*, `sibmating.LMC` = *sib-mating/LMC*, `helper.sex` = *helper sex ratio*, `SRbias.ability` = *labile sex ratio*, `type.bias` = $2\,|helper\,sex\,ratio - 0.5|$. The `eusoctreepruned` structure corresponds to our inferred phylogeny (Fig. M11), while the `inphyl` dataframe is the subset of the `help` dataframe included in the phylogeny.

Taxonomic models

Helper sex ratio

```
prior1 <- list(
  B=list(mu=c(0,0,0,0),
    V=gelman.prior(~sex.ability+genetic.sys+sibmating.LMC,
      data=help, scale=1+pi^2/3)),
  R=list(V=1, fix=1),
  G=list(G1=list(V=diag(1)*0.1, nu=1),
```

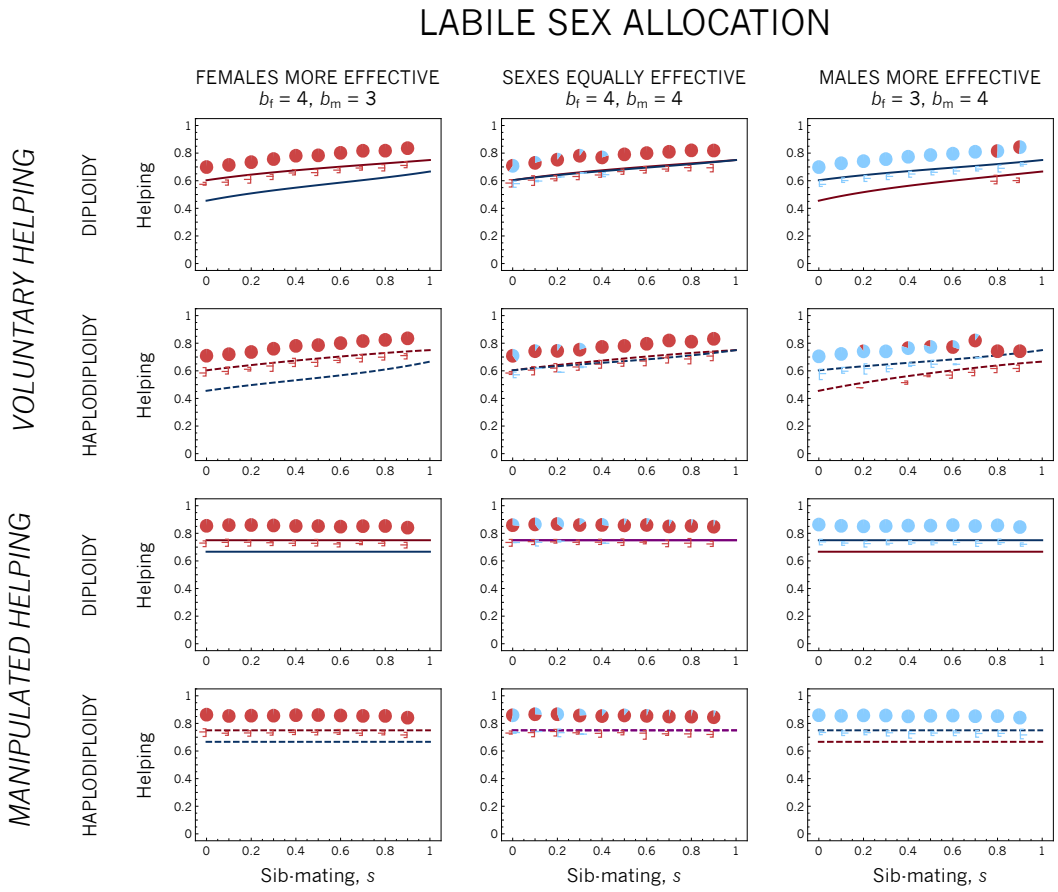


Figure M10: Validation of analytical results with stochastic individual-based model. For each plot, analytical results are given by the solid and dashed lines (labile sex allocation, this page) or filled areas (fixed sex allocation, next page). The overlaid whisker plots and pie charts summarize results for 10 runs of the individual-based model each. Whisker plots give the minimum, maximum, and mean for the average amount of helping (*i.e.*, $h = (1–z)x + zy$) over the last 100 generations of each run. Where there are separate red and blue whiskers for a given set of runs (under labile sex allocation, this page) the runs for which females helped more (red) or males helped more (blue) are summarized separately. (Continued on next page.)

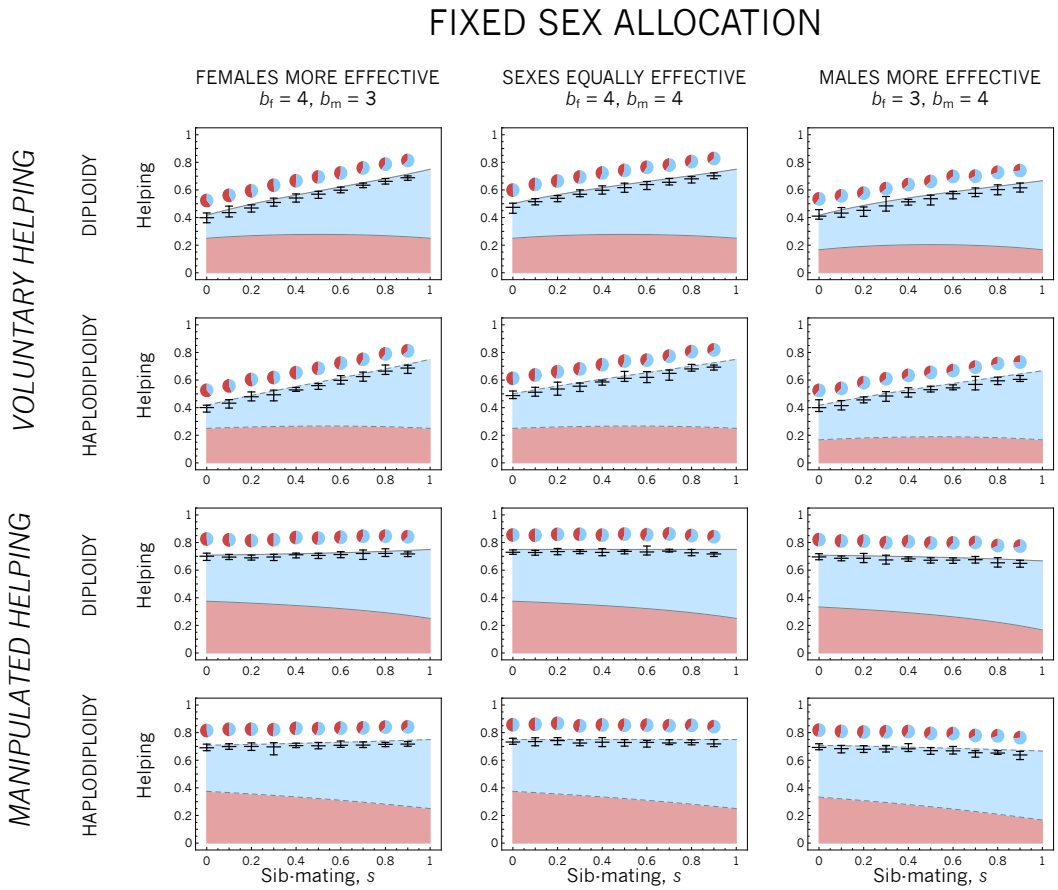


Figure M10, *continued*: Pie charts give the average sex ratio of helpers over the last 100 generations of each run, with females in red and males in blue. Note that for labile sex allocation (previous page), this largely indicates the proportion of runs that terminated with predominantly-female versus predominantly-male helpers, while for fixed sex allocation (this page), this largely indicates the composition of a mixed-sex caste that was repeatable across runs. Results are shown for $M = 200$, $K = 100$, $\mu = 0.01$, $\sigma = 0.01$; simulations were run for 60,000 generations for labile sex allocation (after an initial 10,000 generations of permitting only sex allocation, and not helping, to evolve; previous page) and 30,000 generations for constrained sex allocation (this page).

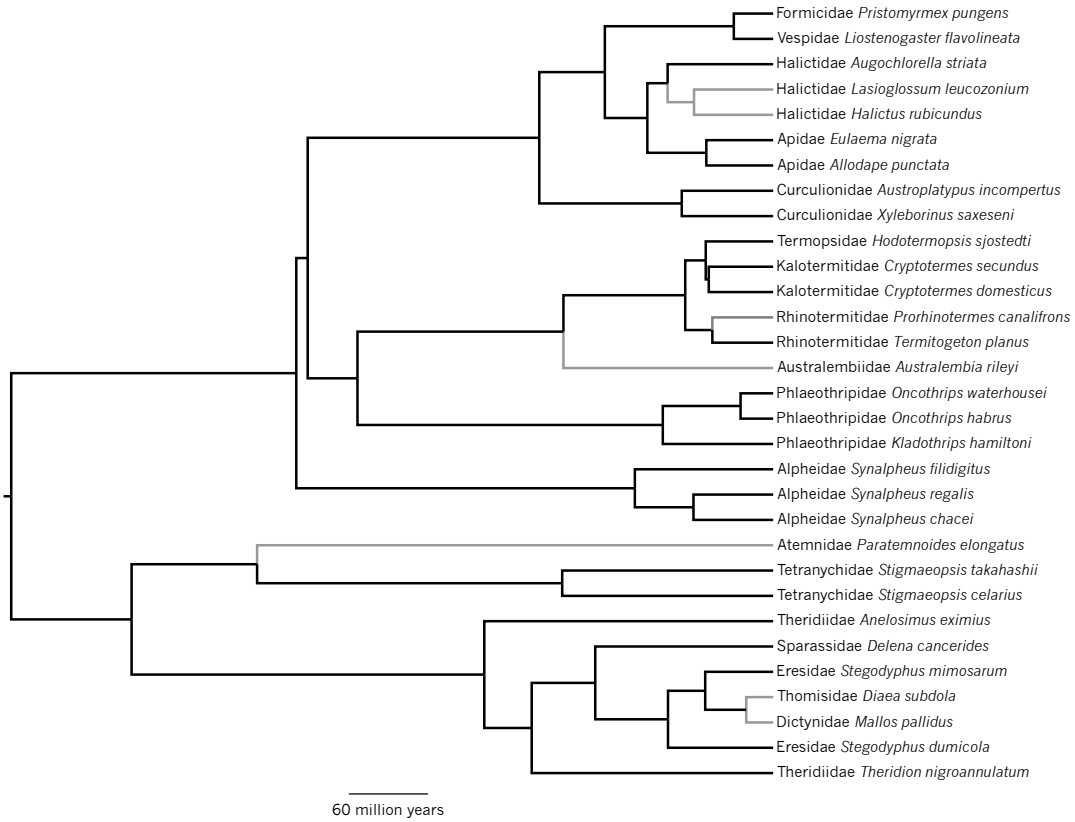


Figure M11: Phylogram inferred for species in comparative survey. Grey branches indicate species for which sequence data were unavailable, and for which sequences from another species of the same genus were substituted. Note that this inferred phylogeny largely corresponds to the consensus phylogeny illustrated in Fig. 1 of the main text, with the exception of the position of the Thysanoptera.

```
    G2=list(V=diag(1)*0.1, nu=1),
    G3=list(V=diag(1)*0.1, nu=1)))

m1 <- MCMCglmm(helper.sex~sex.ability+genetic.sys+sibmating.LMC,
  random=~Order+Family+genus, prior=prior, data=help, family="threshold",
  verbose=FALSE, nitt=13000*100, thin=100, burnin=3000*100)
```

	post.mean	l-95% CI	u-95% CI	eff.samp	pMCMC	
(Intercept)	-1.78065	-3.02628	-0.65280	1039.3	<0.001	***
sex.ability	16.16842	9.53451	23.46285	1000.0	<0.001	***
genetic.syshaplodiploidy	0.01895	-1.31792	1.18770	1207.4	0.996	
sibmating.LMCyes	-0.62559	-1.68962	0.55452	777.8	0.252	

Extent of sex bias in sib-rearing

```
prior2 <- list(
  B=list(mu=c(0,0,0),
    V=gelman.prior(~sex.ability+SRbias.ability, data=help, scale=1+pi^2/3)),
  R=list(V=1, fix=1),
  G=list(G1=list(V=diag(1)*0.1, nu=1),
    G2=list(V=diag(1)*0.1, nu=1),
    G3=list(V=diag(1)*0.1, nu=1)))

m2 <- MCMCglmm(type.bias~sex.ability+SRbias.ability,
  random=~Order+Family+genus, prior=prior2, data=help, family="threshold",
  verbose=FALSE, nitt=13000*100, thin=100, burnin=3000*100)
```

	post.mean	l-95% CI	u-95% CI	eff.samp	pMCMC	
(Intercept)	2.4333	1.1479	3.7063	1000	<0.001	***
sex.ability	-4.9699	-6.9721	-2.9656	1000	<0.001	***
SRbias.ability	1.0180	-0.1678	2.0768	1062	0.07	.

Phylogenetic models assuming $\lambda = 1$

Helper sex ratio

```
prior1a <- list(
  B=list(mu=c(0,0,0,0),
    V=gelman.prior(~sex.ability+genetic.sys+sibmating.LMC,
      data=inphyl, scale=1+pi^2/3)),
  R=list(V=1,fix=1),
  G=list(G1=list(V=1, fix=1)))


m1a <- MCMCglmm(helper.sex~sex.ability+genetic.sys+sibmating.LMC,
  random=~animal, pedigree=eusoctreepruned, prior=prior1a, data=inphyl,
  reduced=TRUE, family="threshold", nitt=13000*100, thin=100,
  burnin=3000*100, pl=TRUE)
```

	post.mean	l-95% CI	u-95% CI	eff.samp	pMCMC	
(Intercept)	-2.4879	-4.9395	-0.1104	906.0	0.0178	*
sex.ability	15.0413	5.5682	25.4804	219.8	<1e-04	***
genetic.syshaplodiploidy	-0.6888	-2.6641	1.2989	2423.9	0.4954	
sibmating.LMCyes	0.6245	-1.2157	2.5216	2357.3	0.5080	

Extent of sex bias in sib-rearing

```
prior2a <- list(
  B=list(mu=c(0,0,0),
    V=gelman.prior(~sex.ability+SRbias.ability,
      data=inphyl, scale=1+pi^2/3)),
  R=list(V=1,fix=1),
  G=list(G1=list(V=1, fix=1)))


m2a <- MCMCglmm(type.bias~sex.ability+SRbias.ability, random=~animal,
  pedigree=eusoctreepruned, prior=prior2a, data=inphyl, reduced=TRUE,
  family="threshold", nitt=13000*100, thin=100, burnin=3000*10, pl=TRUE)
```

```
                 post.mean   l-95% CI   u-95% CI   eff.samp    pMCMC
 (Intercept)         0.8689    -0.4583     2.2444       9003   0.2064
 sex.ability        -3.5985    -6.3021    -1.3055       5838   0.0002   ***
 SRbias.ability      1.4011     0.2276     2.6770       6468   0.0214   *
```

Phylogenetic models estimating λ from the tree

Helper sex ratio

```
prior1b <- list(
  B=list(mu=c(0,0,0,0),
    V=gelman.prior(~sex.ability+genetic.sys+sibmating.LMC,
      data=inphyl, scale=1+pi^2/3)),
  R=list(V=1,fix=1),
  G=list(G1=list(V=1, nu=1000, alpha.mu=0, alpha.V=1)))


m1b <- MCMCglmm(helper.sex~sex.ability+genetic.sys+sibmating.LMC,
  random=~animal, pedigree=eusoctreepruned, data=inphyl, family="threshold",
  nitt=13000*100, thin=100, burnin=3000*100, prior=prior1b, pl=TRUE)
```

```
                           post.mean   l-95% CI   u-95% CI   eff.samp     pMCMC
 (Intercept)                 -2.9977    -5.6264    -0.4047       4763    0.0068   **
 sex.ability                 17.9893     8.7704    28.5621       1755    <1e-04   ***
 genetic.syshaplodiploidy    -1.3863    -4.0466     1.0142       7400    0.2544
 sibmating.LMCyes             0.6078    -1.7164     2.9446       7933    0.6072
```

Extent of sex bias in sib-rearing

```
prior2b <- list(
  B=list(mu=c(0,0,0),
    V=gelman.prior(~sex.ability+SRbias.ability,
      data=inphyl, scale=1+pi^2/3)),
  R=list(V=1,fix=1),
  G=list(G1=list(V=1, nu=1000, alpha.mu=0, alpha.V=1)))
```

```
m2b <- MCMCglmm(type.bias~sex.ability+SRbias.ability, random=~animal,
  pedigree=eusoctreepruned, data=inphyl, family="threshold", nitt=13000*100,
  thin=100, burnin=3000*100, prior=prior2b, pl=TRUE)

                 post.mean   l-95% CI   u-95% CI   eff.samp    pMCMC
 (Intercept)       1.98468   -0.24521    4.24272      10000   0.0894   .
 sex.ability      -6.40142  -11.07199   -2.27306       7077   0.0010   ***
 SRbias.ability    2.03909    0.03248    4.48851       7014   0.0282   *
```

Acknowledgments

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Chapter 4

Worker sterility, population genetics, and inclusive fitness

Abstract

Inclusive-fitness theory highlights monogamy as a key driver of altruistic sib-rearing. Accordingly, monogamy should promote the evolution of worker sterility in social insects when sterile workers make for better helpers. However, a recent population-genetics analysis (Olejarz *et al.* 2015) found no clear effect of monogamy on worker sterility. Here, we revisit this analysis. First, we relax genetic assumptions, considering not only alleles of extreme effect—encoding either no sterility or complete sterility—but also alleles with intermediate worker-sterility effects. Second, we broaden the stability analysis—which focused on the invasibility of populations where either all workers are fully-sterile or all workers are fully-reproductive—to identify where intermediate pure or mixed evolutionarily-stable states may occur. Finally, we consider additional, demographically-explicit ecological scenarios relevant to worker non-reproduction. This extended analysis demonstrates that an exact population-genetics approach strongly supports the prediction of inclusive-fitness theory that monogamy promotes sib-directed altruism in social insects.

Introduction

Altruism among animals is epitomized by the workers of insect societies, who sacrifice their personal reproductive success to promote their siblings' welfare. This remarkable self-abnegation—seemingly at odds with the "survival of the fittest"—is traditionally explained by kin selection: a gene causing workers to share provisions or defend the communal nest can spread if the workers' sacrifice increases the survival of their siblings, who are likely to carry copies of the same gene. Higher genetic relatedness between the altruist and her beneficiaries would therefore—all else being equal—promote selection for altruism (Hamilton 1964). Accordingly, monogamy is often highlighted as a key promoter of sibling altruism, since maternal promiscuity decreases relatedness between siblings, diminishing the inclusive-fitness benefits of sib-rearing (Hamilton 1972; Charlesworth 1978; Charnov 1978; Boomsma 2007, 2009, 2013; Gardner *et al.* 2012; Davies *et al.* 2016). A wealth of empirical evidence supports this view, revealing a strong association between monogamy and sib-directed altruism in arthropods (Hughes *et al.* 2008), birds (Cornwallis *et al.* 2010), and mammals (Lukas & Clutton-Brock 2012).

A conspicuous example of sib-directed altruism in the social Hymenoptera (wasps, bees, and ants) is worker sterility. In many hymenopteran species, female workers lay unfertilized eggs in their natal colony, which develop into males on account of their haplodiploid mode of sex determination. But in some species, workers have partly or entirely stopped making sons to focus their efforts on helping instead. A standard account of inclusive-fitness theory would predict that—as with other forms of sibling altruism—monogamy should promote helpful worker sterility.

However, this prediction has recently been challenged by Olejarz *et al.*'s (2015) mathematical analysis of worker sterility in haplodiploid insect colonies, which uses an intricate population-genetics model to derive exact conditions for the invasion and stability of a worker-sterility allele. Surprisingly, this analysis could not identify a consistent effect of monogamy on the evolution of non-reproductive workers. Here, we revisit this analysis, exploring alternative assumptions concerning the genetics, evolution, and ecology of worker sterility. We find that a more-comprehensive inves-

tigation of Olejarz *et al.*'s (2015) exact population-genetics approach strongly supports the view that monogamy promotes helpful worker sterility in insect societies and corroborates inclusive-fitness theory more generally.

Unconstrained allelic effects: monogamy promotes worker sterility

Olejarz *et al.* (2015) investigated the spread of an allele that renders workers carrying the allele—who would otherwise produce sons through arrhenotokous parthenogenesis, substituting them for the queen's sons—completely sterile. As the proportion z of sterile workers in a colony increases, the proportion p_z of males produced by the queen rather than by workers also increases, while overall colony productivity r_z may increase or decrease. Following these assumptions, they found that—in a seeming challenge to inclusive-fitness theory—worker sterility sometimes invades under single mating ($n = 1$) only, sometimes under double mating ($n = 2$) only, sometimes under both single and double mating, and sometimes under neither, suggesting no clear effect of monogamy on the invasion of sterility (Olejarz *et al.* 2015).

To explore the generality of this unexpected finding, we take up a suggestion by Olejarz *et al.* (2015, p. 13) and extend their analysis to consider alleles with intermediate effects on worker sterility (as was done for a similar model by Olejarz *et al.* 2016). Intermediate-effect alleles may exhibit incomplete penetrance (such that each carrier has an intermediate probability of being sterile), or may encode intermediate phenotypes (such that each carrier divides her resources between colony tasks and personal reproduction); these scenarios are mathematically equivalent, but for simplicity, we focus on the former. This suggested extension seems particularly apt, as the incomplete penetrance of sterility has been shown to be important for the evolution of reduced worker reproduction both in theory and in empirical practice (Charlesworth 1978; Ratnieks *et al.* 2006; Wenseleers & Ratnieks 2006b; Ronai *et al.* 2016); indeed, some form of incomplete penetrance is required to preserve the fecundity of queens carrying the sterility allele. Accordingly, we have derived exact conditions for the invasion of a recessive or dominant sterility allele with arbitrary

penetrance (see *Methods*). When we require mutant worker-sterility alleles to show full penetrance, our analysis exactly recovers Olejarz *et al.*'s (2015) results (Fig. 1a). However, when we allow mutant worker-sterility alleles to show incomplete penetrance, we find that—strikingly—monogamy always promotes the invasion of helpful worker sterility (Fig. 1b). (Note that monogamy may inhibit worker sterility when sterility is harmful; see *Methods*.)

Why does allowing intermediate effects make such a categorical difference? The population genetics of invasion is the key. For example, a recessive sterility allele, when rare, is almost always expressed in colonies founded by a heterozygous female who has mated with one mutant male and $n-1$ wild-type males. Other colony types occur, but are either comparatively rare (because they require more copies of the mutant allele among mating partners), or exhibit exactly the same phenotype as wild-type colonies (because sterility is expressed only when both parents pass the recessive mutant allele to their daughters). Therefore, sterility can only invade if these "mutant" colonies—in which a proportion $z = \frac{v}{2n}$ of workers are sterile, where v is the allele's penetrance—succeed in spreading the sterility allele. If we only permit alleles with full penetrance ($v = 1$) to arise, this allelic constraint may overpower the altruism-promoting effect of higher relatedness: for example, double mating ($n = 2$) may facilitate sterility's invasion over single mating ($n = 1$) if colony efficiency is relatively high when $z = 1/4$ and relatively low when $z = 1/2$ (Fig. 1c). In contrast, if we permit alleles with incomplete penetrance ($0 < v \leq 1$) to arise, mutant colonies may exhibit any one of a range of phenotypes, depending on v (namely, $0 < z \leq 1/2$ for single mating, and $0 < z \leq 1/4$ for double mating), and monogamy always promotes the invasion of helpful worker sterility over promiscuity, by both maximizing sibling relatedness and allowing a wider range of phenotypes to be explored (Fig. 1d; see *Methods* for the corresponding analysis assuming dominant sterility).

Beyond invasion: monogamy promotes worker sterility

These results explain why promiscuity sometimes promotes the invasion of helpful sterility over monogamy under specific genetic constraints. But to only consider

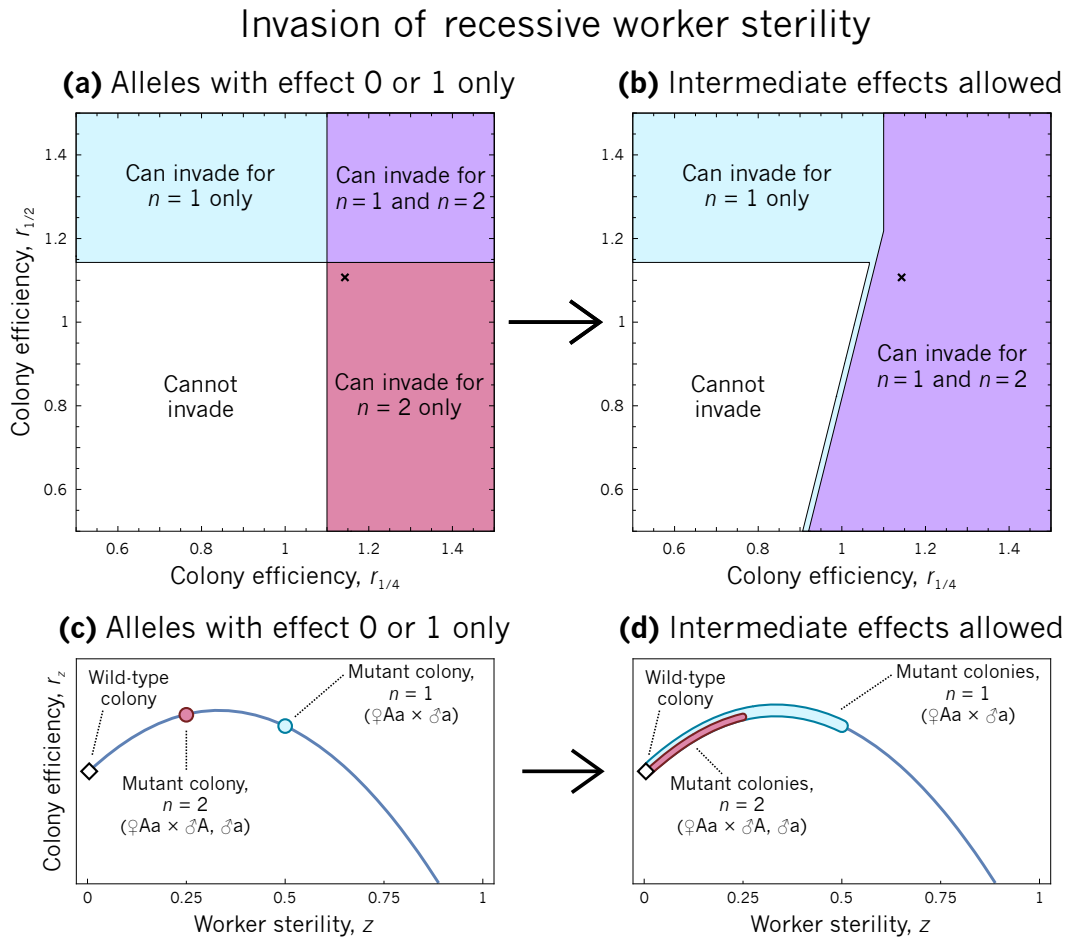


Figure 1: The invasion of worker sterility under recessive genetics, exploring the regions of parameter space where sterility can invade under single mating only, double mating only, both, or neither. **(a)** If we assume that only full-sterility alleles can arise, double mating sometimes promotes the invasion of sterility over single mating. But **(b)** if we assume that alleles encoding intermediate worker sterility may arise, double mating never promotes the invasion of sterility over single mating, depending on the colony efficiency values $r_0 = 1$, $r_{1/4}$, and $r_{1/2}$. This is because **(c)** for a rare allele encoding full sterility, mutant colonies have the phenotype $z = 1/2$ under single mating and $z = 1/4$ under double mating. Therefore, sterility may invade more easily under double mating if colony efficiency is relatively peaked near $z = 1/4$. But **(d)** for a rare allele encoding intermediate sterility, mutant colonies may express any phenotype $0 < z \leq 1/2$ under single mating and $0 < z \leq 1/4$ under double mating, depending on the allele's effect, and so mutant phenotypes are less constrained by the population's mating number. In order to facilitate comparison with Fig. 3A of Olejarz *et al.* (2015), we assume $p_z = 0.2 + 0.8z$, and for r_z we use the unique quadratic curve passing through the points specified by $r_0 = 1$, $r_{1/4}$, and $r_{1/2}$.

whether sterility invades may be misleading, for two reasons. First, that a sterility allele spreads from rarity says little about its equilibrium frequency, which may be a more-relevant measure of monogamy's impact on worker altruism than mere invasion. Indeed, although promiscuity sometimes promotes sterility's invasion *per se* under constrained penetrance, we find that monogamy typically promotes equilibrium sterility under the same conditions (Fig. 2).

Second, if we do allow intermediate-effect alleles, then considering only a single invasion is inadequate, because long-term evolution is likely to involve multiple successive invasions (*cf.* Hammerstein 1996). How can we predict the outcome without knowing in advance which alleles may arise, and when? The solution is that, over the long term, populations exposed to sufficient genetic variation will converge on an evolutionarily-stable strategy (ESS; Maynard Smith & Price 1973)—a level of sterility that cannot be invaded by an allele encoding any other level of sterility. To identify a candidate ESS for sterility, we further extend Olejarz *et al.*'s (2015) population-genetics analysis to derive an exact condition for the invasion of an allele encoding a small increase to average sterility, z:

$$-\frac{1}{1-z}(1-p_z)(3n-2)+\frac{r'_z}{r_z}\left(4+3n(1+p_z)\right)-p'_z(2-n)>0\,, \tag{1}$$

where r'_z and p'_z are the slopes of the r_z and p_z functions at z, respectively. Remarkably, this exact condition holds for both recessive and dominant genetics. Using this condition and a global stability analysis, we find that the ESS for helpful sterility is always highest under single mating—that is, over long-term evolution, monogamy always promotes helpful worker sterility (Fig. 3; see *Methods*).

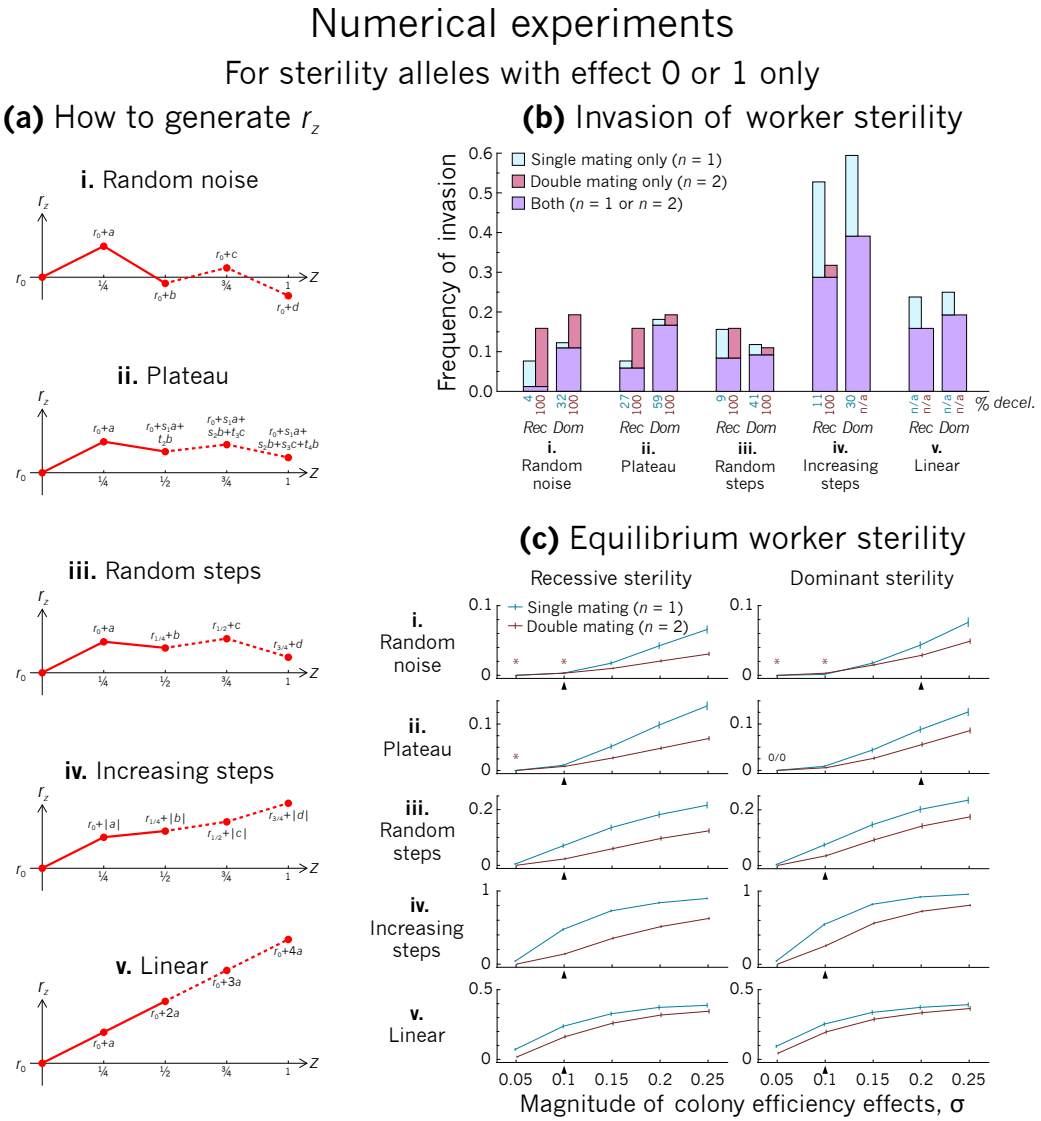


Figure 2: Here, we compare the evolution of worker sterility under single versus double mating by revisiting the numerical experiments of Olejarz *et al.* (2015). **(a)** There are many possible ways to construct the colony efficiency function r_z based on picking random numbers from a normal distribution. Five alternatives are shown here, including the two procedures used by Olejarz *et al.* ("Random noise", their Procedure 1, and "Plateau", their Procedure 2). For testing whether sterility invades, only two points are needed (solid lines), but this can be extended to four points (dashed lines) for measuring sterility at equilibrium. **(b)** We record the frequency of invasion of a full-sterility allele under single ($n = 1$) versus double mating ($n = 2$), running 10 million experiments for each scenario. Percentages beneath the bar chart show that an initially-decelerating r_z is required for sterility to invade under double mating only (see *Methods*). **(c)** We record the average worker sterility at equilibrium over 5000 experiments for each scenario. Except when r_z is constructed using the "random noise" or "plateau" procedure and the magnitude of efficiency effects is small (asterisks), single mating tends to promote average worker sterility at equilibrium over double mating (the $0/0$ denotes no worker sterility under either single or double mating). This can happen even if sterility is more likely to invade under double mating (for example, compare results of procedures i-iii in panel **(b)** versus panel **(c)**). Arrowheads beneath the x-axis show where parameters coincide with those used in panel **(b)**. The "magnitude of colony efficiency effects" is the standard deviation of normally-distributed variates used for constructing r_z. For panels **(b)** and **(c)**, we assume $p_z = 0.2 + 0.8z$. See *Methods* for details.

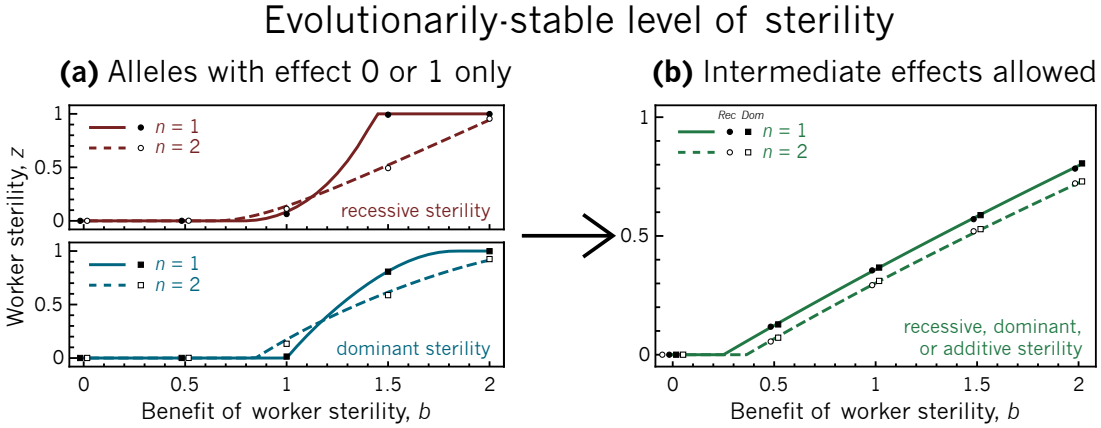


Figure 3: The evolutionarily-stable level of sterility under single versus double mating, for **(a)** constrained allelic variation, with recessive (top) versus dominant (bottom) sterility and **(b)** unconstrained allelic variation, regardless of whether sterility is recessive, dominant, or additive. **(a)** When allelic variation is constrained, double mating (dashed lines) can sometimes promote sterility over single mating (solid lines). But **(b)** when allelic variation is unconstrained, single mating always promotes sterility. Overlaid markers show results of a stochastic individual-based model (see *Methods*), matching well with the predicted evolutionarily-stable levels of worker sterility. To illustrate a scenario where constraints on heritable variation may lead to promiscuity promoting worker sterility over monogamy, we use the colony efficiency function $r_z = 1 + bz - z^2$, with a "benefit of worker sterility" term bz and a "decelerating" term $-z^2$. For the proportion of male eggs laid by the queen, we again use $p_z = 0.2 + 0.8z$.

Intuition for this exact population-genetics result may be obtained by recasting condition 1 in terms of inclusive fitness (Hamilton 1964). Accordingly, natural selection favours an increase to average sterility, z, when

$$\underbrace{-\frac{1-p_z}{1-z}R_{\text{son}}}_{\text{sacrifice effect}} + \underbrace{\frac{r'_z}{r_z}\left(R_{\text{sis}} + p_z R_{\text{bro}} + (1-p_z)R_{\text{neph}}\right)}_{\text{efficiency effect}} + \underbrace{p'_z R_{\text{bro}} + \left(\frac{1-p_z}{1-z} - p'_z\right)R_{\text{neph}}}_{\text{male production effect}} > 0\,, \tag{2}$$

where $R_{\text{son}} = \frac{1}{2}$, $R_{\text{neph}} = \frac{2+n}{8n}$, $R_{\text{sis}} = (1+p_z)\frac{2+n}{8n}$, and $R_{\text{bro}} = \frac{1}{4}$ are the life-for-life relatedness of a worker to her son, her nephew (a random worker's son), her reproductive sister, and her brother, respectively (Hamilton 1972). Note that promiscuity decreases worker relatedness to sisters and nephews, but not to sons or brothers. The left-hand side of condition 2 can be interpreted as the inclusive-fitness effect experienced by a focal worker who stops laying male eggs. The "sacrifice effect" captures the direct cost of her sterility, in that she forfeits her relative share $\frac{1-p_z}{1-z}$ of all worker-laid males. The "efficiency effect" captures her impact on colony efficiency, which increases by a relative amount $\frac{r'_z}{r_z}$, augmenting the production of her sisters and of colony-produced

males, a proportion p_z of whom are her brothers, and a proportion $1 - p_z$ of whom are her nephews. And the "male production effect" captures her impact on the proportion of male eggs produced by the queen versus workers: her relative gain of brothers is p'_z, while her relative gain or loss of nephews exactly balances her forfeited sons and gained brothers.

Condition 2 clarifies the impact of monogamy upon helpful worker sterility: by increasing a worker's relatedness to her nephews and sisters, monogamy increases her inclusive-fitness benefit of promoting colony efficiency, and by increasing a worker's relatedness to her nephews, it increases her inclusive-fitness benefit of augmenting her fellow workers' production of sons. Hence, overall, monogamy promotes helpful worker sterility. Condition 2 also clarifies how Olejarz *et al.*'s (2015) model differs from Boomsma's (2007, 2009, 2013) model for the evolution of eusociality: in Boomsma's model, females trade away offspring for siblings as dispersers evolve into a non-totipotent worker caste, while in Olejarz *et al.*'s model, an existing non-totipotent worker caste trades away sons for brothers and nephews. Conditions 1 and 2 are exactly equivalent, are valid for recessive, dominant, or additive genetics, and can be obtained using standard kin-selection methodology (see *Methods*).

Alternative ecological scenarios: monogamy promotes worker sterility

Finally, we consider some alternative scenarios for the evolution of worker non-reproduction, using a demographically-explicit model of queen-worker competition over egg-laying. Whether we investigate sex-blind egg replacement by workers, soldier sterility in claustral inbreeders, or the evolution of eusociality via female non-dispersal, we find that monogamy always promotes helpful worker sterility (Fig. 4). This conclusion also holds if we alternatively consider a diploid mode of inheritance (see *Methods*).

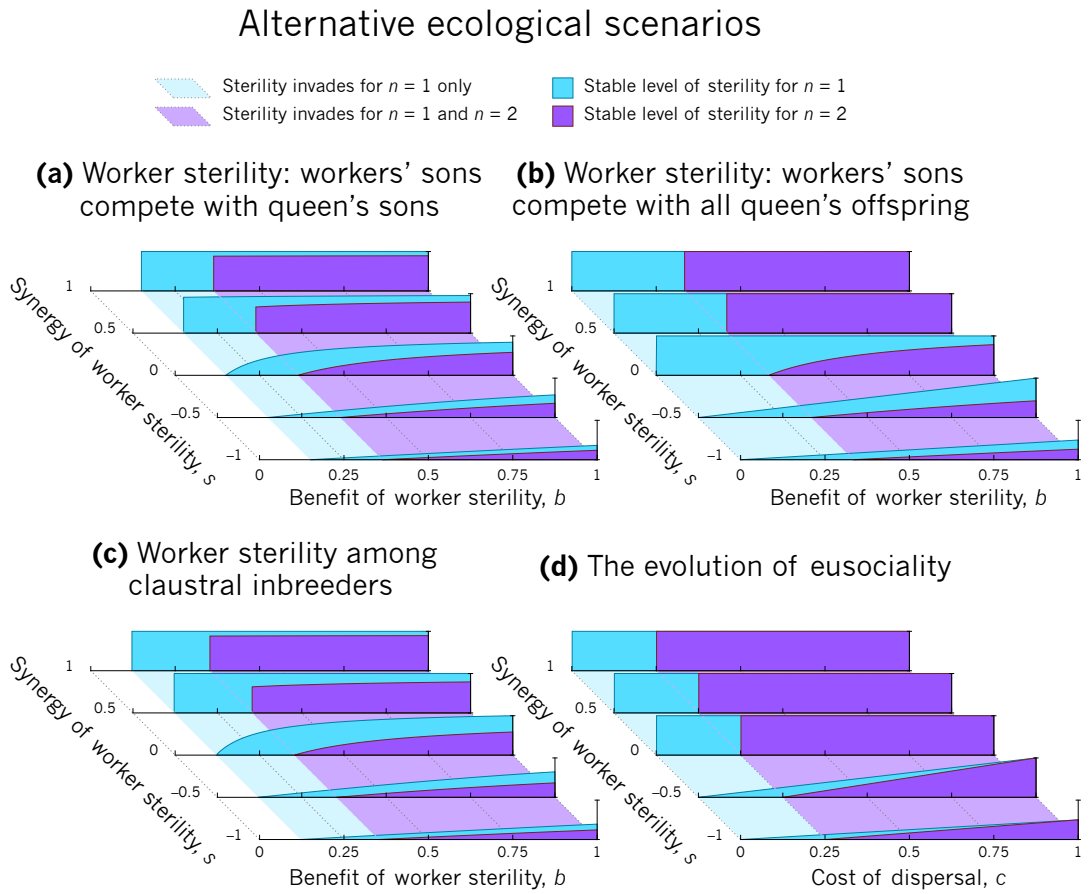


Figure 4: The evolution of worker sterility under alternative ecological scenarios. Here, we determine the stable level of worker sterility under four demographically-explicit models of worker sterility; see *Methods* for full details. **(a)** One possible assumption is that worker-laid males only compete with the queen's sons (*cf.* Olejarz *et al.* 2015). In this case, monogamy promotes worker sterility over promiscuity. **(b)** It is also possible to assume that worker-laid males compete with the queen's offspring of both sexes, and not just with the queen's sons. In this case, monogamy promotes worker sterility over promiscuity. **(c)** In the gall-forming thrips, the foundress produces an initial brood of female and male soldiers, who may produce part of the next brood by inbreeding amongst themselves (Chapman *et al.* 2002). Female soldiers can sacrifice part of their reproductive potential to invest more in defending their nestmates. In this case, monogamy promotes worker sterility over promiscuity. **(d)** A possible model for the evolution of eusociality involves dispersing, fully-reproductive females evolving into sterile workers, who stay in the nest to help, producing no offspring (Boomsma 2007, 2009, 2013). In this case, monogamy promotes worker sterility over promiscuity. We show results for $k = 4$ in **(a)** and $k = 2$ in **(b)** and **(c)**, where k is the workers' initial share in offspring production relative to the queen (see *Methods* for details).

Conclusion: monogamy promotes worker sterility

In seeming contrast to the predictions of inclusive-fitness theory, Olejarz *et al.*'s (2015) exact population-genetics analysis could not identify a consistent effect of monogamy on the evolution of worker sterility. This surprising result, if robust, would have not only overturned a considerable theoretical consensus, but would also have left a number of empirically-described patterns bereft of a predictive, explanatory framework. Happily, we have shown that by relaxing constraints on genetic variation (Fig. 1), considering the consequences of invasion rather than just its occurrence (Fig. 2), describing long-term evolutionarily-stable states (Fig. 3), and exploring a wide range of ecological scenarios (Fig. 4), a clear sterility-promoting effect of monogamy consistently emerges. Moreover, we have shown that the long-term evolutionary outcome is readily described, conceptualized, and explained by standard inclusive-fitness theory. In sum, a more comprehensive analysis based on Olejarz *et al.*'s (2015) exact population-genetics approach supports inclusive-fitness theory and its prediction that monogamy promotes the evolution of worker sterility.

Methods

1. Helpful versus harmful worker sterility and policing

Throughout the main text, our focus is on helpful worker sterility, where giving up some or all of her reproductive potential allows a worker to provide more help within her colony, as this biological assumption underpins most work on altruistic sib-rearing in social insects. However, the model of Olejarz *et al.* (2015), despite making strong genetic assumptions, makes few ecological assumptions about worker sterility, which means it may also describe harmful worker sterility. If worker sterility is harmful—namely, if worker sterility reduces colony efficiency and/or reduces other workers' personal fitness—monogamy may inhibit worker sterility, depending on the overall impact of sterility on a worker's inclusive fitness.

In this model, harmful worker sterility may occur via two routes—one operating through colony efficiency, r_z, and one operating through the queen's production of

males, p_z. The first case occurs when an increase in average worker sterility decreases colony efficiency—for example, if the sterility allele has a pleiotropic effect on worker condition which results in less-efficient work. In such a case, monogamy will inhibit the evolution of worker sterility relative to promiscuity, since promiscuity decreases relatedness between relatives, thereby lessening the harmful impact of sterility upon a worker's inclusive fitness via colony efficiency.

The second case occurs when an an increase in a focal worker's sterility harms the reproductive success of other workers. In the main text, we assume that when a worker becomes sterile, her forfeited sons are replaced partly by the queen's sons and partly by her sisters' sons, such that by forfeiting sons she gains both nephews and brothers. But if, due to the shape of the p_z function, the queen gains a larger proportion of sons than the worker forfeits (that is, when $p'_z > \frac{1-p_z}{1-z}$), this "outsized gain" by the queen must be balanced by *decreased* male production by other workers, such that, by becoming sterile, the focal worker loses nephews overall. If the focal worker loses nephews by becoming sterile (*i.e.*, when $\frac{1-p_z}{1-z} - p'_z < 0$; see condition 2), then promiscuity, by decreasing the worker's relatedness to nephews, may promote this harmful form of worker sterility over monogamy, unless this relative cost of sterility is countered by a colony efficiency benefit of sterility, which would be largest in magnitude under monogamy.

This second form of harmful worker sterility is connected with worker policing—that is, when workers invest resources in preventing other workers from laying eggs (Ratnieks 1988; Ratnieks & Visscher 1989). If worker sterility is harmful, then a worker gives up part of her personal fitness in order to decrease the reproduction of her fellow workers; this is analogous to costly worker policing. Standard inclusive-fitness theory (Ratnieks 1988; Ratnieks & Visscher 1989; Ratnieks *et al.* 2006) and empirical evidence (Wenseleers & Ratnieks 2006a, 2006b) have emphasized that promiscuity promotes worker policing, so the result that this harmful form of worker sterility may be promoted by promiscuity is not at all surprising.

For non-incremental increases in sterility, the condition for harmful sterility becomes $\frac{p_v-p_u}{v-u} > \frac{1-p_u}{1-u}$, where u is the level of worker sterility in the monomorphic population before the mutant allele is introduced, and v is the level of worker sterility

encoded by the mutant allele.

2. Explicit population-genetics analysis

In section 8, we extend the methods of Olejarz *et al.* (2015) to consider the invasion of an allele with an arbitrary effect on worker sterility; the results of this analysis are presented here. We find that a recessive allele encoding worker sterility v can invade a population monomorphic for sterility u when

$$\frac{r_{\frac{(2n-1)u+v}{2n}}}{r_u} > \frac{2\left(2n(1-u)+u-v\right)\left(2+n(1+p_u)\right)}{\left(\begin{array}{l} n(8+4n(1-u)-3u-5v)+2(u-v) \\ +(2+n)(2n(1-u)+u-v)p_u \\ -2n(2-u-v-n(1-u))p_{\frac{(2n-1)u+v}{2n}} \end{array}\right)}. \tag{3}$$

Similarly, we find that a dominant allele encoding worker sterility v can invade a population monomorphic for sterility u when

$$\frac{r_{\frac{u+v}{2}}}{r_u}\left(\begin{array}{l} 1+\left(\frac{(1-u)p_{\frac{u+v}{2}}}{2-u-v}+\frac{(1-v)\left((2-n)(u-v)+n(2-u-v)p_{\frac{(n-1)u+v}{n}}\right)}{2(n(1-u)+u-v)(2-u-v)}\right)\frac{r_{\frac{(n-1)u+v}{n}}}{r_u} \\ \\ +\frac{n(1-v)(1-p_{\frac{(n-1)u+v}{n}})}{n(1-u)+u-v}\frac{r_{\frac{(n-1)u+v}{n}}}{r_{\frac{u+v}{2}}} \end{array}\right) > 2. \tag{4}$$

Note that conditions 3 and 4 give both the invasion and stability of a given level of sterility: that is, if a sterility allele with effect v can invade a population monomorphic for sterility u, then this is the same as saying that a population monomorphic for sterility u is not stable to invasion by a sterility allele with effect v. For example, substituting $n = 1$, $u = 0$, $v = 1$ into condition 3 yields the condition for the invasion of a recessive sterility allele under single mating from Olejarz *et al.* (2015; their condition 1), while substituting $n = 1$, $u = 1$, $v = 0$ into condition 4 yields the condition for the stability of a recessive sterility allele under single mating from Olejarz *et al.* (2015; their condition 3).

In order to find when natural selection will favour a small increase in sterility δz, we make the substitution $v = u + \delta z$ into conditions 3 and 4 above. Then, by linearizing

r_z and p_z around the point $z = u$, we can recast these conditions in terms of the value and slope of r_z and p_z at this point. More specifically, for a recessive sterility allele, substituting $v = u + \delta z$ into condition 3 yields

$$\frac{r_{u+\frac{\delta z}{2n}}}{r_u} > \frac{2\left(2n(1-u)-\delta z\right)\left(2+n(1+p_u)\right)}{\left(\begin{array}{l} 4n(2+n)(1-u)-(2+5n)\delta z \\ +(2+n)(2n(1-u)-\delta z)p_u \\ -2n(2-n-(2-n)u-\delta z)p_{u+\frac{\delta z}{2n}} \end{array}\right)}.$$

Linearizing r_z and p_z around $z = u$, we replace $r_{u+\frac{\delta z}{2n}}$ with $r + \frac{\delta z}{2n} r'$, where $r = r_u$ and $r' = \frac{\mathrm{d}r}{\mathrm{d}z}|_{z=u}$. Similarly, we replace $p_{u+\frac{\delta z}{2n}}$ with $p + \frac{\delta z}{2n} p'$, where $p = p_u$ and $p' = \frac{\mathrm{d}p}{\mathrm{d}z}|_{z=u}$. This yields

$$\frac{r + \frac{\delta z}{2n} r'}{r} > \frac{2\left(2n(1-u)-\delta z\right)\left(2+n(1+p)\right)}{\left(\begin{array}{l} 4n(2+n)(1-u)-(2+5n)\delta z \\ +(2+n)(2n(1-u)-\delta z)p \\ -2n(2-n-(2-n)u-\delta z)(p+\frac{\delta z}{2n}p') \end{array}\right)}.$$

Eliminating the fractions on both sides, discarding terms of order δz^2 or higher, substituting z for u and simplifying yields

$$-\frac{1}{1-z}(1-p_z)(3n-2) + \frac{r'_z}{r_z}\left(4+3n(1+p_z)\right) - p'_z(2-n) > 0\,,$$

which is condition 1 of the main text.

Similarly, for a dominant sterility allele, substituting $v = u + \delta z$ into condition 4 yields

$$\frac{r_{u+\frac{\delta z}{2}}}{r_u}\left(\begin{array}{l} 1+\left(\frac{(1-u)p_{u+\frac{\delta z}{2}}}{2-2u-\delta z}+\frac{(1-u-\delta z)\left(n(2-2u-\delta z)p_{u+\frac{\delta z}{n}}-(2-n)\delta z\right)}{2(n(1-u)-\delta z)(2-2u-\delta z)}\right)\frac{r_{u+\frac{\delta z}{n}}}{r_u} \\ \\ +\frac{n(1-u-\delta z)\left(1-p_{u+\frac{\delta z}{n}}\right)}{n(1-u)-\delta z}\frac{r_{u+\frac{\delta z}{n}}}{r_{u+\frac{\delta z}{2}}} \end{array}\right) > 2\,.$$

By linearizing r_z and p_z around $z = u$ as above, we obtain

$$\frac{r+\frac{\delta z}{2}r'}{r}\left(\begin{array}{l} 1+\left(\frac{(1-u)(p+\frac{\delta z}{2}p')}{2-2u-\delta z}+\frac{(1-u-\delta z)\left(n(2-2u-\delta z)(p+\frac{\delta z}{n}p')-(2-n)\delta z\right)}{2(n(1-u)-\delta z)(2-2u-\delta z)}\right)\frac{r+\frac{\delta z}{n}r'}{r} \\ \\ +\frac{n(1-u-\delta z)\left(1-p-\frac{\delta z}{n}p'\right)}{n(1-u)-\delta z}\frac{r+\frac{\delta z}{n}r'}{r+\frac{\delta z}{2}r'} \end{array}\right) > 2\,.$$

Expanding all terms, discarding terms of order δz^2 or higher, substituting z for u and simplifying yields

$$-\frac{1}{1-z}(1-p_z)(3n-2)+\frac{r'_z}{r_z}\left(4+3n(1+p_z)\right)-p'_z(2-n)>0\,,$$

which, again, is condition 1 of the main text.

3. Invasion of a dominant sterility allele

In the main text, we discuss why promiscuity can sometimes favour the invasion of a recessive worker sterility allele. The invasion of a dominant worker sterility allele is similar, but in this case there are two "mutant" mating types which determine whether sterility can invade: a heterozygous mutant female mating with n wild-type males, and a wild-type female mating with one mutant male and $n-1$ wild-type males. These mating types produce colonies with a proportion $z = \frac{v}{2}$ and $z = \frac{v}{n}$ of sterile workers, respectively. Hence, for a sterility allele with full penetrance, under single mating ($n = 1$), it is the relative success of colonies with 50% and 100% sterile workers which determines whether a sterility allele with full penetrance can invade, while under double mating ($n = 2$), only the relative success of colonies with 50% sterile workers determines whether a sterility allele with full penetrance can invade. Therefore, if the relative success of colonies with 100% sterile workers is low, this could be enough to disfavour the invasion of a fully-penetrant worker sterility allele into a wild-type population under single but not double mating. Nonetheless, for the scenario investigated by Olejarz *et al.* (2015, their Fig. 8), we find that single mating always promotes the invasion of dominant sterility over double mating (Fig. 5). Moreover, allowing for helpful-sterility alleles showing the full range of degrees of penetrance and domi-

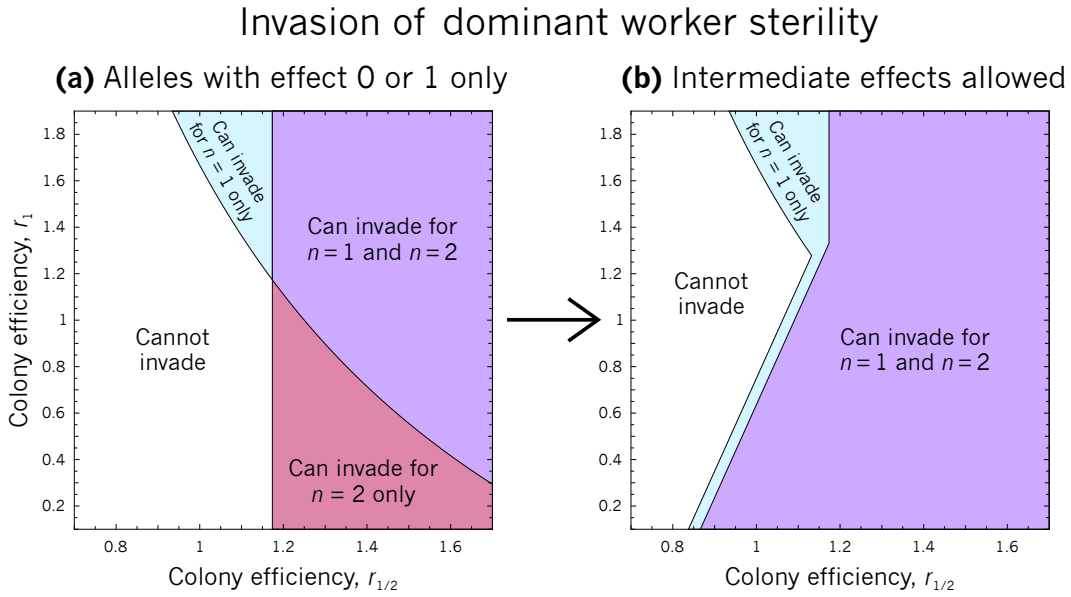


Figure 5: The invasion of worker sterility under dominant genetics, exploring the regions of parameter space where sterility can invade under single mating only, double mating only, both, or neither. **(a)** If we assume that only full-sterility alleles can arise, double mating sometimes promotes the invasion of sterility over single mating. But **(b)** if we assume that alleles encoding intermediate worker sterility can arise, double mating never promotes the invasion of sterility over single mating. In order to facilitate comparison with Figure 8 of Olejarz *et al.* (2015), we assume $p_z = 0.2 + 0.8z$, and for r_z we use the unique quadratic curve passing through the points specified by $r_0 = 1$, $r_{1/2}$, and r_1.

nance/recessivity, there is no scenario under which *some* sterility alleles can invade under double mating, and yet *no* sterility allele can invade under single mating.

4. Numerical experiments

Olejarz *et al.* (2015) performed numerical experiments to see whether sterility was more likely to invade under single mating or double mating. To do so, they constructed randomly-generated r_z functions according to one of two procedures. Here, we add to these procedures, bringing the number of possible methods for constructing the r_z function to five (Fig. 2a). Each involves drawing four random variates—here, notated as a, b, c, and d—from a normal distribution with mean 0 and standard deviation σ. In all cases, we assume $r_0 = 1$, and use the random variates to generate $r_{1/4}$, $r_{1/2}$, $r_{3/4}$, and r_1, which suffice to numerically integrate the evolutionary dynamics of worker sterility using the system of ODEs described by Olejarz *et al.* (2015). We restrict our attention here to the invasion of an allele encoding full sterility in its carriers, under either recessive or dominant genetics.

The first procedure, "random noise", is equivalent to Procedure 1 in Olejarz *et al.* (2015). Here, we set $r_{1/4} = r_0 + a$, $r_{1/2} = r_0 + b$, $r_{3/4} = r_0 + c$, and $r_1 = r_0 + d$. Note that the four values are completely uncorrelated with each other; sequential values of r_z are independent from previous values, which is why we have named this procedure "random noise". This procedure might generate plausible r_z functions for a population where every colony-level increase in worker sterility were to completely erase the effect of any previous increase in worker sterility, replacing it with a new, random effect.

The second procedure, "plateau", is equivalent to Procedure 2 in Olejarz *et al.* (2015). Here, the values $r_{1/4}$, $r_{1/2}$, $r_{3/4}$, and r_1 are drawn from a correlated multivariate normal distribution. This can be simulated by transforming four uncorrelated normal variates; one way of doing this is by using the matrix

$$\begin{bmatrix} 1 & \rho & \rho & \rho \\ \rho & 1 & \rho & \rho \\ \rho & \rho & 1 & \rho \\ \rho & \rho & \rho & 1 \end{bmatrix},$$

where ρ is the desired correlation between each variate. By multiplying the vector of uncorrelated variates by the Cholesky decomposition of this matrix, one obtains four correlated variates

$$\begin{aligned}
a' &= a \\
b' &= a\rho + b\sqrt{1-\rho^2} \\
c' &= a\rho + b\frac{\rho\sqrt{1-\rho}}{\sqrt{1+\rho}} + c\sqrt{3-2\rho-\frac{2}{1+\rho}} \\
d' &= a\rho + b\frac{\rho\sqrt{1-\rho}}{\sqrt{1+\rho}} + c\frac{\rho\sqrt{3-2\rho-\frac{2}{1+\rho}}}{1+2\rho} + d\sqrt{\frac{1+\rho(2-3\rho)}{1+2\rho}}\,.
\end{aligned}$$

Now, we set $r_{1/4} = r0 + a'$, $r_{1/2} = r0 + b'$, $r_{3/4} = r0 + c'$, and $r_1 = r_0 + d'$. Note that, because the variables are correlated, the first "step" (from r_0 to $r_{1/4}$) tends to be larger in magnitude than subsequent "steps" (*i.e.*, from $r_{1/4}$ to $r_{1/2}$, $r_{1/2}$ to $r_{3/4}$, or $r_{3/4}$ to r_1), which is why we have named this procedure "plateau". This procedure might generate plausible r_z functions for a population in which worker sterility brings diminishing returns to colony productivity, where these diminishing returns happen to set in near $z = 1/4$.

Note that both the "random noise" and "plateau" procedures tend to produce r_z functions that disadvantage single mating relative to double mating. For the "random noise" procedure, this is because although the procedure is just as likely to produce a peak at $z = 1/2$ (which would favour single mating) as at $z = 1/4$ (which would favour double mating), workers at $z = 1/2$ are typically "trading away" more male production than workers at $z = 1/4$ (since $p_{1/2} \geq p_{1/4}$), yet, on average, they are receiving the same expected increase in productivity; hence, single mating is relatively disfavoured. And since the "plateau" procedure tends to produce colony efficiency functions with diminishing returns on worker sterility for colonies with $z > 1/4$, it is much more likely to produce an r_z function with a relative peak at $z = 1/4$ rather than a relative peak at $z = 1/2$.

The third procedure, "random steps", sets each point in r_z to the value of the previous point plus a random perturbation: $r_{1/4} = r_0 + a$, $r_{1/2} = r_{1/4} + b$, $r_{3/4} = r_{1/2} + c$, and $r_1 = r_{3/4} + d$. This procedure might generate plausible r_z func-

tions if each increase in worker sterility had a random increasing or decreasing effect on colony productivity. The fourth procedure, "increasing steps", is similar, except steps are constrained to be positive: $r_{1/4} = r_0 + |a|$, $r_{1/2} = r_{1/4} + |b|$, $r_{3/4} = r_{1/2} + |c|$, and $r_1 = r3/4 + |d|$. This procedure might generate plausible r_z functions if each increase in worker sterility added a random increase to colony productivity. The fifth procedure, "linear", uses a single normal variate to establish a constant step size for r_z: $r_{1/4} = r_0 + a$, $r_{1/2} = r_{1/4} + a$, $r_{3/4} = r_{1/2} + a$, and $r_1 = r_{3/4} + a$. This procedure might generate plausible r_z functions if each increase in worker sterility had a consistent increasing or decreasing effect on colony productivity. For each of these new procedures, later points in r_z depend on earlier points, but there is no tendency for "steps" between points in r_z to change in average magnitude.

In Fig. 2, we test each of these 5 procedures to see whether single or double mating promotes the invasion (Fig. 2b) or equilibrium level of sterility (Fig. 2c) more, for recessive versus dominant sterility. The form of p_z we use ($p_z = q + (1 - q)z$, with $q = 0.2$), chosen for comparison with the numerical experiments of Olejarz *et al.* (2015, their Table 1), prevents worker sterility from resulting in a net loss of nephews (see *Helpful versus harmful worker sterility and policing*, above). Beneath the bar charts in Fig. 2d, we show the percentage of experiments for which the exclusive invasion of sterility under either single or double mating occurred with an initially-decelerating r_z (*i.e.*, where $r_{1/2} - r_{1/4} < r_{1/4} - r_0$). Note that, for these values of p_z, double mating only promotes the invasion of sterility over single mating when r_z is initially-decelerating. In Fig. 2c, error bars show bootstrapped 95% confidence intervals for average worker sterility.

5. ESS analysis

By setting the left-hand side of condition (2) to zero, it is possible to find a convergence-stable point (Davies *et al.* 2016) for worker sterility. At these points, natural selection will not favour the invasion of an allele encoding either a small increase or a small decrease to worker sterility (*i.e.*, convergence-stable points are stable to small perturbations); moreover, for a population playing a strategy that is close to a convergence-stable point, natural selection will favour the invasion of strategies between the

population strategy and the convergence-stable point (*i.e.*, convergence-stable points are reachable from nearby states). However, a convergence-stable point is only an evolutionarily-stable strategy (ESS) if *no* alternative allele can invade at this point. Therefore, in order to find a true ESS, we treat convergence-stable points as "candidate ESSs", then use conditions 3 and 4 to determine whether any alternative allele can invade a population monomorphic for the candidate ESS under the appropriate regime of dominance or recessivity. If no alternative allele can invade, the candidate ESS is a true ESS. In Fig. 3, true ESSs are shown.

Note that it is possible for an ESS to *not* be convergence-stable, and this method will not identify such states. However, we are only interested in ESSs that are reachable, *i.e.*, both convergence-stable and evolutionarily-stable. Such strategies are called "continuously-stable strategies" (CSSs; Eshel 1983).

6. Demographically-explicit ecological scenarios

In section 9, we develop a general kin-selection model for the evolution of worker sterility. This analysis can be used to investigate a variety of ecological scenarios. Here, we present four such scenarios for the evolution of worker sterility.

6.1 Scenario A. Workers' sons replace queen's sons

In this scenario, we assume that non-sterile workers replace the queen's sons with their own sons, as in the model of Olejarz *et al.* (2015). Following these assumptions, we find that natural selection will favour an increase to worker sterility, z, when

$$\underbrace{-\frac{1-p_z}{1-z}R_{\text{son}}}_{\text{sacrifice effect}}+\underbrace{\frac{r'_z}{r_z}\left(R_{\text{sis}}+p_zR_{\text{bro}}+(1-p_z)R_{\text{neph}}\right)}_{\text{efficiency effect}}+\underbrace{p'_zR_{\text{bro}}+\left(\frac{1-p_z}{1-z}-p'_z\right)R_{\text{neph}}}_{\text{male production effect}}>0\,, \tag{5}$$

where $R_{\text{son}}=\frac{1}{2}$, $R_{\text{neph}}=\frac{2+n}{8n}$, $R_{\text{sis}}=(1+p_z)\frac{2+n}{8n}$, and $R_{\text{bro}}=\frac{1}{4}$. As explained in the main text, the left-hand side of condition 5 can be interpreted as the inclusive-fitness effect experienced by a worker who stops laying male eggs. The "sacrifice effect" captures the direct cost of her sterility, in that she forfeits her relative share $\frac{1-p_z}{1-z}$ of all worker-laid males. The "efficiency effect" captures her impact on colony efficiency,

which increases by a relative amount $\frac{r'_z}{r_z}$, augmenting the production of her sisters and of colony-produced males, a proportion p_z of whom are her brothers, and a proportion $1 - p_z$ of whom are her nephews. And the "male production effect" captures her impact on the proportion of male eggs produced by the queen versus workers: her relative gain of brothers is p'_z, while her relative gain or loss of nephews exactly balances her forfeited sons and her gained brothers.

Similarly, natural selection favours an increase to the queen's sex allocation, x (her proportion of resources allocated to daughters), when

$$\frac{1}{x} - \frac{1}{1-x} > 0\,. \tag{6}$$

That is, natural selection favours an increased investment into daughters when $x < 1/2$, and a decreased investment into daughters when $x > 1/2$, such that an even sex ratio is favoured overall, regardless of worker sterility.

6.2 Scenario B. Workers' sons compete with all queen's offspring

It is also possible to assume that, rather than only displacing the queen's sons, workers' sons compete with the queen's sons and daughters equally. This scenario may apply if workers do not discern between fertilized and unfertilized eggs when they replace the queen's eggs with their own; alternatively, it may apply if rather than replacing the queen's eggs, the workers simply lay their eggs in the communal nest, and all queen-produced and worker-produced offspring have the same expected survival. Following these assumptions, we find that natural selection will favour an increase to worker sterility, z, when

$$\underbrace{-\frac{1-p_z}{1-z}R_{\text{son}}}_{\text{sacrifice effect}} + \underbrace{\frac{r'_z}{r_z}\left(xp_zR_{\text{sis}} + (1-x)p_zR_{\text{bro}} + (1-p_z)R_{\text{neph}}\right)}_{\text{efficiency effect}}$$
$$\underbrace{+xp'_zR_{\text{sis}} + (1-x)p'_zR_{\text{bro}} + \left(\frac{1-p_z}{1-z} - p'_z\right)R_{\text{neph}}}_{\text{juvenile production effect}} \;>\; 0\,, \tag{7}$$

where p_z is the proportion of all juveniles on the patch that are produced by the queen, $R_{\text{son}} = \frac{1}{2}$, $R_{\text{neph}} = \frac{2+n}{8n}$, $R_{\text{sis}} = \frac{1+(1-2x)p_z}{xp_z}\frac{2+n}{8n}$, and $R_{\text{bro}} = \frac{1}{4}$. In this model, queen sex allocation alters the relative reproductive value of a female compared to that of a male, $\frac{1+(1-2x)p_z}{xp_z}$ (the product of the relative reproductive value of all females compared to that of all males, $\frac{1+(1-2x)p_z}{1-xp_z}$, and the number of females relative to the number of males, $\frac{1-xp_z}{xp_z}$), which comes into the expression for R_{sis}. Similarly to condition 5, the left-hand side of condition 7 can be interpreted as the inclusive-fitness effect experienced by a worker who stops laying male eggs. Here, the "sacrifice effect" captures the direct cost of her sterility, in that she forfeits her relative share $\frac{1-p_z}{1-z}$ of all worker-laid males. The "efficiency effect" captures her impact on colony efficiency, which increases by a relative amount $\frac{r'_z}{r_z}$, a proportion xp_z of which goes towards sisters, $(1-x)p_z$ towards brothers, and $1-p_z$ towards nephews. And the "juvenile production effect" captures her impact on the proportion of eggs produced by the queen versus workers: her relative gain of sisters is xp'_z, and her relative gain of brothers is $(1-x)p'_z$, and so her relative gain of nephews exactly balances her lost sons, less her gained brothers and sisters.

In this scenario, queen sex allocation is not independent of worker sterility. We find that natural selection favours an increase to the queen's investment in daughters, x, when

$$\frac{1+p_z}{2x} - \frac{p_z}{1-x} > 0\,; \tag{8}$$

hence, when all colony offspring are queen-laid ($p_z = 1$), the queen favours an even sex ratio ($x = 1/2$), but as the proportion of colony offspring laid by workers increases, the queen favours an increasingly female-biased sex ratio. Specifically, the equilibrium sex ratio is $x^* = \frac{1+p_z}{1+3p_z}$.

6.3 Scenario C. Worker sterility among claustral inbreeders

Here, we assume that the queen produces a first brood of female and male soldiers, who mate amongst themselves; the second brood of female and male dispersers is partly produced by the queen and partly produced by the soldiers, as in the gall-forming social thrips (Chapman 2002). For simplicity, we assume here that queens

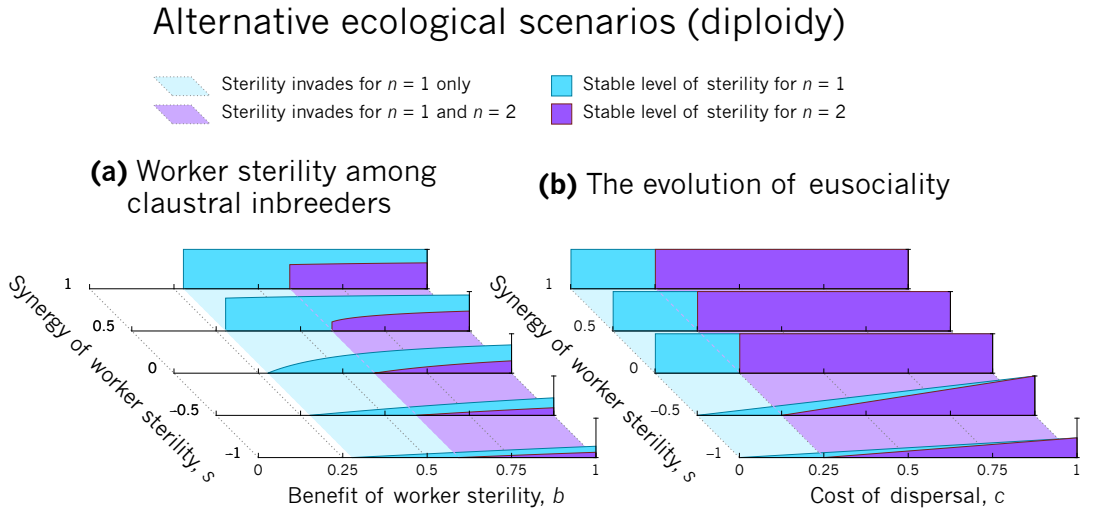


Figure 6: The evolution of worker sterility under alternative ecological scenarios, for diploidy. Here, we determine the stable level of worker sterility under two demographically-explicit models of worker sterility; see *Methods* for full details. **(a)** For claustral inbreeders under diploidy, monogamy promotes worker sterility over promiscuity; we show results for $k = 4$ here. **(b)** For the evolution of eusociality via non-dispersing female workers under diploidy, monogamy promotes worker sterility over promiscuity.

and soldiers produce an even sex ratio for the second brood, but allowing sex ratio evolution does not change the results qualitatively (not shown). Following these assumptions, we find that natural selection favours an increase to the sterility of female soldiers, z, when

$$\underbrace{-\frac{1-p_z}{1-z}\left(R_{\text{dau}}+R_{\text{son}}\right)}_{\text{sacrifice effect}}+\underbrace{\frac{r'_z}{r_z}\left(p_z\left(R_{\text{sis}}+R_{\text{bro}}\right)+\left(1-p_z\right)\left(R_{\text{niece}}+R_{\text{neph}}\right)\right)}_{\text{efficiency effect}}$$
$$\underbrace{+p'_z\left(R_{\text{sis}}+R_{\text{bro}}\right)+\left(\frac{1-p_z}{1-z}-p'_z\right)\left(R_{\text{niece}}+R_{\text{neph}}\right)}_{\text{juvenile production effect}} \;>\; 0\,, \tag{9}$$

where, under haplodiploidy, $R_{\text{dau}} = \frac{5+p_z}{6}$, $R_{\text{son}} = \frac{3+p_z}{6}$, $R_{\text{niece}} = \frac{3+6n+p_z}{12n}$, $R_{\text{neph}} = \frac{3+2n+p_z}{12n}$, $R_{\text{sis}} = \frac{3+2n+p_z}{6n}$, and $R_{\text{bro}} = \frac{1}{3}$. Because this scenario does not require arrhenotokous parthenogenesis of males, it also applies to diploid populations. Under diploidy, $R_{\text{dau}} = R_{\text{son}} = \frac{11+p_z}{16}$ and $R_{\text{niece}} = R_{\text{neph}} = R_{\text{sis}} = R_{\text{bro}} = \frac{1+n}{4n}$ (Fig. 6a). Similarly to condition 7, the left-hand side of condition 9 can be interpreted as the inclusive-fitness effect experienced by a worker who stops laying eggs; but in condi-

tion 9, the female worker's "sacrifice effect" involves giving up both daughters and sons; the "efficiency effect" involves an increase in both niece and nephew production as well as sister and brother production; and the "juvenile production effect" involves the focal worker gaining both sisters and brothers, while her gain or loss of nieces and nephews balances her forfeited offspring and her gained siblings.

6.4 Scenario D. The evolution of eusociality

Here, we assume that the queen produces and provisions a first brood of females, and then produces a second batch of female and male eggs. Each first-brood female can either disperse—leave the nest, mate, and produce female and male offspring on her own—or work—stay in the nest and help to raise the queen's second-brood offspring without producing any offspring of her own. We assume that each worker can raise b siblings, on average, in her natal nest, and that each disperser can raise $b(1-c)$ offspring, on average, in her newly-founded nest, where c represents the cost of dispersal; and, additionally, that workers may synergistically or antagonistically interact according to the parameter s, such that if the total number of female workers is Kz, then in total workers can raise $Kzb(1+sz)$ of the queen's second-brood offspring. This model is similar to the one considered by Boomsma (2007, 2009, 2013) for the evolution of eusociality. Following these assumptions, we find that natural selection will favour an increase to worker sterility, z, when

$$\underbrace{-b(1-c)\left(R_{\text{dau}}+R_{\text{son}}\right)}_{\text{sacrifice effect}}+\underbrace{b(1+2sz)\left(R_{\text{sis}}+R_{\text{bro}}\right)}_{\text{efficiency effect}}>0\,, \tag{10}$$

where $R_{\text{dau}}=R_{\text{son}}=\frac{1}{2}$, $R_{\text{sis}}=\frac{2+n}{4n}$, and $R_{\text{bro}}=\frac{1}{4}$. As with scenario C, this scenario also applies to diploid populations; under diploidy, $R_{\text{dau}}=R_{\text{son}}=\frac{1}{2}$ and $R_{\text{sis}}=R_{\text{bro}}=\frac{1+n}{4n}$ (Fig. 6b). When $z=0$, this condition reduces to

$$c>\frac{n-1}{2n}$$

under both haplodiploidy and diploidy; that is, under strict monogamy ($n=1$), any marginal benefit of rearing siblings over offspring (for example, any non-zero cost of

dispersal, mating, or nest founding) suffices to favour the invasion of sterile workers, regardless of the level of worker synergy, s; but with any level of multiple mating ($n > 1$), a threshold dispersal cost of at least $\frac{n-1}{2n}$ is required for natural selection to favour the invasion of sterile workers (Fig. 4d; Fig. 6b). In other words, only marginal efficiency gains are needed for worker sterility to invade under strict monogamy (Boomsma 2007, 2009, 2013).

6.5 Explicit forms for r_z and p_z

Scenarios A, B, and C above are independent of the particular r_z and p_z functions used. However, for preparing Figs. 4 and 6, we used the explicit forms

$$\begin{aligned} r_z &= 1 + bz + sz^2 \text{ and} \\ p_z &= \frac{1}{1 + k(1-z)}. \end{aligned}$$

The r_z function above has three components: a baseline efficiency of 1; bz, representing a linear fitness benefit for each sterile worker; and sz^2, representing an "interaction effect" of worker sterility. We use the parameter s to examine scenarios where multiple sterile workers results in either synergy ($s > 0$) or diminishing returns ($s < 0$) to colony productivity.

The p_z function given above corresponds to a model in which the queen and $k(1-z)$ reproductive workers each take an equal share of offspring production. Alternatively, k can capture not only the total number of workers but also their ability to control offspring production relative to the queen; for example, halving k could represent either a halving in the number of workers or a halving of their relative ability to control offspring production, keeping the number of workers constant.

A function of this form can also model more complicated demographic processes: for example, if we assume that there are N workers, each of whom replaces a random egg with their own at rate W, while the queen can replace a workers' egg with her own at rate Q, then the form above gives the proportion of eggs produced by the queen at equilibrium when $k = \frac{NW}{Q}$. In models where worker-laid and queen-laid individuals compete equally, regardless of their sex, production of eggs and replacement of

eggs will often be equivalent processes: that is, the form given above for p_z also holds if workers, rather than replacing the queen's eggs, simply lay their own eggs in the communal nest without replacement. In that case, the r_z function would capture the overall production and survival of eggs.

6.6 Stable level of sterility

For Fig. 4, we determine the convergence-stable point (Davies *et al.* 2016) for sterility by numerically integrating the selection gradients for sterility and sex allocation (left-hand sides of conditions 5–10). First, we set the sex ratio to $x = \bar{x} = 1/2$ and allow it to evolve in the absence of worker sterility ($Z = z = \bar{z} = 0$) until it reaches its equilibrium value. Then, we allow both the sex ratio and sterility to coevolve, until equilibrium is reached for both traits.

7. Stochastic individual-based model

To verify the results of our kin selection analysis (Fig. 3), we implemented a stochastic individual-based model in C++. Here, each individual comprises a locus encoding their breeding value for worker sterility, Z. The locus comprises one or two genes, depending on whether the individual is haploid or diploid, and each gene is represented by a real number $\gamma \in [0, 1]$. Breeding values are determined by averaging genic values: hence, a haploid individual with genotype γ has breeding value $Z = \gamma$, while a diploid individual with genotype γ_1, γ_2 has breeding value $Z = (\gamma_1 + \gamma_2)/2$.

At the beginning of each generation, M mated females each produce K female workers on their home patch. Each worker has a probability Z of being sterile. The patch average sterility z determines the colony productivity r_z and the proportion of males produced by the queen p_z. The next generation of breeders is then produced: first, a patch is randomly selected from the population with probability proportional to its colony efficiency, r_z, and a female is produced by the queen on that patch; then, another n patches are randomly selected with replacement, with probability proportional to their colony efficiency, and each of these n patches produces a male (from the queen with probability p_z, or from a random reproductive worker on that patch with

probability $1 - p_z$); the female mates with these n males, and this process is performed M times, at which point all the M mated females replace the foundresses of existing patches. All other individuals on each patch die, returning the population to the beginning of the life cycle.

Simulations start with a monomorphic population in which all $\gamma = 0$, and hence $Z = 0$ for each individual. A gene in a newly-produced individual has a 1% probability of mutating, in which case its genic value changes from γ to $\gamma' = \max(0, \min(\gamma + \delta, 1))$, where δ is drawn from a normal distribution with mean 0 and standard deviation 0.01. We validated this stochastic individual-based model by using it to verify the analytical conditions of Olejarz *et al.* (2015; Fig. 7).

8. Explicit population-genetics analysis

Here, we analyse the invasion of a sterility allele into a wild-type population. The population is initially monomorphic for an allele A encoding sterility with penetrance $0 \leq u \leq 1$, and a rare mutant allele a is introduced which encodes sterility with penetrance $0 \leq v \leq 1$. Throughout, we closely follow the approach of Olejarz *et al.* (2015), whose analysis is equivalent to ours with the assumptions that u and v are restricted to either 0 or 1.

We denote colony types by the genotype of the queen and the genotypes of her mating partners. Hence, $X_{AA,m}$ is the frequency of colonies with an AA queen, m mutant (a) males, and $n - m$ wild-type (A) males; similarly for $X_{Aa,m}$ and $X_{aa,m}$. At any given time step, we also keep track of the number of reproductive females of each genotype—x_{AA}, x_{Aa}, and x_{aa}—and the number of reproductive males of each genotype—y_A and y_a. Matings between reproductives lead to the establishment of new colonies; hence, the evolutionary dynamics of colony types are captured by:

$$\begin{aligned}
\dot{X}_{AA,m} &= x_{AA}\binom{n}{m} y_A^{n-m} y_a^m - \phi X_{AA,m} \\
\dot{X}_{Aa,m} &= x_{Aa}\binom{n}{m} y_A^{n-m} y_a^m - \phi X_{Aa,m} \\
\dot{X}_{aa,m} &= x_{aa}\binom{n}{m} y_A^{n-m} y_a^m - \phi X_{aa,m} \,. \qquad (11)
\end{aligned}$$

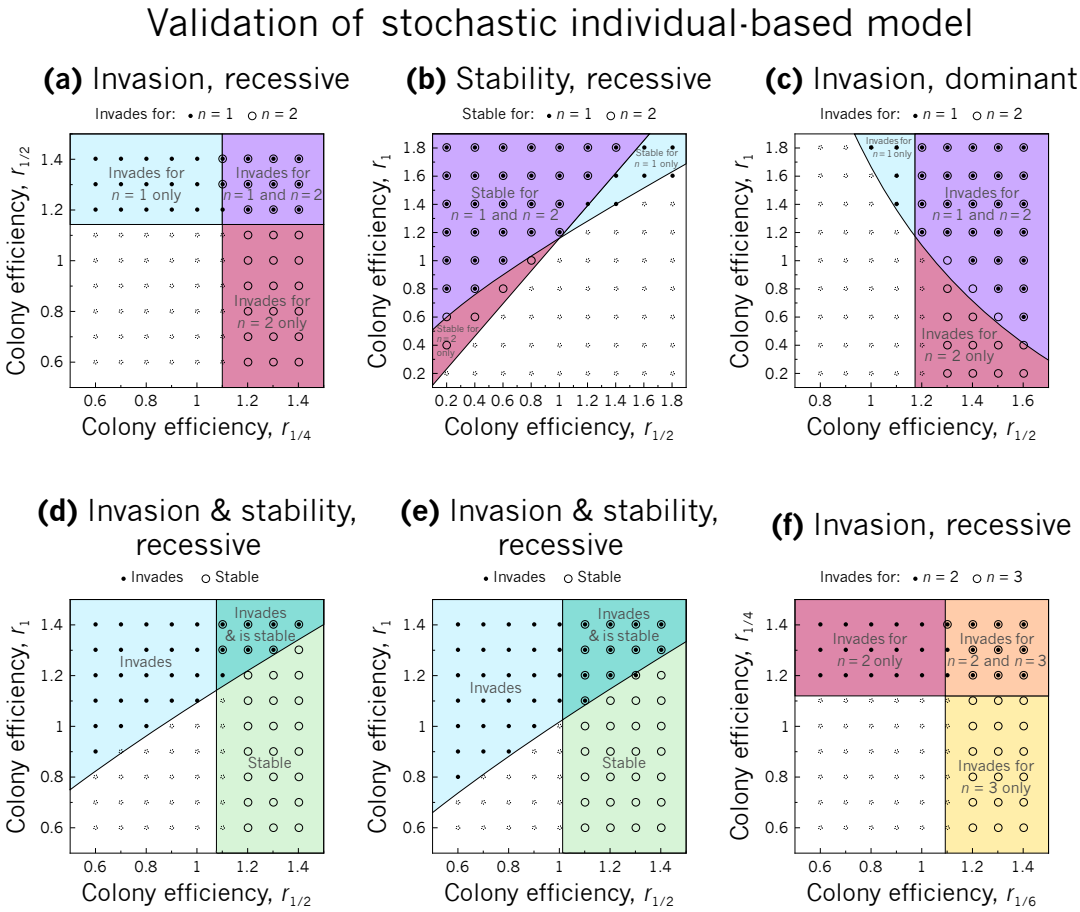


Figure 7: Here we validate our stochastic individual-based model by testing the analytical predictions of Olejarz *et al.* (2015) against the individual-based model with constrained allelic variation (such that alleles can only encode sterility 0 or 1). Analytical predictions are shown as shaded regions; model results are shown as markers overlaid, with the meaning of each marker shown above each plot; dashed open circles indicate that neither annotated outcome occurred. For each parameter combination, populations either started with no sterility (for testing the invasion of sterility) or full sterility (for testing the stability of sterility), and the rare allele was introduced at frequency 0.01 every 1000 generations. After 18 such introductions (to reduce the chance of genetic drift eliminating the rare allele), the population was left without further introduction of rare alleles for 3,000 generations. The outcome of a given experiment depends on whether, at the end of these 20,000 generations, the population had returned to its initial state or not. Parameters were chosen to match **(a)** Figure 3A, **(b)** Figure 3B, **(c)** Figure 8, **(d)** Figure 5A, **(e)** Figure 5B, and **(f)** Figure 9 of Olejarz *et al.* (2015).

That is, the rate of establishment of new AA, m colonies is proportional to the frequency of reproductive AA females, multiplied by their probability of mating with exactly $n - m$ wild-type males and m mutant males; similarly for Aa, m and aa, m colonies.

The death rate of existing colonies, ϕ, is defined as

$$\phi = (x_{AA} + x_{Aa} + x_{aa})(y_A + y_a)^n \tag{12}$$

in order to enforce a density constraint, namely:

$$\sum_{m=0}^{n} (X_{AA,m} + X_{Aa,m} + X_{aa,m}) = 1\,. \tag{13}$$

8.1 Reproductives if the mutant allele is dominant

When the mutant allele is dominant, the production of each type of reproductive female (x_{AA}, x_{Aa}, x_{aa}) and male (y_A, y_a) is:

$$x_{AA} = \sum_{m=0}^{n} \left\{ \frac{n-m}{n} r_{\frac{(n-m)u+mv}{n}} X_{AA,m} + \frac{n-m}{2n} r_{\frac{(n-m)u+(n+m)v}{2n}} X_{Aa,m} \right\}$$

$$x_{Aa} = \sum_{m=0}^{n} \left\{ \frac{m}{n} r_{\frac{(n-m)u+mv}{n}} X_{AA,m} + \frac{1}{2} r_{\frac{(n-m)u+(n+m)v}{2n}} X_{Aa,m} + \frac{n-m}{n} r_v X_{aa,m} \right\}$$

$$x_{aa} = \sum_{m=0}^{n} \left\{ \frac{m}{2n} r_{\frac{(n-m)u+(n+m)v}{2n}} X_{Aa,m} + \frac{m}{n} r_v X_{aa,m} \right\}$$

$$y_A = \sum_{m=0}^{n} \left\{ \begin{array}{l} \left(p_{\frac{(n-m)u+mv}{n}} + \frac{\frac{n-m}{n}(1-u)+\frac{1}{2}\frac{m}{n}(1-v)}{\frac{n-m}{n}(1-u)+\frac{m}{n}(1-v)} \left(1 - p_{\frac{(n-m)u+mv}{n}}\right) \right) r_{\frac{(n-m)u+mv}{n}} X_{AA,m} \\ + \left(\begin{array}{l} \frac{1}{2} p_{\frac{(n-m)u+(n+m)v}{2n}} \\ \\ + \frac{\frac{n-m}{2n}(1-u)+\frac{1}{4}(1-v)}{\frac{n-m}{2n}(1-u)+\frac{1}{2}(1-v)+\frac{m}{2n}(1-v)} \left(1 - p_{\frac{(n-m)u+(n+m)v}{2n}}\right) \end{array} \right) r_{\frac{(n-m)u+(n+m)v}{2n}} X_{Aa,m} \\ \\ + \left(\frac{1}{2}\frac{n-m}{n}\right)(1-p_v) r_v X_{aa,m} \end{array} \right\}$$

$$y_a = \sum_{m=0}^{n} \left\{ \begin{array}{l} \left(\frac{\frac{1}{2}\frac{m}{n}(1-v)}{\frac{n-m}{n}(1-u)+\frac{m}{n}(1-v)} \left(1 - p_{\frac{(n-m)u+mv}{n}}\right)\right) r_{\frac{(n-m)u+mv}{n}} X_{AA,m} \\ \\ + \left(\begin{array}{l} \frac{1}{2} p_{\frac{(n-m)u+(n+m)v}{2n}} \\ \\ + \frac{\frac{1}{4}(1-v)+\frac{m}{2n}(1-v)}{\frac{n-m}{2n}(1-u)+\frac{1}{2}(1-v)+\frac{m}{2n}(1-v)} \left(1 - p_{\frac{(n-m)u+(n+m)v}{2n}}\right) \end{array} \right) r_{\frac{(n-m)u+(n+m)v}{2n}} X_{Aa,m} \\ \\ + \left(p_v + \left(\frac{1}{2}\frac{n-m}{n} + \frac{m}{n}\right)(1-p_v)\right) r_v X_{aa,m} \end{array} \right\} . \quad (14)$$

These equations can be understood as follows. First, note that in an AA, m colony, a fraction $z = \frac{n-m}{n}u + \frac{m}{n}v = \frac{(n-m)u+mv}{n}$ of workers will be sterile (AA workers with probability u, and Aa workers with probability v); in an Aa, m colony, a fraction $z = \frac{n-m}{2n}u + \frac{1}{2}v + \frac{m}{2n}v = \frac{(n-m)u+(n+m)v}{2n}$ of workers will be sterile (AA workers with probability u, and Aa and aa workers with probability v); and in an aa, m colony, a fraction $z = \frac{n-m}{n}v + \frac{m}{n}v = v$ of workers will be sterile (Aa and aa workers with probability v). That is why these values of z as subscripts to the r_z and p_z functions are always associated, above, with their associated colony frequencies, $X_{AA,m}$, $X_{Aa,m}$, and $X_{aa,m}$, respectively.

For female reproductives, each separate term within the curly braces above combines three elements; we will take the first term in curly braces in the x_{AA} line,

$$\frac{n-m}{n} r_{\frac{nu+m(v-u)}{n}} X_{AA,m} ,$$

as an example. The three elements are the frequency of a given colony type (*i.e.*, $X_{AA,m}$); the productivity of that colony type, as a function of the fraction of sterile workers within colonies of that type (*i.e.*, $r_{\frac{nu+m(v-u)}{n}}$); and the fraction of females and/or males produced by that colony type with the corresponding genotype (*i.e.*, a fraction $\frac{n-m}{n}$ of females produced in AA, m colonies have genotype AA, which is why they add to the quantity x_{AA}). Each term within equation 14 can be broken down in this way.

Accordingly, the production of female reproductives can be understood as follows: AA, m colonies produce $\frac{n-m}{n}$ AA females and $\frac{m}{n}$ Aa females; Aa, m colonies produce

$\frac{n-m}{2n}$ AA females, $\frac{1}{2}$ Aa females, and $\frac{m}{2n}$ aa females; and aa, m colonies produce $\frac{n-m}{n}$ Aa females and $\frac{m}{n}$ aa females.

Male production is more complicated, since both queens and workers produce males, but the principle is the same. We will take the first term in curly braces in the y_A line,

$$\left(p_{\frac{(n-m)u+mv}{n}} + \frac{\frac{n-m}{n}(1-u) + \frac{1}{2}\frac{m}{n}(1-v)}{\frac{n-m}{n}(1-u) + \frac{m}{n}(1-v)} \left(1 - p_{\frac{(n-m)u+mv}{n}}\right) \right) r_{\frac{(n-m)u+mv}{n}} X_{AA,m} \,,$$

as an example. Here, the overall productivity of AA, m colonies (*i.e.*, $r_{\frac{(n-m)u+mv}{n}} X_{AA,m}$) goes towards the production of both the queen's sons and workers' sons. In particular, the queen is AA, so all her sons have genotype A, and the queen produces a fraction $p_{\frac{(n-m)u+mv}{n}}$ of males in the colony. Simultaneously, the workers—whose sons comprise a fraction $1 - p_{\frac{(n-m)u+mv}{n}}$ of colony male production—are $\frac{n-m}{n}$ AA and $\frac{m}{n}$ Aa; in the former group, workers are reproductive with probability $1 - u$, while in the latter group, workers are reproductive with probability $1 - v$; and all the sons of the first group will be A, while only half of the sons of the second group will be A. Hence, overall, a fraction $\left(\frac{\frac{n-m}{n}(1-u)+\frac{1}{2}\frac{m}{n}(1-v)}{\frac{n-m}{n}(1-u)+\frac{m}{n}(1-v)}\right)\left(1 - p_{\frac{(n-m)u+mv}{n}}\right)$ of males produced in AA, m colonies are A males produced by workers. Note that the expressions for y_A and y_a can be further simplified, but we have left them in the form above to maximize clarity.

Accordingly, the production of male reproductives can be understood as follows. In AA, m colonies, the queen's sons are all A; all of the sons of AA workers and half of the sons of Aa workers are A, while the other half of the sons of Aa workers are a. In Aa, m colonies, the queen's sons are half A and half a; all of the sons of AA workers and half of the sons of Aa workers are A, while the other half of the sons of Aa workers and all of the sons of aa workers are a. Finally, in aa, m colonies, the queen's sons are all a; half of the sons of Aa workers are A, while the other half of the sons of Aa workers and all the sons of aa workers are a.

8.2 Reproductives if the mutant allele is recessive

Along similar principles, when the mutant allele is recessive, the production of each type of reproductive female and male is:

$$x_{AA} = \sum_{m=0}^{n} \left\{ \frac{n-m}{n} r_u X_{AA,m} + \frac{n-m}{2n} r_{\frac{(2n-m)u+mv}{2n}} X_{Aa,m} \right\}$$

$$x_{Aa} = \sum_{m=0}^{n} \left\{ \frac{m}{n} r_u X_{AA,m} + \frac{1}{2} r_{\frac{(2n-m)u+mv}{2n}} X_{Aa,m} + \frac{n-m}{n} r_{\frac{(n-m)u+mv}{n}} X_{aa,m} \right\}$$

$$x_{aa} = \sum_{m=0}^{n} \left\{ \frac{m}{2n} r_{\frac{(2n-m)u+mv}{2n}} X_{Aa,m} + \frac{m}{n} r_{\frac{(n-m)u+mv}{n}} X_{aa,m} \right\}$$

$$y_A = \sum_{m=0}^{n} \left\{ \begin{array}{l} \left(p_u + \left(\frac{n-m}{n} + \frac{1}{2}\frac{m}{n}\right)(1-p_u)\right) r_u X_{AA,m} \\ \\ + \left(\begin{array}{l} \frac{1}{2} p_{\frac{(2n-m)u+mv}{2n}} \\ \\ + \frac{\frac{n-m}{2n}(1-u)+\frac{1}{4}(1-u)}{\frac{n-m}{2n}(1-u)+\frac{1}{2}(1-u)+\frac{m}{2n}(1-v)} \left(1 - p_{\frac{(2n-m)u+mv}{2n}}\right) \end{array} \right) r_{\frac{(2n-m)u+mv}{2n}} X_{Aa,m} \\ \\ + \left(\frac{\frac{1}{2}\frac{n-m}{n}(1-u)}{\frac{n-m}{n}(1-u)+\frac{m}{n}(1-v)} \right) (1 - p_{\frac{(n-m)u+mv}{n}}) r_{\frac{(n-m)u+mv}{n}} X_{aa,m} \end{array} \right\}$$

$$y_a = \sum_{m=0}^{n} \left\{ \begin{array}{l} \left(\frac{1}{2}\frac{m}{n}\right)(1-p_u) r_u X_{AA,m} \\ \\ + \left(\begin{array}{l} \frac{1}{2} p_{\frac{(2n-m)u+mv}{2n}} \\ \\ + \frac{\frac{1}{4}(1-u)+\frac{m}{2n}(1-v)}{\frac{n-m}{2n}(1-u)+\frac{1}{2}(1-u)+\frac{m}{2n}(1-v)} \left(1 - p_{\frac{(2n-m)u+mv}{2n}}\right) \end{array} \right) r_{\frac{(2n-m)u+mv}{2n}} X_{Aa,m} \\ \\ + \left(p_{\frac{(n-m)u+mv}{n}} + \left(\frac{\frac{1}{2}\frac{n-m}{n}(1-u)+\frac{m}{n}(1-v)}{\frac{n-m}{n}(1-u)+\frac{m}{n}(1-v)} \right) (1 - p_{\frac{(n-m)u+mv}{n}}) \right) r_{\frac{(n-m)u+mv}{n}} X_{aa,m} \end{array} \right\}. \quad (15)$$

These equations can be understood similarly to equation 14; in fact, they are identical, except for two general changes. First, the subscripts to r_z and p_z are different, because the mutant allele is recessive instead of dominant, which results in different proportions of sterile workers in colonies of each type: in an AA, m colony, a frac-

tion $z = \frac{n-m}{n}u + \frac{m}{n}u = u$ of workers will be sterile; in an Aa, m colony, a fraction $z = \frac{n-m}{2n}u + \frac{1}{2}u + \frac{m}{2n}v = \frac{(2n-m)u+mv}{2n}$ of workers will be sterile; and in an aa, m colony, a fraction $z = \frac{n-m}{n}u + \frac{m}{n}v = \frac{(n-m)u+mv}{n}$ of workers will be sterile. Second, because of these differing proportions of sterile workers, the production of sons by workers is different, so the coefficients of $1 - p_z$ in the fourth and fifth lines are different.

8.3 Condition for invasion of a dominant mutant sterility allele

Continuing to follow the approach of Olejarz *et al.* (2015): for a dominant mutant sterility allele, whether the allele increases in frequency from rarity is governed by the behaviour of $AA, 0$, $AA, 1$, and $Aa, 0$ colonies. Colony types with more copies of the mutant allele are rarer, and so will have a negligible effect on invasion. Therefore, from equation 11, we need only consider:

$$\begin{aligned} \dot{X}_{AA,0} &= x_{AA}y_A^n - \phi X_{AA,0} \\ \dot{X}_{AA,1} &= n x_{AA} y_A^{n-1} y_a - \phi X_{AA,1} \\ \dot{X}_{Aa,0} &= x_{Aa}y_A^n - \phi X_{Aa,0}\,. \end{aligned} \tag{16}$$

We start with a wild-type population ($X_{AA,0} = 1$) and introduce a small perturbation of magnitude $\epsilon \ll 1$. Considering the density constraint (equation 13), and only keeping terms up to order ϵ, this gives

$$\begin{aligned} X_{AA,0} &= 1 - \epsilon(\delta_{AA,1}^{(1)} + \delta_{Aa,0}^{(1)}) - \mathcal{O}(\epsilon^2) \\ X_{AA,1} &= \epsilon\delta_{AA,1}^{(1)} + \mathcal{O}(\epsilon^2) \\ X_{Aa,0} &= \epsilon\delta_{Aa,0}^{(1)} + \mathcal{O}(\epsilon^2)\,, \end{aligned} \tag{17}$$

which implies that

$$\begin{aligned} \dot{X}_{AA,1} &= \epsilon\dot{\delta}_{AA,1}^{(1)} + \mathcal{O}(\epsilon^2) \\ \dot{X}_{Aa,0} &= \epsilon\dot{\delta}_{Aa,0}^{(1)} + \mathcal{O}(\epsilon^2)\,. \end{aligned} \tag{18}$$

Substituting 17 into 14, and keeping terms only up to order ϵ, gives

$$\begin{aligned}
x_{AA} &= r_u + \epsilon\left(-r_u(\delta^{(1)}_{AA,1}+\delta^{(1)}_{Aa,0}) + \frac{n-1}{n} r_{\frac{(n-1)u+v}{n}}\delta^{(1)}_{AA,1} + \frac{1}{2} r_{\frac{u+v}{2}}\delta^{(1)}_{Aa,0}\right) + \mathcal{O}(\epsilon^2)\\
x_{Aa} &= \epsilon\left(\frac{1}{n} r_{\frac{(n-1)u+v}{n}}\delta^{(1)}_{AA,1} + \frac{1}{2} r_{\frac{u+v}{2}}\delta^{(1)}_{Aa,0}\right) + \mathcal{O}(\epsilon^2)\\
x_{aa} &= 0 + \mathcal{O}(\epsilon^2)\\
y_A &= r_u + \epsilon\left(\begin{array}{l} -r_u(\delta^{(1)}_{AA,1}+\delta^{(1)}_{Aa,0}) \\ \\ + \frac{2n(1-u)+2u-1-v+(1-v)p_{\frac{(n-1)u+v}{n}}}{2(n(1-u)-(v-u))} r_{\frac{(n-1)u+v}{n}}\delta^{(1)}_{AA,1} \\ \\ + \frac{3-2u-v-(1-u)p_{\frac{u+v}{2}}}{2(2-u-v)} r_{\frac{u+v}{2}}\delta^{(1)}_{Aa,0}\end{array}\right) + \mathcal{O}(\epsilon^2)\\
y_a &= \epsilon\left(\frac{(1-v)(1-p_{\frac{(n-1)u+v}{n}})}{2\left(n(1-u)-(v-u)\right)} r_{\frac{(n-1)u+v}{n}}\delta^{(1)}_{AA,1} + \frac{1-v+(1-u)p_{\frac{u+v}{2}}}{2(2-u-v)} r_{\frac{u+v}{2}}\delta^{(1)}_{Aa,0}\right) + \mathcal{O}(\epsilon^2)\,.
\end{aligned} \tag{19}$$

Finally, substituting 12, 18, and 19 into 16 and discarding powers of ϵ^2 or higher gives

$$\begin{aligned}
\epsilon\dot{\delta}_{AA,1} &= \epsilon r_u^n\left(-r_u\delta^{(1)}_{AA,1} + n\left(\begin{array}{l}\frac{(1-v)(1-p_{\frac{(n-1)u+v}{n}})}{2(n(1-u)-(v-u))} r_{\frac{(n-1)u+v}{n}}\delta^{(1)}_{AA,1} \\ \\ + \frac{1-v+(1-u)p_{\frac{u+v}{2}}}{2(2-u-v)} r_{\frac{u+v}{2}}\delta^{(1)}_{Aa,0}\end{array}\right)\right)\\
\epsilon\dot{\delta}_{Aa,0} &= \epsilon r_u^n\left(\frac{1}{n} r_{\frac{(n-1)u+v}{n}}\delta^{(1)}_{AA,1} - r_u\delta^{(1)}_{Aa,0} + \frac{1}{2} r_{\frac{u+v}{2}}\delta^{(1)}_{Aa,0}\right).
\end{aligned}$$

This can be rewritten in matrix form as

$$\begin{bmatrix}\dot{\delta}^{(1)}_{AA,1}\\ \dot{\delta}^{(1)}_{Aa,0}\end{bmatrix} = \begin{bmatrix} r_u^n\left(-r_u + n\frac{(1-v)(1-p_{\frac{(n-1)u+v}{n}})}{2(n(1-u)-(v-u))} r_{\frac{(n-1)u+v}{n}}\right) & r_u^n n\frac{1-v+(1-u)p_{\frac{u+v}{2}}}{2(2-u-v)} r_{\frac{u+v}{2}} \\ r_u^n\frac{1}{n} r_{\frac{(n-1)u+v}{n}} & r_u^n\left(-r_u + \frac{1}{2} r_{\frac{u+v}{2}}\right)\end{bmatrix}\begin{bmatrix}\delta^{(1)}_{AA,1}\\ \delta^{(1)}_{Aa,0}\end{bmatrix}.$$

If the dominant eigenvalue of the above matrix is greater than zero, then a dominant sterility allele with penetrance v can invade a population monomorphic for

sterility with penetrance u. This condition, after simplification, is

$$\frac{r_{\frac{u+v}{2}}}{r_u}\left(\begin{array}{l} 1+\left(\frac{(1-u)p_{\frac{u+v}{2}}}{2-u-v}+\frac{(1-v)\left((2-n)(u-v)+n(2-u-v)p_{\frac{(n-1)u+v}{n}}\right)}{2(n(1-u)+u-v)(2-u-v)}\right)\frac{r_{\frac{(n-1)u+v}{n}}}{r_u} \\ \\ +\frac{n(1-v)(1-p_{\frac{(n-1)u+v}{n}})}{n(1-u)+u-v}\frac{r_{\frac{(n-1)u+v}{n}}}{r_{\frac{u+v}{2}}} \end{array}\right) > 2\,. \quad (20)$$

8.4 Condition for invasion of a recessive mutant sterility allele

For a recessive mutant sterility allele, whether the allele increases in frequency from rarity is governed by the behaviour of $AA,0$, $AA,1$, $Aa,0$, $AA,2$, $Aa,1$, and $aa,0$ colonies. Colony types with more copies of the mutant allele are rarer, and so will have a negligible effect on invasion. Therefore, from equation 11, we need only consider:

$$\begin{aligned} \dot{X}_{AA,0} &= x_{AA}y_A^n - \phi X_{AA,0} \\ \dot{X}_{AA,1} &= nx_{AA}y_A^{n-1}y_a - \phi X_{AA,1} \\ \dot{X}_{Aa,0} &= x_{Aa}y_A^n - \phi X_{Aa,0} \\ \dot{X}_{AA,2} &= \frac{n(n-1)}{2}x_{AA}y_A^{n-2}y_a^2 - \phi X_{AA,2} \\ \dot{X}_{Aa,1} &= nx_{Aa}y_A^{n-1}y_a - \phi X_{Aa,1} \\ \dot{X}_{aa,0} &= x_{aa}y_A^n - \phi X_{aa,0}\,. \end{aligned} \quad (21)$$

We start with a wild-type population ($X_{AA,0} = 1$) and introduce a small perturbation of magnitude $\epsilon \ll 1$. Considering the density constraint (equation 13), and only keeping terms up to order ϵ^2 (since terms of order ϵ alone are not sufficient to determine whether the recessive allele invades), this gives

$$\begin{aligned} X_{AA,0} &= 1-\epsilon\delta_{AA,0}^{(1)} - \epsilon^2\delta_{AA,0}^{(2)} - \mathcal{O}(\epsilon^3) \\ &= 1-\epsilon(\delta_{AA,1}^{(1)}+\delta_{Aa,0}^{(1)}) - \epsilon^2(\delta_{AA,1}^{(2)}+\delta_{Aa,0}^{(2)}+\delta_{AA,2}^{(2)}+\delta_{Aa,1}^{(2)}+\delta_{aa,0}^{(2)}) - \mathcal{O}(\epsilon^3) \\ X_{AA,1} &= \epsilon\delta_{AA,1}^{(1)} + \epsilon^2\delta_{AA,1}^{(2)} + \mathcal{O}(\epsilon^3) \\ X_{Aa,0} &= \epsilon\delta_{Aa,0}^{(1)} + \epsilon^2\delta_{Aa,0}^{(2)} + \mathcal{O}(\epsilon^3) \end{aligned}$$

$$\begin{aligned} X_{AA,2} &= \epsilon^2 \delta^{(2)}_{AA,2} + \mathcal{O}(\epsilon^3) \\ X_{Aa,1} &= \epsilon^2 \delta^{(2)}_{Aa,1} + \mathcal{O}(\epsilon^3) \\ X_{aa,0} &= \epsilon^2 \delta^{(2)}_{aa,0} + \mathcal{O}(\epsilon^3)\,, \end{aligned} \tag{22}$$

which implies that

$$\begin{aligned} \dot{X}_{AA,0} &= -\epsilon \dot{\delta}^{(1)}_{AA,0} - \epsilon^2 \dot{\delta}^{(2)}_{AA,0} - \mathcal{O}(\epsilon^3) \\ &= -\epsilon(\dot{\delta}^{(1)}_{AA,1} + \dot{\delta}^{(1)}_{Aa,0}) - \epsilon^2(\dot{\delta}^{(2)}_{AA,1} + \dot{\delta}^{(2)}_{Aa,0} + \dot{\delta}^{(2)}_{AA,2} + \dot{\delta}^{(2)}_{Aa,1} + \dot{\delta}^{(2)}_{aa,0}) - \mathcal{O}(\epsilon^3) \\ \dot{X}_{AA,1} &= \epsilon \dot{\delta}^{(1)}_{AA,1} + \epsilon^2 \dot{\delta}^{(2)}_{AA,1} + \mathcal{O}(\epsilon^3) \\ \dot{X}_{Aa,0} &= \epsilon \dot{\delta}^{(1)}_{Aa,0} + \epsilon^2 \dot{\delta}^{(2)}_{Aa,0} + \mathcal{O}(\epsilon^3) \\ \dot{X}_{AA,2} &= \epsilon^2 \dot{\delta}^{(2)}_{AA,2} + \mathcal{O}(\epsilon^3) \\ \dot{X}_{Aa,1} &= \epsilon^2 \dot{\delta}^{(2)}_{Aa,1} + \mathcal{O}(\epsilon^3) \\ \dot{X}_{aa,0} &= \epsilon^2 \dot{\delta}^{(2)}_{aa,0} + \mathcal{O}(\epsilon^3)\,. \end{aligned} \tag{23}$$

Substituting equation 22 into equation 15, and keeping terms only up to order ϵ^2, gives

$$\begin{aligned} x_{AA} &= r_u + \epsilon\left(-\frac{1}{n} r_u \delta^{(1)}_{AA,1} - \frac{1}{2} r_u \delta^{(1)}_{Aa,0}\right) \\ &\quad + \epsilon^2 \left(\begin{array}{l} -\frac{1}{n} r_u \delta^{(2)}_{AA,1} - \frac{1}{2} r_u \delta^{(2)}_{Aa,0} - \frac{2}{n} r_u \delta^{(2)}_{AA,2} \\ + \left(\frac{n-1}{2n} r_{\frac{(2n-1)u+v}{2n}} - r_u\right) \delta^{(2)}_{Aa,1} - r_u \delta^{(2)}_{aa,0} \end{array} \right) + \mathcal{O}(\epsilon^3) \\ x_{Aa} &= \epsilon\left(\frac{1}{n} r_u \delta^{(1)}_{AA,1} + \frac{1}{2} r_u \delta^{(1)}_{Aa,0}\right) + \\ &\quad \epsilon^2\left(\frac{r_u}{n} \delta^{(2)}_{AA,1} + \frac{1}{2} r_u \delta^{(2)}_{Aa,0} + \frac{2}{n} r_u \delta^{(2)}_{AA,2} + \frac{1}{2} r_{\frac{(2n-1)u+v}{2n}} + r_u \delta^{(2)}_{aa,0}\right) + \mathcal{O}(\epsilon^3) \\ x_{aa} &= \epsilon^2\left(\frac{1}{2n} r_{\frac{(2n-1)u+v}{2n}} \delta^{(2)}_{Aa,1}\right) + \mathcal{O}(\epsilon^3) \end{aligned}$$

$$
\begin{aligned}
y_A &= r_u + \epsilon\left(-\frac{1-p_u}{2n}r_u\delta^{(1)}_{AA,1} - \frac{1+p_u}{4}r_u\delta^{(1)}_{Aa,0}\right) \\
&\quad + \epsilon^2\begin{pmatrix} -\frac{2-p_u}{2n}r_u\delta^{(2)}_{AA,1} - \frac{1+p_u}{4}r_u\delta^{(2)}_{Aa,0} - \frac{1-p_u}{n}r_u\delta^{(2)}_{AA,2} \\ + \left(\frac{(3n-2)(1-u)+(2-n(1-u)-u-v)p_{\frac{(2n-1)u+v}{2n}}}{2n(1-u)-(v-u)}r_{\frac{(2n-1)u+v}{2n}} - r_u\right)\delta^{(2)}_{Aa,1} \\ -\frac{1+p_u}{2}r_u\delta^{(2)}_{aa,0}\end{pmatrix} + \mathcal{O}(\epsilon^3) \\
y_a &= \epsilon\left(\frac{1-p_u}{2n}r_u\delta^{(1)}_{AA,1} + \frac{1+p_u}{4}r_u\delta^{(1)}_{Aa,0}\right) \\
&\quad + \epsilon^2\begin{pmatrix} \frac{1-p_u}{2n}r_u\delta^{(2)}_{AA,1} + \frac{1+p_u}{4}r_u\delta^{(2)}_{Aa,0} + \frac{1-p_u}{n}r_u\delta^{(2)}_{AA,2} \\ + \frac{2+n(1-u)-2v-(2-n(1-u)-u-v)p_{\frac{(2n-1)u+v}{2n}}}{2(2n(1-u)-(v-u))}r_{\frac{(2n-1)u+v}{2n}}\delta^{(2)}_{Aa,1} \\ +\frac{1+p_u}{2}r_u\delta^{(2)}_{aa,0}\end{pmatrix} + \mathcal{O}(\epsilon^3)\,. \qquad (24)
\end{aligned}
$$

Substituting equations 12, 23, and 24 into equation 21 and discarding powers of ϵ^2 or higher gives, in matrix form,

$$
\begin{bmatrix} \dot{\delta}^{(1)}_{AA,1} \\ \dot{\delta}^{(1)}_{Aa,0} \end{bmatrix} = r_u^{n+1}\begin{bmatrix} -\frac{1+p_u}{2} & \frac{n(1+p_u)}{4} \\ \frac{1}{n} & -\frac{1}{2} \end{bmatrix}\begin{bmatrix} \delta^{(1)}_{AA,1} \\ \delta^{(1)}_{Aa,0} \end{bmatrix}.
$$

The dominant eigenvalue is 0, and its corresponding eigenvector is $\begin{bmatrix} n \\ 2 \end{bmatrix}$, which gives

$$
\begin{aligned}
\delta^{(1)}_{AA,1} &= \frac{n}{n+2}\delta^{(1)}_{AA,0} \\
\delta^{(1)}_{Aa,0} &= \frac{2}{n+2}\delta^{(1)}_{AA,0}\,. \qquad (25)
\end{aligned}
$$

(In other words, this tells us how to "distribute" the first-order perturbation to $X_{AA,0}$ over the first-order perturbations to $X_{AA,1}$ and $X_{Aa,0}$.)

Substituting equations 12, 23, 24, and 25 into equation 21, and keeping terms up to order ϵ^2, gives

$$
\begin{aligned}
-\dot{\delta}^{(2)}_{AA,0} &= \frac{2-n-np_u}{4n} r_u^{n+1}\left(-2\delta^{(2)}_{AA,1}+n\delta^{(2)}_{Aa,0}\right)+\frac{(-2+np_u)}{n} r_u^{n+1}\delta^{(2)}_{AA,2} \\
&\quad + r_u^n \left(r_u + \frac{\left(\begin{array}{l} v-u \\ -n\left(2+n^2(1-u)-u-v+2n(2-u-v)\right) \\ +n^2\left(2-n(1-u)-u-v\right) p_{\frac{(2n-1)u+v}{2n}} \end{array} \right)}{2n\left(2n(1-u)-(v-u)\right)} r_{\frac{(2n-1)u+v}{2n}} \right) \delta^{(2)}_{Aa,1} \\
&\quad - \frac{1}{2} n\left(1+p_u\right) r_u^{n+1}\delta^{(2)}_{aa,0} + \frac{n(3+n)r_u^{n+1}}{2(2+n)^2}\left(\delta^{(1)}_{AA,0}\right)^2 \\
\\
\dot{\delta}^{(2)}_{AA,1} &= \frac{1}{4}\left(1+p_u\right) r_u^{n+1}\left(-2\delta^{(2)}_{AA,1}+n\delta^{(2)}_{Aa,0}\right)-\left(p_u-1\right) r_u^{n+1}\delta^{(2)}_{AA,2} \\
&\quad + \frac{\left(n\left(\begin{array}{l} n(u-1)+2(v-1) \\ +(2+n(u-1)-u-v)p_{\frac{(2n-1)u+v}{2n}} \end{array} \right) r_u^n r_{\frac{(2n-1)u+v}{2n}} \right)}{2(2n(u-1)-u+v)} \delta^{(2)}_{Aa,1} \\
&\quad + \frac{1}{2} n\left(1+p_u\right) r_u^{n+1}\delta^{(2)}_{aa,0} - \frac{\left(n(1+n)r_u^{n+1}\right)}{(2+n)^2}\left(\delta^{(1)}_{AA,0}\right)^2 \\
\\
\dot{\delta}^{(2)}_{Aa,0} &= -\frac{r_u^{n+1}}{2n}\left(-2\delta^{(2)}_{AA,1}+n\delta^{(2)}_{Aa,0}\right)+\frac{2r_u^{n+1}}{n}\delta^{(2)}_{AA,2} \\
&\quad + \frac{1}{2} r_u^n r_{\frac{(2n-1)u+v}{2n}}\delta^{(2)}_{Aa,1} + r_u^{n+1}\delta^{(2)}_{aa,0} - \frac{2nr_u^{n+1}}{(2+n)^2}\left(\delta^{(1)}_{AA,0}\right)^2 \\
\\
\dot{\delta}^{(2)}_{AA,2} &= -r_u^{n+1}\delta^{(2)}_{AA,2} + \frac{(n-1)nr_u^{n+1}}{2(2+n)^2}\left(\delta^{(1)}_{AA,0}\right)^2 \\
\\
\dot{\delta}^{(2)}_{Aa,1} &= -r_u^{n+1}\delta^{(2)}_{Aa,1} + \frac{2nr_u^{n+1}}{(2+n)^2}\left(\delta^{(1)}_{AA,0}\right)^2 \\
\\
\dot{\delta}^{(2)}_{aa,0} &= -r_u^{n+1}\delta^{(2)}_{aa,0} + \frac{r_u^n r_{\frac{(2n-1)u+v}{2n}}}{2n}\delta^{(2)}_{Aa,1}\,. \qquad (26)
\end{aligned}
$$

Now, each of these equations must be solved.

The equation for $\dot{\delta}^{(2)}_{AA,2}$ can be directly integrated, yielding:

$$\delta^{(2)}_{AA,2} = \frac{n(n-1)}{2(n+2)^2}\left(\delta^{(1)}_{AA,0}\right)^2\left(1-\exp(-r_u^{n+1}t)\right). \tag{27}$$

The same can be done for $\dot{\delta}^{(2)}_{Aa,1}$, yielding:

$$\delta^{(2)}_{Aa,1} = \frac{2n}{(n+2)^2}\left(\delta^{(1)}_{AA,0}\right)^2\left(1-\exp(-r_u^{n+1}t\right). \tag{28}$$

Equation 28 can be used to solve for $\delta^{(2)}_{aa,0}$, yielding:

$$\delta^{(2)}_{aa,0} = \frac{r_{\frac{(2n+1)u+v}{2n}}}{(2+n)^2 r_u}\left(\delta^{(1)}_{AA,0}\right)^2\left(1-(1+r_u^{n+1}t)\exp(-r_u^{n+1}t)\right). \tag{29}$$

The equations for $\dot{\delta}^{(2)}_{AA,1}$ and $\dot{\delta}^{(2)}_{Aa,0}$ can be manipulated to yield

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}(-2\delta^{(2)}_{AA,1}+n\delta^{(2)}_{Aa,0}) &= -\frac{(2+p_u)r_u^{n+1}}{2}(-2\delta^{(2)}_{AA,1}+n\delta^{(2)}_{Aa,0})+2p_u r_u^{n+1}\delta^{(2)}_{AA,2}\\ &\quad -\frac{n\left(4-u-3v-2\left(2-n(1-u)-u-v\right)p_{\frac{(2n-1)u+v}{2n}}\right)}{2\left(2n(1-u)-(v-u)\right)}r_u^n r_{\frac{(2n-1)u+v}{2n}}\delta^{(2)}_{Aa,1}\\ &\quad -np_u r_u^{n+1}\delta^{(2)}_{aa,0}+\frac{2n}{(2+n)^2}r_u^{n+1}\left(\delta^{(1)}_{AA,0}\right)^2,\end{aligned}$$

which can be integrated to give

$$-2\delta^{(2)}_{AA,1}+n\delta^{(2)}_{Aa,0} =$$

$$\begin{aligned}&\begin{pmatrix}\frac{2n(2+(n-1)p_u)}{(2+n)^2(2+p_u)}-\frac{2np_u r_{\frac{(2n-1)u+v}{2n}}}{(2+n)^2(2+p_u)r_u}\\ -\frac{2n^2\left(4-u-3v-2(2-n(1-u)-u-v)p_{\frac{(2n-1)u+v}{2n}}\right)r_{\frac{(2n-1)u+v}{2n}}}{(2+n)^2(2n(1-u)-(v-u))(2+p_u)r_u}\end{pmatrix}\left(\delta^{(1)}_{AA,0}\right)^2\\ &+\begin{pmatrix}-\frac{2n\left(r_u\left(n-1-tr_u^n r_{\frac{(2n-1)u+v}{2n}}\right)-r_{\frac{(2n-1)u+v}{2n}}\right)}{(2+n)^2 r_u}\\ -\frac{2n\left((3n-2)(v-u)+2n(2-n(1-u)-u-v)p_{\frac{(2n-1)u+v}{2n}}\right)r_{\frac{(2n-1)u+v}{2n}}}{(2+n)^2(2n(1-u)-(v-u))p_u r_u}\end{pmatrix}\left(\delta^{(1)}_{AA,0}\right)^2\exp\left(-r_u^{n+1}t\right)\\ &+\begin{pmatrix}\frac{4n(n-2)}{(2+n)^2(2+p_u)}\\ +\frac{4n\left((3n-2)(v-u)+2n(2-n(1-u)-u-v)p_{\frac{(2n-1)u+v}{2n}}\right)r_{\frac{(2n-1)u+v}{2n}}}{(2+n)^2(2n(1-u)-(v-u))p_u(2+p_u)r_u}\end{pmatrix}\left(\delta^{(1)}_{AA,0}\right)^2\exp\left(-\frac{2+p_u}{2}r_u^{n+1}t\right).\end{aligned} \tag{30}$$

We solve for $\dot{\delta}^{(2)}_{AA,0}$ by substituting equations 27–30 into equation 26. In doing so, we

permit t to become relatively large, such that all the time-dependent terms in equations 27–30 approach zero. Accordingly, the sign of $\dot{\delta}^{(2)}_{AA,0}$ tells us that the mutant sterility allele will invade if:

$$\lim_{t\to\infty} \dot{\delta}^{(2)}_{AA,0} > 0$$

That is, after substitution and simplification, a recessive sterility allele with penetrance v will invade a population monomorphic for sterility with penetrance u if

$$\frac{r_{\frac{(2n-1)u+v}{2n}}}{r_u} > \frac{2\left(2n(1-u)+u-v\right)\left(2+n(1+p_u)\right)}{\left(\begin{array}{l} n(8+4n(1-u)-3u-5v)+2(u-v) \\ +(2+n)(2n(1-u)+u-v)p_u \\ -2n(2-u-v-n(1-u))p_{\frac{(2n-1)u+v}{2n}} \end{array}\right)}. \tag{31}$$

9. Kin-selection analysis

Here, we develop a general model of the evolution of wholly or partly non-reproductive workers using standard kin selection methodology (Taylor & Frank 1996, Frank 1998). In this model, a mated queen founds a colony by producing an initial brood of females and/or males. Depending on the model scenario, first-brood females may either mate with first-brood males—from their own or from a different colony—or remain unmated. Then, according to the level of worker sterility z, a focal first-brood female (*i.e.*, a worker) invests a proportion of her resources into helping to raise the colony's next brood—which consists partly of queen-produced offspring (queen-laid females, notated f, and queen-laid males, notated m) and partly of worker-produced offspring (worker-laid females, notated ϕ, and worker-laid males, notated μ)—and a proportion of her resources into producing her own offspring. Individuals of the second brood disperse and mate, with each female mating with n males, and mated females then found new patches, restarting the cycle.

In this model, we denote a focal worker's sterility by Z, the average sterility on a focal patch by z, and the average sterility in the population by $\bar{z}$. A focal queen's sex ratio strategy for her second brood is denoted by x, and the average sex ratio strategy among all queens in the population is denoted by $\bar{x}$. The production of queen-laid second-brood females on a focal patch is $f = f(z,x)$; the production of queen-laid

second-brood males on a focal patch is $m = m(z, x)$; the production of worker-laid females by a focal worker is $\phi = \phi(Z, z, x)$; and the production of worker-laid males by a focal worker is $\mu = \mu(Z, z, x)$. We denote by $\bar{f} = f(\bar{z}, \bar{x})$, $\bar{m} = m(\bar{z}, \bar{x})$, $\bar{\phi} = \phi(\bar{z}, \bar{z}, \bar{x})$, and $\bar{\mu} = \mu(\bar{z}, \bar{z}, \bar{x})$ the population-average production of each of these four classes, respectively, and by $\tilde{f} = f/\bar{f}$, $\tilde{m} = m/\bar{m}$, $\tilde{\phi} = \phi/\bar{\phi}$, and $\tilde{\mu} = \mu/\bar{\mu}$ the relative production of each of these four classes.

For a gene increasing worker sterility to spread, its carriers, on average, should leave more descendants than other members of the population. Accordingly, natural selection will favour an increase in worker sterility, z, when

$$\frac{\partial \tilde{f}}{\partial z} R_{\text{sis}} + \frac{\partial \tilde{m}}{\partial z} R_{\text{bro}} + \frac{\partial \tilde{\phi}}{\partial Z} R_{\text{dau}} + \frac{\partial \tilde{\phi}}{\partial z} R_{\text{niece}} + \frac{\partial \tilde{\mu}}{\partial Z} R_{\text{son}} + \frac{\partial \tilde{\mu}}{\partial z} R_{\text{neph}} > 0 \,. \tag{32}$$

Above, R_{sis}, R_{bro}, R_{dau}, R_{niece}, R_{son}, and R_{neph} are the (life-for-life) relatedness between a focal female worker and her sister, brother, daughter, niece, son, and nephew, respectively, and all derivatives are evaluated at $Z = z = \bar{z}$.

Each term on the left-hand side of condition 32 captures how a small increase in worker sterility impacts upon the fitness of different individuals in the population, weighted by the life-for-life relatedness between those individuals and a focal worker, which combines both (i) the reproductive value of those individuals (*i.e.*, their capacity for projecting genes into future generations) and (ii) the extent to which those individuals themselves carry the gene increasing worker sterility. Alternatively, each term can be read as an inclusive-fitness effect experienced by a focal worker who gives up reproduction to become sterile. These interpretations are mathematically equivalent, but we focus on the inclusive-fitness interpretation here, as it is conceptually simpler.

Similarly, natural selection will favour an increase in the queen's sex allocation strategy (her investment in daughters), x, when

$$\frac{\partial \tilde{f}}{\partial x} R_{\text{dau|Q}} + \frac{\partial \tilde{m}}{\partial x} R_{\text{son|Q}} + \frac{\partial \tilde{\phi}}{\partial x} R_{\text{gdau|Q}} + \frac{\partial \tilde{\mu}}{\partial x} R_{\text{gson|Q}} > 0 \,. \tag{33}$$

Above, $R_{\text{dau|Q}}$ is the relatedness between a focal queen and her daughter, $R_{\text{son|Q}}$ is the relatedness between a focal queen and her son, $R_{\text{gdau|Q}}$ is the relatedness between

a focal female and her granddaughter (her daughter's daughter), $R_{\text{gson|Q}}$ is the relatedness between a focal female and her grandson (her daughter's son), and all derivatives are evaluated at $x = \bar{x}$. Each term on the left-hand side of condition 33 captures how a small increase in the queen's investment in daughters, as opposed to sons, impacts upon the fitness of different individuals in the population; alternatively, each term can be read as an inclusive-fitness effect experienced by a focal queen who gives up one of her sons to raise an extra daughter.

For scenario A, the production of queen-laid females, queen-laid males, worker-laid females, and worker-laid males is $f = xr_z$, $m = (1-x)r_z p_z$, $\phi = 0$, and $\mu = (1-x)r_z(1-p_z)\frac{1-Z}{1-z}$, respectively. For scenario B, we use $f = xr_z p_z$, $m = (1-x)r_z p_z$, $\phi = 0$, and $\mu = r_z(1-p_z)\frac{1-Z}{1-z}$. For scenario C, we use $f = xr_z p_z$, $m = (1-x)r_z p_z$, $\phi = yr_z(1-p_z)\frac{1-Z}{1-z}$, and $\mu = (1-y)r_z(1-p_z)\frac{1-Z}{1-z}$. And for scenario D, we use $f = x(z+sz^2)$, $m = (1-x)(z+sz^2)$, $\phi = y(1-Z)(1-c)$, and $\mu = (1-y)(1-Z)(1-c)$. Substituting these definitions into conditions 32 and 33 recovers conditions 5–10 above.

9.1 Relatedness calculations

The life-for-life relatedness of individual A to individual B is $R_{\text{AB}} = \frac{F_{\text{AB}}}{F_{\text{AA}}}\frac{c_{\text{B}}}{c_{\text{A}}}$, where F_{AB} is the consanguinity of individual A and individual B, F_{AA} is the consanguinity of individual A to herself, c_{B} is the class reproductive value of individual B, and c_{A} is the class reproductive value of individual A (Bulmer 1994). Note that since individual A is always the same individual within a given condition above, we can instead use $R_{\text{AB}} = F_{\text{AB}}c_{\text{B}}$ or any multiple thereof without affecting the resulting conditions.

Accordingly, consanguinities needed for the conditions above can be found in Table 1. The consanguinities for a female worker under claustral inbreeding are obtained by first calculating the coefficient of inbreeding for a foundress in this mating system (the probability that her two genes at a given locus are identical by descent). Suppose that a juvenile is foundress-laid with probability Q, and soldier-laid with probability $1-Q$. If foundress-laid, her coefficient of consanguinity is zero, because patch founders are unrelated. If worker-laid, then her paternally-inherited gene comes from her grandmother, and her maternally-inherited gene comes, with equal probability, either from her grandfather—who is unrelated to her grandmother—or from her grandmother;

For outbreeders

Relationship	Notation	Haplodiploidy	Diploidy
female to daughter	F_{dau}	$\frac{1}{4}$	$\frac{1}{4}$
female to son	F_{son}	$\frac{1}{2}$	$\frac{1}{4}$
female to sister	F_{sis}	$\frac{2+n}{8n}$	$\frac{1+n}{8n}$
female to brother	F_{bro}	$\frac{1}{4}$	$\frac{1+n}{8n}$
female to niece	F_{niece}	$\frac{2+n}{16n}$	$\frac{1+n}{16n}$
female to nephew	F_{neph}	$\frac{2+n}{8n}$	$\frac{1+n}{16n}$
female to daughter's daughter	F_{gdau}	$\frac{1}{8}$	$\frac{1}{8}$
female to daughter's son	F_{gson}	$\frac{1}{4}$	$\frac{1}{8}$

For claustral inbreeders

Relationship	Notation	Haplodiploidy	Diploidy
female worker to daughter	$F_{\text{dau}\|\text{c}}$	$\frac{5+Q}{4(3+Q)}$	$\frac{11+Q}{8(3+Q)}$
female worker to son	$F_{\text{son}\|\text{c}}$	$\frac{1}{2}$	$\frac{11+Q}{8(3+Q)}$
female worker to sister	$F_{\text{sis}\|\text{c}}$	$\frac{3+2n+Q}{4n(3+Q)}$	$\frac{1+n}{2n(3+Q)}$
female worker to brother	$F_{\text{bro}\|\text{c}}$	$\frac{1}{3+Q}$	$\frac{1+n}{2n(3+Q)}$
female worker to niece	$F_{\text{niece}\|\text{c}}$	$\frac{3+6n+Q}{8n(3+Q)}$	$\frac{1+n}{2n(3+Q)}$

Table 1: Consanguinities used in inclusive-fitness models.

in the latter case, her two genes are either copies of the "same" gene in her grandmother, in which case they are identical by descent with probability 1, or are copies of "different" genes from her grandmother, in which case they are identical by descent with probability G, where G is the juvenile's grandmother's coefficient of inbreeding. That is, overall, the probability that these two genes are identical by descent is $F = (1-Q)\frac{1}{2}\left(\frac{1+G}{2}\right)$, and at equilibrium, $G = F$, which gives $F = \frac{1-P}{3+P}$. A similar argument gives the same result under diploidy.

9.2 Class reproductive values

To determine the class reproductive value of each of the four juvenile classes (queen-laid females, class f; queen-laid males, class m; worker-laid females, class ϕ; and worker-laid males, class μ), we first solve for the total reproductive value of all females, $c_{\mathrm{F}} = c_{\mathrm{f}} + c_{\phi}$, and the total reproductive value of all males, $c_{\mathrm{M}} = c_{\mathrm{m}} + c_{\mu}$. Defining $Q = \frac{\bar{f}}{\bar{f}+\bar{\phi}}$ as the probability that a random female is queen-laid, and $P = \frac{\bar{m}}{\bar{m}+\bar{\mu}}$ as the probability that a random male is queen-laid, note that a random female inherits half of her genes from a female in the previous census if she is queen-laid, and three quarters of her genes from a female in the previous census if she is worker-laid; and a random male inherits all his genes from a female in the previous census if he is queen-laid, and half of his genes from a female in the previous census if he is worker-laid. Hence, the recurrence relation $c_{\mathrm{F}} = (\frac{Q}{2} + \frac{3(1-Q)}{4})c_{\mathrm{F}} + (P + \frac{1-P}{2})c_{\mathrm{M}}$, with the constraint that $c_{\mathrm{M}} = 1 - c_{\mathrm{F}}$, can be solved to give $c_F = \frac{2(1+P)}{3+2P+Q}$ and $c_M = \frac{1+Q}{3+2P+Q}$. Since an individual's mating success is not affected by whether they are queen- or worker-laid, we have $c_{\mathrm{f}} = Qc_{\mathrm{F}}$, $c_{\phi} = (1-Q)c_{\mathrm{F}}$, $c_{\mathrm{m}} = Pc_{\mathrm{M}}$, and $c_{\mu} = (1-P)c_{\mathrm{M}}$, which, overall, gives

$$\begin{aligned} c_{\mathrm{f}} &= \frac{2(1+P)Q}{3+2P+Q} \\ c_{\mathrm{m}} &= \frac{P(1+Q)}{3+2P+Q} \\ c_{\phi} &= \frac{2(1+P)(1-Q)}{3+2P+Q} \\ c_{\mu} &= \frac{(1-P)(1-Q)}{3+2P+Q}. \end{aligned}$$

When all second-brood juveniles are queen-laid ($P = Q = 1$), this yields the expected result that $c_\mathrm{f} = 2/3$, $c_\mathrm{m} = 1/3$, $c_\phi = 0$, and $c_\mu = 0$; when all second-brood juveniles are worker-laid ($P = Q = 0$), this yields the expected result that $c_\mathrm{f} = 0$, $c_\mathrm{m} = 0$, $c_\phi = 2/3$, and $c_\mu = 1/3$ (Price 1970, Taylor 1996).

It is illustrative to examine a special case. When all second-brood females are queen-laid ($Q = 1$), this reduces to

$$\begin{aligned} c_\mathrm{f} &= \frac{1+P}{2+P} \\ c_\mathrm{m} &= \frac{P}{2+P} \\ c_\phi &= 0 \\ c_\mu &= \frac{1-P}{2+P}. \end{aligned}$$

In this case, when $P = 1$, we have the expected result that the total value of juvenile females is $2/3$ and the total value of juvenile males is $1/3$, because of the usual asymmetries of haplodiploidy. But when $P = 0$, the total value of juvenile females is $1/2$ and the total value of juvenile males is $1/2$. This is because new juvenile females get half their genes from their mother and half from their father, while new juvenile males are parthenogenetically produced by worker females, and so ultimately get half their genes from their mother's mother and half their genes from their mother's father. In this way, juvenile females and males have an equal share in producing the next generation of juveniles (*cf.* Boomsma & Grafen 1991).

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Part II

Cellular ecology and conflict within genomes

Chapter 5

The tragedy of the transposons

Abstract

Transposons are major drivers of eukaryotic evolution, implicated in development, genome complexity, rewiring regulatory networks, biodiversity, and genetic disease. But our understanding of the coevolution of transposons and their hosts is limited; in particular, we still rely heavily on population genetics papers from the 1980s that do not allow adaptation by transposable elements. Here we show that allowing transposon adaptation fundamentally changes evolutionary predictions. We find that "privately-duplicating" elements—transposons that can invest directly into their personal reproduction—are under continual selection to increase their replication rate, to the point where they drive their host population to extinction, as in the classic "tragedy of the commons". Exploring host–element and element–element interactions that could prevent this outcome, we find that neither selection against transposons' deleterious effects nor exploitation by parasitic elements can plausibly prevent host extinction. Instead, our model predicts that only active suppression by hosts can prevent the collapse of the population: in particular, suppression must act post-transcriptionally in order to permanently prevent extinction. We test our model of transposon ecology with an empirical survey of suppression allocation over different transposon classes in three eukaryotic species, showing that host suppression is concentrated on privately-duplicating elements. Our model brings transposon evolution into the canon of social evolution theory and shows that, like other selfish

agents, transposons are under natural selection to exploit their environment to the point of collapse. We conclude that only by host suppression of transposons is it possible for complex life to exist.

Introduction

Transposons—genetic elements that duplicate themselves within host genomes—have had a pivotal impact upon eukaryotic evolution. Not only are transposons major sources of genetic variation, including half of all spontaneous mutations in *Drosophila* (Drake *et al.* 1998), but they also originate junk DNA, including up to two thirds of the human genome (de Koning *et al.* 2011); they alter gene expression patterns, rewiring regulatory networks (Sundaram *et al.* 2014; Rebollo *et al.* 2012); and they contribute to population divergence and hybrid incompatibility, promoting species diversity (Blumenstiel & Hartl 2005; Josefsson *et al.* 2006; Abbott *et al.* 2013; Ågren 2013; Carbone *et al.* 2014). From a clinical perspective, transposon activity has been implicated in cancer (Belancio *et al.* 2010; Scott *et al.* 2016), neurological disorders (Reilly *et al.* 2013), and numerous hereditary diseases (Ostertag & Kazazian 2001). In response to their deleterious effects, eukaryotes have evolved various counter-adaptations to suppress transposon proliferation, including a highly-conserved "genomic immune system"—the Piwi-piRNA pathway—that performs sequence-specific silencing of transposons and targeted degradation of their gene products (Aravin *et al.* 2007; Senti & Brennecke 2010), in addition to other lineage-specific mechanisms for controlling particular transposon families (*e.g.* Jacobs *et al.* 2014; Reichmann *et al.* 2013). Yet transposons remain enormously successful, comprising the most abundant class of gene in sequenced genomes (Aziz *et al.* 2010).

In spite of its importance, the coevolution of transposons and their hosts remains poorly understood from a theoretical perspective. In the 1980s, when mechanistic details were still sparse, seminal population-genetics models (*e.g.*, Charlesworth & Charlesworth 1983; Charlesworth & Langley 1986; Langley *et al.* 1988) outlined the basic population dynamics of transposons. While duplication increases transposon copy number, selection against individuals carrying the most transposons decreases

copy number, leading to an equilibrium in population transposon abundance. These models assume that transposons have a fixed proliferation rate which is not permitted to freely evolve. However, it is now clearer that individual transposons' rates of proliferation may evolve, and our knowledge of transposon biology has advanced significantly, revealing a complex web of interactions between transposons of different families and their hosts. Hence, although our knowledge of transposon biology has advanced significantly, our understanding of what underpins the basic conflict between transposons and their hosts—at an evolutionary and ecological level—has not.

Here we show that incorporating the key details of transposon ecology transforms our understanding of how transposons and their genomes evolve. We develop a model of transposon–host coevolution, considering different modes of transposition, different potential costs of transposition, interactions between transposons, and finally, modes of host suppression. Building on the classic model of Charlesworth and Charlesworth 1983), we demonstrate the key importance of what we call "public" versus "private" transposon duplication—and show that, under the latter regime, transposons will be selected to increase their rate of duplication until their host population is driven to extinction, playing out a "tragedy of the commons" (Hardin 1968; Rankin *et al.* 2007). This highlights the importance of understanding transposons as agents of genetic conflict (Burt & Trivers 2006). Applying concepts from social evolution theory, we show which strategies of host defence are crucial for preventing the collapse of the genome, and how these will be differentially deployed against transposon families with different mechanisms of duplication. Finally, we support our key predictions with an empirical survey of transposon suppression. A key conclusion of our work is that the integrity of genomes rests on the active suppression of transposon-mediated conflict.

Results and discussion

The model

Our model is based on the classic analysis of Charlesworth and Charlesworth (1983). Our model has three key features in common with theirs: (i) a diploid host population in which chromosomal sites can be either empty or occupied by a transposon (Fig. 1a); (ii) transposons proliferating within genomes, with individual transposons duplicating at rate u and being lost at rate $v < u$ each generation (Fig. 1b); and (iii) sexual reproduction between hosts in discrete generations, with host reproductive success w a decreasing function of transposon number n (Fig. 1c). In this model, there are three possible outcomes: (i) elimination, where selection prevails and all transposons are lost; (ii) extinction, where transposon proliferation overwhelms selection, killing or sterilizing the host population; or (iii) equilibrium, when transposon proliferation is balanced by selection against their deleterious effects, resulting in the maintenance of a stable average copy number $\bar{n}$ (Fig. 1d).

We implement our model using two methods: a deterministic population-genetics model, which allows us to track the invasion of a mutant transposon into a population of wild-type transposons, and a stochastic individual-based model, which permits us to simulate an arbitrary number of transposon types and their interaction with host suppression; this is not possible with the deterministic model, owing to the increased complexity of simulating an arbitrary number of transposon types and host suppression phenotypes. Regardless of which model we use, when we set the duplication rate u to a fixed value, we find that populations reach a stable equilibrium for transposon number (Fig. 1e), as predicted by classic analyses (*e.g.* Charlesworth & Charlesworth 1983).

The existence of a copy number equilibrium requires the transposon duplication rate to be stable over time. However, there is no reason to believe that transposon duplication rates are not subject to evolution. Transposons typically harbour internal promoter sites (Burt & Trivers 2006), the affinity of which to host transcription factors impact upon their expression level, and it has been shown that sequence variation between members of a transposon family correlates with their activity (Brouha *et al.*

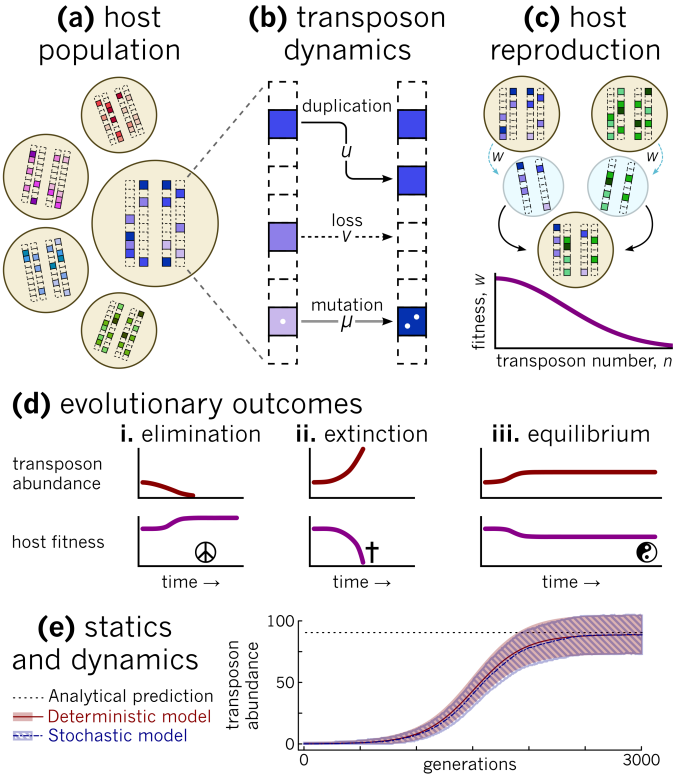


Figure 1: Cartoon of key processes in the model. **(a)** Diploid host chromosomes consist of distinct sites, either unoccupied (empty squares) or occupied by transposons (solid squares). **(b)** Every generation, transposons duplicate at rate u, are lost with probability v, and mutate with probability μ. **(c)** Sexual reproduction replaces half of a given transposon's genetic context each generation, rapidly dissociating transposons from their direct descendants. Host reproductive success w decreases with transposon number n. **(d)** The model has three possible outcomes: (i) elimination, where all transposons are lost; (ii) extinction, where transposon proliferation kills or sterilizes all hosts; or (iii) equilibrium, where transposon load is stably maintained. **(e)** Both the deterministic population-genetics model and stochastic individual-based model conform to analytical predictions for equilibrium transposon abundance. Shaded areas show 5% and 95% quantiles.

2003). As a result, the expression level of a given transposon—and consequently, its duplication rate (Curcio *et al.* 1990)—will be at least partly affected by mutations to the transposon sequence. Therefore, although existing theory explains how natural selection maintains equilibrium for a given fixed duplication rate, we do not yet understand how the transposition rates observed in nature evolve in the first place. In order to understand this process, it will be necessary to consider how the mechanistic details of transposon duplication—and hence, an element's role in cellular ecology—differ between various classes of elements. Moreover, as we show next, allowing transposition rates to mutate fundamentally shifts evolutionary predictions.

Public versus private elements and the tragedy of the transposons

In the terminology of social evolution, fully-autonomous transposons can be broadly classified into two types, according to their mode of duplication: public elements and private elements. In eukaryotes, these two strategies are exhibited by DNA transposons and by RNA transposons, respectively. Public elements—DNA transposons, in eukaryotes—encode a transposase which, once translated, localizes to the nucleus, searches the genome for a transposon-family–specific transposase binding site, and transposes the associated sequence (Fig. 2a). Because the transposase's affinity for duplicating a particular locus is uncorrelated with that locus's investment in producing transposases, public elements are—in social evolution terminology—cooperative: they invest in replicative enzymes that benefit others in the same family, pooling their replicative resources with the sequence to be transposed selected randomly from among them.

In contrast, for private elements—RNA transposons, in eukaryotes—the transcription rate of a given element is directly correlated with its duplication rate. For example, LINE elements encode a reverse transcriptase which binds preferentially to its own mRNA transcript in order to use that transcript as a template for reverse transcription (Fig. 2b); this affinity for self-transcription is known as *cis*-preference (Burt & Trivers 2006), and is mediated both by proximity between the nascent polypeptide and the RNA message, and by recognition of particular sequences at the 3′ end of the transcript. The reverse transcriptase of the L1 retrotransposon in mammals binds to

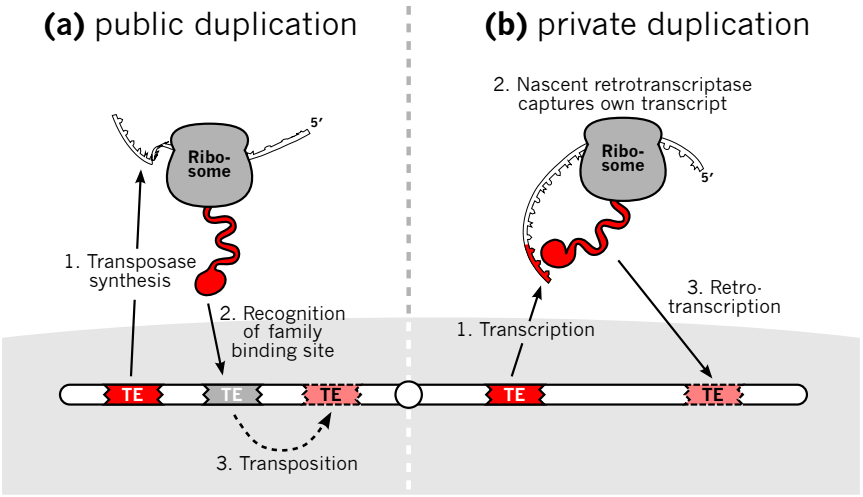


Figure 2: Transposon ecology: public versus private duplication. **(a)** Public elements pool their resources, such that the benefit of each element's investment into reproduction is divided amongst all transpositionally-competent elements in a given family. In this schematic, a DNA element encodes a transposase that localizes to the nucleus and transposes a different DNA element of the same family, irrespective of the target element's investment into family reproduction. **(b)** Private elements keep their resources to themselves, such that the benefit of each element's investment into reproduction accrues solely to that element. In this schematic, a LINE element encodes a retrotranscriptase that captures its own transcript, using it as a template for reverse transcription. The source element's investment into transcription is an investment into its own reproduction.

polyadenylated sequences, allowing it to capture its own messenger RNA's poly-A tail as it emerges from the ribosome (Doucet *et al.* 2015); other LINE elements have various conserved sequences at their 3′ end which mediate *cis*-preference (Okada *et al.* 1997). Because of their direct link between transcription and retrotranscription, private elements are inherently selfish: their investment into transcription is for their own benefit, and is not shared with other elements of the same family.

We now investigate the consequences of allowing the duplication rate to evolve using both our deterministic population-genetics model and our stochastic individual-based model. We assign each transposon an activity a, a measure of expression level that determines its duplication rate u. For private elements, we simply assume $u = a$; for public elements, we assume $u = \frac{1}{n}\sum a$, where the sum is taken over all n public elements in the same host, and hence each element receives an equal share in the total duplication rate of its family.

When we allow transposon activity to evolve in our model, the distinction between private and public elements has a crucial impact upon the evolutionary outcome. For

public elements, transposons exhibiting a fixed duplication rate reach a stable equilibrium, as we have seen (Fig. 3a). When we introduce a mutant transposon into the population, we see that transposons exhibiting different activities can stably coexist (Fig. 3b). And, when we allow transposon activity to evolve freely in our stochastic individual-based model, we see a similar pattern: transposons adopt a wide range of strategies, eventually settling on a stable distribution of activities, and equilibrium is reached (Fig. 3c). In fact, a significant fraction of transpositionally-competent elements contribute nothing at all to the family's success: for example, in the simulation shown in Fig. 3c, roughly 9% of the elements are completely inactive at equilibrium.

With private duplication, we see a different ultimate outcome. Again, when transposons are limited to a fixed duplication rate, a stable equilibrium is reached, as seen with public duplication and in classic analyses (Fig. 3d; Charlesworth & Charlesworth 1983). However, when we now allow transposition rate to vary—introducing mutants with a higher activity—these mutants always displace the wild-type transposons, driving host fitness lower and lower (Fig. 3e). And when we allow privately-duplicating transposons to freely evolve in our individual-based model, we find that they are continually selected to increase their activity—with more-prolific transposons repeatedly outcompeting less-prolific transposons—until the number of new insertions each generation is too great to be counteracted by natural selection, and the host population goes extinct (Fig. 3f). Analogously to the classic tragedy of the commons (Hardin 1968, Rankin *et al.* 2007), private transposons prosper in exploiting a common resource—in this case, the host population—even though this eventually results in its collapse and their own demise.

Why does changing the mode of duplication—public versus private—lead to such divergent outcomes? Like other parasites, transposons face a trade-off between transmission and virulence (Frank 1996): they can proliferate more within hosts by duplicating faster—which, all else being equal, promotes their spread through the population—but this comes at a cost of doing more harm to their host, which inhibits their transmission through the population. In order to conceptualize this fundamental tradeoff, we cast it here in analytical terms. For a rare mutant transposon, we formally describe the tension between these two opposing forces—proliferation within the

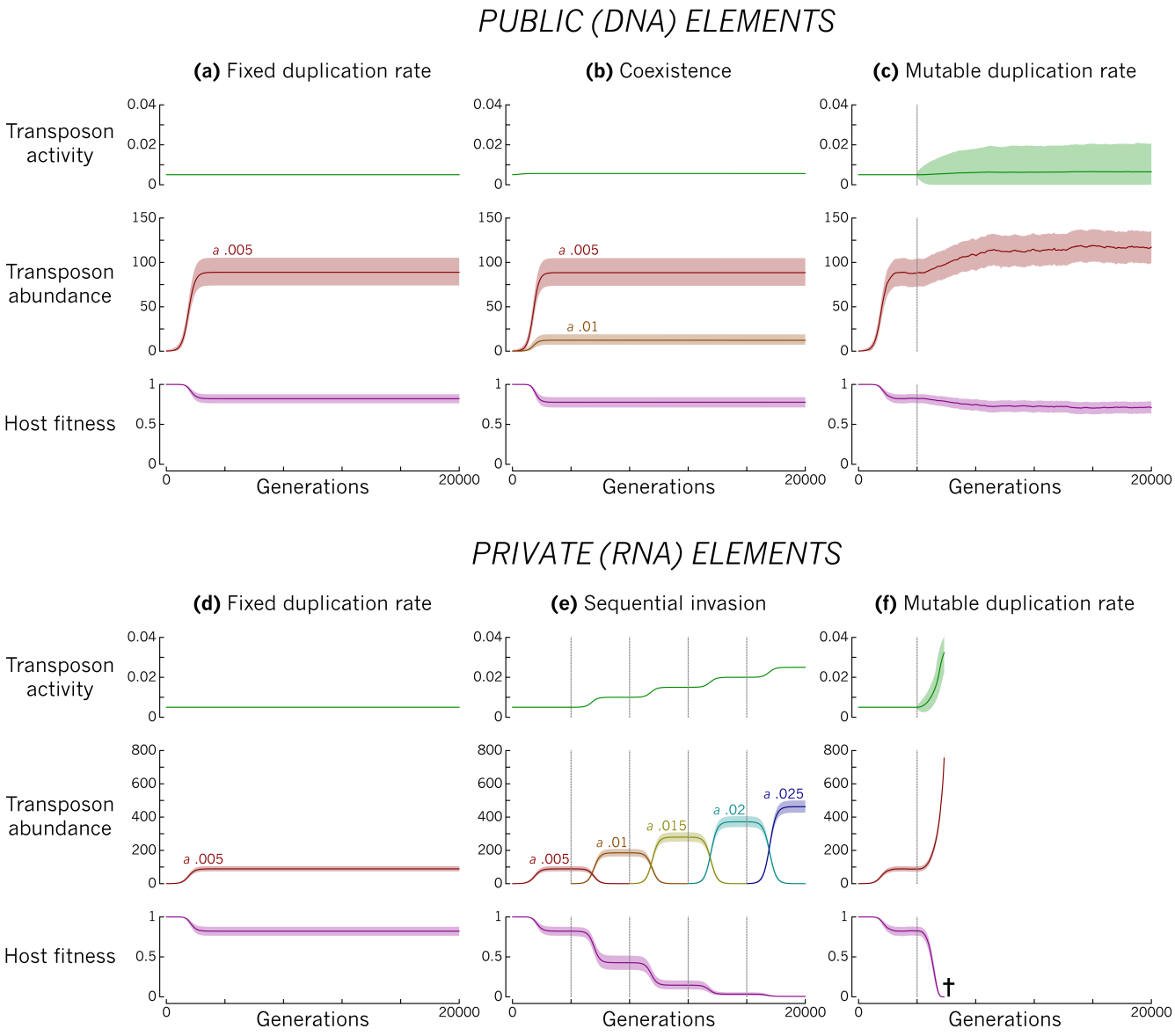


Figure 3: The tragedy of the transposons. A transposon family's mode of duplication—public or private—crucially determines the behaviour of transposons when they can freely mutate. Public duplication: **(a)** A fixed duplication rate results in equilibrium. **(b)** When a more prolific transposon is introduced, coexistence of the two types is observed. **(c)** When transposon activity can freely evolve, polymorphism in activity is maintained, and the system reaches a new equilibrium. Public elements do not have to invest in reproduction in order to spread: 9% of actively-duplicating elements in this panel have no transcriptional activity. Private duplication: **(d)** A fixed duplication rate results in equilibrium. **(e)** When a more prolific transposon is introduced, it displaces slower-duplicating transposons. These reach a higher equilibrium abundance, gradually lowering population fitness. **(f)** When activity can freely evolve, transposons are selected to be increasingly prolific, to the point of driving the host population extinct,—analogously to the classic "tragedy of the commons".

genome versus selection between genomes—with the inequality

$$\delta u > -\delta W\,. \tag{1}$$

The left-hand side of inequality 1 captures the mutant transposon's increase in within-genome proliferation, equal to the change in its duplication rate u, while the right-hand side of this equation captures the associated decrease in between-genome transmission, equal to the resultant change in its host's relative fitness W. Conceptually, when the increase in a mutant transposon's duplication rate exceeds the expected decrease in its hosts' relative fitness that results, the mutant element can invade.

For private elements, when a mutant transposon is introduced with its activity increased by δa, inequality 1 becomes

$$\delta a > -\frac{\mathrm{d}W}{\mathrm{d}a}\delta a\,; \tag{2}$$

that is, the increase to the transposon's duplication rate is simply δa, while the decrease to its host's relative fitness is equal to the slope of host relative fitness on the focal transposon's activity, times its increase in activity. Compare this to the trade-off for public elements: in this case, when a mutant transposon is introduced with its activity increased by δa, the increase in replication is shared amongst all members of the transposon family in the same genome, and so inequality 1 becomes approximately

$$\frac{1}{\bar{n}}\delta a > -\frac{\mathrm{d}W}{\mathrm{d}a}\delta a\,; \tag{3}$$

that is, under public duplication, a mutant transposon only experiences a fraction (approximately equal to $\frac{1}{\bar{n}}$) of its increased activity as an increase to its within-genome proliferation; therefore, the within-genome benefit of increased activity is smaller under public than private duplication. Since this fraction is inversely proportional to average copy number, publicly-duplicating transposons are subject to a "copy number brake" which makes increases to the transposition rate less beneficial as a given transposon family becomes more abundant within a host population.

This simple model also makes it clear that sex is ultimately the key to the tragedy

of the transposons, because sexual recombination limits the scope for selection against more active transposons (Bestor 1999; Lee 2010). Specifically, a mutant transposon suffers the selective consequences of producing more descendant transposons only to the extent that it stays associated with them. From a transposon's perspective, sex takes away half its genetic context each generation and replaces it with genetic material from its host's mate. This new genetic material may itself bear a number of transposons, but this contribution is uncorrelated with the behaviour of the focal transposon (see Fig. 1d). Because sex takes away half of a transposon's associates each generation, the average length of time that two transposons share a genome is approximately two generations (see *Methods*). As a result, there is almost no scope for relatedness between transposable elements to build up on the timescale relevant for duplications—with typical duplication rates being less than on the order of 10^{-5}–10^{-3} per generation (Burt & Trivers 2006). A more permanent association between a transposon and its relatives might provide them with an incentive to limit their activity, but regular recombination allows mutant prolific transposons to act with relative impunity, leveraging their transmissive advantage to displace wild-type transposons. Even if such an invasion lowers host fitness, this cost rapidly diffuses through the host population, such that the prolific transposons ultimately bear little penalty.

Clearly, retrotransposons in the wild do not always drive their host populations extinct, and yet this is what we observe in our model above. A major question raised by our results, therefore, is: how do natural populations avoid transposon-driven extinction? We next examine three hypotheses: (i) that stronger selection against transposons can counteract selection for increased activity; (ii) that parasitic elements can lead to the collapse of retrotransposon populations; and (iii) that host suppression can prevent extinction.

Two currencies of cost

For selection on transposon activity to lead to a tragedy of the commons, the transmissive advantage within genomes of greater activity must regularly exceed the selective disadvantage between genomes of greater activity. Is our finding that evolving duplication rates lead to a tragedy of the commons simply a result of unrealisti-

cally weak selection against transposons? Alternatively, could a different mode of selection against transposons prevent a tragedy of the commons? Above, following Charlesworth and Charlesworth (1983), we assume that damage to the host does not depend on transposon activity *per se*, only on the number of insertions in the host genome. But if the host suffers a fitness penalty which increases with transposon activity—for example, if increased activity means increasing the expression level of a transposon, which incurs an energetic cost to the host—then is it possible that selection could favour transposons that limit their duplication rate?

To investigate these possibilities systematically, we identified six potential mechanisms from the literature for how transposons may harm their hosts (Fig. 4): (i) that dispersed transposon sequences cause ectopic recombination (Langley *et al.* 1988); (ii) that insertion into coding sequences disrupts genes (Charlesworth & Charlesworth 1983); (iii) that the proliferation of transposon sequences incurs a DNA synthesis penalty for the host (Charlesworth *et al.* 1994; Charlesworth & Charlesworth 2010); (iv) that the expression of transposon genes incurs an energetic cost for the host (Nuzhdin 1999); (v) that massive overproduction of transposon gene products leads to protein aggregation, misfolding, and ribosomal stalling (Dong *et al.* 1995); and (vi) that unrepaired double-strand breaks may be caused by "cut-and-paste" transposition (Brookfield 1991). (This final hypothesis applies only to DNA transposons in eukaryotes, but we include it here for comparison.) Hypotheses i–iii are copy number costs, increasing with the number of insertions but not with activity *per se*. Hypotheses iv–vi are activity costs, increasing with the product of copy number and average transposon activity. We parameterized each fitness model with data collected from the literature, and also constructed a "combined" fitness model incorporating all five hypotheses relevant for RNA transposons (see *Methods* for full details).

Regardless of which cost model we use, we find that in no case is selection against transposons sufficient to prevent host extinction (Fig. 5). Even under the combined cost model, costs must be increased 30-fold relative to the most plausible estimates (see Methods) in order to prevent a tragedy of the commons, and the resulting equilibrium duplication rate of approximately one duplication every other generation is significantly higher than typical estimates for u of $\sim 10^{-4}$. That is, if we assume that

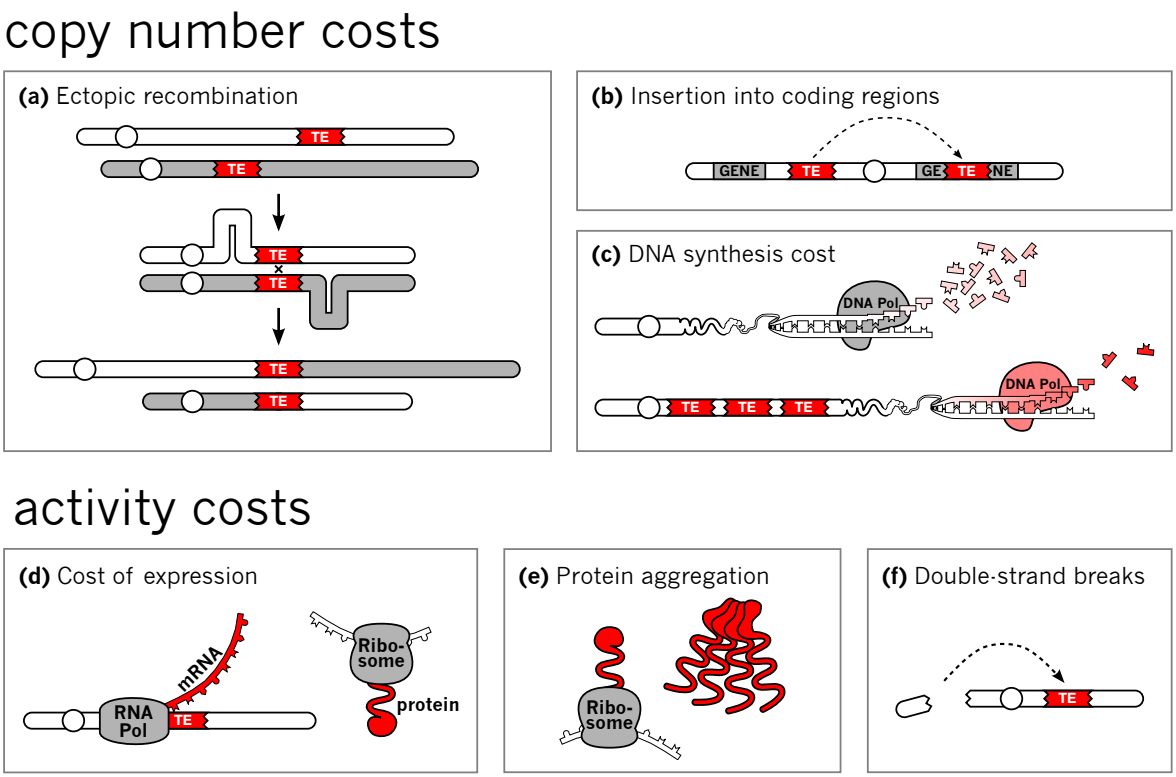


Figure 4: Transposon ecology: potential costs of transposon activity. Transposons can incur costs for their hosts in many ways. These can broadly be classified into two categories: copy number costs, in which the mere presence of the transposon is harmful, and hence the total cost increases with the number of transposons; and activity costs, in which attempts at replication by the transposon are harmful, and hence the total cost increases with overall transposon activity. These two models may yield different predictions when the activity of transposons can evolve, which decouples copy number costs from activity costs. Copy number costs: **(a)** pairing between dispersed elements leading to ectopic recombination; **(b)** insertions disrupting coding or regulatory regions; **(c)** energetic burden of synthesizing transposon DNA. Activity costs: **(d)** energetic burden of synthesizing RNA and protein; **(e)** protein aggregation and misfolding caused by excessive transposon activity; **(f)** double-strand breaks induced by elements transposing via a "cut and paste" mechanism (DNA elements only).

each insertion is 30–50 times more harmful than plausible estimates suggest it should be, it is possible to prevent population collapse, but transposons still settle on duplication rates that are vastly higher than what we observe, and host fitness is severely compromised in the process. We conclude that plausible selection against transposons' effects is not in itself sufficient to prevent the extinction of host populations.

How does the introduction of activity costs alter selection for transposon proliferation? Analytically, to capture the effect of both copy number costs and activity costs, we can decompose the derivative $\frac{dW}{da}$ from inequalities 2 and 3 into two components, one reflecting a direct activity cost, and one reflecting an indirect copy number cost which results from the increase in transposon number caused by the increase in activity:

$$\frac{dW}{da} = \frac{\partial W}{\partial a} + \frac{\partial W}{\partial n}\frac{dn}{du}\frac{du}{da}. \quad (4)$$

The first term on the right-hand side of equation 4, $\frac{\partial W}{\partial a}$, is the expected direct cost of increased transposon activity, while the second term, $\frac{\partial W}{\partial n}\frac{dn}{du}\frac{du}{da}$, is the product of (i) the slope of host relative fitness on copy number; (ii) the increase in average copy number caused by an increase to a focal transposon's duplication rate; and (iii) the increase in the focal transposon's duplication rate associated with its increase in activity. For small u, $\frac{dn}{du}$ is approximately equal to $2u$, because when sexual recombination dissociates a transposon from half of its descendants each generation, the average amount of time that a transposon inhabits the same genome as its descendants is two generations (see *Methods*).

Since an activity cost results in a direct disincentive for transposons to increase their proliferation rate, why doesn't imposing a fitness cost on transposon activity improve outcomes? Although gene expression, gene product aggregation, and the induction of double-strand breaks do incur a cost to the host, this cost is simply not large enough in magnitude to make a significant difference. To illustrate this point, we have calculated the expected equilibrium copy number for a given duplication rate under the various cost regimes we have tested (Fig. 6). Compared with estimates of duplication rate and abundance for seven different transposon families from *Drosophila melanogaster*, this illustration shows that ectopic recombination is likely the main cause

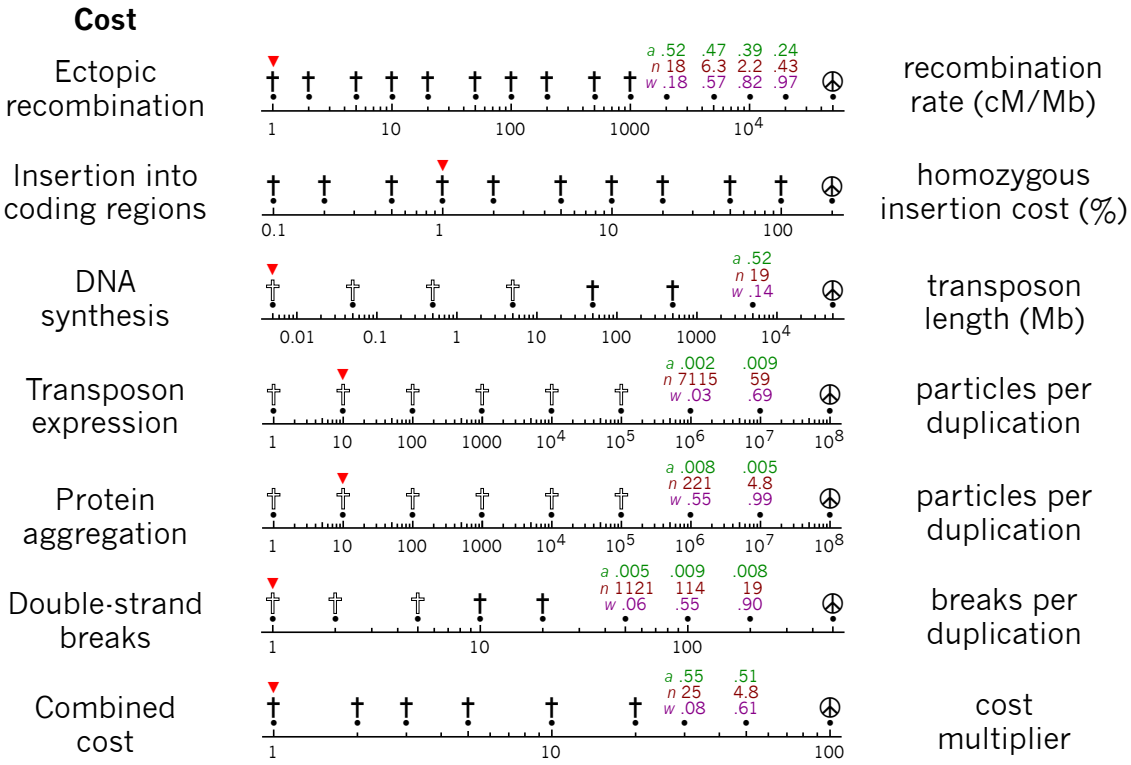


Figure 5: Realistic selection on transposons cannot prevent a tragedy of the commons for privately-duplicating elements such as RNA transposons. Six mechanisms are analysed (Fig. 4) as possible costs that would result in natural selection on transposons to limit their own activity, plus a seventh that combines all five relevant harms for RNA (privately-duplicating) transposons (RNA transposons are not thought to cause double-strand breaks, but that cost is shown for comparison). In each case, an estimate from literature for the magnitude of the cost is highlighted in red (see *Methods* for details); the strength of selection on each element increases exponentially towards the right on each scale. Crosses show where a run of the individual-based model with the corresponding cost resulted in extinction of the host population (filled crosses) or putative extinction (more than 10,000 active elements accumulated; simulations could not be run beyond this point). Peace signs represent the elimination of transposons from the population. Where equilibrium was attained for a given cost model, the equilibrium activity (a), copy number (n), and host fitness (w) are shown. Costs would need to be several orders of magnitude higher than expected in order to prevent population extinction. Moreover, even when costs are increased to such a point in the "combined cost" model, the predicted transposon duplication rate is much higher than is commonly observed.

of selection against transposable elements: other putative costs are simply too weak to keep equilibrium copy number out of the millions or even billions.[1] In particular, the three activity costs we investigate—gene expression, gene product aggregation and misfolding, and the induction of double-strand breaks—are relatively weak. From this point forward, we assume that the cost of transposons is due to ectopic recombination, combined with the costs of gene expression and gene product misfolding; although these latter costs are small, they will become relevant again when we explore host suppression below.

Given that transposons exhibit the duplication rates observed in natural populations, it seems that ectopic recombination is the most important force acting to keep their copy number in check. However, we still need to explain why duplication rates are as low as they are observed to be, since we have shown that selection against their deleterious effects alone cannot account for this.

A role for parasites?

In natural populations, LINE RNA elements can sometimes themselves be parasitized by sequences that interfere with *cis*-preference. For example, the mammalian L1 retrotransposon is parasitized by Alu elements, which bind to the signal recognition particle proteins SRP9/14, attach themselves to the ribosome with their poly-A tail exposed, and compete with the L1 mRNA for binding with the nascent L1 ORF2p retrotranscriptase (Fig. 7). These sequences encode no proteins of their own, but duplicate themselves solely by parasitizing autonomous elements. Because the presence of nonproductive elements contributes to the maintenance of a stable duplication rate in public elements, and competition between different transposable element families has been suggested as a factor contributing to the extinction of transposon lineages (Burt & Trivers 2006), we set out to investigate whether parasitizing elements might prevent the collapse of the host population in private elements by reducing their transposition rate. We do not expect parasitism of private transposons to be a general solution for

[1] The cost of insertion into coding regions could be sufficient to strongly select against unprolific transposon families, but its strength falls off rapidly with increasing duplication rate. Rather, it is likely that transposons avoid inserting into coding regions as a matter of course, with selection rapidly acting against any deleterious insertions that occur, such that selection against ectopic recombination is a longer-lasting, more gradual, and more important force.

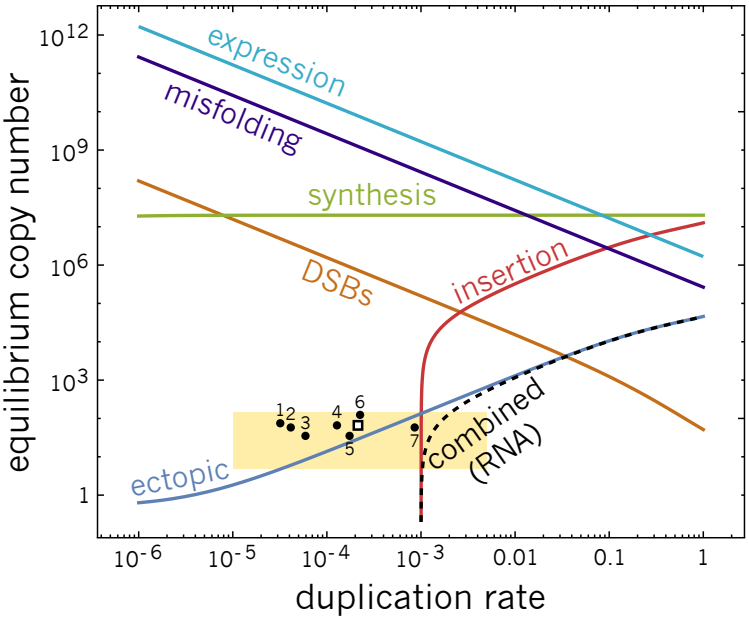


Figure 6: The cost of ectopic recombination is probably responsible for selection against transposons in natural populations. Here we show the relationship between duplication rate and expected equilibrium copy number for seven different possible cost regimes. Only selection against ectopic recombination and insertion into coding sequences are likely to keep transposons within the observed range for duplication rate and copy number, and the latter cost requires a fine-tuning of the duplication rate that is probably unrealistic. The shaded yellow region shows the region in which most transposon families in sexually-reproducing eukaryotes fall (between 5 and 150 actively-transposing copies, with an average duplication rate between 1×10^{-5} and 5×10^{-3}). Numbered dots show estimates from transposon families in *D. melanogaster*: (1) jockey, (2) Doc, (3) opus, (4) 297, (5) mdg-1, (6) roo, (7) copia. The square shows the average over all families. Estimates are from Maside *et al.* 2000 and Nuzhdin & Mackay 1994; points 6 and 7 are averages for roo and copia over both studies.

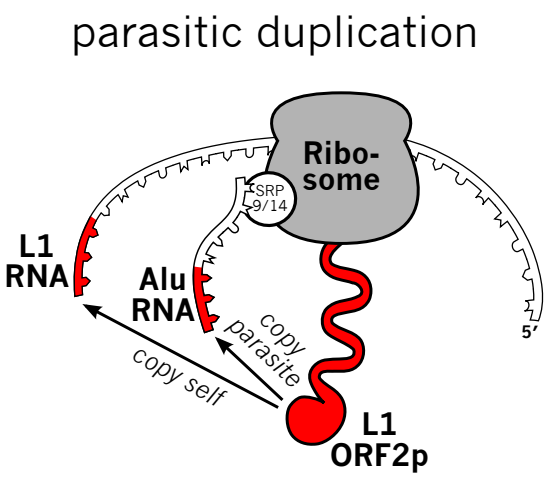


Figure 7: Transposon ecology: parasitic elements. LINE elements, such as L1 in mammals, are vulnerable to parasitism by non-autonomous SINE elements, such as Alu in primates. Here, an Alu RNA, complexed with signal recognition particle SRP9/14 proteins, binds to the ribosome with its poly-A tail exposed, thereby competing with the L1 RNA for retrotranscription by the nascent L1 ORF2p protein. The parasitic element still must invest energy into parasitizing the "target" element; this is what distinguishes this type of element from inactive public elements, which invest nothing into transcription but can still be copied.

preventing host extinction, because it does not seem that all RNA transposon families have a corresponding parasitic element. But could parasites be responsible in some cases for creating a stable transposition rate? (To avoid terminological confusion with the larger host population, we refer to the privately-duplicating transposon "hosts" of parasitic elements as "targets".)

A crucial parameter for parasitic elements is how their cytoplasmic concentration translates into efficiency at being inserted into the genome. Clearly, as the production of parasitic sequence increases, they should be retrotranscribed with increasing frequency. But does this frequency reach a plateau? Considering mechanistic details can help answer this question. As stated previously, the Alu element in primates parasitizes the L1 transposon by binding to ribosomes with its poly-A tail hanging off the ribosomal surface, such that the extending L1 polypeptide may bind to the parasitic sequence instead of to its own transcript (Fig. 7; Bennett *et al.* 2008; Ahl *et al.* 2015; Doucet *et al.* 2015). However, this mechanism suggests a limit to the efficacy of parasitism. Only one Alu element can be bound to a given ribosome at one time, and there may be no guarantee that the extending polypeptide would always select the Alu RNA rather than its own RNA, given the chance. In humans, about 50% of new inserts by

L1 machinery are Alu elements, which suggests that their parasitism may be subject to limits (Burt & Trivers 2006). We use the parameter ϕ to define the maximum efficiency of parasitic elements—*i.e.*, the frequency with which the elongating reverse transcriptase binds to the parasitic RNA rather than its own RNA, when confronted with both. We find that only for highly-effective parasites ($\phi > 98\%$) can parasitism result in the collapse of a transposon population and prevent host extinction (Fig. 8). Given that parasitized elements are likely to evolve to increase their own self-specificity in response to parasitism, and given the moderate level of parasitism observed with L1 and Alu, such a high level of parasite specificity does not seem likely. Finally, we turn to host suppression as a potential mechanism for preventing the collapse of the host population.

Choosing targets in the transposon–host arms race

In natural populations, mechanisms of host suppression may target genes, gene products, or both (Fig. 9). For example, KRAB zinc-finger genes target DNA associated with SVA elements, marking them for suppression in primates (Jacobs *et al.* 2014); and the Piwi-piRNA pathway, widely conserved across eukaryotes, is also capable of silencing transposon-associated DNA (Senti & Brennecke 2010). This mode of suppression targets the genes themselves, "turning off" transcription at the source. However, the Piwi-piRNA pathway also targets and degrades the mRNA transcripts of the transposons that it silences (Senti & Brennecke 2010). This mode of suppression targets gene products, breaking down mRNAs as they are transcribed.

In order to model these two possibilities, we introduced into our model a host suppression locus encoding a continuous trait s, where each unitary increase to s results in a decrease in transposon activity and an increase in the cost of host suppression (see *Methods* for details). We find that the target of host suppression—genes versus gene products—results in crucially different outcomes. In our model, when host suppression targets genes, the outcome is an ever-escalating evolutionary arms race: in response to increased suppression, transposons increase their activity even more, until the cost of maintaining suppression itself grows too severe and populations are driven extinct (Fig. 10a). In contrast, when suppression targets gene products,

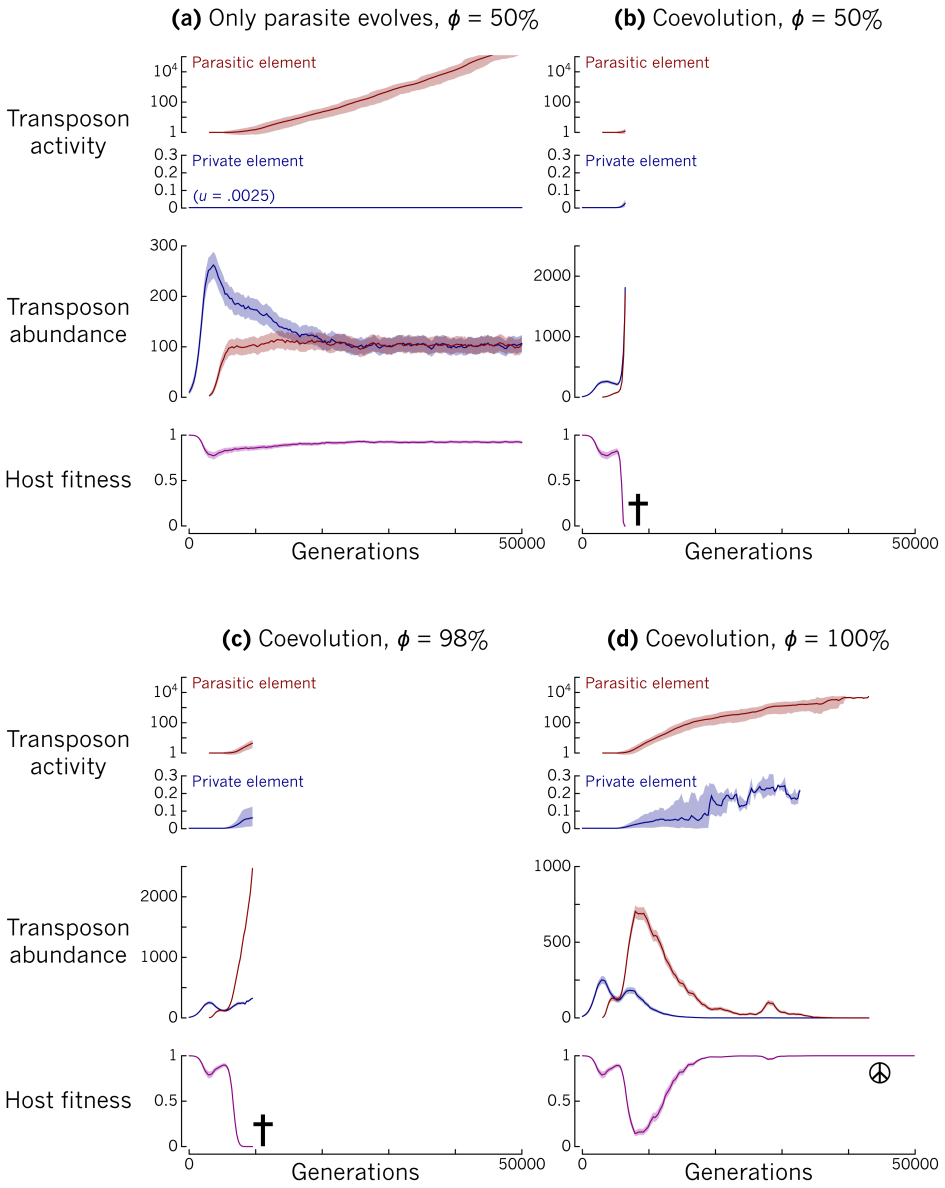


Figure 8: Dynamics of parasitic elements and the private elements they exploit. **(a)** When the target element's activity is fixed, parasites as a whole rapidly saturate opportunities for duplication, but still compete amongst themselves for access to reproduction. **(b)** When the target element is permitted to evolve, a tragedy of the commons results despite its partial exploitation by a parasite. This occurs when the maximum rate of parasitism is relatively low ($\phi = 50\%$), but also **(c)** when it is relatively high ($\phi = 98\%$). **(d)** When the parasite is permitted to completely exploit its target element ($\phi = 100\%$), it will eliminate that element completely, and then die off itself. However, complete exploitation may not be common; in the best-studied system of parasitic elements (L1/Alu in primates), about 50% of new insertions are L1 and 50% are Alu (Burt & Trivers 2006).

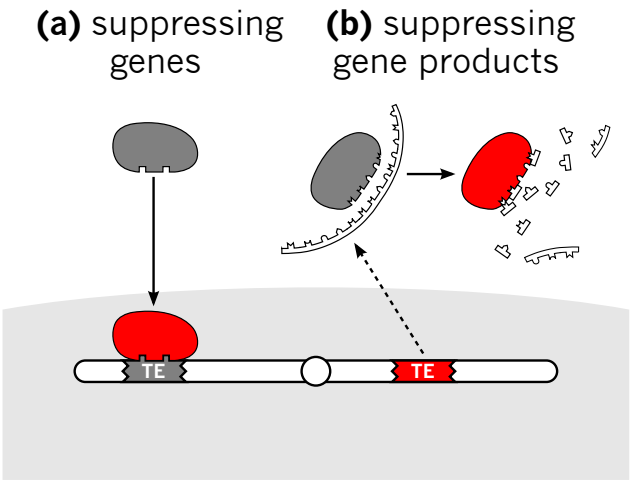


Figure 9: Transposon ecology: targets of host suppression. **(a)** When suppression targets genes, transcription is "turned off" at the source. In this case, suppression dampens both the transposon's duplication rate and its expenditure of cellular resources. **(b)** When suppression targets gene products, transcripts are degraded as they are produced. Here, suppression dampens the transposon's duplication rate, but the transposon still spends as much host energy transcribing itself.

a stable equilibrium is eventually reached—that is, the arms race reaches a point at which neither party is selected to further escalate the conflict (Fig. 10b).

To conceptualize these results, we turn to our analytical framework again. The effect of suppression is to make it such that an increase in transposon activity results in only a fractional increase to the duplication rate. When suppression targets genes, the cost to the host of increased transposon activity also decreases. For a mutant transposon with an increase δa in activity, the fundamental tradeoff of inequality 1 becomes

$$S\delta a > -S\left(\frac{\partial W}{\partial a} + \frac{\partial W}{\partial n}\frac{\mathrm{d}n}{\mathrm{d}u}\frac{\mathrm{d}u}{\mathrm{d}a}\right)\delta a\,, \tag{5}$$

where $0 < S \leq 1$ is the factor by which suppression decreases the activity of transposons. Here, S tempers both the transposon's increase in within-genome proliferation, δa, and the associated fitness cost to the host, $\left(\frac{\partial W}{\partial a} + \frac{\partial W}{\partial n}\frac{\mathrm{d}n}{\mathrm{d}u}\frac{\mathrm{d}u}{\mathrm{d}a}\right)\delta a$. Since the factor S enters into both sides of the inequality, it cancels out; thus, suppression of genes can only slow the collapse of the genome, not prevent it altogether. However, when

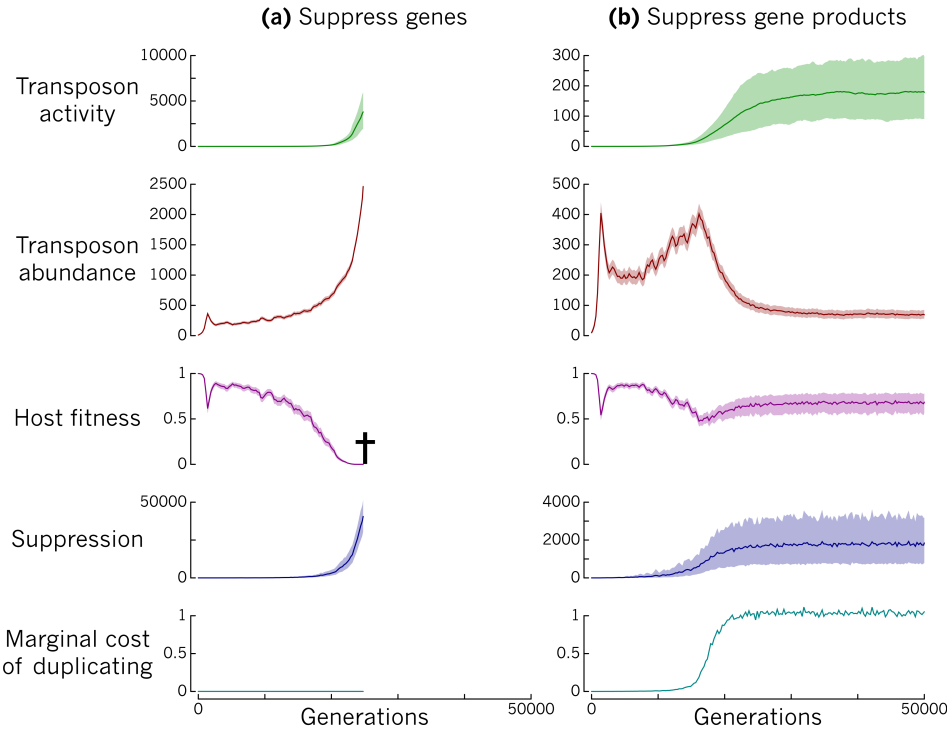


Figure 10: Dynamics of suppression. **(a)** When transposon suppression targets genes, an arms race is instituted whereby transposons compensate for suppression by increasing their activity. Eventually the cost of maintaining suppression overwhelms the host, leading to extinction. **(b)** When transposon suppression targets gene products such as RNA, this has the effect of increasing a transposon's cost of duplication. Increasing costs can lead to a stable equilibrium for transposon activity because, as the marginal cost of duplicating increases (expressed in units of relative host fitness), the transposon's incentive to invest more in reproduction decreases. While the cost of expression limits transposon activity, the cost of ectopic recombination limits transposon copy number. The result is stability in activity, duplication rate, transposon abundance, host fitness, and host suppression.

suppression targets gene products, the inequality becomes

$$S\delta a > -\left(\frac{\partial W}{\partial a} + S\frac{\partial W}{\partial n}\frac{\mathrm{d}n}{\mathrm{d}u}\frac{\mathrm{d}u}{\mathrm{d}a}\right)\delta a\,; \tag{6}$$

that is, suppression succeeds in dampening any copy number cost associated with an increase in the actual duplication rate u, but the host—and by extension, the mutant transposon itself—still pays the full cost $\frac{\partial W}{\partial a}$ of increased activity, because it is still investing host resources in its increased activity. Hence, the marginal cost to a transposon of duplicating itself gradually rises as the suppression of gene products increases, until the point at which the benefit of increased activity no longer exceeds its cost (Fig. 10b)—thus averting the tragedy of the commons.

If this model is correct, we should expect–especially for privately-duplicating RNA transposons—a high level of transposon expression in natural populations, but also strong suppression, resulting overall in low duplication rates. There is evidence for transposons being highly-transcribed: even with suppression in place, a significant proportion of transcribed RNA in mammalian germlines—3% in mice, and 15% in humans—comes from transposons (Faulkner *et al.* 2009). Moreover, the strength of suppression appears to be significant, especially through the primary eukaryotic line of defence against transposons, the Piwi-piRNA pathway. When this pathway is knocked out in mice, for example, spermatogenesis becomes impossible owing to extreme proliferation of transposons in germline cells (Kuramochi-Miyagawa *et al.* 2004); when PIWI activity is knocked down in *Drosophila*, measured abundances of transposon RNA in the ovary increase up to 30-fold (Sytnikova *et al.* 2014). The Piwi-piRNA pathway works partly through degradation of transposon gene products, which is consistent with our model. However, there is also ample evidence for suppression of genes rather than gene products. For example, in the Piwi-piRNA pathway, the initial degradation of RNA transcripts acts as a trigger which targets further suppression to the genome. And one of the most striking illustrations of the transposon–host arms race comes from the ZNF93 transposon suppressor gene in primates: the L1 transposons that ZNF93 suppresses—by binding to L1 DNA, inhibiting its transcription—can escape suppression via an internal deletion of the

binding sequence, though this comes at the cost of a reduced expression level for the transposon (Jacobs *et al.* 2014). Overall, the evidence suggests that both suppression of transposon genes and of their gene products may be important for genomic stability.

A test of the model: suppression and arms races in natural populations

Our model predicts that host suppression of transposons has been the key to the evolution of stable genomes. This prediction is supported by the widespread occurrence of suppression. This is a relatively weak test of our model as, to our knowledge, there are no sexually-reproducing eukaryotes without suppression for comparison. However, a key corollary of suppression's importance is that it should be targeted to the type of transposon that threatens genome stability the most. We examined this in our simulations by allowing transposons from public, private, and parasitic families to evolve in the same host population, permitting hosts to allocate suppression to each type independently. We find that, as expected, hosts suppress private transposons the most, while they disburse less energy suppressing the self-limiting public transposons, and the least energy of all suppressing parasitic elements (Fig 11a).

In order to evaluate whether this pattern of suppression allocation holds true for natural populations, we analysed piRNA expression data from the germlines of three species—*Homo sapiens*, *Drosophila melanogaster*, and *Danio rerio*—to evaluate which type of transposon is most suppressed. These piRNAs are 26–31 nt fragments used by the Piwi-piRNA pathway to direct suppression towards complementary sequences in RNA or DNA. In turn, piRNAs are themselves generated from specially-demarcated piRNA clusters, segments of the host genome which act as "traps" for mobile elements that insert themselves there. A null hypothesis might be that hosts allocate suppression according to which type of transposon is the most abundant in the genome, since that type ought to appear most frequently in piRNA clusters. Therefore, we measured the "suppression bias" of each transposon type by normalizing piRNA abundance to the fraction of the host genome occupied by the corresponding transposon type. We find—as predicted—that private elements are the most highly-suppressed in all three species, with public elements suppressed less, and parasitic elements suppressed less still (Fig 11b).

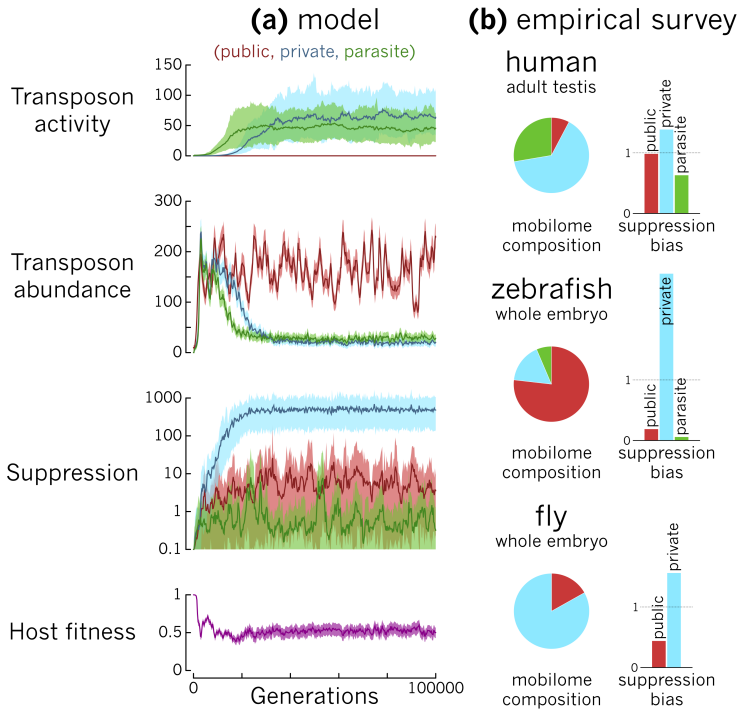


Figure 11: Suppression allocation in the model and in natural populations. **(a)** When a host population is simultaneously invaded by public, private, and parasitic elements, hosts allocate most of their suppressive resources to the private elements, some to the public elements, and very little to the parasitic elements. **(b)** Germline cells preferentially suppress private elements, while public elements receive less suppression and parasitic elements receive the least amount of suppression. Pie charts show the proportion of each species' mobilome (the total of all mobile elements in the genome) that can be classified as public, private, or parasite is shown, with proportions relative to total sequence length. Suppression bias bar charts show the number of piRNA sequences mapping to each type of element relative to mobilome composition, normalized such that the average suppression bias is 1.

Conclusions

Here we have shown that existing models of equilibrium between transposons and their hosts break down in the face of transposon evolution. We suggest that transposons come in three types—public, private, and parasitic—which adopt different ecological roles within the genome. When private transposons are allowed to evolve, they are selected to continually increase their activity, causing a "tragedy of the commons" that results in the death of transposons and of their hosts. Neither selection against transposons' deleterious effects nor parasitism can plausibly prevent this outcome. Instead, host suppression is crucial to prevent population extinction, and we suggest that only a suppression strategy targeting gene products—as opposed to silencing the genes themselves—can result in a long-term equilibrium in transposon duplication rate.

More generally, our study emphasizes the importance of viewing transposon evolutionary dynamics not merely as a negotiation between opposing, static forces of proliferation and selection, but also as a fundamentally adaptive process—of selfish genetic elements adapting to their hosts, and of hosts adapting to selfish genetic elements in turn. This approach is aided by taking an ecological view of the genome, to consider the roles transposons adopt in interacting with their social and physical environment. Such a view fundamentally changes predictions for long-term genome evolution, and helps to explain empirical findings concerning host suppression and transposon activity.

Our model suggests that only the suppression of gene products is a viable long-term solution to unchecked transposon proliferation. However, suppression systems which act directly on genes are common (*e.g.* Aravin *et al.* 2007; Reichmann *et al.* 2013; Jacobs *et al.* 2014). It is possible that a dual host strategy—suppressing both genes and gene products—is more effective than the suppression of gene products alone. Alternatively, it might be possible that there are fundamental limits to transposon evolution—perhaps imposed by some fundamental cap on transcription rates, or an uneven distribution of mutational effects which make it difficult for transposons to reliably increase their expression beyond a certain point. Such limits as these could result

in host genes being fundamentally more powerful than selfish genetic elements, which might simply allow the host to win its arms race with transposons even in the absence of a true equilibrium in transposon activity. This possibility could be investigated with a more mechanistically-explicit model of the evolution of transposon expression level.

In order to keep our focus primarily on differences between transposon families according to their ecological role, we have assumed in our simulations that different transposon types—public, private, and parasitic—differ only in their mode of duplication. However, transposons of these types in natural populations also differ in other ways. For example, parasitic elements tend to be much shorter than average, while private elements are longer, which reflects the differences in complexity between the three types of element. In turn, element length may have an impact upon: (i) selection against transposons, as longer elements have been shown to be more deleterious (Petrov *et al.* 2003); (ii) the maximum activity of transposons, as longer elements may be able to contain more transcription-promoting sequences; and (iii) suppression, as longer sequences should generate more piRNAs. A more precise accounting of these and other differences between types may result in a deeper understanding of the system.

The genome is a complex place, home to a vast number of selfish genetic elements whose interactions with its host population have a wide range of micro- and macro-evolutionary effects. In this arena, it is clear that the accumulation of transposon-derived DNA is central to many major features of genome organization and complexity—a problem which has recently attracted vociferous debate (Lynch 2007; ENCODE Project Consortium 2012; Graur *et al.* 2013; Palazzo & Gregory 2014). Here we hope to have shown the power of a social evolution approach for understanding transposon and genome evolution. The evolution of genomes is the earliest, and arguably the most important, example of social evolution in the history of life. Through this exploration of transposon–host coevolution, we hope to update our theoretical understanding to match the growing mechanistic knowledge of social life in the genome.

Methods

1. Mathematical analysis

1.1 Deterministic population-genetics model

We model an infinite, diploid, monoecious host population with non-overlapping generations. The host population is infected by transposons of two types, A and B; we use A to represent the wild-type transposons and B to represent mutant transposons. We notate the frequency of individuals with i type-A transposons and j type-B transposons as f_{ij}; the transposon types differ in their activities, a_{A} and a_{B}, but have the same rate of loss v. The population goes through two stages: (1) updating of genomes with respect to transposon duplication and loss, and (2) reproduction with respect to differential fitness. In the updating stage, the probability that there will be k_{e} excisions among m transposons is $E(k, m, v) = \mathbb{B}(k|n = m, p = v)$ and the probability that there will be k_{d} duplications among m transposons is $D(k_{\mathrm{d}}, m, u) = \mathbb{P}(k|\lambda = mu)$, where $\mathbb{B}(k|n, p) = \binom{n}{k} p^k (1-p)^{n-k}$ is the binomial probability mass function and $\mathbb{P}(k|\lambda) = \frac{\lambda^k}{k!} e^{-\lambda}$ is the Poisson probability mass function. The duplication rate u is equal to a_{A} for private transposons of type A, a_{B} for private transposons of type B, and $\frac{n_{\mathrm{A}} a_{\mathrm{A}} + n_{\mathrm{B}} a_{\mathrm{B}}}{n_{\mathrm{A}} + n_{\mathrm{B}}}$ for public transposons in a genome with n_{A} type-A transposons and n_{B} type-B transposons. For simplicity, we assume that over the course of a generation, each transposon is "marked" for excision with probability v and generates transcripts at rate a_{A} or a_{B}, and immediately prior to host reproduction, the marked transposons are removed and all transcripts are converted into new transposons. That is, an individual can go from hosting i to $i + \delta$ transposons by undergoing zero excisions and δ duplications, or one excision and $\delta + 1$ duplications, or two excisions and $\delta + 2$ duplications, *etc*. Therefore, after updating, the frequency of individuals with i type-A transposons and j type-B transposons is $F_{ij} = \sum_{I,J} f_{IJ} A(I \to i) B(J \to j)$, where $A(I \to i) = \sum_{k=\max(0,I-i)}^{I} E(k, I, v) D(i - I + k, I, a_{\mathrm{A}})$ and $B(J \to j) = \sum_{k=\max(0,J-j)}^{J} E(k, J, v) D(j - J + k, J, a_B)$ when transposons duplicate privately, and $A(X \to x) = B(X \to x) = \sum_{k=\max(0,X-x)}^{X} E(k, X, v) D(x - X + k, X, \frac{n_{\mathrm{A}} a_{\mathrm{A}} + n_{\mathrm{B}} a_{\mathrm{B}}}{n_{\mathrm{A}} + n_{\mathrm{B}}})$ when transposons duplicate publicly. In the reproduction stage, a genotype-ij indi-

vidual generates a relative proportion w_{ij} of all gametes, where w_{ij} is the fitness of a genotype-ij individual, and no linkage is assumed between transposons; therefore, the frequency of gametes with i type-A transposons and j type-B transposons is $g_{ij} = \sum_{I,J} \frac{w_{IJ} F_{IJ}}{\bar{w}} \mathbb{B}(i|n = I, p = \frac{1}{2}) \mathbb{B}(j|n = J, p = \frac{1}{2})$, where $\bar{w} = \sum_{I,J} f_{IJ} w_{IJ}$ is the average host fitness; then, gametes fuse to form the next offspring generation, $f'_{ij} = \sum_{I=0}^{i} \sum_{J=0}^{j} g_{IJ} g_{i-I,j-J}$, replacing the parental generation and returning the population to the beginning of the life cycle. The model is phrased above in terms of supporting a potentially-infinite number of transposons in any given genome, but we only simulate genotype frequencies up to a number $n_{\max}$ sufficient to accurately capture population dynamics.

1.2 Stochastic individual-based model

There are N hosts, each with $2C$ linear chromosomes comprising L sites. Sites can be empty or occupied by a transposon. Indexing an individual's n transposons by $i = \{1, 2, \ldots, n\}$, we denote the activity of the ith transposon by a_i. Every generation, each genome is updated by k_{d} duplications, k_{e} excisions, and k_{m} mutations, where k_{d} is drawn from a Poisson distribution with mean $\sum_i a_i$, k_{e} is drawn from a binomial distribution with n trials each with success probability v, and k_{m} is drawn from a binomial distribution with n trials each with success probability μ_a. Then k_{d} elements are randomly drawn with replacement, where the probability of element i being drawn is proportional to a_{i} for private elements and proportional to $\frac{1}{n_{\mathrm{F}}} \sum_f a_f$ for public elements of family F, where the index f ranges over all elements in family F, and properties of these elements are saved for later insertion; k_{m} elements are selected, with replacement, and their activity a_i is changed to $a'_i = \max(0, \min(a_{\max}, a_i + \delta a))$, where δa is drawn from a normal distribution with mean 0 and standard deviation $\sigma_{\mathrm{A}} + \sigma_{\mathrm{a}} a_i$ (that is, there is both an absolute and a relative component of transposon mutation); k_{e} elements are selected, without replacement, and replaced by empty sites; and then the elements selected for duplication are inserted into randomly-selected empty sites. Each individual also bears two suppressor alleles for each transposon family f, s_{f1} and s_{f2}, each of which is randomly mutated with probability μ_{s} to a new value $s' = \max(0, \min(s_{\max}, s + \delta s))$, where δs is drawn from a normal distribution

with mean 0 and standard deviation $\sigma_S + \sigma_s s$. Individuals reproduce with probability proportional to their fitness w; gametes are generated via meiosis of parental chromosomes, where the number of chiasmata is drawn from a Poisson distribution with mean χ; suppressor loci are unlinked to transposon loci and to each other, and one of each pair of suppressor alleles is passed on randomly to each gamete. Gametes fuse to form N new individuals, returning the population to the beginning of the life cycle.

1.3 Simulation details

For simulations of the deterministic population-genetics model and the stochastic individual-based model, parameters are given in Table 1, with the following parameters varying between runs:

Fig. 1e (deterministic): $N = \infty$, $w =$ ectopic recombination model with $\varepsilon = 1$; $a_0 = 0.005$.

Fig. 1e (stochastic): $N = 50,000$; $w =$ ectopic recombination model with $\varepsilon = 1$; $w_{ext} = 10^{-6}$; $a_0 = 0.005$.

Fig. 3a, b, d, e: $N = \infty$, $w =$ ectopic recombination model with $\varepsilon = 1$; $a_0 = 0.005$.

Fig. 3c, f: $N = 50,000$; $w =$ ectopic recombination model with $\varepsilon = 1$; $w_{ext} = 10^{-6}$; $a_0 = 0.005$.

Fig. 5: $N = 5000$; $w =$ as indicated in figure; $w_{ext} = 10^{-6}$; $a_0 = 0.005$, unless higher activity is needed to avoid the immediate clearance of transposons from the host population.

Fig. 6: fitness functions as given in section 2 below, with number of VLPs per successful duplication $k = 10$ and number of DSBs per successful duplication (for DNA transposons) $j = 10$.

Fig. 8: $N = 5000$; $w =$ ectopic recombination model with $\varepsilon = 0.15$; $w_{ext} = 10^{-10}$; $a_0 = 0.0025$.

Fig. 10: $N = 10,000$; $w =$ ectopic recombination model with $\varepsilon = 0.15$ + cost of expression with $k = 100$ + cost of misfolding with $k = 100$ + cost of suppression (see below); $w_{ext} = 10^{-10}$; $a_0 = 0.005$.

Fig. 11: $N = 5000$; $w =$ ectopic recombination model with $\varepsilon = 0.15$ + cost of expression with $k = 100$ + cost of misfolding with $k = 100$ + cost of suppression;

$w_{\text{ext}} = 0$; $a_0 = 0.005$.

Any parameters not given here are as indicated elsewhere in the text.

1.4 Association between transposons

The average association between a transposon and its descendants, ignoring the effect of selection, is the stationary process resulting from excision, loss, and recombination. This yields a transition matrix

$$A_{ij} = \sum_{k=1}^{\infty} \mathbb{B}\left(j|n=k, p=\frac{1}{2}\right) \left[\sum_{l=\max(0,i-j)}^{i} \mathbb{B}\left(l|n=i, p=v\right) \mathbb{P}\left(l+j-i|\lambda=iu\right)\right],$$

where $\mathbb{B}$ is the binomial probability mass function and $\mathbb{P}$ is the Poisson probability mass function. The mean over the stationary distribution of this transition matrix is $\frac{1+2u-v^2}{1-(u-v)^2}$: this is the expected asymptotic number of identical-by-descent clones a transposon with duplication rate u and excision rate v is associated with in the long run, including itself. For small u and v, this is approximately equal to $1+2u$. That the average number of descendants a given transposon shares its genome with is approximately $2u$ can be understood in light of the fact that u is the rate at which a focal transposon creates a descendant in the same genome, and that because sexual recombination changes roughly half of a given transposon's genetic context each generation, a transposon will remain in the same genome as its descendants for, on average, approximately 2 generations.

1.5 Analytical calculation of equilibrium copy number

Following Charlesworth & Charlesworth (1983), the equilibrium copy number $\bar{n}$ can be calculated by solving the equation

$$V_n \frac{\mathrm{d}\ln w}{\mathrm{d}n} = \bar{u} - v\,,$$

where V_n is the variance in copy number between individuals and the derivative is evaluated at $n = \bar{n}$. When $\bar{u}$ is small, copy number roughly follows a Poisson distribution and so $V_n = \bar{n}$. However, when the duplication rate is higher, copy number is

Parameter	Notation	Value
population size	N	*
chromosome number (haploid)	C	2
chromosome length (Morgans; stochastic model only)	χ	0.9
host fitness model	w	*
extinction threshold	w_{ext}	*
transposon activity		
initial value	a_0	*
mutation rate (per transposon per generation)	μ_{a}	0.01
mutation magnitude	σ_{A}, σ_{a}	0.001, 0.05
host suppression		
half-saturation constant	K	0.1
initial value of each allele	s_0	0
mutation rate (per allele per generation)	μ_{s}	0.025
mutation magnitude	σ_{S}, σ_{s}	0.05, 0.25
parasitism		
half-saturation constant	κ	100
maximum parasite efficiency	ϕ	*

Table 1: Key parameters used in the deterministic population-genetics and stochastic individual-based models. Parameters marked with an asterisk are given in the text or in figures. The extinction threshold is the fitness below which a population is assumed to be in terminal decline.

overdispersed relative to a Poisson distribution. We have found that a more accurate solution for equilibrium copy number (*i.e.*, one that matches both the deterministic population-genetics model and the stochastic individual-based model) can be determined by taking $V_n = \bar{n}\left(1 + \frac{8}{3}(\bar{u} - v) - \frac{3}{4}(\bar{u} - v)^2\right)$. This is an approximate relationship, which gives reasonable estimates when $0 < \bar{u} - v < 1$.

2. Host fitness models

Selection against transposon virulence—and, hence, the outcome of transposon–host coevolution—depends crucially on how transposons harm their hosts. However, the difficulty of measuring transposon-mediated effects on host fitness means that it is uncertain which mechanisms of harm are actually responsible for selection against transposon proliferation. Here, we investigate six hypotheses for selection against TEs, parameterizing a host fitness function for each using published data wherever possible. In contructing these fitness functions, we assume a "typical" transposon family and a "typical" multicellular eukaryote host. These are broad categories, but our focus here is on reaching plausible cost estimates. We focus on estimates from *D. melanogaster*, not least because this is the best-studied model organism for most of our purposes.

Three of the selective forces invoked depend primarily on transposon copy number: the risk of ectopic recombination, insertions into genes or regulatory regions, and the energetic burden of synthesizing transposon DNA. The three remaining selective forces depend primarily on overall transposon activity, the product of copy number and average expression level: the energetic cost of transposon expression, the cellular cost of massive overexpression of identical proteins, and the production of double-strand breaks by DNA transposons.

2.1 Ectopic recombination

Because transposons are homologous sequences dispersed throughout the genome, two transposons of the same family at heterologous loci may undergo ectopic exchange. If this occurs during meiosis, crossing over between nonhomologous

loci may occur, resulting in massive gene loss or duplication in gametes, rendering them inviable (Langley *et al.* 1988; Lee & Langley 2010; Charlesworth & Charlesworth 2010). Ectopic recombination may also occur during mitosis, but since the frequency of mitotic recombination is much lower than that of meiotic recombination—for example, 10^4–10^5-fold lower in *Saccharomyces cerevisiae* (Lee *et al.* 2009)—we ignore this possibility here. Ectopic exchange between transposons has been directly observed in yeast, *D. melanogaster*, and humans (Charlesworth & Charlesworth 2010), and because the risk of ectopic recombination scales quadratically with the number of dispersed homologous sequences, this cost naturally increases faster than log-linearly with transposon number. Consequently, it has been suggested to be the most important transposon-related cost to host fitness (Charlesworth & Charlesworth 2010).

If each pair of dispersed transposons is equally probable to cause an ectopic recombination event, and host fitness scales with the proportion of viable gametes produced, this suggests a fitness function

$$w = \left(1 - \frac{\lambda\varepsilon\rho}{100}\right)^{\frac{1}{2}\left(n(n-1)-n_\mathrm{g}\right)},$$

where: λ is the length of the transposon in Mb; ε is the efficiency of ectopic recombination relative to allelic recombination; ρ is the allelic rate of recombination in cM / Mb; n is transposon copy number; and n_g is the number of transposon copies that are homozygous, making $\frac{1}{2}\left(n(n-1)-n_\mathrm{g}\right) = \binom{n}{2} - \frac{n_\mathrm{g}}{2}$ the number of possible pairs of dispersed transposons.

We take $\lambda = 5$ kb as a representative transposon length—this is the median length over all RNA transposon families in *D. melanogaster* (from a range of approximately 1–10 kb). For the recombination rate between dispersed elements, studies in yeast suggest that ectopic recombination is approximately 15% as frequent as allelic recombination, although the rate can be elevated if the ectopic sequences occur very close together (within ~300 kb) on homologous chromosomes (Goldman & Lichten 1996). The allelic recombination rate is approximately 1 cM/Mb in *Drosophila* (Singh *et al.* 2005; Fiston-Lavier *et al.* 2010) and humans (McVean *et al.* 2004); more generally, the median recombination rate in mammals (Dumont & Payseur 2000) and plants

(Jaramillo-Correa *et al.* 2010) is approximately 1 cM/Mb. Overall, then, we adopt ρ = 1 cM/Mb and $\varepsilon = 0.15$ as an estimate of the rate and relative efficiency of ectopic recombination. Silencing of transposons may result in their adopting a heterochromatic state, and the rate of recombination in heterochromatin is generally lower than that in euchromatin; however, for the sake of simplicity, we ignore the possibility of suppression directly tempering the copy number cost of transposons.

2.2 Insertions

To model the insertional effects of transposons, at least three aspects need to be considered. First, the effect of individual insertions; second, the relative magnitude of heterozygous versus homozygous effects; and third, interactions between insertions. Taken together, this could be modelled as an effect for each homozygous insertion, a lesser effect for each heterozygous insertion, and synergistic effects for each possible pair of insertions (of three possible types: homozygous + homozygous, homozygous + heterozygous, and heterozygous + heterozygous). Together, this yields a fitness function of the form

$$w = (1-s)^{\frac{n_\mathrm{g}}{2}} (1-Hs)^{n_\mathrm{h}} (1-ts)^{\frac{1}{8}n_\mathrm{g}(n_\mathrm{g}-2)} (1-Hts)^{\frac{1}{2}n_\mathrm{g}n_\mathrm{h}} \left(1-H^2ts\right)^{\frac{1}{2}n_\mathrm{h}(n_\mathrm{h}-1)} .$$

Above, n_g and n_h are the number of homozygous and heterozygous insertions, respectively; s is the average effect of a homozygous insertion; H is the relative effect of a heterozygous insertion compared to a homozygous insertion; and t is the relative epistatic effect for each pair of homozygous insertions.

Note that effects of deleterious mutations are commonly expressed using an exponential function of the number of mutations and the number of mutations squared (*e.g.*, of the form $w = \exp(-\alpha n - \beta n^2)$). For an expression of this form, note that when s is small, the above function is approximately equal to

$$w = \exp\left(-\alpha_\mathrm{g} n_\mathrm{g} - \alpha_\mathrm{h} n_\mathrm{h} - \beta_\mathrm{g} n_\mathrm{g}^2 - \beta_\mathrm{gh} n_\mathrm{g} n_\mathrm{h} - \beta_\mathrm{h} n_\mathrm{h}^2\right) ,$$

where $\alpha_\mathrm{g} = \frac{s}{2}(1-\frac{t}{2})$, $\alpha_\mathrm{h} = Hs(1-\frac{Ht}{2})$, $\beta_\mathrm{g} = \frac{st}{8}$, $\beta_\mathrm{gh} = Hts$, and $\beta_\mathrm{h} = \frac{H^2ts}{2}$.

Many studies have estimated the fitness effects of homozygous or hemizygous transposon insertions. Eanes *et al.* (1988), investigating P element insertions on the X chromosome in *D. melanogaster* males, estimated an average fitness cost of 1.1% per hemizygous insertion. Houle and Nuzhdin (2004), studying copia insertions in *D. melanogaster* females, estimated an average offspring viability cost of 0.76% per homozygous insertion. Costs of a larger magnitude were estimated by Mackay *et al.* (1992), who studied the insertional effects of a modified P element, estimating an average viability cost of 12.2% per homozygous insertion. However, Houle & Nuzhdin note that the applicability of the estimates made by Mackay *et al.* may be limited because the modified P element used was four times longer than the natural element and may have differed in target-site specificity; additionally, mutational effects may have been overestimated due to the fact that aborted transpositions were undetectable (Houle & Nuzhdin 2004). To put these figures into a wider context, it is helpful to compare them to a study of mini-Tn10 insertions in *E. coli* by Elena and Lenski (1997), who estimated an average fitness cost of 2.73% per insertion, which is roughly consistent with the lower estimates for insertional effects above if an adjustment is made for the fact that only 48% of the *D. melanogaster* genome is genic, compared with nearly all of the *E. coli* genome (Alexander *et al.* 2010). Overall, an average insertional effect of $s \approx 1\%$ for a typical multicellular eukaryote seems appropriate.

Note that fitness effects in these experiments will not have been limited to insertional effects, but will also have included other effects of transposon insertions. However, it is likely that these other costs will have been small relative to insertional effects. Ectopic recombination would not be a significant issue for *E. coli*; and even if it may have occurred in the Eanes *et al.* (1988) *Drosophila* experiment—recombination does not normally occur in *Drosophila* males, but it can be induced by P elements (Chen *et al.* 1998)—the maximum cost due to ectopic recombination of 10 homozygous insertions, for the 2,907-bp P element and a 1 cM/Mb rate of recombination, would yield a total fitness reduction of only 0.017%, 3 orders of magnitude below the measured effect for 10 insertions. Copy numbers were higher in the Houle and Nuzhdin (2004) study (from 31–67 copies), which for the 5,146-bp copia element could have incurred up to a 1.6% fitness reduction from zero elements, or 0.047% per

element over the range examined, again significantly lower than the estimated fitness effect per element. Estimates from Eanes *et al.* (1988) and Elena and Lenski (1997) may include the effects of double-strand breaks, as P and Tn10 are DNA elements, but the Houle and Nuzhdin (2004) experiment would not have, as copia is an RNA element. DNA synthesis costs could not have been significant in any of the studies (see below). Finally, expression and/or misfolding costs could have played a role in the experiments of Eanes *et al.* (1988) and of Houle and Nuzhdin (2004). However, their rough congruence with the data of Elena and Lenski (1997) suggests overall that insertions are the main effect being measured here.

As for synergistic effects, the studies cited above do not support a statistically-significant synergistic effect of insertions. However, the study with the most data points was that by Elena and Lenski (1997), which found a synergistic effect roughly equivalent to 0.025 (adjusted for the above form for w); this was not significantly different from zero in their analysis, but since it is known that a synergistic cost is necessary for equilibrium, we adopt the assumption that $t = 0.025$, which is not inconsistent with measured fitness effects.

Finally, we are left with estimating the heterozygous effect of an insertion. Estimates for the average heterozygous effect of a mutation vary, but overall, $H = 0.1$ seems to be taken as an acceptable value (García-Dorado & Caballero 2000). The aforementioned Mackay *et al.* (1992) study found an average heterozygous insertion effect of 5.5%, compared to the average homozygous insertion effect of 12.2%, which suggests an average heterozygous effect closer to $H = 0.45$. However, interestingly, in this study, the average heterozygous effect of an insertion was not correlated with the average homozygous effect. Overall, the picture that emerges is that insertions have highly variable effects. However, as average values, the parameters $s = 0.01$, $t = 0.025$, $H = 0.1$ may not be too far off.

Although we adopt uniform parameters, insertional effects are likely to be highly variable between sites, depending on the target-site specificity of the transposon family as well as the ratio of coding to non-coding DNA in the genome. Because of this insertion-site dependency, insertions have naturally more variable effects than other costs; highly-deleterious insertions will be more strongly selected against than nearly-

neutral insertions, resulting in an accumulation of transposons at sites with smaller fitness effects (Charlesworth & Charlesworth 2010). Preliminary experiments with a two-site-class model of insertional effects (high cost and low cost) did not lead to significantly different qualitative outcomes (not shown). Therefore, we ignore this variation here for the sake of simplicity, only noting that increasing the variability among insertional effects will generally lead to a higher transposon number at equilibrium than a fixed insertional effect would suggest.

2.3 DNA synthesis

In most higher eukaryotes, a substantial fraction of the genome (20–80%) is transposon-derived. The fitness cost of duplicating this "junk" DNA comes down to the energetic cost of DNA synthesis relative to the cell's overall "energy budget". The energy budget of a rapidly-growing cell is approximately $26.92V^{0.97} \times 10^9$ P, where the unit P represents ATP $\rightarrow$ ADP hydrolysis events (Lynch & Marinov 2015), or 5.322×10^{13} P for a "typical" multicellular eukaryote with a cell volume of 2,500 μm^3 (the median of measured cell volumes; Lynch & Marinov 2015). This implies a fitness cost per P of 1.9×10^{-14}. The synthesis cost of DNA is 526 P per base (Lynch & Marinov 2015), suggesting a fitness function of the form

$$w = 1 - Y\lambda n\,,$$

where λ is the length of the transposon in Mb and $Y \approx 1 \times 10^{-5}$ Mb^{-1} is the cost of synthesizing 1 Mb of DNA. Note that, although collectively, the cost of synthesizing junk DNA can be large (for example, on the order of 1% for humans), the cost of synthesizing any single transposon, with a typical length of 5 kb, is insignificant.

2.4 Gene product synthesis

In order to do the work of transposing, transposons need to be transcribed and translated. At high copy numbers and expression levels, these costs may become significant for the cell. As above, we assume that each unit of hydrolysed ATP carries a fitness cost of 1.9×10^{-14}. In the limit of fast generation time, transcription in a higher eukaryote

carries a fitness cost

$$s_{\mathrm{RNA}} = 46 L_{\mathrm{r,mat}} N_{\mathrm{r}} \cdot 1.9 \times 10^{-14}$$

(Lynch & Marinov 2015), where $L_{\mathrm{r,mat}}$ is the length of the mature RNA transcript—that is, the length of the coding sequence plus approximately 100 bases for the poly-A tail—and N_{r} is the number of transcripts. Similarly, translation carries a fitness cost

$$s_{\mathrm{p}} = 24 L_{\mathrm{p}} N_{\mathrm{p}} \cdot 1.9 \times 10^{-14} ,$$

where L_{p} is the length of the protein in amino acid residues, and N_{p} is the protein copy number (Lynch & Marinov 2015).

Functional RNA transposons assemble into virus-like particles (VLPs), which require the production of multiple protein subunits. For example, the Ty1 retrotransposon in yeast, which is highly similar to copia in *D. melanogaster*, assembles into a ~14.1 MDa VLP (Burns *et al.* 1992) consisting largely of a multi-copy capsid protein surrounding a core containing the template and reverse transcriptase. For an average mass of 110 Da per amino acid, this suggests that the production of approximately 128,000 amino acids is required for assembly of a functional transposon. Overall, this yields an approximate cost for a Ty1/copia-like element of $c = 6 \times 10^{-8}$ per assembled particle. Finally, we denote by k the number of VLPs required, on average, for one successful duplication. This value is difficult to estimate, however, it may be significantly higher than 1. In *Saccharomyces cerevisiae*, multiple clusters of ~10 VLPs can be imaged in wild-type cells (Checkley *et al.* 2010), even though duplication rates for transposons in this species are on the order of one every thousand generations or less. We adopt k = 10–100 as a conservative estimate. The resulting fitness function is then

$$w = 1 - kc\bar{a}n ,$$

where $\bar{a}n$ is the total activity of transposons.

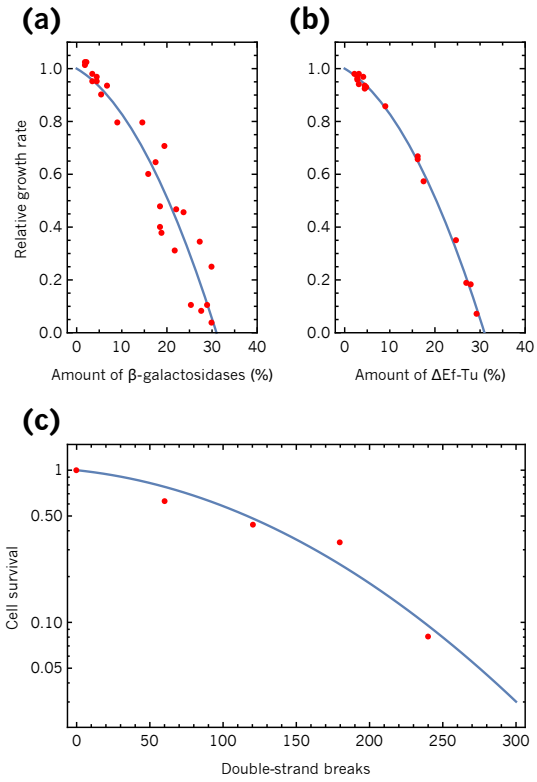


Figure 12: Host fitness models fit to data from: **(a, b)** Dong *et al.* (1995) on overexpression of proteins; **(c)** Rothkamm *et al.* (2003) on the induction of double-strand breaks.

2.5 Misfolding

A further possibility is that overexpression of a given protein may trigger ribosomal stalling and breakdown of cell function. For example, Dong *et al.* (1995) found that, when overexpressing protein in *E. coli*, expressing a given protein at a level of roughly 30% of overall protein production caused cell death.

The data from Dong *et al.* (1995) fit an equation for cell fitness (growth rate) of

$$g = 1 - x - Mx^2 ,$$

(Fig. 12a&b), where $M = 7.1865$ and x is the fraction of overall protein production consumed by the overexpressed gene. We set the coefficient on x in the above function to 1 on the supposition that this term represents the energetic cost of synthesizing a fraction x of the cell's proteins, while the term $-Mx^2$ represents the cost of aggregation and misfolding, which is proportional to the rate x^2 at which two proteins of the same type will interact in a cell. We ignore the x term representing the energetic cost of

synthesis, which we have calculated above, and use a fitness function

$$w = 1 - Mx^2 \,.$$

In order to determine the fraction x of overall protein production for a given gene, we first need to know the total amount of protein produced in a typical cell. Lynch & Marinov (2015) give the formula

$$N_{\text{tot,p}} = (1.6 \times 10^6) V^{0.93}$$

for the total number of proteins in a given cell with volume V in μm^3. For a cell with size 2,500 μm^3, this gives $N_{\text{tot,p}} \approx 2.31 \times 10^9$; if we assume that a typical eukaryotic protein comprises 400 amino acids (Milo 2013), this gives a total amino acid production of 9.25×10^{11} amino acids per cell in the limit of fast cell growth. Assuming, as above, 128,000 amino acids per fully-assembled transposon particle, this gives

$$x = 1.4 \times 10^{-7} k\bar{a}n \,,$$

where k is the number of transposon particles produced per successful duplication and $\bar{a}n$ is the total transposon activity.

Alternatively, Tomala and Korona (2013) report that overexpressing proteins to an average level of 1% of the total protein expression in yeast yields an average fitness cost of 2.3% for well-structured, cytosolic proteins (disordered or trans-membrane proteins carry an additional cost when overexpressed). This suggests an initially larger cost to aggregation and misfolding than the above formula would predict. However, we use the function derived from the data of Dong *et al.* (1995) as it gives a fitness curve for multiple values of x rather than the fitness effect when $x = 1\%$ only.

2.6 Unrepaired double-strand breaks

The cut-and-paste mechanism of DNA transposons leaves behind a double-strand break (DSB), which, if unrepaired, can imperil host fitness by leading to incorrect segregation of chromosomes during cell division. The magnitude of this risk is

difficult to assess directly; however, a study by Rothkamm *et al.* (2003) measured the survival of Chinese hamster ovary (CHO) cells following ionizing radiation, which causes double-strand breaks; under these conditions, 1 Gy of radiation induces approximately 30 DSBs (Rothkamm *et al.* 2003). The data fit a function

$$w = \exp\left(-Bd - Cd^2\right) ,$$

with $B = 2.32074 \times 10^{-3}$ and $C = 3.11734 \times 10^{-5}$ (Fig. 12c). The experiment above administered the irradiation over a period of approximately 1 minute, which is much shorter than the generation time of CHO cells, ~1 day. The majority of DSBs were repaired after 1 day. Based on this information, we make the assumption that this "acute" cost of DSBs can be interpreted as the number of DSBs per cell cycle which cause death. However, this is likely to significantly overestimate the strength of selection against double-strand breaks if cells can tolerate double-strand breaks spread over a longer period of time better than they can all at once. The number of breaks, d, is equal to $j\bar{a}n$, where $j \geq 1$ is the number of "cut-and-paste" transpositions per successful copy. This value will vary depending on whether transposition occurs randomly with respect to the cell cycle, or whether transposons are adapted to transpose more frequently during the phases of the cell cycle in which transposition is more likely to result in a successful copy (*e.g.* after the replication fork has passed through the transposon's locus of origin, but not its destination locus). We adopt $j = 10$ as a rough estimate.

3. Parasitic duplication and host suppression

For parasitic elements, we use the stochastic individual-based model detailed above, assuming that the binding of parasites to ribosomes follows Michaelis-Menten dynamics, and hence the proportion of all duplication events from a family of private elements that are parasitized is given by $\phi\frac{\bar{a}n}{\kappa+\bar{a}n}$, where $\bar{a}n$ is the total activity of the parasitic family, κ is a half-saturation constant, and ϕ is the saturating rate of parasitism. For host suppression, we also assume that suppressor dynamics follow Michaelis-Menten dynamics, such that, when the amount of suppression acting

on a particular family is s, a given family's activity is multiplied by a factor $\frac{K}{K+s}$, where K is a half-saturation constant. We assume that suppression acting on parasitic elements decreases their activity a, affecting their access to parasitic duplication; hence, suppression of a parasitic family will, all else being equal, increase the proliferation of its target family by reducing the proportion $\phi\frac{\bar{a}n}{\kappa+\bar{a}n}$ of its replication that is parasitized. The cost of host suppression is difficult to determine *a priori*. Therefore, we simply assign a fitness cost of 10^{-5} for each unit of suppression, which is enough to select against needless suppression without preventing hosts from adequately suppressing transposons.

4. Empirical survey of host suppression

To analyse the composition of genomes by transposon type, we downloaded RepeatMasker (Smit *et al.* 2013) annotated genomes for *Homo sapiens* (release GRCh38 / hg38) and *Danio rerio* (release GRCz10 / danRer10), and transposon annotations from FlyBase version 5.32 (Attrill *et al.* 2010) for *Drosophila melanogaster*. We calculated the total sequence length of all transposon annotations for each genome, classifying repetitive sequences marked as DNA or RC (RepeatMasker) and Foldback or IR (Flybase) as publicly-duplicating transposons, repetitive sequences marked as LINE or LTR (RepeatMasker) and non-LTR or retroviral (FlyBase) as privately-duplicating transposons, and repetitive sequences marked as SINE or SVA (RepeatMasker) as parasitic transposons. Sequences marked as "SINE-like" in the Flybase annotations are now thought to be Helitron-class DNA elements, not parasitic RNA elements (Yang & Barbash 2008), so we classified them as public elements as well. We discounted any non-mobile repetitive elements or sequences with uncertain or unknown classification.

To assess the amount of suppression expended by hosts on each type of transposon, we analysed piRNA abundance data from each organism of interest. For *H. sapiens*, we used Ha *et al.*'s (2014) data on the total abundance of piRNA sequences mapping to DNA (public), LINE & LTR (private), and SINE & SVA (parasitic) transposons. For *D. rerio*, we used data from Wei *et al.* (2012) on the total abundance of piRNA sequences mapping to DNA (public), LINE & LTR (private), and SINE (parasitic) transposons, taking the total number of transcripts over the four surveyed stages

of embryonic development—256-cell, sphere, shield, and 1 day post-fertilization—and ignoring any mappings to non-mobile repetitive sequences or sequences with uncertain or unknown classification. For *D. melanogaster*, we used modENCODE (Celniker *et al.* 2009) genomic alignments of small RNAs sequenced from *Drosophila* Canton S strain embryos, specifically sets 745 & 755 (0–1 hr), 740 & 752 (2–6 hr), 753 & 754 (6–10 hr), and 2741 (12–24 hr). These datasets include fragments sized 18–30 nt, which are not exclusively piRNAs; however, excluding all fragments of 25 nt or less to obtain a piRNA-enriched dataset did not significantly change results. By comparing the alignments from these data sets to the FlyBase-annotated genome, we were able to determine the total number of small RNAs mapping to IR, Foldback and SINE-like (public; see above) and non-LTR & retroviral (private) transposons. The suppression bias is the abundance of piRNAs mapping to a given transposon type divided by the total genomic content of that transposon type, normalized such that element types have an average bias of 1.

5. Effects of ping-pong amplification

The Piwi-piRNA system uses "ping-pong amplification" to target silencing towards active transposons (Senti & Brennecke 2010). It is possible that this positive feedback loop—which uses degraded fragments of suppressed RNA as templates for further suppression, both of genes and gene products—might interact with transposon activity in such a way that there is a point beyond which an increase in transcription is actually counter-productive for the transposon, *i.e.*, results in fewer successful duplications. We investigated this possibility using an ordinary differential equation (ODE) model based on current biochemical understanding of the Piwi-piRNA system (Aravin *et al.* 2007; Senti & Brennecke 2010). Within a host cell, we model five species: active (unsilenced) transposons, N; silenced transposons, M; transposon transcripts, T; suppressors, S; and silencers, L. At time zero there are n_0 active transposons and no silenced transposons. Active transposons generate transcripts at rate aN; silenced transposons generate no transcripts. Transcripts generate new active transposons at rate dT, which consumes the transcript. Suppressors are generated at rate b, degrading transcripts at rate cST. As a model of "ping-pong amplification", the degradation of a transcript trig-

gers the recruitment of q silencers and r suppressors. Finally, silencers convert active transposons into silenced transposons at rate mLN; this process consumes the silencer. This yields the following system of ODEs with respect to time, t:

$$\begin{aligned}
\frac{\mathrm{d}N}{\mathrm{d}t} &= dT - mLN \\
\frac{\mathrm{d}M}{\mathrm{d}t} &= mLN \\
\frac{\mathrm{d}T}{\mathrm{d}t} &= aN - cST - dT \\
\frac{\mathrm{d}S}{\mathrm{d}t} &= b + rcST \\
\frac{\mathrm{d}L}{\mathrm{d}t} &= qcST - mLN
\end{aligned}$$

In order to test the prediction over a wide range of parameter values, we integrated this system of equations over time $t = 0$ to 1, varying the transcription rate a over $0.1, 1, 10, 100$. We performed this manipulation over all possible parameter combinations of $b, c, d, m \in 0.1, 1, 10$ and $q, r \in 0.5, 1, 5$, as well as all possible combinations of $b, c, d, m \in 0.01, 1, 100$ and q, r as above. In no case did an increase in a, holding other parameters constant, result in a decrease in the number of duplications made (the sum of M and N, minus n_0, at $t = 1$). However, the system did exhibit diminishing returns for transposons, *i.e.*, a tenfold increase in a usually, but not always, resulted in a less than tenfold increase in the number of duplications made.

Chapter 6

Discussion and conclusions

Both the physical organization of life and its evolutionary structure originate from successive syntheses of once-independent units. These major evolutionary transitions are important events because they establish the fundamental modes through which living things are shaped by natural selection. Many of the recurrent patterns exhibited by these evolutionary alliances—which are subject to both reinforcement by tightened cooperation and weakening by new conflict—are attributable to features of the social environment in which they arise. The work collected in this thesis explores some of these ecological factors that shape the making and maintaining of evolutionary alliances, focusing on cooperation in arthropod societies and conflict within genomes. In this final chapter, I summarize and discuss my findings, suggest avenues for ongoing investigation, and review some themes that emerge.

Chapter summary and discussion

The evolution of paternal care (chapter 2)

Among the solitary Hymenoptera, maternal care is widespread, while paternal care is rare. According to the "preadaptation hypothesis" (Ross *et al.* 2013), this ancestral pattern explains the exclusively-female worker caste of the social Hymenoptera, since the origins of worker behaviour lie in brood care redirected towards siblings. The preadaptation hypothesis thus stands as an alternative to Hamilton's (1964, 1972) haplodiploidy hypothesis, which attributes female-biased sib-rearing in the social

Hymenoptera to asymmetries in relatedness stemming from haplodiploid inheritance, and which has now fallen out of favour for both theoretical and empirical reasons (Gardner *et al.* 2012; Ross *et al.* 2013). However, haplodiploidy could still explain why workers in the social Hymenoptera are female if it were itself responsible for the female-biased expression of parental care in their solitary ancestors (Wade 2001). Gardner (2012) showed that haplodiploidy does not promote maternal care relative to diploidy, but a similar pattern could arise if haplodiploidy relatively inhibited paternal care.

Using a mathematical kin-selection model, I show that haplodiploidy neither promotes nor inhibits paternal care in outbred populations relative to diploidy. And under inbreeding—for example, owing to mating between siblings, a common trait among solitary Hymenoptera—haplodiploidy actually promotes paternal care relative to diploidy. In symmetry with the conclusions of Gardner (2012), I show that haplodiploidy—if it has an effect upon the evolution of parental care at all—ought to promote paternal care and inhibit maternal care relative to diploidy, an effect which is in discord with the overabundance of maternal-only care in the solitary Hymenoptera. Hence, relatedness asymmetries stemming from haplodiploidy are unlikely to have especially preadapted females for sib-rearing in the social Hymenoptera. Rather, sex biases in the expression of brood care in the solitary Hymenoptera probably owe to other sexual asymmetries that extend more generally over the terrestrial animal kingdom—for example, uncertain paternity and variable mating success among males (Queller 1997b).

The ecology of sex and who helps in arthropod societies (chapter 3)

The eusocial Hymenoptera and the higher termites—the pinnacles of arthropod sociality—have long been held up as representing contrasting approaches to societal organization (*e.g.* Wheeler 1934): while the bees, wasps, and ants employ an exclusively-female workforce, the termites employ both sexes. Limited to these taxa, the genetics of sex determination (haplodiploidy for the Hymenoptera, diploidy for the termites) seemed to provide an elegant explanation for the sex composition of worker castes (Hamilton 1964, 1972). However, a broader view of arthropod

sociality—including not only the social Hymenoptera and termites, but also the social spider mites, spiders, pseudoscorpions, shrimp, webspinners, thrips, and beetles—reveals extensive diversity in the sex of helpers that does not appear to be readily explained by ploidy (Fig. 1, chapter 3). If relatedness asymmetries stemming from ploidy do not explain variation in who helps, what does?

My mathematical model suggests that the ecology of sex plays an important role in determining the sex of helpers and the taxonomic distribution of arthropod societies. Focusing on five aspects of arthropod sexual ecology—sex-specific preadaptation, the evolutionary lability of the sex ratio, the rate of sib-mating, the level of promiscuity, and reproductive autonomy—I show that, while diploidy versus haplodiploidy *per se* has almost no effect on the sex and abundance of helpers, sexual ecology strongly shapes patterns of helping. Preadaptation strongly influences who helps, while an evolutionarily-labile sex ratio can drive an evolutionary feedback that promotes extreme sex biases in sib-rearing. Sib-mating and local mate competition can promote sociality, while promiscuity inhibits it. Testing these predictions with a phylogenetically-controlled empirical survey, I confirm that ploidy has no significant impact upon the sex of helpers, while sex-specific preadaptation has a highly-significant impact, and a labile sex ratio does appear to magnify sex biases in helping.

Overall, I suggest that while haplodiploidy may have had a strong impact upon the evolution of sex-specific helping, this impact does not owe to asymmetries in relatedness between the sexes. Rather, I argue that haplodiploidy may promote helping for two reasons. First, it allows a ready means of adjusting the sex ratio, which promotes helping because it allows the mother to allocate more resources to the more helpful sex (*cf.* Gardner & Ross 2013). Second, by purging recessive alleles associated with inbreeding depression, and by allowing a sex-ratio response to local mate competition, it preadapts taxa for sib-mating, which I show may promote sociality.

Worker sterility, population genetics, and inclusive fitness (chapter 4)

Ecological and genetic factors interact to drive the evolution of social behaviour. All else being equal, worker sterility in the social Hymenoptera should be promoted

by monogamy because this mating system increases sibling relatedness, thereby increasing the indirect benefits of the increased investment by workers into colony productivity that non-reproduction allows (Boomsma 2007, 2009, 2013). A recent mathematical analysis (Olejarz *et al.* 2015) has challenged this prediction of inclusive-fitness theory with an explicit population-genetics model, finding that monogamy sometimes promotes and sometimes inhibits the invasion of a worker sterility allele relative to promiscuity, depending on the choice of parameter values. While debates about the validity of inclusive fitness theory are often conducted at a conceptual level, Olejarz *et al.* claim to give a concrete and biologically-relevant example where inclusive fitness theory fails, and so its results are worth careful appraisal.

Reanalysing the model of Olejarz *et al.*, I find that its conclusions rest on unrealistic assumptions concerning the genetics, ecology, and evolution of worker sterility. I show that instances in this model where promiscuity promotes the invasion of worker sterility rely on the imposition of strong genetic constraints—namely, full allelic penetrance—and that when this assumption is relaxed, the prediction of inclusive-fitness theory that monogamy promotes worker sterility is supported. I highlight a crucial distinction between helpful and harmful worker sterility; I argue that focusing only on the invasion of worker sterility is an inapposite metric for measuring adaptation; and I demonstrate that adopting more demographically-plausible assumptions concerning the effect of worker sterility upon colony productivity supports the predictions of inclusive fitness theory even in the face of strong genetic constraints. Finally, I explore alternative ecologies, showing that monogamy promotes altruism between siblings in a variety of scenarios.

The price of inclusive fitness theory's conceptual simplicity and explanatory power is that the elegant accounting of marginal fitness effects, relatedness, and reproductive value embodied by Hamilton's rule can only be made exact when costs and benefits are linear—for example, in the limit of weak selection—or when some of these terms are recast in regression forms that may be more difficult to apply and interpret. The weak-selection assumption that evolution proceeds in small steps along a phenotypic continuum abstracts away genetic details, but for the vast majority of social traits, these genetic details are unknown. Unless there is a strong reason to believe that the genetics

of a given social behaviour will be constrained in a particular way, a model built on such constraints may not beget generally-applicable conclusions. To be certain, any theory of biological adaptation must ultimately be consistent with population genetics. But population genetics is a theory of microevolution, while inclusive fitness theory is a theory of adaptation (*cf.* Hammerstein 1996). Answering fundamentally different questions, each has been empirically successful in its respective domain.

The tragedy of the transposons (chapter 5)

Transposons may represent not only the most abundant class of genes on the planet (Aziz *et al.* 2010), but also the most influential. Since their discovery by McClintock (1950), awareness of their potential as mutagens, master regulators, sources of genetic disease, and tools for molecular biology has steadily grown. In a landmark paper, Charlesworth and Charlesworth (1983) established how selection against transposons' deleterious effects could counter their proliferation. This equilibrium requires a constant rate of duplication on one side, and—equal and opposite in magnitude—a constant rate of removal by natural selection on the other. Since then, our knowledge of host lines of defence against transposons has increased, and several lines of evidence have pointed towards a dynamic arms race between transposon and host (*e.g.* Blumenstiel 2011; Jacobs *et al.* 2014). However, our theoretical understanding of transposon–host coevolution has not been updated to reflect these developments.

Building on the analysis of Charlesworth and Charlesworth (1983), I have developed a model of transposon–host coevolution. Applying concepts from social evolution theory, I show that transposons fall into three distinct categories based on their mode of duplication—public, private, or parasitic—which map onto DNA transposons, autonomous RNA transposons, and non-autonomous RNA transposons, respectively. The category in which a transposon falls has crucial importance for its evolution; in the absence of host suppression, and under a realistic model of the cost of transposons, privately-duplicating transposons will be selected to increase their duplication rate to the point of driving their host population extinct, analogously to the classic tragedy of the commons (Hardin 1968). Host suppression can prevent this outcome, but a permanent equilibrium between host and transposon is only

possible if host suppression targets gene products, not genes, thereby accruing the cost of proliferation to transposons, rather than to the host population they exploit. Our theoretical predictions for the extent of host suppression directed against public, private, and parasitic transposons are borne out by an empirical survey assessing the germline allocation of host suppression in *Homo sapiens*, *Drosophila melanogaster*, and *Danio rerio*. Finally, in an attempt to resolve lingering uncertainty over the fitness cost of transposons, I suggest that only the risk of ectopic recombination adequately explains observed transposon frequencies in natural populations.

In evolutionary biology, selection favouring "the good of the species" is discredited (Fisher 1958). Few would invoke "old" group selection to explain animal behaviour today. Yet at the level of the genome, this line of thinking is rife. Numerous papers repeat the claim to have finally debunked the concept of transposable elements as mere "selfish genes" or "useless junk" by highlighting a particular case of co-option by a host species. It is clear that transposon-derived DNA often furnishes the material for host adaptation—nearly always, once it is no longer capable of transposition. But this does not mean that transposons are anything other than parasites. We should not be surprised by the widespread co-option of transposons given that transposon-derived DNA comprises 20–80% of most multicellular eukaryotes' genomes. What else are eukaryotic genomes supposed to do with all that junk? Only an understanding of transposons as selfish genetic elements explains the major features of their evolution.

Ongoing work

Here, I identify some potential topics for future investigation. In chapter 3, I investigate patterns of sex-biased sib-rearing in arthropods. Some of these taxa have made the major transition to eusociality, while others have attained only a primitive level of sociality. In this latter form, individuals invest in sibling care, but there is no division into reproductive and unmated helper castes exhibiting complementary totipotency—*i.e.*, full dependence on each other for reproduction. Why do some societies make the transition to eusociality and not others? And to what extent can we consider these primitive societies as evolutionary steps on the way to eusociality, as opposed to alter-

native paths? One potential clue lies in the distribution of sib-mating and local mate competition over origins of primitive sociality versus eusociality. Eusocieties appear less likely to have emerged from sib-mating ecologies than primitive societies (Fig 1, chapter 3). One possibility is that in sib-rearing taxa, personal reproduction will require less of an investment than in other taxa, because individuals do not need to pay the cost of dispersing and finding a mate. In such a scenario, full commitment to a sterile helper caste may not bring sufficient benefits to outweigh the cost of giving up reproduction completely. Alternatively, if helpers in sib-mating populations tend to produce offspring within their natal nest, then they will inhabit different population structures than those found in outbreeding societies, and this may have an impact upon the evolution of social behaviour. This question could be investigated with a mathematical model that allows for these differences in population structure and which models an individual's investment into personal reproduction more explicitly.

The model of transposon–host coevolution developed in chapter 5 was primarily used to investigate the dynamics of one or two transposon families co-evolving with a host population at a time. But natural populations may host transposons from dozens of different families simultaneously. How do interactions between different transposon families impact upon transposon–host coevolution, if at all? Could transposons from different families evolve to compete with each other directly? A related question involves competition within transposon families. Transposon particles feature an outer shell constructed from capsid proteins. Previous work by Nee and Maynard Smith (1990) suggests that, if transposons parasitize the production of capsid proteins, they may be selected to invest increasingly more in parasitism rather than capsid production. How might this selection pressure affect selection on transposon duplication rate?

Many features of host ecology also remain to be fully explored as factors in transposon–host coevolution. Here, the frequency of sexual reproduction is a key factor; in the absence of genetic recombination, relatedness can build up between transposon copies. Simultaneously, selection against transposons' deleterious effects will become much stronger. These selection pressures may have opposing effects on publicly-duplicating transposons: on one hand, they may be selected to increase their duplication rate because they are more likely to be helping their relatives; on the other

hand, they may be selected to decrease their duplication rate because they will feel the selective effects of any damage done to their hosts more keenly. In host populations that reproduce partly asexually—either through selfing or clonal reproduction—how will these opposing selection pressures be resolved—will one outweigh the other, or will they perhaps cancel (*cf.* Taylor 1992)? Additionally, host population size has been highlighted as a crucial factor in genome size evolution (Lynch 2007), with larger populations better able to select against wasteful increases to genome size, such as those generated by transposons. But any increase to host population size will, all else being equal, increase the transposon population size as well, which may result in opposing selective pressures on transposon proliferation and suppression. These questions could be addressed by further developing the model presented in chapter 5.

Finally, predictions concerning the evolution of a social trait may differ depending on the genetic architecture underlying the trait and on any constraints on allelic variation (Fig. 3, chapter 4). Under what conditions should we expect patterns predicted by analytical tools that assume "unconstrained" genetics—such as the direct fitness maximization techniques used in many social evolution models—to hold in the natural world? It should be possible to theoretically quantify the average error in predictions for social traits as a function of genetic constraints—the rate of appearance of new alleles, population size, over- or underdominance, nonlinearities in the genotype–phenotype map, and so on—for a series of possible underlying genetic architectures. Although the use of these analytical techniques has been amply justified empirically, quantifying the expected error could help strengthen theoretical justification for their use as predictive and explanatory tools, and could identify scenarios in which additional caution is warranted.

Concluding remarks

Two main themes emerge from the work presented here. One is that explicitly considering coevolution between different classes of individuals may lead to novel predictions. When different classes are permitted to adopt different strategies, or when these classes interact asymmetrically such that their strategy sets are funda-

mentally different, complexities can arise. In chapter 3, my model analyses biased sex allocation as a response to sex-biased helping—a classic problem of local resource enhancement—but also allows sex-biased helping to evolve in response to changes in sex allocation. It is well known that mothers may be selected to bias their sex allocation in favour of the more helpful sex (*e.g.* Trivers & Willard 1973; Emlen *et al.* 1986; Pen & Weissing 2000), but allowing helpers to respond to changes in sex allocation (*cf.* Gardner & Ross 2013) yielded the unexpected result that single-sex helping is expected when the sex ratio is labile. In chapter 5, I build upon a classic model of transposon dynamics (Charlesworth & Charlesworth 1983), allowing both transposon proliferation and host suppression to evolve. Introducing these complexities leads to new predictions for the system's evolutionary dynamics, and considering both together is necessary to explain the long-term stability of eukaryotic genomes.

Perhaps the main theme of this thesis is the importance of ecology, which comes out in three ways. First, being explicit about ecology can help to clarify the relevance of a mathematical model. The model of sex-biased helping in chapter 3 was partly motivated by the observation that arthropod societies often evolve in the context of inbreeding. The impact upon sex-biased helping of inbreeding *per se*—that is, increased genetic relatedness between mating partners—had been considered many times before: for example, because inbreeding reduces the asymmetry in relatedness between a haplodiploid female and her siblings of either sex (Hamilton 1972; Crozier & Pamilo 1996); or because inbreeding can promote the spread of altruistic alleles under particular genetic architectures (Wade & Breden 1981; Craig 1982b; Uyenoyama 1984; Michod 1993). Many authors have argued that because inbreeding increases relatedness to siblings by the same amount as it increases relatedness to offspring, it should have no overall effect upon the evolution of sib-rearing (Trivers & Hare 1976; Craig 1982b; Pamilo 1991; Bourke & Franks 1995). However, inbreeding in arthropod societies typically happens in the specific ecological context of sib-mating and local mate competition, which has simultaneous impacts upon relatedness, mating ecology, and population structure; taken together, these impact upon sex-biased helping in ways that are difficult to predict from a consideration of inbreeding devoid of its ecological context.

Second, being explicit about ecology helps to focus the input to a model. While the flexibility of mathematical models can be an advantage, total agnosticism towards the range of possible inputs to a model can lead to misleading conclusions. In chapter 4, I reanalyse a mathematical model of the evolution of worker sterility by Olejarz *et al.* (2015). One of the model's parameters is a function r_z specifying how the proportion of sterile workers in an insect colony influences that colony's productivity. Arguing that this function could take any shape—while simultaneously imposing strong constraints on the genetics of worker sterility—led Olejarz *et al.* (2015) to the conclusion that monogamy had no consistent effect on the evolution of worker sterility. Going further, they were able to suggest that, in contrast to the predictions of inclusive fitness theory, monogamy typically inhibits worker sterility, because when they tested a large number of randomly-generated r_z functions, they found that worker sterility invaded more often under double mating than single mating (Olejarz *et al.* 2015). However, the procedures used to generate r_z were both biologically nonsensical and subject to systematic bias (see chapter 4). When these procedures are replaced with more biologically-sensible ones, the opposite conclusion follows. Parameters of a mathematical model can, in principle, be assigned any value. But in order to maximize explanatory power, it is important to tell the difference between setting biologically-meaningful limits and imposing arbitrary constraints. Here, thinking explicitly about ecology will often be a helpful guide.

Finally, considering ecology will often reveal unexpected connections between behaviours in seemingly different systems. In chapter 5, I use concepts from social evolution theory to analyse transposon–host coevolution, and this reveals some interesting ecological analogies. For example, from the perspective of a transposon, there is a similarity between genetic recombination and dispersal. Sex between hosts encourages transposons to deplete their local environment and harm their neighbours; this can be understood as a similar process to how increased dispersal decreases the importance of kin competition (Taylor 1992). Additionally, public duplication bears similarities to the production of public goods: in the same way that the production of public goods leaves a group vulnerable to cheaters (Frank 1998), public duplication reduces the incentive for individual transposons to increase their duplication rate,

which is why only privately-duplicating transposons threaten to cause a tragedy of the commons.

The major transitions view has shown how natural selection shapes the design of biological systems at all stages of complexity, from genes to cells, from bodies to societies. Understanding the ecological forces experienced by individuals at all levels not only enriches our understanding of particular systems, but also deepens our knowledge of the evolutionary process that unifies all living things.

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