

Experimental Community and Thermal Ecology of Rainforest *Drosophila* and Their Parasitoids



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“土蜂，今一种黑色，腰甚细……其生子如粟米大，乃捕取草上青蜘蛛十余枚，仍塞口，以拟其子大为粮也……其一种入芦竹管中者，亦取草上青虫，一名螺赢。诗人云：‘螟蛉有子，螺赢负之。’言细腰物无雌，皆取青虫，教祝便变成己子，斯为谬矣。”

----- 陶弘景《本草经集注》

Translation: "There is one type of black wasps with very thin waists…… It lays eggs the size of a grain of millet, catches dozens of green spiders on grass, and then uses them to feed its offspring…… There is another wasp of this kind, called "GuoLuo", which flies into reeds and catches caterpillars. "*Shi Jing*" (1046 BC – 771 BC) wrote that "butterfly has kids, which GuoLuo helps to raise". According to that, people say that the thin-waist wasps do not have females. They only catch caterpillars and then educate the caterpillars to be like themselves. (According to my research,) "*Shi Jing*" is wrong."

----- Tao, HongJing (456 AD – 536 AD). "*Ben Cao Jing Ji Zhu*"

“螟蛉有子，螺赢负之” (“Butterfly has kids, which GuoLuo helps to raise”) has become a very famous line that many Chinese pieces of literature, including ancient masterpieces, have used. Tao wrote, “all the books were just copying each other”. He then set out to observe the phenomenon by himself. Later he found that these wasps laid eggs on the body of caterpillars they caught, and their offspring developed by feeding on the caterpillars. Often partial observation can deceive us. Experiments will lead us in searching for causes and mechanisms.

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Abstract

Climate change is altering global biodiversity patterns and the ecological functions provided by nature. Species do not react to climate change independently: changes in one species' distribution, abundance and performance influence, and sometimes reverse, the fundamental reaction of other species to climate change. Consequently, the observed shift in distribution and abundance of co-occurring species within a community vary widely. Understanding the causes of the varied responses is essential for predicting biodiversity changes and their ecological impact. Using a tropical *Drosophila*-parasitoid system in the lab, I investigated the determinants of species turnover on elevation-driven thermal gradients, as well as how species interactions alter responses of population and community to rising temperatures.

First, I compare the thermal traits and competitive ability of *Drosophila* species found along tropical elevational gradients. I demonstrate that reproduction limits imposed by high temperatures set the warmer boundaries, and that competitive exclusion explains the absence of lowland species at high elevations. Next, I study the impact of parasitism on the heat tolerance of host populations. I show that the interactive effects of parasitism and heatwaves on the mortality in host populations can be additive, antagonistic and synergistic, depending on the availability of protein resources and host identity. I then use mesocosms to investigate how different modes of rising temperatures (i.e. extremes vs. general warming) influence the range expansion of lowland *Drosophila* species, and their combined impact on the composition of the high-elevation *Drosophila*-parasitoid community. I show heatwaves but not general warming facilitate the establishment of the lowland species in the high-elevation community, which surprisingly increases the resident *Drosophila* abundance through its suppression of parasitoids. Finally, I focus on the contribution of parasitism to the coexistence of host species. This collaborative work highlights that the impact of a generalist parasitoid on host coexistence is idiosyncratic among different host pairs. Overall, this dissertation shows that rising temperatures, particularly through high-temperature extremes, have a significant impact on species range and community composition; and parasitism serves as a key species interaction that mediates the indirect impact of climate and novel species on populations and communities.

Author Contributions

Jinlin Chen had independent intellectual input into all aspects of the thesis. She is the primary author of General Introduction (Chapter 1), Chapter 2, Chapter 3, Chapter 4, and General Discussion (Chapter 6). For Chapters 2, 3, and 4, she initiated the research project, conducted all the experimental work, and analysed the data. Then she led the writing of the three chapters and incorporated co-author comments to produce the final manuscript. She is the co-first author of Chapter 5. She developed the research idea together with Chris Terry and led the design and execution of the experimental work. She conducted the new analysis of the species competition model during the resubmission of the manuscript and she contributed to the writing and revision of the manuscript.

Owen T. Lewis (supervisor, and co-author of Chapters 2 - 5) secured funding and provided intellectual input. He offered guidance during the development of the research ideas and contributed substantially to revisions.

Chris Terry (co-first author of Chapter 5) initiated the research idea, conducted the experiment with Jinlin Chen, and led the data analysis and writing of Chapter 5.

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1

General Introduction

Graphical Abstract

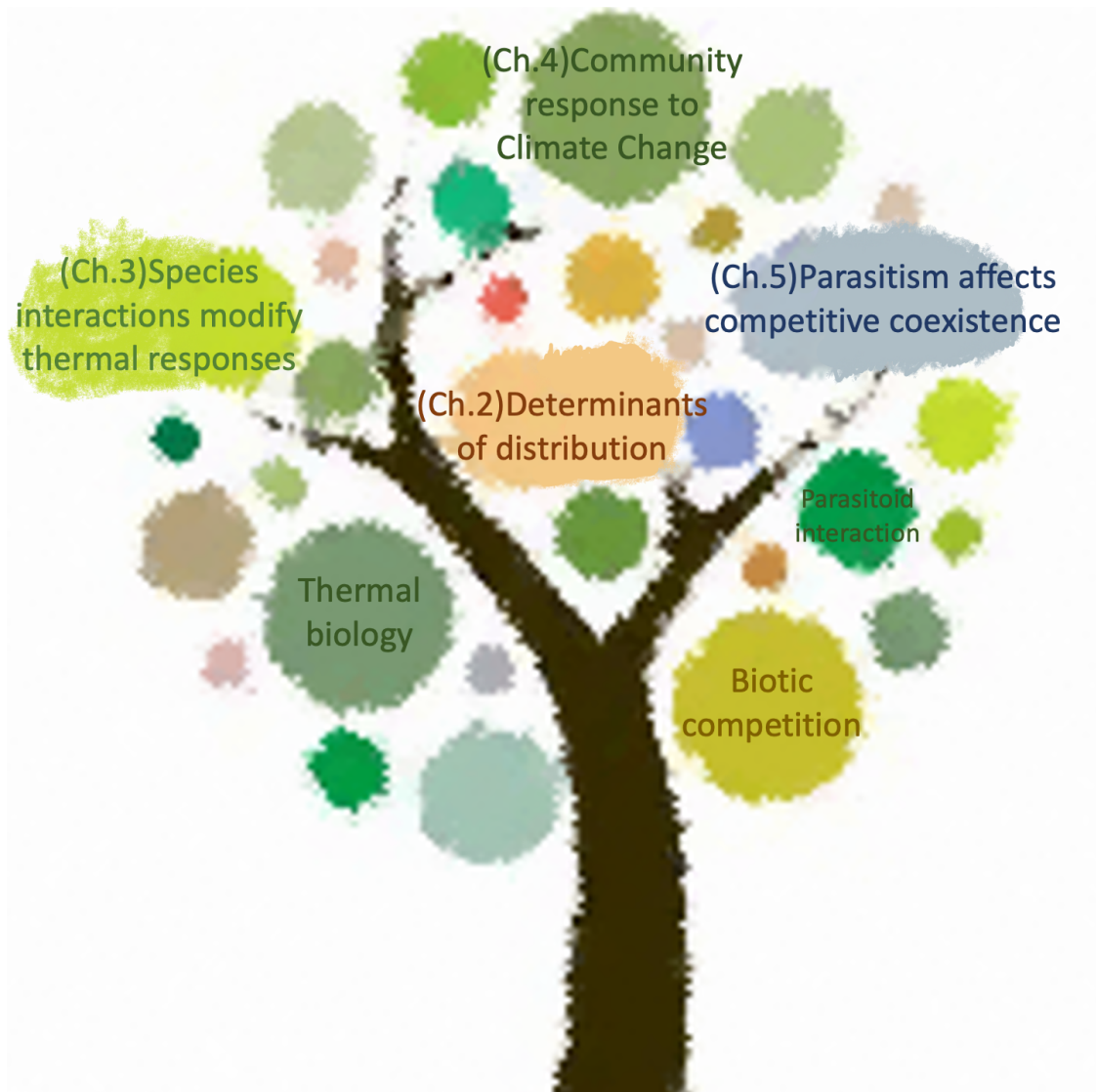


Figure 1.1 The progression of my four result chapters.

Temperature is a fundamental regulator of all living processes, from flowers blooming to the sex determination of sea turtles. Specifically, body temperature regulates all metabolic activities underpinning movement, growth, reproduction, and behaviour in organisms (Huey and Kingsolver, 1989; Huey and Stevenson, 1979). Ectotherms rely on environmental heat sources directly; hence, a change in the average or variability of environmental temperature can have an immediate effect on their performance (Huey and Kingsolver, 1989). Endotherms have gained the ability to regulate their body temperature through costly internal metabolism (Crawshaw, 1980; Scholander et al., 1950). However, they will suffer from an extra physiological burden on thermoregulation due to the shift in the temperature differential between the body and the environment (du Plessis et al., 2012), as well as the direct damage by extreme temperature stress (Sales et al., 2018; Stillman, 2019).

Temperature-dependent performance of wildlife links tightly with human society. For example, suitable habitats for many endangered species are threatened by climate change (Hoffmann et al., 2019; Preston et al., 2008). Disease vectors may spread to new locations, putting pressure on local public health (Medlock and Leach, 2015). As a result of global warming, agricultural pests are increasing their metabolic rate and range or may become free from natural enemies, causing significant economic harm (Deutsch et al., 2018; Jiang et al., 2022; Thomson et al., 2010). Extreme climatic fluctuation has been associated with pest outbreaks (Chaves et al., 2014; Salih et al., 2020). The loss in biodiversity is also associated with a massive loss in ecological functions such as pollination, decomposition and so on, especially those delivered by specialised species interactions (Vanbergen et al., 2013; Wagner, 2020). All aspects of human society, including urban infrastructure, agricultural practices, and natural disaster preventative measures, are built upon the present climate and depend on the ecological services currently provided by nature.

Unfortunately, biodiversity and its ecosystem functions are being challenged by global warming. The relationship between organism performance and body temperature is usually asymmetric (Buckley et al., 2022; Huey and Kingsolver, 1989): performance increases relatively slowly with body temperature when below

the optimal temperature for peak performance; once the body temperature exceeds the optimal, performance decreases dramatically with accelerating speed. This distinctive nature of thermal biology will result in an accelerating decline of biodiversity even by gradual warming of constant speed, notwithstanding more frequent heat extremes (Genevier et al., 2019; McPherson et al., 2021). Understanding the mechanisms allows us to more accurately predict when and how thresholds are reached, as well as to design strategies for mitigation and adaptation.

Species are responding to climate change, but not in a uniform way. Parmesan et al. (1999) showed a century of poleward range expansion of speckled-wood butterflies in the United Kingdom, drawing research attention in the past two decades on the range shift across various taxonomic groups and geographic regions (Chen et al., 2011; Parmesan et al., 1999; Parmesan and Yohe, 2003). Nearly all multiple-species studies documenting how species' distributions and/or abundances react to climate change reveal that species within the same biological community behave differently (Cheng et al., 2019; Michielini et al., 2021; Moritz et al., 2008; Tingley et al., 2012). These observations raise an essential question: what biological and ecological mechanisms underlie the variations of species' responses (e.g. distribution, abundance, and behaviour) to climate change?

1.1 Challenges of a changing world

Global warming consists of both an increasing trend of average temperature and increasingly severe extreme temperature events. Compared with the 1850-1900 baseline, the atmosphere has warmed by an average of 1.09°C (0.95°C to 1.20°C) over the past 150 years (IPCC, 2022). Each of the last four decades has been successively warmer than any preceding post-industrial decade. Temperature is rising at a rate not witnessed in the last two thousand years (IPCC, 2022). With atmospheric CO₂ concentrations higher than ever before in the previous two million years, the inertia of global warming is still strong even if the "extremely low greenhouse gas emission" goals were met. At the end of the 21st century, the global mean temperature will increase by an additional 1.0°C to 4.7°C (IPCC, 2022). Together with a rising

mean, extreme heat events have also become more frequent and longer-lasting, contributing significantly to the excess heat experienced by nature and human society (IPCC, 2022; Perkins-Kirkpatrick and Lewis, 2020). At 4°C of warming, once-in-a-hundred-year pre-industrial extreme temperatures are expected to occur every one to two years over most of the world (Suarez-Gutierrez et al., 2020). Even with moderate warming, such as the 2°C goals set by the Paris agreement, extreme heat will exceed critical thresholds for health, agriculture, and other sectors much more frequently by the mid-21st century (IPCC, 2022; Dunne et al., 2013), rendering the old definition of "extremes" and our societal infrastructure incompatible with the new conditions of the ecosystem. Adapting to new conditions of global and regional climate has become an undeniable need for the public, policymakers and ecologists.

In parallel with climate change, anthropogenic activities are causing significant shifts in the biological environment. Mismatches in range shift and phenology are altering the strength of the existing species interactions (Schweiger et al., 2008; Visser and Gienapp, 2019). Economic and agricultural activities are consuming pristine habitats (Barbier, 2020; Wilcox et al., 2017). Invasive species are also radically changing the composition and structure of biological communities (Clavero et al., 2009; Mollot et al., 2017). Furthermore, the excessive use of pesticides, antibiotics, and microplastics causes wildlife to experience additional physiological stress (Saaristo et al., 2018). In summary, the changing biotic interactions and intensifying environmental stress will impact how organisms and communities respond to thermal stress (França et al., 2020).

Tropical regions have contributed the least to climate change and have the least societal capability to address natural disasters, but will experience the greatest increase in variability of climate (Bathiany et al., 2018) and an uncertain future for biodiversity (Wright and Muller-Landau, 2006). The thermal shifts are likely to occur concurrently with more frequent cyclones or heavy precipitation, resulting in a "one-two punch impact" (Liao et al., 2021; Lin, 2019). Tropical regions often undergo lower seasonal or inter-annual changes in temperature, causing resident species to have a narrow thermal range and little potential to evolve tolerance to a

higher temperature (Stevens, 1989). The swift and great changes, especially in the extremes, raise the concern of whether tropical animals can adapt in time to the new climate (van Heerwaarden and Sgrò, 2021; Huey et al., 2009). Moreover, dramatic habitat alteration, fuelled by human population growth, lifestyle change, and economic exploitation, is drastically altering the biotic environment in the tropics which the highest species diversity relies upon (Laurance et al., 2014). Species interactions and network structure significantly influence a community's stability in response to abiotic stress (Grilli et al., 2017; Sentis et al., 2013). However, species and their interactions are disappearing in the tropics even before being discovered (Joppa et al., 2011). Due to a lack of information and a rapid change in abiotic and biotic habitats (Gardner et al., 2007), tropical wildlife is one of the most worrying and uncertain portions of biodiversity threatened by climate change.

1.2 Challenges in studying climate change ecology

The macroecology approach has contributed significantly to determining where, what, and when biodiversity has reacted to climate change or will react in the future. The climate envelope model is still the popular methodology for macroecology projections (Trisos et al., 2020). It is based on the assumption that the realised thermal niche of a species would remain unchanged throughout time and space, an assumption that has received strong criticism (Araújo and Peterson, 2012). Such studies of correlations are limited in their ability to disentangle the relative contribution of two or more confounding variables. However, climate change encompasses several climate components that are changing (IPCC, 2022). Without an understanding of causes, it is unreliable to apply macroecological methods to explain and predict the vast differences in species' responses. Moreover, it is problematic to estimate the thermal niche of tropical species based on their existing distributions, as the estimated niche breadth may be truncated compared with their true tolerance because of the absence of hotter areas where tropical species have the potential to persist (Feeley and Silman, 2010). Therefore, it is essential, especially for tropical species, to understand their fundamental thermal biology (Bozinovic and Pörtner, 2015).

Species that dwell in the same location may have distinct fundamental thermal responses (Anderson and Falkenberg, 2021). Thermal biology research helps to reveal the ultimate threat of rising temperatures and lethal extremes to different species (?). However, species are typically not most abundant at their optimal temperature for population growth (?), indicating that species distribution and abundance are not determined entirely by temperature. None of the species exists in isolation from the others. Antagonistic interactions (e.g. predation, competition) can contribute to the biotic resistance against the colonisation of novel species expanding their ranges following the changing climate (DeRivera et al., 2005). A lack of biotic resources or mutualists (due to range shift, phenological mismatch, habitat modification, etc.) may fuel the retraction of the dependent species to a greater extent than the direct impact of climate (Preston et al., 2008). Specifically, the tropics are home to numerous species. Their well-connected interaction networks raise the possibility of indirect effects (Thierry et al., 2019), which increases the unpredictability of the response of any component species to climate change.

With varying speed and scale of range shift of co-existing and neighbouring species, species compositions have been permutated by climate change (Blois et al., 2013). The establishment of novel species in a biological community will alter the nature of the interacting networks (Alexander et al., 2015; Descombes et al., 2020; Giannini et al., 2015). Montane communities, with their elevational gradients in temperature and directional range shifts in recent decades (Chen et al., 2009; Freeman et al., 2021), are exceptional research systems. For instance, Alexander et al. (2015) demonstrated that, if the focal species fails to track climate, the impacts of competition with already-coexisting upland species or potential lowland invaders were different. Descombes et al. (2020) showed that lowland herbivore insects, when transferred to high elevations to simulate range expansion, promoted highland plant coexistence by selective feeding. However, it is yet unclear on a broad scale if there exist any general properties of climate-change-facilitated novel interactions.

Complicating matters further, the impact of species interactions is not static but relies on climatic conditions (Alexander et al., 2015; Sala and Gherardi, 2019).

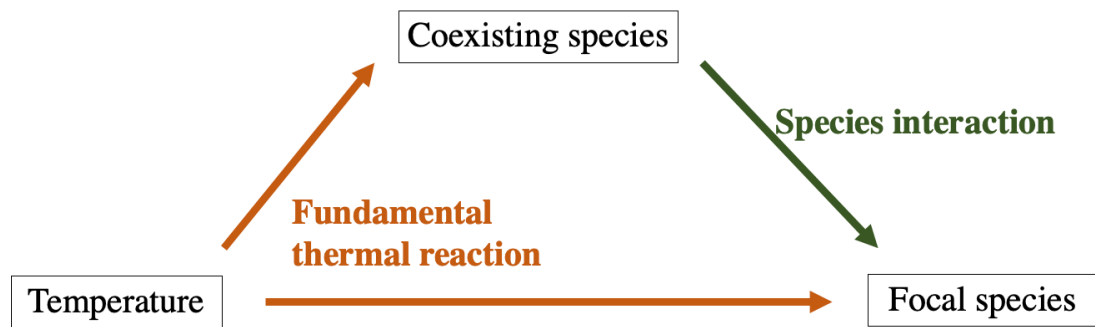


Figure 1.2 The direct effect of temperature and the indirect effect through species interactions.

Climate conditions can change the behaviour (including phenology) of the interacting species and how the focal species respond to others, hence modifying the impact per capita of species interactions. Climate can further act through demographic changes of the relevant species, altering the total impact strength. For example, Sala and Gherardi (2019) demonstrated with a desert grassland system that recurrent extreme droughts eventually drove desert shrubs to collapse. This abrupt change in the biotic setting completely changed the original trajectory of grasses' response to the drought treatment. The abiotic and biotic processes summarized in Figure 1.2 are simultaneous in the era of climate change. Therefore, an old-fashioned view that sees abiotic and biotic processes as sequential drivers of community composition is no longer practical (HilleRisLambers et al., 2012).

1.3 Approaches: Integrating thermal biology and community ecology through experiments

The study of climate change ecology is characterised by context dependence between abiotic and biotic elements (Chown et al., 2010; Gilman et al., 2010; Huey and Stevenson, 1979; Jeffs and Lewis, 2013). As shown in Figure 1.3, the impacts of climatic factors on the resident community and novel species depend on the novel species and the resident community, respectively. Additionally, the abiotic environment modifies the interactions between the novel species and the natives. These interactive effects between multiple factors result in the considerable variations

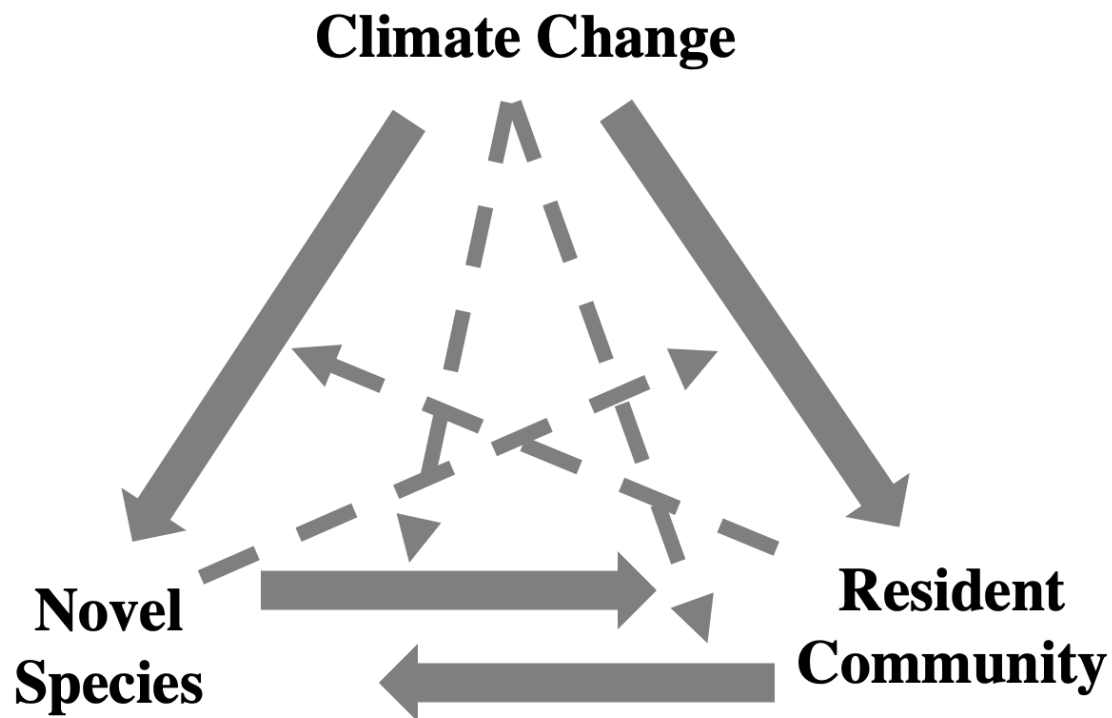


Figure 1.3 The close interdependence of climate change, the resident ecological community, and novel species.

of reactions of a species or community to climate change. In my thesis, I intend to combine experimental community ecology and thermal biology to identify the sources of variations in biodiversity's responses to climate change.

Experimental approaches are crucial for understanding how species would behave outside of their previous abiotic and biotic conditions. First, fundamental thermal niches need to be experimentally measured, particularly for tropical species (Feeley and Silman, 2010). Second, the effects of novel stressors or species are still challenging to predict from the current situation (Alexander et al., 2016). It remains an active area of research whether there exist predictable differences between the impacts of existing species interactions and novel species interactions. There is an urgent need for empirical studies for any general principles to emerge in the future. Third, manipulative experiments help to identify the true impact of a species in complex biological networks containing numerous types of interactions and multiple trophic levels (Novak and Wootton, 2010). Lastly, experiments serve as the ultimate test for

any mechanisms proposed to explain species distribution or community structure.

The integration of thermal biology and community ecology forms the link between the physiology of individual species and the biodiversity of the landscape (Hoffmann, 2010; Huey and Stevenson, 1979). By comparing the temperature reactions across biological organisational levels (i.e. individuals, populations, communities), new species interactions and ecological mechanisms can be discovered, as demonstrated in four result chapters of my dissertation. In addition, quantitative community ecology theory (Chesson, 2000), such as the modern coexistence theory utilised in this dissertation, can help integrate measurements of thermal niche and species interactions into a unified framework.

1.4 Study system

We study the community of *Drosophila* flies and their natural enemies, parasitoid wasps, inhabiting rainforest in the Wet Tropics bioregion of Queensland, north-eastern Australia. The rainforest has high value for biodiversity, particularly because of the endemic species occupying cool, wet highland refuges (Williams et al., 2003). Nonetheless, the region is under threat of temperature extremes. Since 1950, the average maximum temperature has risen by 0.8°C (0.14°C per decade) and is projected to rise by an additional $0.5^{\circ}\text{C} - 1.4^{\circ}\text{C}$ by 2030, or by an extra $1.0^{\circ}\text{C} - 2.4^{\circ}\text{C}$ by 2070 (Suppiah et al., 2007). Moreover, heatwaves are increasing in frequency, duration, and onset time (Hilbert et al., 2014; Jyoteeshkumar reddy et al., 2021; Perkins-Kirkpatrick and Lewis, 2020). The tropical mountains have natural species turnovers along the climatic gradient driven by elevation, offering a useful system for assessing the vulnerability of tropical species to temperature change (Corlett, 2011).

Parasitoids are a group of insects whose larvae develop on, or in, the bodies of other insects and eventually kill their hosts. The majority of parasitoids are wasps (Hymenoptera) and flies (Diptera). They make up one of the most diverse species groups among documented insects (Godfray, 1994). Parasitoids are numerically very abundant and ecologically important in nearly all terrestrial ecosystems (Godfray and Shimada, 1999; Hassell and Waage, 1984). They are also environmental-friendly

alternatives to pesticides for controlling forestry and agriculture pests (Waage and Hassell, 1982). It has been suggested that parasitoids have a smaller thermal range than their hosts, prompting concern over how their ecological functions will change with climate change (Furlong and Zalucki, 2017; Jeffs and Lewis, 2013).

Fruit or vinegar flies (*Drosophila* spp., Diptera) are another species-rich taxon with a wide geographic distribution in both anthropogenic and natural ecosystems (Markow and O’Grady, 2008). They use a wide range of resources, including fruits, sap, flowers, mushrooms and cacti (Markow and O’Grady, 2008). *Drosophila* flies are sensitive to temperature (Batista et al., 2018), and their population sizes are also influenced by humidity, food availability, competition, and natural enemies (e.g. parasitoid wasps) (Fletcher, 1973; Krebs and Barker, 1991; Mitsui et al., 2007). The relative abundance of *Drosophila* flies can be easily surveyed through baiting (Jeffs et al., 2021). Multiple laboratory measurements of their thermal performance have been developed in the past two decades (Hoffmann, 2010; Overgaard et al., 2014), as well as the quantification of their interactions with other species (Kraaijeveld and Godfray, 1997). For the above reasons, *Drosophila* species provide a highly practical system for ecological studies (Markow and O’Grady, 2008). Additionally, their long history as a model system for Genetics, Cell Biology and Biochemistry provides valuable knowledge that facilitates the study of their thermal biology and Eco-Evo dynamics.

The numerous direct and indirect biological interactions are challenging to study in ecological networks. Due to the intimate relationship between parasitoids and hosts, a host-parasitoid network can often be viewed as a relatively tractable, strongly-interacting unit containing a diversity of interactions (Hrček and Godfray, 2015). The network can also be expanded vertically with more trophic levels, such as host plants, hyperparasitoids and symbionts. Field examinations of host-parasitoid networks along environmental gradients have offered insight into the ecological processes structuring communities (Maunsell et al., 2015), as well as the impact of habitat degradation (Tylianakis et al., 2007). Compared to other laboratory model systems such as moth-parasitoid, beetle-parasitoid, or aphid-parasitoid networks,

the *Drosophila*-parasitoid system benefits from the fast life cycle of *Drosophila*. More importantly, the convenience in culturing and manipulation of a relatively high number of species of both *Drosophila* hosts and parasitoid species allows for the examination of two-and-higher-order species interactions in biological networks.

Our laboratory *Drosophila*-parasitoid system contains eleven *Drosophila* species and four parasitoid species, outnumbering the species diversity used in other laboratory host-parasitoid communities. The *Drosophila* species we have in culture represent more than 95% of *Drosophila* abundance found in our study sites. Our *Drosophila* species show distinctive patterns in their elevational distribution (Table 1.1). In addition, our *Drosophila* species differ in development time, body sizes, aggressiveness, mobility and other traits. The four parasitoid wasp species (Table 1.2) include three larval parasitoid species and one pupal parasitoid species that attack hosts in their larval or pupal stage, respectively. All parasitoids emerge from the pupa stage of their hosts, facilitating sampling and standardized quantification of rates of parasitism. Parasitoids also differ in their development time and host specificity (Chia-Hua Lue, unpublished results).

Table 1.1 Species information of the *Drosophila* species collected from the Australian rainforest and cultured in our laboratory.

Species name	Species subgroup	Development time at 25°C	Distribution category*
<i>D. pseudotakahashii</i>	takahashii	10 days	highland-specific
<i>D. pallidifrons</i>	nasuta	10 days	highland-specific
<i>D. birchii</i>	montium	12 days	widespread
<i>D. sulfurigaster</i>	nasuta	10 days	widespread
<i>D. rubida</i>	hypocausta	17 days	widespread
<i>D. pseudoananassae</i>	ananassae	10 days	widespread
<i>D. pandora</i>	ananassae	10 days	lowland-specific
<i>D. bipectinata</i>	ananassae	10 days	lowland-specific
<i>D. bunnanda</i>	montium	12 days	lowland-specific
<i>D. simulans</i>	melanogaster	10 days	lowland-specific
<i>D. melanogaster</i>	melanogaster	10 days	lab strain Dah

* Distribution categories were determined by where isofemale lines have been collected and were further confirmed from a standardized field survey of community structure along the elevational gradient of two mountains (Jeffs et al., 2021).

Table 1.2 Species information of the parasitoid species collected from the Australian rainforest and cultured in our laboratory. The parasitoid species are as-yet unnamed. Their genus, unique species identifiers curated in the DROP database (Lue et al., 2021), and the names of lab strains are provided.

Genus	Species identifier	Lab strain name	Development time at 25°C	Susceptible stage of hosts	Known host range (<i>Drosophila</i> species subgroup)
<i>Asobara</i>	drop-Aso-sp8	PV20; KHB	2 weeks	second instar larva	montium, nasuta, hypocausta, melanogaster
<i>Leptopilina</i>	drop-Fig124-sp2	106BPL	3 weeks	second instar larva	montium, nasuta, hypocausta, melanogaster
<i>Ganaspis</i>	drop-Gan1-sp1	96BKHL; 69B	4 weeks	second instar larva	ananassae, takahashii, montium, nasuta, hypocausta, melanogaster
<i>Trichopria</i>	drop-Dia127-sp9	66LD	3 weeks	young pupa	ananassae, takahashii, montium, nasuta, hypocausta, melanogaster

1.5 Thesis outlines

My research reveals the variations in thermal traits, competitive ability, and susceptibility to parasitoids within the local species pool, thereby identifying the mechanisms that maintain range limits and species coexistence. I then demonstrate how species interactions influence the response of populations and communities to thermal stress.

Species distribution is fundamentally governed by the tolerance of its constituent individuals to abiotic factors (e.g., temperature, precipitation, oxygen, etc.) and by the species with which it interacts (e.g., host, prey, competitor, predator, mutualists, etc.). A long-held assumption is that abiotic stress has a predominant impact on cold distribution limits, whereas species interactions determine warm distribution limits (Paquette and Hargreaves, 2021). However, evidence from the tropics has produced notably mixed results (Cahill et al., 2014). Therefore, I investigated in **Chapter 2** the abiotic and biotic factors influencing species turnover along elevational gradients. After measuring the reproductive thermal performance curves and physiological thermal limitations of the *Drosophila* species, I analysed the correlation between the thermal traits and distribution patterns of different species. Then, I analysed the single- and multi-generational impacts of competition between *Drosophila* species pairs at lowland (warm) and highland (cold) temperatures.

Trophic interactions can influence the thermal responses of prey or host populations (Sentis et al., 2013). Despite the pervasiveness of host-parasitoid interactions, the non-demographic effect of parasitoids on hosts is poorly studied. Nevertheless, knowing how trophic interaction with parasitoids affects the temperature tolerance of hosts is a crucial link between lab-measured, organism-level thermal performance and real-world predictions of natural arthropod communities. In **Chapter 3**, I designed experiments to examine the effect of parasitoids on the responses of *Drosophila* populations to heatwaves under varying nutritional conditions. In particular, I treated four distinct host species to combinations of the presence or absence of parasitoids and heatwaves on diets of five levels of yeast content.

Researchers have been attempting to associate the shifting distribution ranges of species with the increasing global mean temperature (Chen et al., 2011). At the same time, a rising mean temperature is accompanied by increasingly frequent extremes (IPCC, 2022). In the tropics, the rise in mean temperature is predicted to be relatively modest, although the frequency and intensity of extremely high temperatures have and will undergo greater change (Perkins-Kirkpatrick and Lewis, 2020). The precise climatic factors responsible for species range shifts along geographical thermal gradients rely on the mechanisms determining the range limit. Building on the mechanistic knowledge of the *Drosophila* species turnover on the tropical mountains presented in Chapter 2, I hypothesised in **Chapter 4** that extreme heat events, as opposed to a rise in mean temperature, would promote the range expansion of lowland species into highland habitats. I studied further how temperature regimes and the invasion of a novel lowland species affected the resident host-parasitoid community through competition, parasitism, and indirect interactions through a chain of competitive and trophic relationships.

As shown in previous chapters, the response of coexisting species to global warming is influenced by various aspects, including fundamental thermal response, competition within the guild, and interaction with the higher trophic level which is also temperature-dependent. Therefore, in **Chapter 5**, we set out to develop an empirical animal system that quantitatively investigates how parasitism and temperature influence host coexistence. We quantified species competition models between fifteen pairs of six *Drosophila* species in the presence or absence of a generalist parasitoid species, under cold (highland annual mean temperature) and warm (lowland annual mean temperature) conditions. Data for the cold condition were not used in **Chapter 5** for reasons described in the text. Then, we used modern coexistence theory to evaluate the influence of parasitism on the coexistence of hosts. In addition, we investigated the relationship between intrinsic population growth rates and susceptibility to parasitoids. This served to test whether a trade-off between growth and defence may serve as a shortcut for estimating the effect of parasitism on host coexistence.

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2

Climate and Competition Combine to Set Elevational Distributions of Tropical Rainforest *Drosophila*

Graphical Abstract

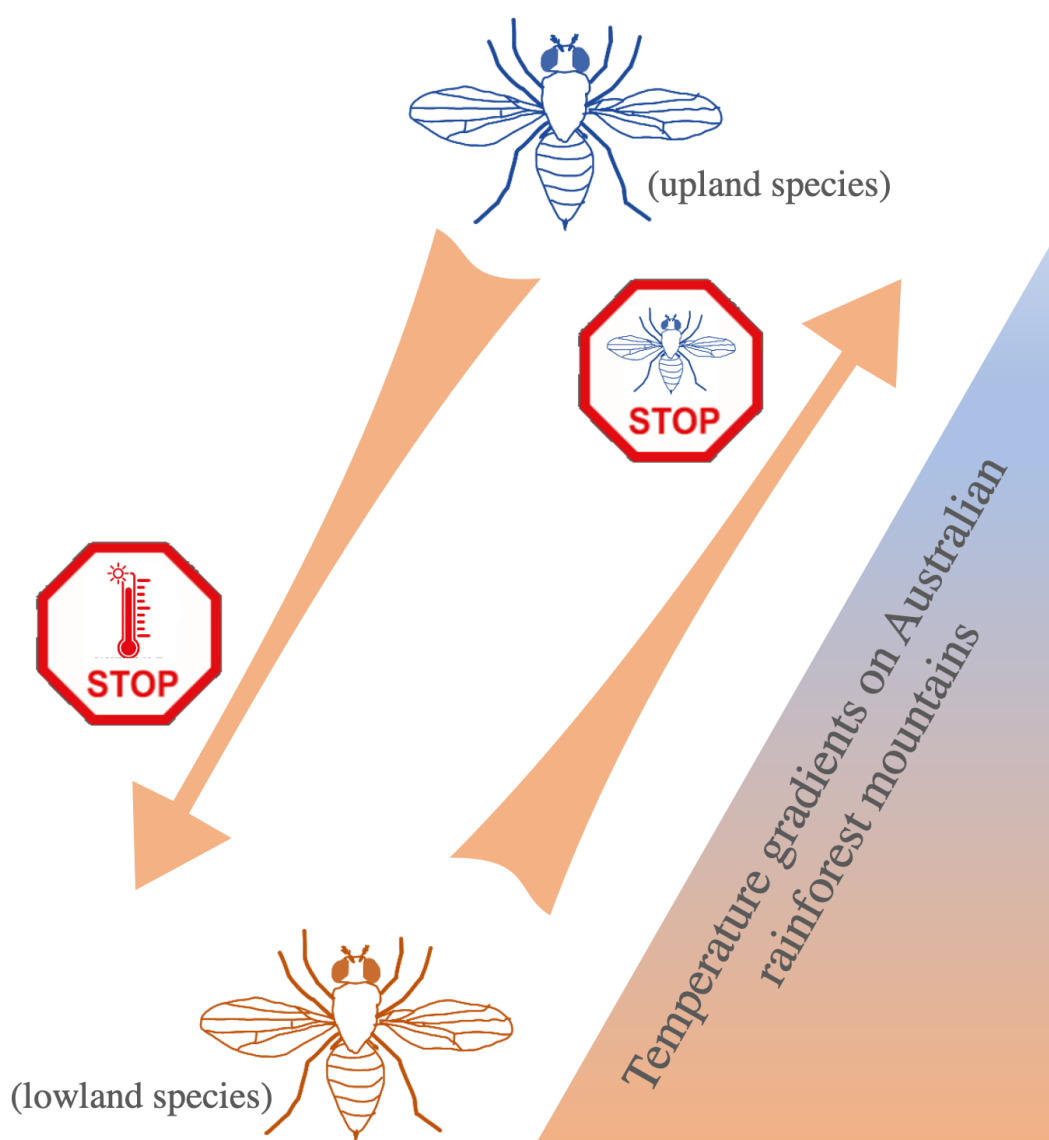


Figure 2.1 Factors setting the range limits of lowland and upland species.

Abstract

Species turnover with elevation is a widespread phenomenon and provides valuable information on why and how ecological communities might reorganize as the climate warms. Tropical mountains typically have pronounced thermal gradients and intense species interactions, providing a testing ground for investigating the relationship between thermal tolerances and biotic interactions as the proximate factors determining species distributions. We investigated temperature and interspecific competition as causes of species turnover and abundance changes in the nine most abundant species of *Drosophila* along elevational gradients in the Australian Wet Tropics. Thermal performance curves revealed that species distributions were better explained by their performance at extreme temperatures, rather than their thermal optima. Upper thermal limits varied less among species than lower thermal limits; nonetheless, at low-elevation sites, these small differences determined the composition of the community through environmental sorting. In contrast, community composition at cool, high-elevation sites was driven by temperature-dependent interspecific competition rather than tolerance to low temperatures. These results run counter to common assumptions about the role of abiotic and biotic factors in structuring communities along thermal gradients, and indicate that tropical insects may be highly vulnerable to future warming. Our study also illustrates the importance of experimental, quantitative tests across biological levels (i.e., individuals to populations) and temporal scales (i.e., within-generation to multi-generation) for understanding the full effects of climate and climate change on guilds of closely-interacting species.

2.1 Introduction

Temperature has a fundamental impact on the reproduction, survival, growth, and behaviour of organisms (Huey and Kingsolver, 1989; Huey and Stevenson, 1979), strongly influencing species' ranges and abundances (Hoffmann and Blows, 1994; Wilson et al., 2005). As a result, estimates of thermal tolerances based on laboratory assays or species' distributions have been used widely to evaluate species' sensitivity to climate change (Kearney and Porter, 2009). Tropical ecosystems comprise many species that live close to their upper thermal limits (Diamond et al., 2012; Huey et al., 2009), and which may not tolerate or adapt to warmer temperatures (Bonebrake and Deutsch, 2012; Kellermann et al., 2012). The narrow thermal ranges of tropical insects (Khaliq et al., 2014) also mean that they will need to undertake relatively large latitudinal or elevational range shifts to track their climate envelopes as the climate warms, increasing the risk of extinction and community disassembly (Colwell et al., 2008; Sheldon et al., 2011).

Despite the high relevance of understanding species' responses to climate change in tropical ecosystems (Corlett, 2012), it remains questioned whether temperature is the major proximate factor setting the position of the 'warm' (low-latitude or low-elevation) limits of species distributions. Global analyses found smaller distribution shifts in response to warming at warm range limits than at cool range limits (Chen et al., 2011; Sunday et al., 2012), and some laboratory assays have found similar upper thermal limits for tropical and temperate species (MacLean et al., 2019; Nowrouzi et al., 2018; Overgaard et al., 2014). Instead, other abiotic factors (e.g. precipitation) or biotic interactions may be critical in determining range limits in the tropics (Engelbrecht et al., 2007; Louthan et al., 2015).

A common expectation, first expressed by Charles Darwin, is that tolerance to low temperatures will set cool range limits, while biotic interactions will more often set warm range limits (O'Brien et al., 2017; Paquette and Hargreaves, 2021). However, a recent analysis of data for 654 taxa found that biotic factors became less important in determining warm limits towards the tropics, while abiotic factors remained consistently important across latitudes (Paquette and Hargreaves, 2021),

highlighting the regional differences in the relative roles of abiotic and biotic factors on local thermal gradients.

There remains limited empirical evidence on the proximate factors setting range limits in species-rich tropical communities (Feeley et al., 2017; Jankowski et al., 2013), and most such studies examine only the correlation between thermal traits (such as critical temperatures and optimal temperatures) and species' distributions (Cahill et al., 2014). A significant correlation between tolerance and distribution does not rule out a role for biotic interactions, which are themselves sensitive to temperature (Gilman et al., 2010). Additionally, the abundance and distribution of a species may still be sensitive to temperature change, even when biotic interaction is the proximate factor determining species composition. To understand the relationship between temperature and distribution, studies quantifying both thermal traits and temperature-dependent biotic interactions are required (Cahill et al., 2014). Such studies can help unify the long-separate concepts of environmental and biotic filters (HilleRisLambers et al., 2012), as well as provide practical information to inform conservation efforts in the face of climate change.

To investigate the roles of thermal tolerances and biotic interactions in determining species' range limits and structuring communities, we focused on the community of *Drosophila* flies occupying mountain rainforest habitats in northeastern Australia. These and other tropical mountains provide natural environmental gradients to test the sensitivity of species to temperature (Corlett, 2011), with pronounced changes in species composition with elevation for many taxa (Williams et al., 2003). We hypothesized that species turnover along the elevational gradient would result from thermal constraints at cool, high-elevation sites, but from competitive exclusion at warmer, lowland sites. Thus, we predicted that cold-tolerant species would become more abundant at higher elevations. We also predicted that species with reduced abundance at low elevations would be those limited by competition with species with lowland-biased distributions, and not necessarily those with lower heat tolerance. To test these predictions, we first examined correlations between species' distributions and multiple thermal tolerance traits. We then

used laboratory experiments mimicking upland and lowland thermal conditions to examine short-term and long-term competitive outcomes for pairs of species with similar or different distribution types.

2.2 Methods

All data are archived on Zenodo (10.5281/zenodo.6400611) and the code of analysis is available on GitHub (<https://github.com/Jinlinc/DistributionRegulators.git>). All statistics mentioned were performed with R statistical software, version 4.0.3 (R Core Team, 2020).

2.2.1 Species distributions along elevational gradients

Field survey. Field data on *Drosophila* communities were collected from rainforest sites at Paluma Range (S18° 59.031' E146° 14.096') and Kirrama Range (S18° 12.134' E145° 53.102'), Queensland, Australia. These sites lie within the Wet Tropics bioregion which has high levels of endemism associated with cool and moist upland refugia (Williams et al., 2003). Abundances of *Drosophila* at these sites peak during the period March to June. *Drosophila* pupae were sampled using bottle traps baited with fermented banana from 11th March – 12th April 2016 for three sites at an elevation of 70m, 350-390m, and 730-880m (subsequently referred to as low-, mid- and high-elevation, respectively) on the two mountains. 182 pupae were sampled at each site and 716 pupae in total were successfully identified to species by DNA metabarcoding (Jeffs et al., 2021), with 86 – 134 pupae at each site. Two infrequent species, *Drosophila serrata* (1 sample) and *D. immigrans* (4 samples), were excluded from analyses.

Distribution analysis. Two methods were used to assess trends in relative abundance with elevation for individual species. First, the probability of detecting a particular *Drosophila* species among other co-occurring *Drosophila* species was modelled as a function of *elevation*, *transect* and their interaction in a generalized linear model (family = “binomial”) using the *stats* package in R. For each species, the response variable was 1 if the pupa was identified as the focal species and 0

if it was identified as any other species. Second, since none of the species had a bimodal distribution with elevation, abundance-weighted mean elevation (*hIndex*) was used as a simple alternative method to quantify distributions. The location of each sample was assigned a value of 0, 0.5, or 1 if it was collected at low-, mid- and high-elevation sites, respectively. *hIndex* was calculated for each species by averaging these values for samples from both transects. Correspondence between the two measures was tested by Spearman’s rank correlation test between the coefficient value for elevation in the regression and *hIndex* across species.

2.2.2 Maintenance of laboratory culture

Isofemale cultures of *Drosophila* were established in 2017 and 2018 from adults collected from high- and low-elevation sites. Cultures were maintained initially at 24°C at the Biology Centre, Czech Academy of Sciences, and then transferred to the Department of Zoology, University of Oxford, UK, in December 2018, where they were maintained at 25°C. Cultures and all experiments mentioned below were maintained under a 12h (light)/12h (dark) cycle. There were approximately 15 to 30 non-overlapping generations in the Czech Republic and four to seven non-overlapping generations in Oxford before cultures were used to establish mass-bred lines (MBLs).

To revive genetic variation, we made MBLs of each *Drosophila* species by combining four isofemale lines (except for *D. pandora*, where only three isofemale lines were available). The four lines were selected from different mountains and different elevations if possible (detailed information in Appendix Table A.1). Each mass-bred population was initiated using two independently-reared MBLs of the same species. Large populations were maintained at 25°C for more than four generations before measuring the thermal traits. The MBLs were maintained at 25°C until 2020 and at 23°C thereafter. In this way, measurements should not have been influenced by maternal effects, acclimation, or isofemale line effects.

MBLs of nine tropical *Drosophila* species (listed in Appendix Table A.1) were included for trait measurements. Of the available species, only *D. rubida* was not included because it was difficult to raise in large numbers and to synchronize

with the other species. Isofemale lines of *D. pseudotakiihashii* were contaminated by another species before measuring the thermal traits. Therefore, its thermal traits were not measured. A new MBL originating from the two available *D. pseudotakiihashii* isofemale lines was used in the competition experiment. *Drosophila melanogaster* does not occur naturally at the study sites, but a laboratory strain (wild type, Dah strain) was measured for thermal performance together with the focal species, as a benchmark for future comparisons.

2.2.3 Reproductive thermal performance

Experimental measurements. We exposed flies to temperatures ranging from 14°C to 32°C and measured how their reproductive success changed with temperature (procedures are detailed in Appendix Figure A.1A). To generate the adult flies to initiate the experiment, fly eggs collected from the population cage were reared at low density (<100 eggs per vial) at 25°C. Emerging adults were separated by sex within 12 hours to guarantee that they were unmated. Rearing was started on different days for different species to synchronize the first day of egg-laying. Two additional vials with five pairs of flies were monitored daily for sexual maturation, indicated by egg laying. Two days after the first observation of egg-laying in both vials, two virgin females and two virgin males were paired in a new vial containing 4ml *Drosophila* medium (weight/volume concentration: 8% corn flour, 4% yeast, 5% sugar, 1% agar, and 1.67% methyl-4-hydroxybenzoate). Vials were randomly assigned to water baths set at one of seven constant temperatures (14°C, 17°C, 20°C, 23°C, 26°C, 29°C, 32°C). For each species and each temperature treatment, eight replicates were evenly split between two experimental blocks, making up a total of 1512 vials. Vials were submerged with the water level kept above the zone within which flies could freely move. The temperature and relative humidity of vials were monitored within two empty tubes placed at the centre and the corner. Observed temperatures varied within 0.5°C around the set temperature. Temperatures in the centre of the water bath were on average 0.5°C higher than at the corners; the average of the former during the experimental period was used

as the corrected temperature in analyses. Relative humidity levels were similar to field conditions, ranging between 80% - 95%.

As temperature influenced how fecundity changed through time (Appendix Figure A.1B), offspring numbers were measured for the 1st – 2nd day and the 7th – 8th day and then averaged to reflect relative fecundity in early adult life. After eight days of exposure to temperature treatments, all flies were returned to 25°C for another four days to examine their recovery of reproduction. Surviving flies were recorded at the beginning and end of each period. Eggs produced during the test periods developed at the same temperature as their parents. Vials were left for 5 – 7 days after the first emergence was observed and then the offspring were counted. The experiment was conducted from May to August 2019.

Thermal performance curve. A multi-level, non-linear piecewise model was fitted to describe how reproductive success changed with temperature for each *Drosophila* species. The average daily fecundity per female was square root transformed, then modelled with the *Briere2* function (Briere et al., 1999):

$$P_i(T) = \begin{cases} a_i T(T - RTmin_i)(RTmax_i - T)^{1/b_i} & \text{for } RTmax_i > T > RTmin_i \\ 0 & \text{for } T \leq RTmin_i \text{ or } T \geq RTmax_i \end{cases} \quad (2.1)$$

where $P_i(T)$ is the reproductive performance of species i in temperature T , $RTmin_i$ and $RTmax_i$ are the minimum and maximum temperatures for the species i to reproduce, a is a scaling factor and b is a shape factor of the curve. Values of $RTmin_i$ of the nine different species were modelled by a *Gaussian* distribution whose mean, μ_{RTmin} , and variance, σ_{RTmin}^2 , were the hyperparameters in the multi-level model. The same applied to $RTmax_i$, a_i , and b_i . $P_i(T)$ was modelled assuming a *Gaussian* distribution of errors. Although a *Gaussian* distribution is not ideal to model the transformed count data, which are all positive, models using untransformed count numbers with *Poisson*, zero-inflated *Poisson*, negative binomial, and lognormal distributions did not adequately converge, potentially due to the piecewise nature of the thermal performance functions. The standard deviations of the *Gaussian* distribution of $P_i(T)$ were assumed different in different temperature treatments and

followed a *Gaussian* distribution, $\sigma_{P(T)} \sim \mathcal{N}(\mu_{\sigma_P}, \sigma_{\sigma_P}^2)$. This generated better fits than assuming the same standard deviation of $P_i(T)$ across temperature treatments, as judged by leave-one-out cross-validation. This was because when the temperature was equal to or close to $RTmin_i$ and $RTmax_i$, $P_i(T)$ was close to zero and its standard deviation was also small.

The prior distributions of the hyperparameters μ_{RTmin} , μ_{RTmax} , μ_a , μ_b , μ_{σ_P} , are *Gaussian* distributions with reasonable means (15°C, 30°C, 0, 0, 0) and relatively large standard deviations (10, 10, 1, 10, 10). The prior distribution of the hyperparameters for variances σ_{RTmin}^2 , σ_{RTmax}^2 , σ_a^2 , σ_b^2 , $\sigma_{\sigma_P}^2$, is inverse-gamma (0.001, 0.001), which is a commonly used non-informative distribution for priors of variance. The values of a_i and b_i were bounded to be positive. The values of $RTmin_i$ were bounded between 0°C to 17°C and the values of $RTmax_i$ were bounded between 26°C to 35°C based on prior knowledge of the range of temperatures under which Australian *Drosophila* can reproduce. The multi-level model was fitted under a Bayesian framework using MCMC sampling within the *rstan* package (Stan Development Team, 2021) in R. Models converged and performance was acceptable in diagnostic plots (Appendix Figure A.2). Medians of the posterior distributions were used as the parameter values to construct the thermal performance curves. Optimal temperatures for peak reproduction were calculated directly from known parameters:

$$RT_{opt} = \frac{2bRTmax + (b + 1)RTmin + \sqrt{4b^2RTmax^2 + (b + 1)^2RTmin^2 - 4b^2RTminRTmax}}{4b + 2} \quad (2.2)$$

Regression analysis. The medians of the posterior distributions of $RTmin$, $RTmax$, and RT_{opt} were modelled by $hIndex$ in a linear model with phylogenetic correction. The phylogenetic tree was modified from Finet et al. (2021) (Appendix Figure A.3). Fecundity at 29°C and 17°C, and recovered fecundity after 29°C and 14°C, were used as direct measurements of performance in high or low temperatures. These fecundities were modelled as a function of $hIndex$ and *block* as fixed effects, and *species* as a random effect in a generalized linear mixed-effect model (family = “negative binomial”) with phylogenetic correction. These regression analyses were

conducted using the *brms* package in R (Bürkner, 2018). *Drosophila melanogaster* and *D. simulans* were not included in the regression because their distribution patterns were unavailable from the field survey.

2.2.4 Thermal knockdown

Experimental measurements. Resistance to extreme cold temperature was measured as the knockdown time for each individual at 5°C and the time for recovery of mobility after a 30-minute exposure to 5°C. The constant temperature chosen for cold stress studies is often around 0°C (Gibert et al., 2001). As tropical species often have significantly weaker cold resistance (Gibert et al., 2001), 5°C was used instead to increase the variation among the tested species after pilot trials. Heat stress was chosen to be 40°C, which follows the common practice for *Drosophila* species (Hoffmann et al., 2003) and is expected to capture the between-species variance in heat tolerance over a time scale which is convenient to measure (Jørgensen et al., 2019). After being knocked down by heat (40°C), most flies did not survive. In this case, only knockdown time was used to evaluate resistance to heat. The knockdown experiments were conducted from May to June 2019.

Virgin adult flies (siblings of those used for reproduction measurements) were kept in same-sex groups at 25°C for 9-10 days before knockdown assays. Assays were conducted for male and female flies separately. An observation rack was divided into nine (3x3) cells. Each cell was randomly assigned one of the nine *Drosophila* species and held seven flat-bottomed 3ml glass vials, each with a randomly-selected individual of the allocated species. One set of observations on such a set-up represents a single block. We repeated measurements for three experimental blocks, and the allocation of species to cells was redrawn for each block. In total, we measured 21 individuals per species per sex. During measurement, the observation rack was moved immediately into the incubator pre-set at 5°C or 40°C. Every tube was examined once every minute and flies that lost their ability to stand in that minute were recorded. After exposure to 5°C for 30 minutes, all flies were in a chill

coma. The observation rack was moved to a 25°C room. Flies were left undisturbed and the time taken until each fly regained its ability to stand was recorded.

Regression analysis. The knockdown time by heat, knockdown time by cold and recovery time from cold of both sexes were first compared among species using ANOVA. When interspecific variation was observed, they were modelled by *hIndex*, *block*, and *cell position* as fixed effects, and *species* as a random effect in linear mixed-effect models with phylogenetic correction using the *brms* package in R. *Species* was included as a random effect to account for repeated measures.

2.2.5 Short-term competition

Experimental design. Pairs of species were reared in the same vials for one generation to evaluate how the densities of intraspecific and interspecific competitors influenced reproductive success under temperature regimes typical of lowland and upland sites. We used incubators set at alternating temperatures mimicking day-time and night-time temperatures in February at high-elevation (23°C / 21°C) and low-elevation sites (28.5°C / 24°C) (Appendix Figure A.4). Adults to establish the competition experiment were reared at moderate density (300 – 500 per bottle) at their testing temperature regimes. After eclosion, individuals that emerged within the same 48 hours were kept together in mixed-sex containers. Two days after the first observation of egg-laying, adults of different sexes were separated, and from the following day were allowed to compete and lay eggs in the 5 ml experimental vials for two days before being discarded.

Five species were chosen as representative species for upland-biased, elevation-generalist and lowland-biased distribution types (see Results for the definition of distribution types). The two-species combinations were: lowland species vs. upland species: *D. bipectinata* vs. *D. pallidifrons*, *D. bipectinata* vs. *D. pseudotakahashii*, *D. pandora* vs. *D. pallidifrons*; lowland species vs. lowland species: *D. bipectinata* vs. *D. pandora*; lowland species vs. elevation-generalist: *D. bipectinata* vs. *D. sulfurigaster*; upland species vs. elevation-generalist: *D. pallidifrons* vs. *D. sulfurigaster*. Each combination was tested at different founding densities in a

factorial design: (4 pairs of species A, 2 pairs of species B), (4A, 4B), (4A, 8B), (2A, 4B) and (8A, 4B). We also included monocultures of each species with 2, 4, and 8 pairs. Each density and species combination was replicated ten times across two or three blocks staggered by two days (two blocks for the *D. pandora* vs. *D. pallidifrons* combination; three blocks for the other five pairs). Offspring that successfully developed to adulthood were identified to species and counted. The experiment on the *D. pandora* vs. *D. pallidifrons* combination was conducted from September to December 2020 and the other five pairs were experimented with from January to March 2021. *Competition models.* We used the *Beverton-Holt* model to describe the population growth of a single generation of flies on discrete and temporary resources:

$$N_{focal,t+1} = \frac{R_0 N_{focal,t}}{1 + a(N_{focal,t} + \beta N_{competitor,t})} \quad (2.3)$$

where $N_{focal,t}$ and $N_{competitor,t}$ are the numbers of focal species and competing species, respectively, at time t . R_0 is the generational reproduction rate and a is a constant defining the form of the density-dependence relationship. β represents the interspecific competition coefficient of the competitor species to the focal species, which defines the equivalence between the two competing species. $N_{focal,t+1}$ was modelled assuming a negative binomial error distribution, under a Bayesian framework using MCMC sampling within the *rstan* package in R (Stan Development Team, 2020), as described by (Terry et al., 2021). Model diagnostics are shown in Appendix Figure A.5A-B. The medians of the posterior distribution were used as the parameter values to infer the equilibrium state of each pair following Hassell and Comins (1976).

2.2.6 Long-term competition

Experimental design. To evaluate the long-term impact of temperature and competition on population size, a lowland species, *D. pandora*, and an upland species, *D. pallidifrons*, were reared in monoculture and mixed-culture environments for

multiple generations under low-elevation and high-elevation temperature regimes (Appendix Figure A.4).

Four monocultures of each species and eight mixed-species cultures were maintained at each temperature regime for 13 weeks, totalling 32 cultures. Monocultures were started with ten pairs of individuals. Mixed-species cultures were started with ten pairs of individuals of each species. The starting density was very low compared to the equilibrium density. The cultures were evenly divided into two blocks starting on different dates. Each was maintained in a series of five bottles following Ayala et al. (1973). At the start of each week, adults surviving in the most recent bottle and adults freshly emerging in the older four bottles were separately collected, photographed and transferred together into a new bottle with fresh food. In this way, adult survival and reproduction were recorded separately. The total population size of each species was counted at the end of the experiment. To avoid pseudo-replication introduced by ‘incubator’ effects, the two incubators were switched between temperature regimes every week, with their contents moved accordingly. Trays were shuffled inside the incubator every two days. Temperature and humidity were recorded and the temperature regimes were confirmed during and at the end of experiments. The experiment was conducted from September to December 2020.

Data analysis. Population sizes were modelled as a function of *temperature*, *species*, *the presence/absence of competitors*, and their interactions, with *culture ID* as a random effect in a generalized linear mixed-effect model (family = “zero-inflated negative binomial”) using the *brms* package in R. Model diagnostics are shown in Appendix Figure A.5C. To visualize the three-way interactive effects, the posterior estimates of the high-temperature effect were plotted against zero for the two species with or without the presence of competitors; the posterior estimates of the effect of the competition were plotted against zero for the two species in each temperature regime.

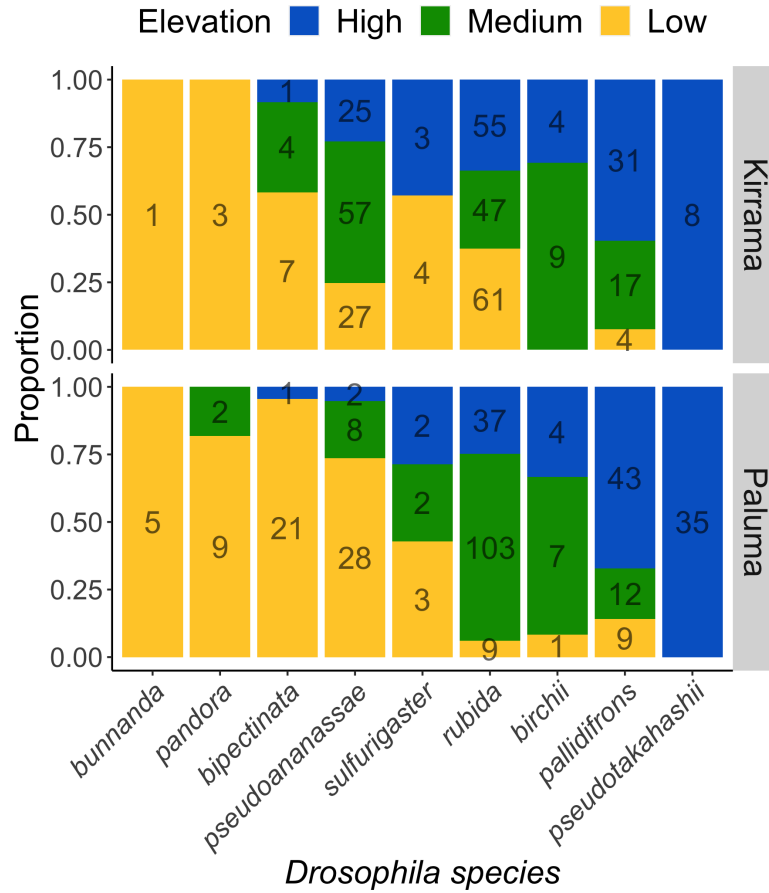


Figure 2.2 Distribution patterns of nine rainforest *Drosophila* species. The proportion of samples found at sites at low (yellow), medium (green) and high (blue) elevations are shown for each species. Numbers on the bars show the counts of samples for each combination of species and sites. Distribution at Mount Kirrama and Mount Paluma were shown separately.

2.3 Results

2.3.1 Field distributions

The numbers of samples found at low-, medium- or high-elevation sites for each of the nine major *Drosophila* species (accounting for 99% of all samples) are shown in Figure 2.2. Distributions quantified using regression and by weighted elevation (*hIndex*) were consistent (Spearman's rank correlation $\rho = 0.93$, p -value = 0.0007). *Drosophila bipectinata* and *D. pandora* had significantly decreased detection probability with increasing elevation and were thus categorized as lowland-biased species with high confidence. *Drosophila pseudoananassae* showed a lowland

bias on one of the two mountains, and a mid-elevation peak on the other. *Drosophila rubida*, *D. sulfurigaster* and *D. birchii* showed no significant change with elevation and were thus defined as elevation generalists. *Drosophila pallidifrons* and *D. pseudotakahashii* were significantly more likely to be found at high elevations and were thus defined as upland-biased species. Coefficients and p values of the regressions and *hIndex* are shown in Appendix Table A.2. For *D. bunnanda*, the six records were insufficient for model fitting but all occurred at lowland sites, and data from a larger-scale study suggest it is a lowland-biased species (Schiffer and McEvey, 2006).

2.3.2 Thermal performance curves

Thermal performance curves of daily fecundity per female vary among species in terms of the range, optimal temperature, peak fecundity, and shape factors (Figure 2.3; Table 2.1; see Appendix Figure A.2C for original data and fitted curves for each

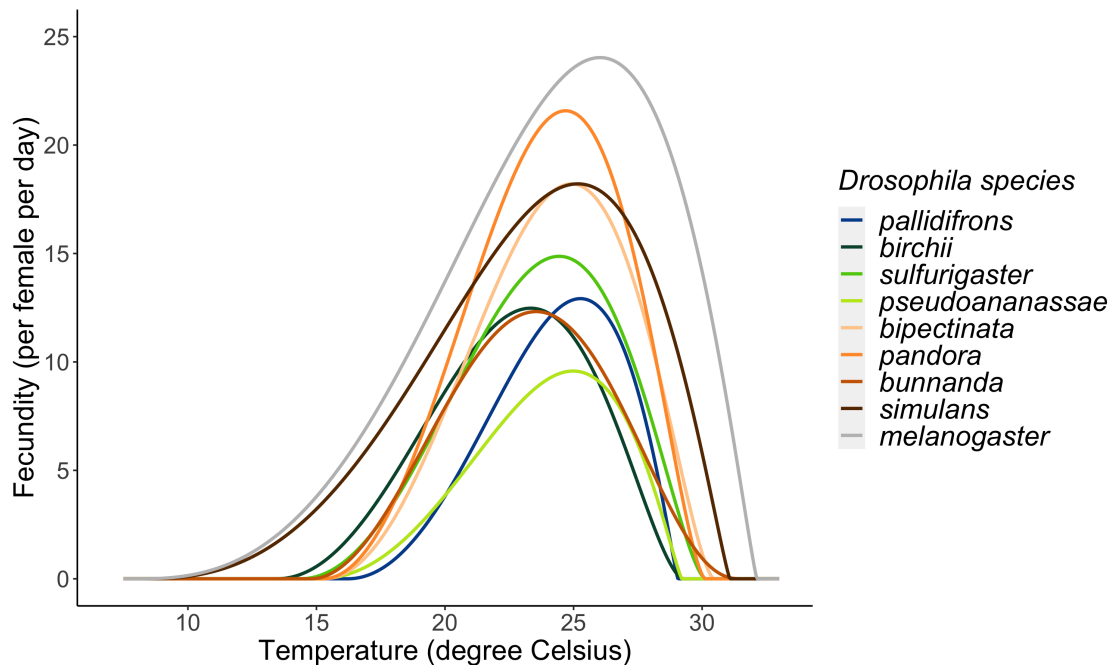


Figure 2.3 Thermal performance curves for the reproduction of nine *Drosophila* species. The fitted numbers of adult offspring produced per female parent per day are plotted against temperature. Line colours indicate species' distribution types, with upland-biased species represented by cold colours and lowland-biased species represented by warm colours.

species). The temperature for optimal reproductive performance, RT_{opt} , did not correlate with distribution patterns (Coefficient = 0.09, 95% credible interval = -2.83 – 3.01; Appendix Figure A.6A). There was no general trade-off between RT_{min} and RT_{max} corresponding to species' distribution types (Spearman's rank correlation $\rho = -0.6$, p-value = 0.10; Appendix Figure A.6B). For example, the lowland-biased species *D. bunnanda* has higher heat tolerance and lower cold tolerance than its elevation-generalist relative, *D. birchii*. In contrast, *D. sulfurigaster* outperforms its upland-biased relative, *D. pallidifrons*, across the temperature range.

2.3.3 Cold tolerance

Values of RT_{min} were not correlated with species distribution patterns (Figure 2.4a. Coefficient = -0.41, 95% credible interval = -4.15 – 3.43). Similarly, upland-biased species did not show higher fecundity at the low temperature, 17°C (Figure 2.4b. Coefficient = -0.27, 95% credible interval -3.80 – 3.05). When exposed to acute sublethal low temperature (5°C), all seven tropical *Drosophila* species showed similarly poor performance compared to *D. simulans* and *D. melanogaster* (Appendix Table A.3 for Tukey multiple pairwise comparisons). All species recovered their fecundity after eight days at 14°C. This recovered fecundity was slightly but non-significantly higher among upland species (Figure 2.4c. Coefficient = 0.33, 95% credible interval = -0.55 – 1.14). It took longer for upland species to regain mobility after chill coma (Figure 2.4d. Male: coefficient = 13.04 (-9.31 – 35.05); female: coefficient = 8.49 (-2.18 – 20.02).)

Table 2.1 Parameters of thermal performance functions and their 90% credible intervals ($ci90$) of the nine species. RT_{min} and RT_{max} are the minimum temperature and maximum temperature for the species to reproduce. a is the scaling factor and b is the shape factor of the *Briere2* function.

Species	a	$ci90_a$	b	$ci90_b$	RT_{min}	$ci90_{RT_{min}}$	RT_{max}	$ci90_{RT_{max}}$
<i>D. bipectinata</i>	0.0046	0.0031 - 0.0060	1.27	1.02 - 1.55	15.28	14.54 - 15.87	30.44	30.08 - 31.05
<i>D. birchii</i>	0.0034	0.0022 - 0.0056	1.17	0.95 - 1.56	13.45	13.07 - 13.77	29.27	28.12 - 29.81
<i>D. bunnanda</i>	0.0016	0.0012 - 0.0025	0.88	0.81 - 1.06	14.57	14.10 - 15.18	31.21	30.60 - 31.78
<i>D. melanogaster</i>	0.0037	0.0032 - 0.0042	1.72	1.48 - 2.03	8.29	6.89 - 9.37	32.13	32.03 - 32.28
<i>D. pallidifrons</i>	0.0073	0.0056 - 0.0098	1.74	1.37 - 2.38	16.23	15.54 - 16.75	29.06	28.19 - 29.38
<i>D. pandora</i>	0.0052	0.0037 - 0.0065	1.26	1.03 - 1.51	15.25	14.58 - 15.80	30.13	29.87 - 30.58
<i>D. pseudoananassae</i>	0.0053	0.0035 - 0.0071	1.68	1.24 - 2.32	15.06	14.17 - 15.89	29.22	28.38 - 29.77
<i>D. simulans</i>	0.0035	0.0027 - 0.0046	1.69	1.36 - 2.21	8.51	6.94 - 9.64	31.09	30.39 - 31.78
<i>D. sulfurigaster</i>	0.004	0.0028 - 0.0051	1.27	1.03 - 1.52	14.37	13.91 - 14.96	30.11	29.84 - 30.63

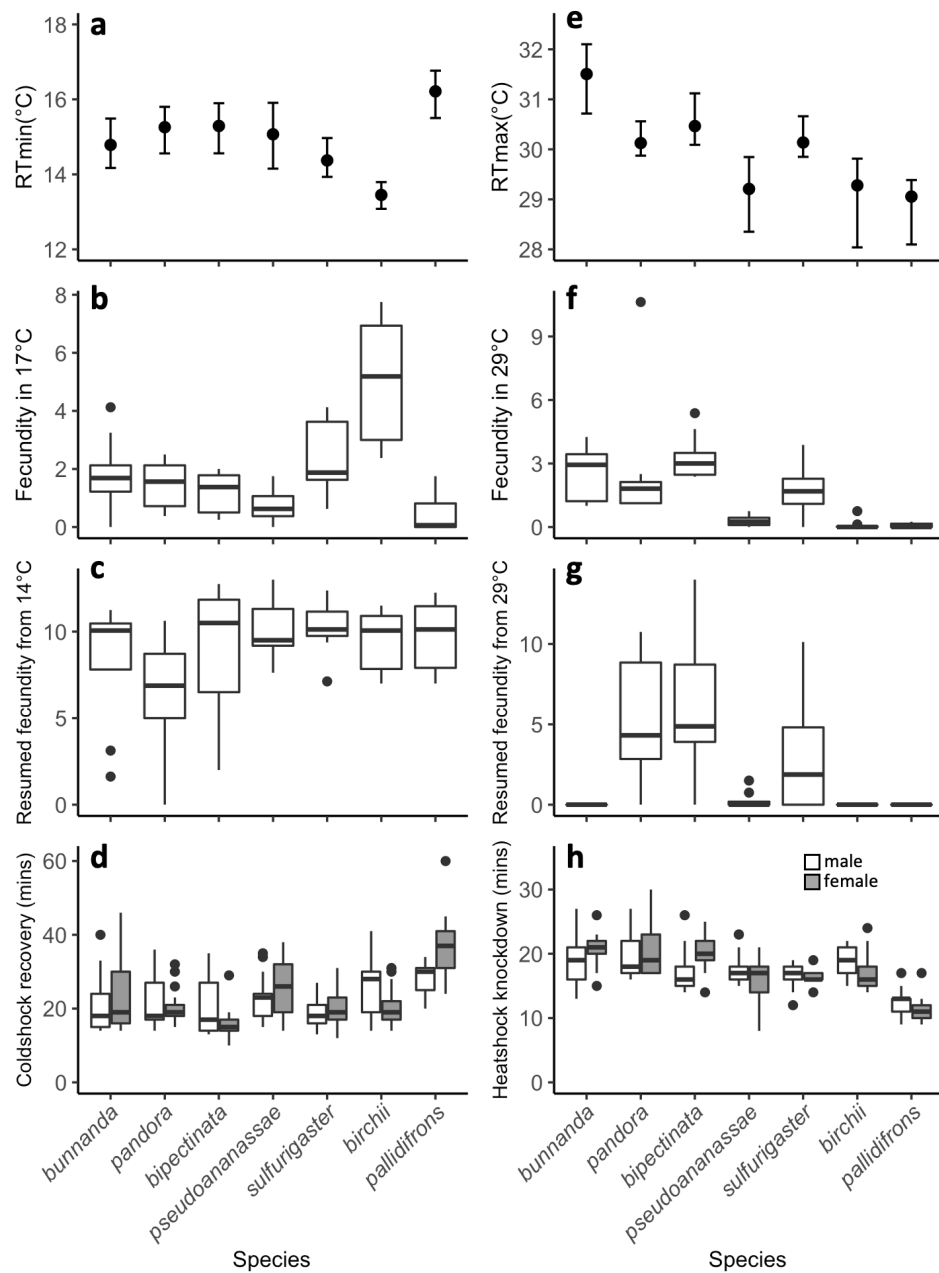


Figure 2.4 Reproductive and physiological thermal tolerance of seven tropical *Drosophila* species. Species are ordered by their distribution patterns, from lowland-biased species (left) to upland-biased species (right). Cold tolerance is represented by RT_{min} (a), fecundity at 17°C (b), recovered fecundity after 14°C (c) and recovery time after chill coma (d). Heat tolerance is represented by RT_{max} (e), fecundity at 29°C (f), recovered fecundity after 29°C (g) and knockdown time at high temperature (h). Graph (a) and (e) show the medians and 90 % credible intervals of the posterior distribution of the estimated parameters. Boxplots in b-d and f-h show the minimum, 25th percentile, median, 75th percentile, maximum and potential outliers. In (d) and (h), data for females (shaded) and males (unshaded) are plotted separately.

2.3.4 Heat tolerance

Regardless of the small variation of RT_{max} (standard deviation = 1.01) compared with RT_{min} (standard deviation = 2.82), RT_{max} was significantly higher among species with more lowland-biased distributions (Figure 2.4e. Coefficient = -3.06, 95% credible interval = -5.30 – -0.88). Reproductive performance at 29°C also decreased with $hIndex$ (Figure 2.4f. Coefficient = -5.80, 95% credible interval = -9.37 – -2.50). After exposure to 29°C for eight days, the upland species, *D. pallidifrons*, could not reproduce when transferred back to 25°C, while four out of the six elevation-generalist and lowland-biased species resumed reproduction (Figure 2.4g). Knockdown time at lethally high temperature (40°C) was shorter among upland species (Figure 2.4h. Male: coefficient = -6.71 (-13.70 – 0.50); female: coefficient = -1.99 (-9.48 – 5.71)), indicating these species lose their mobility faster at high temperatures.

2.3.5 Interspecific competition

When raised in a laboratory condition mimicking the warmer, lowland sites, reproductive success was highest for the two lowland-biased species, followed by the elevation-generalist species, *D. sulfurigaster*. The two upland species could barely reproduce regardless of the presence of competitors (proliferation rates, R_0 , of *D. pallidifrons* and *D. pseudotakahashii* were lower than one; Figure 2.5; Table 2.2). Their competitive effect on lowland species was minimal, indicated by low β values (Table 2.2). The two lowland species, *Drosophila pandora* and *D. bipectinata*, are expected to coexist stably, based on their reproductive and competitive parameters.

When raised under cooler, upland conditions, all species could reproduce and sustain their populations (all R_0 s were higher than one; Table 2.2). Lowland species were strongly affected by the density of *D. pallidifrons*, an upland species, while upland species were significantly less affected by lowland species. Competition with *D. pallidifrons* under upland conditions was predicted to drive *D. pandora* and *D. bipectinata* to exclusion (Table 2.2).

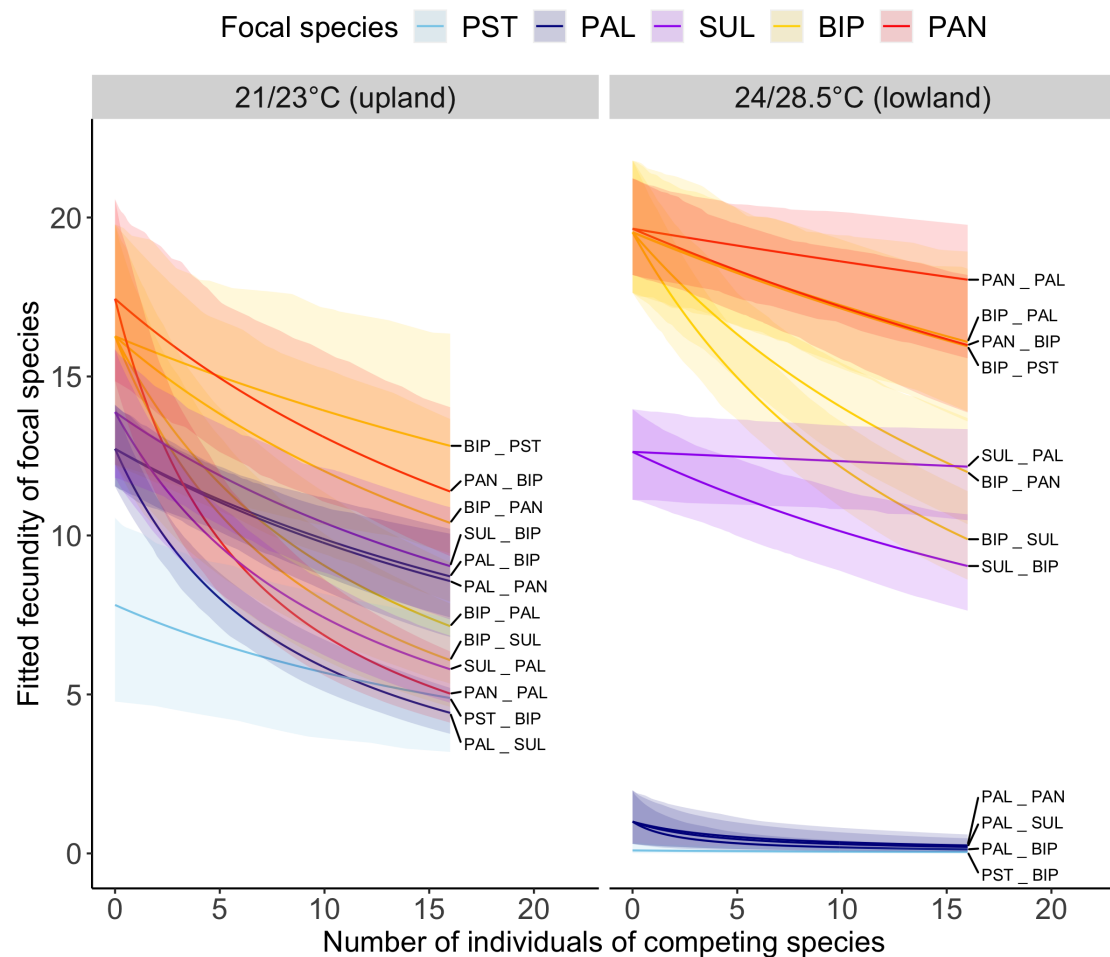


Figure 2.5 Interspecific competition for species pairs under upland and lowland temperature regimes. Each line shows the fecundity of the focal species when its founder number is kept at four pairs while changing the number of individuals of the competing species. The identity of the focal species in each pair is indicated by the colour of the line (see legend) and by the label (e.g., for BIP-PST, the first code BIP is the focal species and the competitor species is PST). The shaded area indicates the 90 % credible interval of the fitted values under the *Beverton-Holt* model for pairwise species competition. PST = *D. pseudotakahashii*, PAL = *D. pallidifrons*, SUL = *D. sulfurigaster*, BIP = *D. bipectinata*, PAN = *D. pandora*.

Table 2.2 Fitted values of the parameters of the competition model and predicted equilibrium states for pairwise interspecific competition. R_0 is the reproductive rate, a is a constant defining the form of the density-dependence relationship, β is the interspecific competition coefficient, and $ci90$ represents the 90% credible intervals of each parameter.

Temperature	Focal species	R_0	$ci90_{R0}$	a	$ci90_a$	Competitor	β	$ci90_\beta$	Equilibrium state of the focal species
Cool (upland)	<i>BIP</i> (<i>D. bipectinata</i>)	11.36	8.06-15.21	0.05	0.02-0.09	PAL	2.26	1.4-4.08	excluded
						PAN	0.99	0.47-1.95	excluded
						PST	0.47	0.12-1.09	stable coexistence
						SUL	2.95	1.91-5.25	excluded
	<i>PAL</i> (<i>D. pallidifrons</i>)	27.94	19.52-38.66	0.42	0.27-0.64	BIP	0.3	0.15-0.49	dominant
						PAN	0.32	0.14-0.52	dominant
						SUL	1.22	0.9-1.62	unstable coexistence
	<i>PAN</i> (<i>D. pandora</i>)	13.68	10.4-17.85	0.07	0.04-0.12	BIP	0.74	0.33-1.36	dominant
						PAL	3.41	2.26-5.59	excluded
	<i>PST</i> (<i>D. pseudotakahashii</i>)	6.27	3.4-10.66	0.08	0.03-0.19	BIP	0.79	0.35-1.76	stable coexistence
Warm (lowland)	<i>SUL</i> (<i>D. sulfurigaster</i>)	20.96	14.27-31.13	0.25	0.14-0.44	BIP	0.41	0.19-0.67	dominant
						PAL	1.05	0.71-1.53	unstable coexistence
						PAL	0.29	0.07-0.63	dominant
	<i>BIP</i> (<i>D. bipectinata</i>)	15.35	12.51-19.05	0.07	0.05-0.11	PAN	0.87	0.54-1.35	stable coexistence
						PST	0.31	0.07-0.63	dominant
						SUL	1.35	0.93-2	excluded
	<i>PAL</i> (<i>D. pallidifrons</i>)	0.99	0.19-2.37	0.12	0.02-0.46	BIP	6.81	2.98-22.27	unable to establish
						PAN	2.99	1.52-8.93	unable to establish
	<i>PAN</i> (<i>D. pandora</i>)	17.18	14.2-21.24	0.09	0.06-0.14	SUL	3.98	1.77-12.83	unable to establish
						BIP	0.27	0.08-0.51	stable coexistence
						PAL	0.11	0.01-0.29	dominant
	<i>PST</i> (<i>D. pseudotakahashii</i>)	0	\	\	\	BIP	\	\	unable to establish
	<i>SUL</i> (<i>D. sulfurigaster</i>)	13.65	10.26-19.03	0.15	0.09-0.24	BIP	0.37	0.18-0.62	dominant
						PAL	0.04	0-0.14	dominant

In the long-term competition experiment, high temperature drove the upland-species *D. pallidifrons* to extinction regardless of the starting species composition (monoculture versus mixed-species culture) (Figure 2.6a). In contrast, the monoculture of the lowland-species *D. pandora* remained abundant at both temperatures (Figure 2.6b: 90% credible interval of temperature effect in monoculture overlaps with zero). The interspecific competition effect on *D. pandora* was only significant in the upland temperature regime (Figure 2.6c), which significantly reduced the population size of *D. pandora* in mixed-species cultures compared to the monocultures.

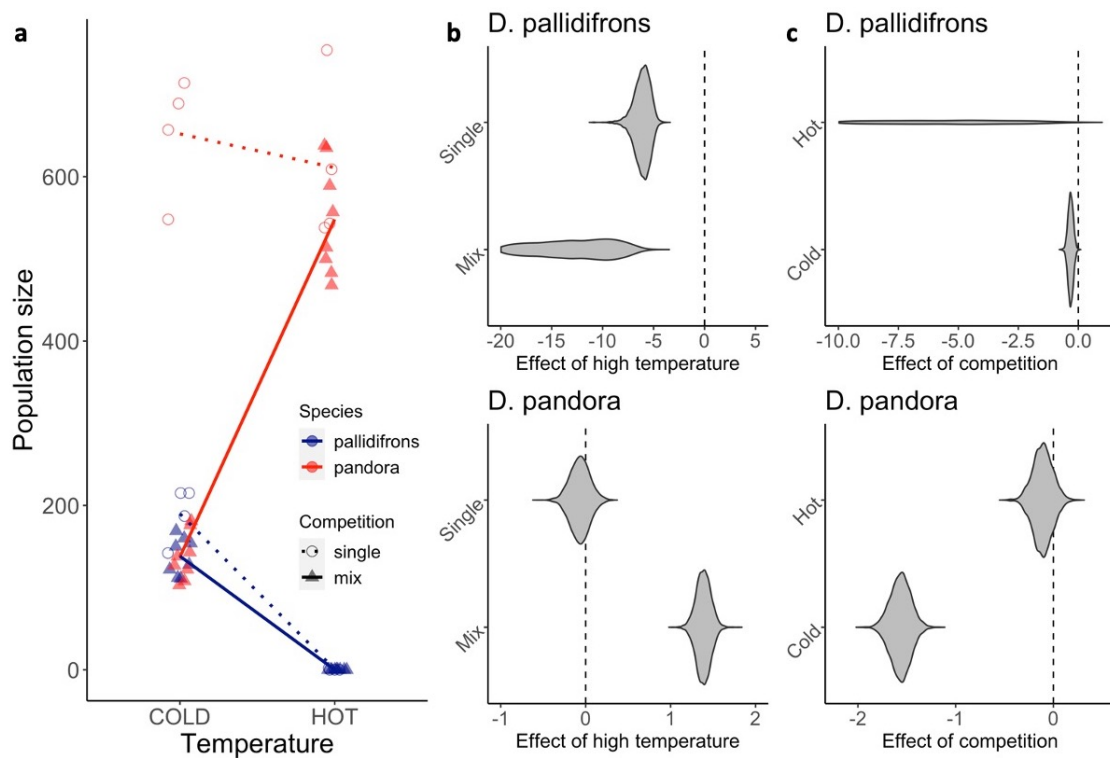


Figure 2.6 Effects of temperature and interspecific competition on population sizes of *D. pallidifrons* and *D. pandora*. a) The ending population sizes of *D. pallidifrons* and *D. pandora* which were initiated in monoculture (single) or mixed-species culture (mix) under upland (cold) and lowland (hot) temperature regimes. b) the posterior distributions of the effect of high temperature (coefficient of temperature) when the indicated species were maintained alone (single) or with the other species (mix). c) the posterior distributions of the effect of competition (coefficient of competition) when the indicated species were maintained at upland (cold) and lowland (hot) temperature regimes.

2.4 Discussion

Counter to expectation, our results do not support the common assumption that cool boundaries to species' ranges are constrained abiotically (reflecting thermal niches), while biotic interactions (such as interspecific competition) define warm boundaries. Instead, we found that maximum temperatures experienced on a daily basis were sufficiently high to serve as an environmental filter at lowland sites. In cooler, high-elevation conditions, lowland species were outcompeted by a certain upland species whose distribution was confined to high elevations as a result of their intolerance to heat.

2.4.1 Contributions of abiotic and biotic factors in determining distributions

The idea that the contributions of abiotic versus biotic factors might differ for species' warm and cool range boundaries can be traced back to Charles Darwin, and remains an area of active debate (Cahill et al., 2014; Hargreaves et al., 2014; Schemske et al., 2009). There is mixed evidence on the importance of upper thermal limits in deciding warm boundaries (positive relationships: (Batista et al., 2018; Duarte et al., 2012; García-Robledo et al., 2016; Kellermann et al., 2012; Merrill et al., 2008); null relationships: (Gaston and Chown, 1999; Huang and Tu, 2008; Kimura, 2004; Nowrouzi et al., 2018)). This inconsistency could arise for at least three reasons. First, data available for synthetic studies are over-represented by systems from temperate latitudes in the northern hemisphere (Feeley et al., 2017) and relate largely to cool limits (Cahill et al., 2014); a recent synthesis finds that the difference in the relative importance of biotic factors to warm versus cool boundaries tends to disappear toward the equator (Paquette and Hargreaves, 2021). Second, mechanisms governing latitudinal or elevational distribution patterns could differ (Chen et al., 2011), and may only become apparent as more datasets accumulate from less-studied locations (Duarte et al., 2012; Freeman et al., 2021). Third, some of the uncertainty might reflect methodological differences when choosing, defining and measuring thermal traits. Studies like the current one that examine a range

of thermal traits and biotic interactions may be needed to understand how and why the governing rules might change under different thermal and biotic regimes (Amundrud and Srivastava, 2020; Srinivasan et al., 2018).

Indeed, different choices of indicators of thermal performance, and different experimental methods may explain why some of our results contrast with some other studies of Australian rainforest *Drosophila*. Overgaard et al. (2014) found no evidence that thermal traits of tropical rainforest *Drosophila* differed among species. Unlike Overgaard et al. (2014), we measured parental fecundity by allowing their eggs to develop to adults under the relevant temperature; we observed that eggs laid at high temperatures never hatched, perhaps as a result of sperm sterilization (Parratt et al., 2021). We also observed different speeds of senescence after exposure to different temperatures (Appendix Figure A.1B), which the three-day period of fecundity measurement in Overgaard’s study would not have captured. Nevertheless, both studies highlight the conserved nature of heat tolerance and suggest small thermal safety margins regarding future warming.

Field experiments on *D. birchii* (O’Brien et al., 2017, 2020), found that its reproduction, when raised alone, was highest at low elevations, where both intraspecific and interspecific competition with its sister species *D. bunnanda* were also high. In our experiments, *D. birchii* was one of the most heat-sensitive species, and its populations were unable to persist at high temperatures typical of low elevations (pilot experiment, unpublished data). This discrepancy can be explained by different temperature regimes used in our study (corresponding to February temperatures in the field, with an average of 26°C and an average daily maximum of 30.6°C at lowland) and theirs (April temperatures, which are around 2°C lower on average temperature and average daily maximum). A further possibility is that single-generation experiments initiated with mated adult flies in their study did not capture the impact of high temperatures before sexual maturation. These observations further highlight the sensitivity of measures of thermal performance to experimental conditions. They also suggest that the relative importance of abiotic and biotic factors in determining species distributions may change seasonally.

2.4.2 Low variations in upper thermal limits

Upper thermal limits vary little among species (Hoffmann, 2010) and have limited adaptive potential (van Heerwaarden and Sgrò, 2021; Kellermann et al., 2012). Consistent with other studies (Goulet et al., 2017; Hangartner and Hoffmann, 2016), we found that heat tolerance is a systematic trait manifested in critical temperatures, fecundities at sub-sterile temperatures, recovered fecundities, and locomotive responses. Modest changes in critical temperature can therefore lead to pronounced differences in overall performance under real and variable temperature regimes.

Such small thermal safety margins suggest a severe threat of biotic attrition in tropical lowlands (Colwell et al., 2008; ?; Duarte et al., 2012; van Heerwaarden and Sgrò, 2021). Laboratory-measured critical temperatures can be sensitive to experimental conditions, making it difficult to relate them to climatological means or maxima, and hence the threat of rising temperatures (Sinclair et al., 2016). In this context, our study benefits from a comparative approach, which reveals that upland species are already constrained by high temperatures, and that a very small difference (about 1°C) distinguishes the upper thermal limits (RT_{max}) of lowland and upland species. Given the low evolutionary potential of heat tolerance (Hoffmann et al., 2013), both lowland species and upland species are likely to be vulnerable to modest temperature increases across the elevation gradient. Thus, lowland biotic attrition and upland range contraction are likely to occur with future warming, leading to cascading effects in lowland communities and threatening endemic upland species on tropical mountains such as those in the Australian Wet Tropics (Freeman and Beehler, 2018).

2.4.3 Daily peaks and extremes as the main abiotic filters

Daily maxima can be more important than mean temperatures in structuring distributions (Lynch et al., 2014). In our study system, the mean temperature during the study season at our lowland sites is around 26°C. At this temperature, all studied species are close to their peak reproductive performance. However, upland and lowland sites differ greatly in the average daily maximum temperature

and the daily duration that the temperature reaches or exceeds stressful levels for *Drosophila* reproduction (Appendix Figure A.7). In a preliminary experiment where populations were maintained for multiple generations in mixed-species cultures at constant 20°C and 26°C (personal observation) *D. pallidifrons* always outnumbered *D. pandora*. This contrasts with the poor performance of *D. pallidifrons* at 24°C/28.5°C documented here. Brief exposure to stressful thermal environments can have similar fitness costs to continuously stressful conditions (Saxon et al., 2018). These results highlight the importance of considering daily temperature variations and extreme temperature events when studying species distributions and projecting responses to climate change (Kingsolver et al., 2013; Ma et al., 2015).

2.4.4 Context-dependence of thermal performance

Inevitably, thermal traits measured under laboratory conditions cannot account for the tendency of thermal tolerances to vary with a range of abiotic factors including precipitation (Bozinovic and Pörtner, 2015), diet, and larval conditions (Bubli et al., 1998). Such variables are to a large extent standardized in our study, allowing dependence on abiotic conditions to be resolved. Our long-term experiment showed that the response of population size to temperature depended on competitive conditions, highlighting the difference between thermal performance curves of organisms and their populations when biotic interactions are present (Davis et al., 1998). There is a growing realisation that sensitivity to temperature depends on the biotic environment (Gilman et al., 2010): it is not only the demographics of the interacting species that are affected by temperature (Huey and Kingsolver, 1989); interactions themselves are altered (Burnside et al., 2014).

Finally, between-population variation and local adaptation (Hoffmann et al., 2002), especially adaptation to abiotic environments at distribution boundaries (Peterson et al., 2019), should not be neglected if studying distributions at large spatial scales (e.g., across latitudes). The relatively restricted spatial scale of our study (within a mountain range), the use of mass-bred lines sourced from different elevations, and the limited plasticity and local adaptation documented

for our study species (MacLean et al., 2019; O'Brien et al., 2017) mean that plastic and evolutionary response to thermal conditions are unlikely to complicate interpretation of our results.

2.4.5 Conclusions

Tropical ecosystems host an exceptional diversity of endemic species (Laurance et al., 2011). Predicting their sensitivity to climate change is a high priority and requires an understanding of the proximate causes of current distributions as well as the interactions between thermal tolerance and other environmental factors, both biotic and abiotic. Our study contributes to the growing literature demonstrating that species can be sensitive to warming at the warm boundaries of their distributions, especially in the tropics. In particular, we highlight the important role of daily maximum temperatures in structuring communities in tropical lowland sites, the essential role of interspecific competition at upland sites, and the vulnerability of species at both upland and lowland to increasing temperatures. These results demonstrate that reliable predictions of the sensitivity of species to rising temperatures may not be achieved using simple correlational approaches; instead, a detailed understanding of multiple thermal traits and interactions with other species may be needed.

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3

A Cryptic Host-Parasitoid Interaction Reduces the Impact of Experimental Heatwaves on Host Populations

Graphical Abstract

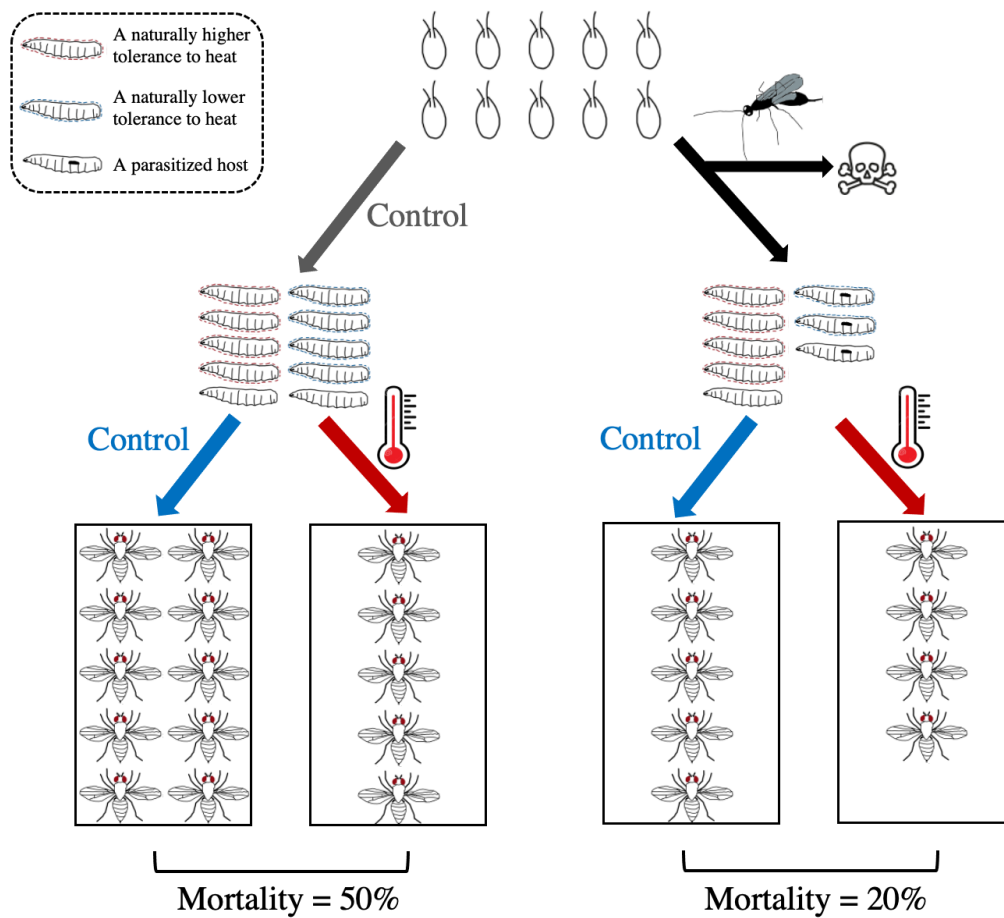


Figure 3.1 Parasitism reduces the demographic impact of heatwaves on host populations

Abstract

Laboratory measures of the thermal performance of organisms are widely used to predict the response of populations to climate change. However, such approaches rarely account for numerous stressors that organisms experience alongside changing temperatures, such as interactions with resources, predators and parasites. Predation has been shown to stabilize prey populations during heatwaves, but the extent to which parasitism modifies the responses of host populations has not been addressed empirically. We hypothesised that parasitism would in contrast lower the heat tolerance of hosts, and that this negative effect would be more pronounced when nutrition was limited due to conflicts in resource allocation to different defensive processes. We, therefore, examined the independent and combined effects of climate extremes (in the form of heatwaves), parasitism, and nutrition on the survival of four *Drosophila* species. Three of these hosts co-occur in nature with the *Asobara* parasitoid species used in the experiment, but the fourth, *D. melanogaster*, does not. We found that nutrition in the form of dietary protein had differing impacts on the ability of populations to tolerate heatwaves and their susceptibility to parasitoids. In the three native hosts, past exposure to parasitoids reduced the impact of heatwaves to host populations, regardless of their underlying susceptibility to parasitism. Meanwhile, parasitism had the opposite effect on the novel host *D. melanogaster*. Particularly, parasitism on unsuitable hosts that resulted in mortality of both host and parasitoid (“aborted parasitism”) also reduced the extent to which host abundances were decreased by heatwaves. Our results highlight the context-dependence of heat tolerance which is crucial for predicting consumer-resource dynamics under an increasingly variable climate.

3.1 Introduction

Record-breaking heatwaves are occurring with increasing frequency around the globe (Meehl and Tebaldi, 2004; Coumou and Rahmstorf, 2012; Perkins-Kirkpatrick and Lewis, 2020). The fitness of organisms tends to decrease at an accelerating rate once optimal temperatures are exceeded (Huey and Kingsolver, 1989; ?). Many species are now experiencing more extended and more frequent exposure to sub-lethal and lethal temperatures (Garrahou et al., 2009; Smale et al., 2019). Fluctuating population dynamics perpetuated by extreme heat events can cause significant disruption to ecosystem functions (Ciais et al., 2005; Baker-Austin et al., 2013; Ma et al., 2015; Harvey et al., 2020).

Meanwhile, biotic interactions have been proposed to stabilise biological communities in the face of disturbances such as heatwaves (Márquez et al., 2007; Harmon et al., 2009; Hector et al., 2022; Ross et al., 2022). Moreover, climate change coincides with shifts in the biotic environment, such as simplified trophic chains, changing abundances of generalist versus specialist species, and the introduction of novel species (Clavel et al., 2011; Estes et al., 2011; Seebens et al., 2021). Even when complete profiles of thermal performance for every species are available, such concurrent changes in the biotic community contribute to substantial uncertainty over how species' distribution and abundance will respond to climate change (Gilman et al., 2010; Mantyka-pringle et al., 2012; Antiqueira et al., 2018). Therefore, understanding the relationship between the processes determining the thermal tolerance and key species interactions is essential for predicting future biodiversity trends.

Host-parasitoid interactions are prevalent in terrestrial ecosystems (Abram et al., 2019). Parasitoids are a group of insects whose larvae develop inside or on the bodies of other arthropods and eventually develop into adults while killing the hosts (Hardy et al., 1994). Though the ecological function of parasitoids in suppressing host populations has often been equated to predation (Hassell and Waage, 1984), host-parasitoid interactions entail more intimate and intricate processes including host immunity and immune evasion by parasitoids (Abram et al., 2019; Yang et al., 2021). Consequently, a parasitoid infection may lead to one of three outcomes:

survival of the host, successful emergence of the parasitoid (with the death of the host), or death of both organisms (“aborted parasitism”: Abram et al. (2019)). For surviving hosts, mounting an immune response can result in negative fitness legacies including a reduction in body size, fecundity and competitive ability (Carton and David, 1983; Kraaijeveld and Godfray, 1997; Lochmiller and Deerenberg, 2000; Niogret et al., 2009). Likewise, the tight physical association of parasitoids with their hosts throughout development is likely to affect the hosts’ ability to withstand other stressors (Hoang, 2001), similar to the effect of parasite and pathogen burden on thermal tolerance (Hector et al., 2022). This non-demographic effect of parasitoid infection has seldom been studied in conjunction with abiotic stress from climate change, let alone the possible differences between hosts with various levels of susceptibility to parasitoids in a natural community.

A fundamental link between an organism’s tolerance to various environmental stressors is the requirement for nutrients. Conflict in resource allocation has been suggested as the underlying mechanism of trade-offs between traits (Lochmiller and Deerenberg, 2000; Garland et al., 2022). Therefore, the amount and quality of certain nutrients will influence how the interactive effects between multiple stressors play out. For example, The availability of protein resources is likely to modulate the strength of trade-offs between resource allocation to thermoregulation versus pathological responses (the cellular and biochemical processes associated with enduring or eliminating the impact of an infection or injury) (Sandland and Minchella, 2003).

We used a *Drosophila*-parasitoid system to examine how interactions with parasitoids alter the tolerance of host populations to heatwaves, and how this tolerance varies among species and under different levels of nutrition. Yeast is the primary source of protein for *Drosophila* larvae (Kutzer and Armitage, 2016). The lack of yeast in the diet resulted in longer development time, smaller adult body size, lower fecundity, lower ability to encapsulate and lower tolerance to abiotic stress (Lee et al., 2008; Colinet and Renault, 2014; Bellutti et al., 2018). Our experiments included *Drosophila* species with varying levels of susceptibility to a focal parasitoid species. We examined how host survival varied depending on

Table 3.1 Definitions of traits of interest and their calculation methods.

Demographic Trait	Measurement	Calculation
Host survival under protein limitation	Host survival rate	$\frac{N_{host}}{N_{egg}}$
Host tolerance to heatwaves	Host mortality rate attributable to heatwave	$1 - \frac{N_{host}}{N_{host_control_temperature}}$
Susceptibility to parasitism	Infestation rate	$\frac{N_{host_no_parasitoid} - N_{host}}{N_{host_no_parasitoid}}$
Parasitism success	Parasitoid emergence rate	$\frac{N_{parasitoid}}{N_{host_no_parasitoid}}$

exposure (or not) to parasitoids and nutritional conditions ranging from a yeast-free to a high-protein diet. This allowed us to test the following hypotheses (visualized in Figure 3.2) on traits of interest defined in Table 3.1:

H1: Low dietary protein levels reduce the tolerance of individual organisms to heatwave conditions, thus decreasing survival rates in host populations.

H2: Susceptibility to parasitism, defined as the fraction of hosts that die from the presence of parasitoids, will increase when the diet is low in protein.

H3: Host organisms infected by parasitoids will have a lower tolerance to heatwaves, because of the cost of repairing damage or mounting an immune response.

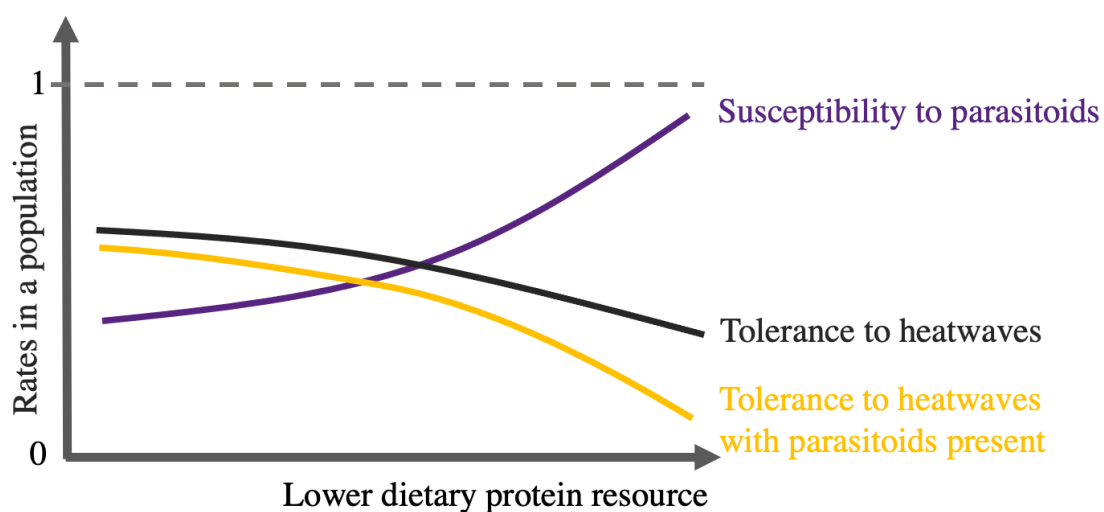


Figure 3.2 Hypotheses. Susceptibility to parasitoids (purple), tolerance to heatwaves (black), and tolerance to heatwaves when parasitoids are present (yellow), ranging from 0 to 1, are plotted against the amount of protein resources provided by the diet.

H4: When protein is scarce, the presence of parasitoids will strengthen the negative impact of heatwaves on host populations.

3.2 Methods

3.2.1 Study system

One parasitoid wasp species and four *Drosophila* species were used in the experiments. We used a specialist larval parasitoid (*Asobara* sp., lab strain KHB (Lue et al., 2021)) from tropical rainforests in Queensland, Australia. *Asobara* wasps are larval parasitoids which attack second-instar *Drosophila* larvae, with a single offspring emerging from each host pupa. Three of the *Drosophila* species originated from the same location, defined as native hosts. They are *D. bipectinata*, *D. sulfurigaster* and *D. birchii*, selected based on their differing susceptibilities to the parasitoid (summarized in Table 3.2). We additionally included the Dahomey laboratory strain of *D. melanogaster*, which originated in Benin, Africa and has been maintained as a large population in captivity since 1970 (Wigby and Chapman, 2004). This *Drosophila* species is not known to co-occur with and act as a natural host for the focal parasitoid species (we subsequently refer to *D. melanogaster* as the “non-native host”). Chapter 2 provided further information on the origin, establishment, and maintenance of laboratory cultures of these hosts and parasitoids.

Table 3.2 Information on the *Drosophila* species and the choices of heatwave and nutrition treatment for each of them.

	<i>Drosophila melanogaster</i>	<i>Drosophila sulfurigaster</i>	<i>Drosophila birchii</i>	<i>Drosophila bipectinata</i>	Reference
Co-occurs naturally with <i>Asobara</i> sp. (KHB strain)	no	yes	yes	yes	(Jeffs et al., 2021)
Proportion of <i>Asobara</i> sp. develop from 50 hosts (24°C)	100%	50%	40%	0%	Chia-Hua Lue, unpublished data
Rank of maximal temperature for reproduction	1 st	3 rd	4 th	2 nd	Chapter 2
Temperature treatment	Control: 24°C(12h) - 24°C(12h)	Control: 22°C(4h) - 26°C(4h) - 22°C(4h) - 18°C(12h)	Control: 22°C(4h) - 26°C(4h) - 22°C(4h) - 18°C(12h)	Control: 22°C(4h) - 26°C(4h) - 22°C(4h) - 18°C(12h)	Based on pilot experiment
	Heatwave: 37.5°C(12h) - 37.5°C(12h)	Heatwave: 30°C(4h) - 34°C(4h) - 30°C(4h) - 24°C(12h)	Heatwave: 30°C(4h) - 34°C(4h) - 30°C(4h) - 24°C(12h)	Heatwave: 30°C(4h) - 38°C(4h) - 30°C(4h) - 24°C(12h)	
Dietary P: C ratio (mass of yeast: mass of cornmeal starch and sucrose)	1:15	1:15	1:15	1:15	
	1:6	1:4	1:4	1:10	(Lee et al., 2006)
	1:4	1:3	1:3	1:6	(Rodrigues et al., 2015)
	1:1	1:1	1:1	1:4	(Bellutti et al., 2018)
	2:1	2:1	2:1	1:1	
Dates of experiments	June-July, 2021	April-May, 2021	April-May, 2021	April-May, 2022	

3.2.2 Experimental design

that group and half of the vials (selected at random) were subjected to a one-day heatwave, while the other half remained at the control temperature. Numbers of adult *Drosophila* and parasitoids that successfully emerged were counted. We performed these procedures (shown in Figure 3.3) twice as blocks. Six replicates, split equally across the two blocks, of each of the 20 treatment combinations were conducted, totalling 120 vials for each *Drosophila* species.

The temperature of the heatwave varied among species and was determined according to the performance of each host species at different heatwave treatments during pilot experiments. Heatwave conditions which induced around 50% host mortality (on standard fly food, without parasitoids) were employed (Table 3.2). The non-native host, *D. melanogaster*, was additionally tested at all the other heatwave regimes designed for the native hosts, which had a negligible impact on the survival of *D. melanogaster*. These results for *D. melanogaster* might therefore indicate *Asobara* parasitoid performance under heatwave conditions that do not cause strong effects on host survival.

Protein-carbohydrate ratios (P: C ratios) were modified by varying the amount of yeast added to the fly food. The protein-carbohydrate ratio is also referred to as ‘protein level’ in the following text. All stock had been fed at a conventional ratio (P: C = 1:3) for at least two years. In a multi-choice experiment, larvae of *D. melanogaster* preferred food with a P: C ratio of 1: 4 to 1: 2 (Rodrigues et al., 2015). Lower yeast levels (yeast-free) and higher yeast levels (maximum P: C = 2: 1) were adopted to produce a gradient of protein levels. Choices of yeast levels were adjusted for differing hosts. For example, the yeast levels employed for *D. bipectinata* were overall lower to increase variations in its susceptibility to the parasitoid.

3.2.3 Data analysis

We calculated several measures of host and parasitoid population performance based on counts of total eggs, emerged adult *Drosophila*, and emerged adult parasitoids (see Table 3.1). All rate measurements were limited between 0 and 1. We then used linear regression to explore how protein levels influenced these measures. All

response variables were arcsine-square-root transformed to satisfy the assumption of Gaussian error distribution, following (Coors and De Meester, 2008). Host survival rate, host mortality rate attributable to heatwaves, infestation rate and parasitoid emergence rate were each modelled as a function of *the rank of the P: C ratio* (treated as a continuous variable), *block*, and the interaction between *the rank of P: C ratio* and *block* if the *block* effect was significant. Analyses of heatwave-induced mortality and infestation rate for *D. bipectinata* were repeated excluding data for the yeast-free treatment because very few *D. bipectinata* survived without yeast. Results were consistent and only the results excluding data from the yeast-free treatment are reported. Additionally, host survival and parasitism success were modelled as a function of the *P: C ratio* as a categorical variable to investigate differences among diets, since nutritional effects on these measures appeared to be non-linear. Post hoc pairwise comparisons were performed using the *emmeans* package upon detecting a significant effect of the *P: C ratio*. All linear models were performed using the *stat* package. All analyses were conducted in R version 4.0.3 (R Core Team, 2020).

We investigated how *heatwave*, *parasitism*, *P: C ratio*, their three-way interactions, and *block* influenced host and parasitoid numbers using a linear model. The response variables were log-transformed counts of adult *D. sulfurigaster* and *D. bipectinata*, and log-transformed total emergence (*Drosophila* and parasitoids) counts of *D. birchii*- and *D. melanogaster*-parasitoid pairs. It was impractical to analyse the host population's response to the heatwave for the latter two species since there were very few host offspring in the parasitism treatment. Log transformation is required to investigate the true interactive effect of two stressors on survival (Dey and Koops, 2021). We fitted these models under a Bayesian approach, using the *brms* package (Bürkner, 2018) to obtain the posterior distributions of the coefficients of the interactive effect between *parasitism* and *heatwave* under different *P: C ratios*.

To study the reduction of total emergence caused by the presence of parasitoids, we fitted generalized mixed-effect linear models with binomial errors. *Parasitism* and *block* were fixed effects, whereas the *P: C ratio* was a random effect that influenced the intercept and slope for *parasitism*. The model was fitted separately

for the control temperature group and heatwave group. Each species was modelled as above using the *lme4* package (Bates et al., 2015).

3.3 Results

3.3.1 Effects of protein resources on survival, heatwave tolerance and susceptibility to parasitoids

The yeast-free treatment significantly decreased the egg-to-adult survival rates of all fly species (Figure 3.4a). A small amount of yeast added to the meal significantly enhanced survival, which remained steady as the P: C ratio rose. In the absence of parasitoids, increasing P: C ratios increased the heatwave-induced mortality of all four fly species (Figure 3.4b; Table 3.3). In contrast, the infestation rate at the control temperature was reduced by increasing P: C ratios (Figure 3.4c; Table 3.3), despite the different overall susceptibility of host species to *Asobara* parasitoids (ranging from 0% to close to 100%). However, the rise in parasitism-induced host mortality did not always result in a corresponding increase in the reproductive success of parasitoids (Figure 3.4d). Parasitoid emergence rates did not differ significantly across P: C ratios in all host species (Table 3.3). Surprisingly, no adult parasitoids developed successfully on *D. bipectinata* despite that host suffered additional mortality in the presence of parasitoids (average across nutrition treatments = 24%; mortality was higher when protein levels were low).

3.3.2 The interactive effects between parasitism and heatwaves

As Figure 3.5 shows, when the P: C ratio reached or exceeded 1:1, the interaction coefficients between parasitism and heatwaves for *D. sulfurigaster* and *D. bipectinata* increased from about zero to significantly higher than zero, meaning that exposure to parasitoids during larval development increased population-wise larva-to-pupa survival rates during heatwaves. As there were too few adult hosts of *D. birchii* and *D. melanogaster* surviving from parasitism, only mortality rates for the total emergence of hosts and parasitoids were tested. *Drosophila birchii*, another native

host, followed a similar pattern to *D. sulfurigaster* and *D. bipectinata*. However, *D. melanogaster*, which is not known to co-occur naturally with the focal parasitoid species, had higher heatwave-induced mortality when parasitized.

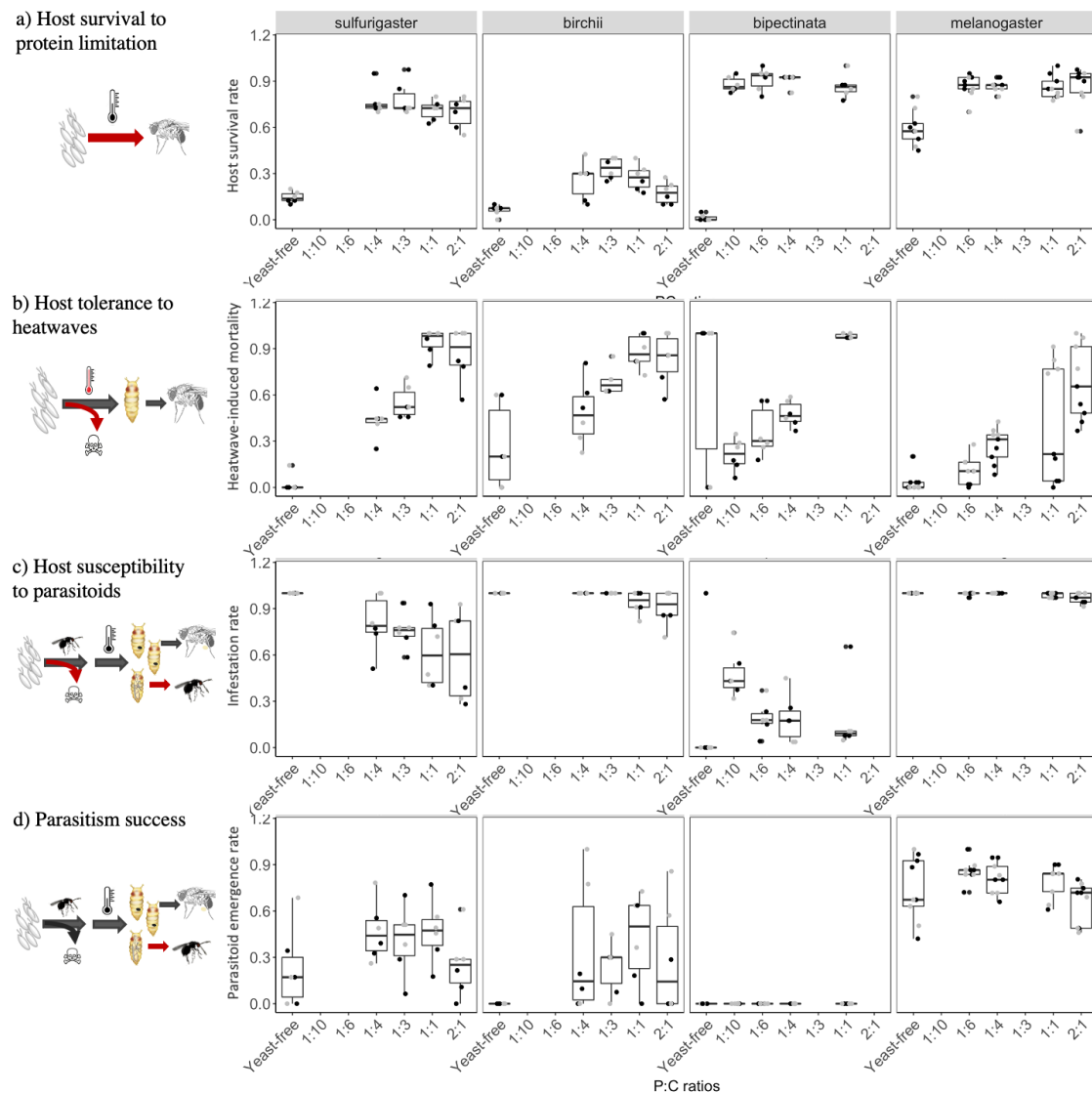


Figure 3.4 The effect of dietary protein on host's or parasitoid's performances: a) egg to adult survival of hosts in an environment without heatwaves or parasitoids; b) the death of hosts attributable to a one-day heatwave in the absence of parasitoids; c) the loss of host adults resulting from the presence of parasitoids at control temperatures; d) the proportion of parasitoid adults developing successfully from available hosts. The diagrams in the left column indicate the processes (red arrows) contributing to each measurement. The x-axis is the P: C ratio of the diet. The colours of data points differ for the two experimental blocks. Data distributions are shown by standard boxplots. Boxplot shows the median, 25% quantile, 75% quantile, and upper and lower limits along with outliers of the data.

Table 3.3 Linear regressions of the effects of nutrition on host survival, heat-induced mortality, infestation rate, and parasitism success. The predictors and their coefficients were shown. Asterisk symbols in a bracket indicate significant coefficients (*: p-value < 0.05, ***: p-value < 0.0001).

Trait	<i>Drosophila sulfurigaster</i>	<i>Drosophila birchii</i>	<i>Drosophila bipectinata</i>	<i>Drosophila melanogaster</i>
Host survival under protein limitation	P: C ratio: 0.1 (***) Block: - 0.02	P: C ratio: 0.04 (***) Block: 0.09	P: C ratio: 0.17 (***) Block: 0.005	P: C ratio: 0.07 (***) Block: 0.1 P: C ratio x block: - 0.05 (*)
Host mortality to heatwaves	P: C ratio: 0.20 (***) Block: - 0.07 P: C ratio x block: - 0.06	P: C ratio: 0.14 (***) Block: - 0.02	P: C ratio: 0.25 (***) Block: 0.07	P: C ratio: 0.07 (***) Block: - 0.31 (*) P: C ratio x block: 0.15 (***)
Susceptibility to parasitism	P: C ratio: - 0.12 (***) Block: - 0.09	P: C ratio: - 0.04 (*) Block: - 0.02	P: C ratio: - 0.08 (*) Block: - 0.03	P: C ratio: - 0.02 (***) Block: - 0.02
Parasitism success	P: C ratio: 0.03 Block: 0.17	P: C ratio: 0.09 (*) Block: 0.30	NA	P: C ratio: 0.02 Block: - 0.07

Table 3.4 Statistical analysis of how total numbers of emergence are affected by the presence of parasitoids in either control or heatwave temperature. The predictors and their coefficients were shown. Asterisk symbols in a bracket indicate a significant coefficient (*: p-value < 0.05, ***: p-value < 0.0001).

	<i>Drosophila sulfurigaster</i>	<i>Drosophila birchii</i>	<i>Drosophila bipectinata</i>	<i>Drosophila melanogaster</i>
Control temperature	parasitism: -1.08 (***) Block: -0.07	parasitism: -0.98 (*) Block: -0.08	parasitism: -1.55 (***) Block: 0.68 (***)	parasitism: -0.99 (***) Block: -0.27 (***)
Heatwave	parasitism: 0.04 Block: -0.26 (*)	parasitism: -0.16 Block: 0.01	parasitism: -0.5 Block: 0.38 (*)	parasitism: -1.49 (**) Block: -0.48 (***)

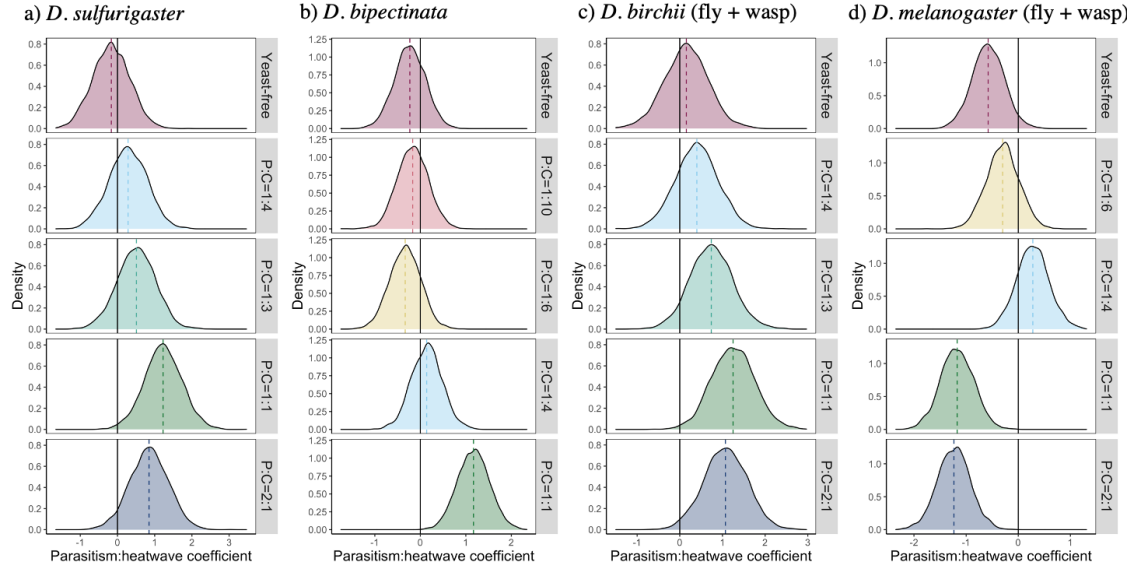


Figure 3.5 The posterior distributions of the interactive effects between parasitism and heatwave treatments at various protein levels. (a) *D. sulfurigaster*, (b) *D. bipectinata*, (c) *D. birchii* and the parasitoid wasps emerging from *D. birchii* hosts, and (d) *D. melanogaster* and the parasitoid wasps emerging from *D. melanogaster* hosts. The dashed line indicates the median of the posterior distribution, and the solid black line indicates a zero coefficient. Colours correspond to P: C ratios as labelled on the right of each plot.

3.3.3 The parasitism-induced mortality of total emergence number

We focus on the parasitism-induced mortality of total emergence to further investigate the mechanisms behind the observed interactive effect. For native hosts (Figure 3.6abc; Table 3.4), the presence of *Asobara* parasitoids resulted in a significant decrease in the total emergence at the control temperature, but had no impact when a heatwave treatment was also applied. In contrast, the detrimental impacts of the *Asobara* parasitoid were amplified for *D. melanogaster* if there was a heatwave (Figure 3.6d; Table 3.4).

3.4 Discussion

As Figure 3.7 summarized, we found that parasitoids altered heatwave-induced mortality in host populations, even for host species that did not obviously interact with the focal parasitoid species (i.e., where no successful parasitism occurred).

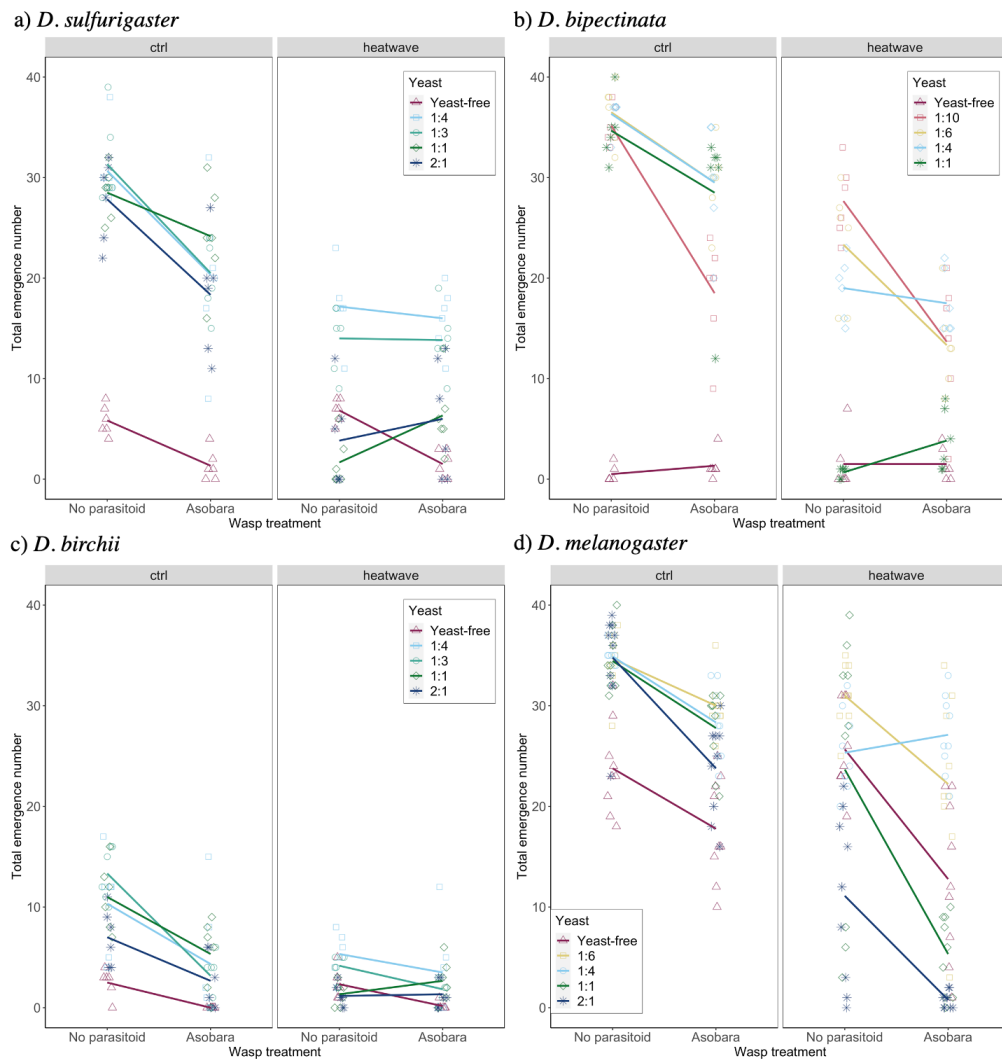


Figure 3.6 The reduction of the total number of *Drosophila* and parasitoid adult offspring due to parasitism treatment under control or heatwave treatments. (a) *D. sulfurigaster*, (b) *D. bipectinata*, (c) *D. birchii*, and (d) *D. melanogaster*. The colours and shapes of the data points indicate the P: C ratios of the diet (values described in the figure legends). Lines connect the mean values of total emergence for a given protein level (indicated by different colours) in non-parasitoid and parasitoid treatments.

Despite differences in the susceptibility to parasitoids, exposure to parasitoids reduced the heatwave's impact on all native host populations. Some of the hosts killed by parasitoid infection likely overlapped with those that would have been killed by heatwaves in the absence of parasitism. In contrast, mortality in the heatwave was higher when the non-native host *D. melanogaster* was parasitized. This synergistic impact could be due to lower tolerance to the heatwave of *Asobara* offspring or parasitoid-carrying hosts compared to parasitoid-free *D. melanogaster*. All the above

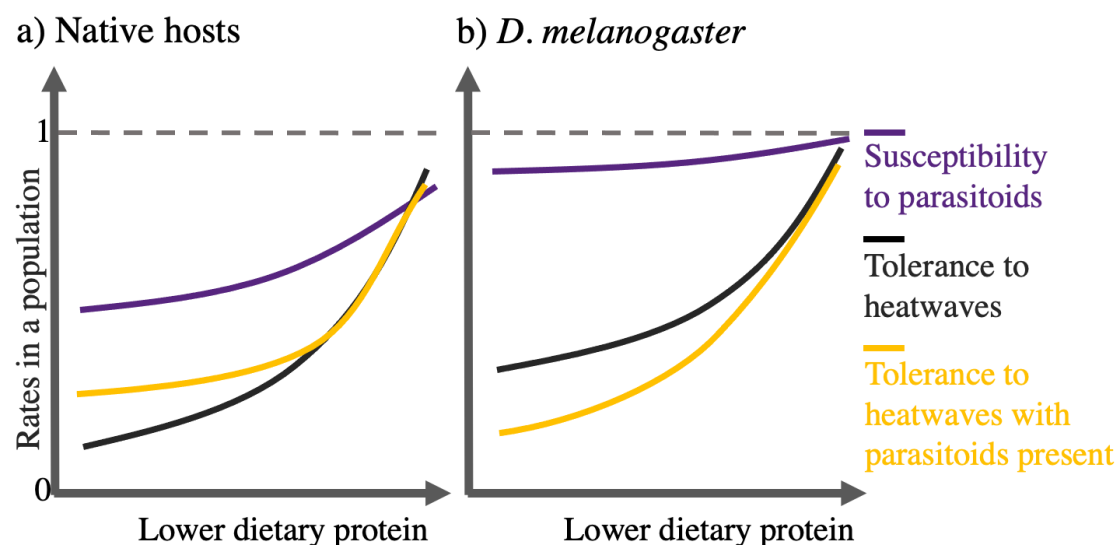


Figure 3.7 Outcomes for the tested hypotheses. The effect of dietary protein on susceptibility to parasitoids (purple), tolerance to heatwave (black), and tolerance to heatwave when parasitoids present (yellow). Native hosts (a) are *D. sulfurigaster*, *D. birchii* and *D. bipectinata*, drawn separately from the non-native host (b), *D. melanogaster*.

interactive effects were only significant when protein-to-carbohydrate ratios were high, which corresponded to low average susceptibility to parasitoids and low average tolerance to heatwaves. These unexpected results (contrary to the predictions of H1, H3, and H4) suggest that susceptibility to parasitoids and tolerance to heatwaves respond differently to dietary protein levels. Our study highlights the importance of distinguishing individual-level and population-level responses: the associated variations in the susceptibility to both stressors among individuals within a population likely result in unexpected antagonist effects of stressors.

3.4.1 Effects of parasitism on responses to heatwaves

Sentis et al. (2013) found that the impact of heatwaves on aphid populations was mitigated when predators were present. We found that parasitoids had a similar effect. Two potential mechanisms could result in this antagonistic effect of the two stressors (Figures 3.8c and d). First, the attack by parasitoids could trigger biochemical pathways that raise the heat tolerance of the host. Second, individuals with higher susceptibility to parasitoids could also be more likely to die from a heatwave (overlapping susceptibility). Supporting the first mechanism,

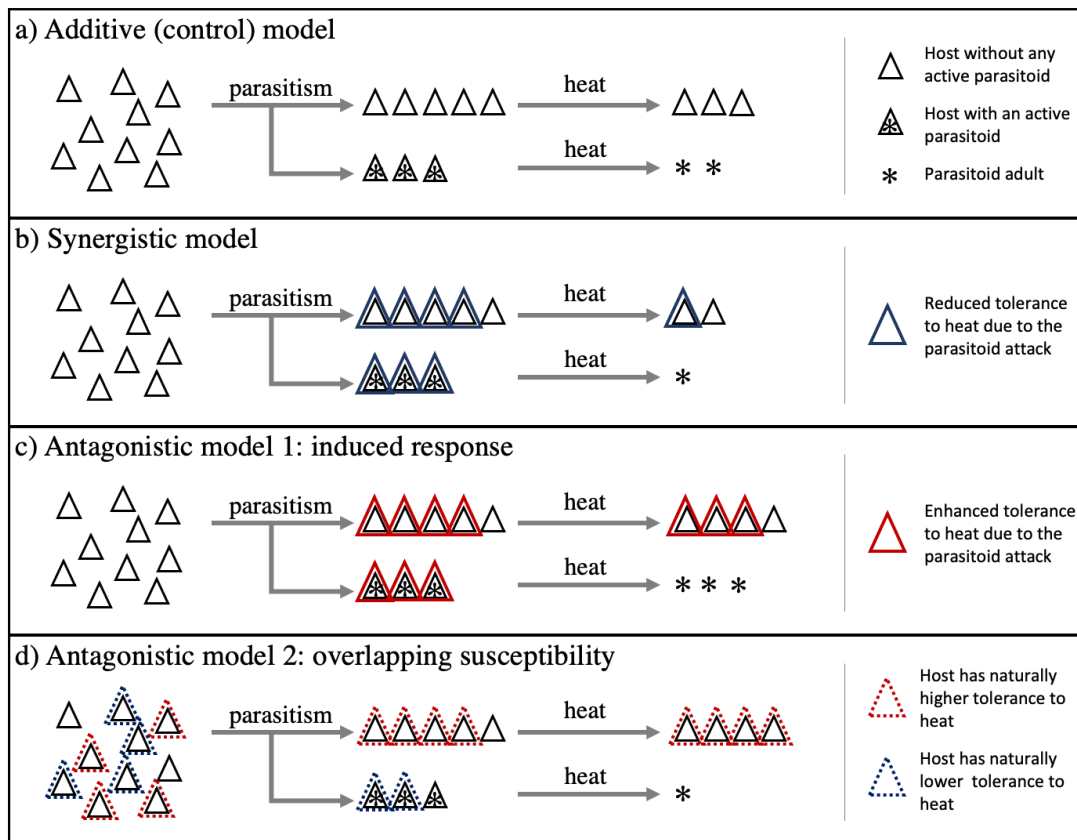


Figure 3.8 Models for different types of interactive effects. (a) The additive model is the null model, with parasitism and heatwave each independently inducing a certain level of host mortality. (b) In the synergistic model, hosts with experience of parasitoid infection are more likely to die from heatwaves than unparasitized hosts. (c) In the first antagonistic model, the parasitoid infection induces higher tolerance to heatwave among hosts. (d) The second antagonistic model suggests that hosts with a naturally higher tolerance to heatwaves are more likely to evade the parasitoid infection (overlapping susceptibility).

Bahar et al. (2013) found that parasitoid-infected diamondback moths expressed more heat shock protein when exposed to high temperatures, although there was no discernible difference between parasitized and non-parasitized diamondback moth larvae in their survival from real heat treatments. Our close examination of the total number of *Drosophila* and parasitoids offspring reveals that overlapping susceptibility may be the primary cause. If there is overlapping susceptibility, the total number of adult offspring of hosts and parasitoids (total emergence) in the "parasitism plus heatwave" treatment is expected to be similar to that in the "heatwave only" treatment (Figure 3.8d), but higher if parasitism induces tolerance to heat (Figure 3.8c). Figure 3.6 shows that in the heatwave treatment, parasitoid-

induced mortality of total emergence disappeared for all native hosts, implying that fly larvae killed by parasitoids would likely die from heatwaves in any case. In Sentis et al. (2013), predators alone also decreased the number of aphids to a level similar to the “predator plus heatwaves” treatment.

As for the non-native host *D. melanogaster*, the presence of parasitoids caused even higher mortality when heatwave treatments were also applied, indicating that parasitoid larvae and/or parasitized host larvae were more likely to die from a heatwave than unparasitized host larvae (Figure 3.8b). As most *D. melanogaster* were successfully parasitized in our study, it is difficult to compare the heat tolerance of parasitoid larvae and that of the parasitoid-carrying hosts. It is likely that the parasitoid species we used has lower thermal tolerance than *D. melanogaster*, as the laboratory strain of *D. melanogaster* can tolerate much higher temperatures than the native hosts.

3.4.2 Effects of nutrition

Our findings are consistent with studies indicating that dietary protein plays an important role in host immunity, including in *Drosophila* (Vass and Nappi, 1998; Lee et al., 2008; Fellous and Lazzaro, 2010; Wilson et al., 2019). The negative correlation between heat tolerance and protein nutrients, on the other hand, runs counter to our original hypothesis. This contradiction emphasises the need to separate food scarcity from a lack of certain nutritional components. Starvation has consistently been shown to result in low tolerance to high temperatures (Nyamukondiwa and Terblanche, 2009; Bujan and Kaspari, 2017; Rusch et al., 2019), while studies focusing on protein-to-carbohydrate ratios produced mixed results (Roeder et al., 2021): Adult *Drosophila* raised on a high-protein diet have greater tolerance to heat shock than those raised on a carbohydrate-rich medium (Andersen et al., 2010; Sisodia and Singh, 2012). On the other hand, high-carbohydrate, low-protein diets are suggested to increase blood trehalose levels (Friedman et al., 1991; Roeder et al., 2021). Trehalose is a key chemical for organismal stress responses (Soto et al., 1999) and is associated with increased thermal tolerance (Broughton et al.,

2005) and desiccation tolerance (Weldon et al., 2019), which is evolutionarily linked with heat tolerance (Bubliy and Loeschcke, 2005).

Only when dietary protein levels were high did the interactive effects between parasitism and heatwave become significant. A plausible cause is that individuals of the native host species only show considerable variation in immunity against parasitoids once dietary protein exceeds a particular level. When dietary protein is low, host larvae will have a low level of defence against parasitoids and will tend to die from parasitoid infection in a relatively random fashion, uncorrelated with their tolerance to heatwave, thus the antagonistic effects are eliminated when dietary protein levels are low. For non-native hosts, however, there are always low variations in defence against the parasitoid. When tolerance to heatwaves is high due to limited dietary protein, parasitoid larvae and parasitoid-carrying hosts might have comparable tolerance to heatwave to uninfected hosts, making the combined effect of parasitism and heatwave for non-native hosts additive.

3.4.3 Caveats and Limitation

To vary the nutritional value of the diet, we adjusted the amount of yeast per volume while retaining the same sugar concentration. Thus, the food's density and consistency also increased with the protein-to-carbohydrate ratio. We were not able to determine whether *Drosophila* consumed different quantities of food based on meal density or consistency. Nevertheless, we reason that adding yeast to food enhanced the nutritional value of food consumed because we observed that our *Drosophila* raised on a higher-yeast diet developed more quickly and grew larger. In addition, denser food could have amplified the impact of the heatwave, since it might retain heat for longer. It might also hinder the ability of parasitoids to locate and lay eggs on hosts. Despite these confounding factors, the aforementioned biomolecular studies at the organism level support the true nutritional effects.

Our experiments were designed in a way that resulted in high parasitism loads on single host species, so we must proceed with caution when extrapolating to natural systems. We exposed a small number (40 eggs) of hosts to two female parasitoids

in a confined space to increase the likelihood of infection by parasitoids. Though a parasitism rate of almost 100% is feasible in some natural situations (Carton et al., 1986), parasitoids in many cases only infect a certain portion of hosts (Carton et al., 1986; Hawkins, 1994). The available hosts are naturally of various species and quality (here quality refers to the general ability to survive stressors, such as parasitoid attack, heatwave, and starvation). The low-quality hosts that would die from parasitoid infection would not contribute to any reproductive success of parasitoids, hence decreasing their fitness. If parasitoids actively avoid low-quality hosts, parasitism-induced mortality and heatwave-induced mortality will not overlap, resulting in an additive rather than antagonistic effect. If parasitoids selectively target highly-susceptible species when they have the choice (Carton et al., 1986), parasitism-induced death among unsuitable hosts such as *D. bipunctata* will be rare. However, egg misplacement into less-suitable hosts may be more widespread than expected even in the presence of other suitable host species (Meisner et al., 2007; Condon et al., 2014; de Bruijn et al., 2022). High egg loads of parasitoids and a low likelihood of encountering hosts in nature could lead to a weak ability to assess host quality (Islam and Copland, 2000). It is necessary to conduct laboratory or field trials with lower parasitoid-to-host ratios and a variety of host species together to inform the impact of parasitoids on the thermal responses of natural host communities.

3.4.4 Ecological implications of the associated intra-specific variations in defensive traits

Our results are consistent with the role of trophic interactions in stabilising food webs in the face of perturbations (Sentis et al., 2013; Ghedini et al., 2015). Our study highlights a little-discussed mechanism: intra-specific variations in susceptibility and correlated susceptibility to different stressors (Vinebrooke et al., 2004; Hawley and Altizer, 2011). Notably, our research cannot determine if parasitism affects the heat tolerance of individual organisms. Nonetheless, in our study, the difference in heat tolerance between parasitized and unparasitized populations of hosts was most likely driven by overlapping susceptibility across host individuals (Figure 3.8d).

Conversely, due to the association between defensive traits, extreme climatic events may change the mean value of the defensive traits of a host population against its parasitoids. In conclusion, knowing the pattern of intra-specific variation is vital for predicting the interactive effect between stressors and for understanding how within-population diversity is shaped by co-occurring stressors.

3.4.5 Ecological effects of the hidden host-parasitoid interaction

Parasitoids attack up to 80% more hosts than the number from which offspring parasitoids will successfully emerge (Campbell, 1963). The physiological consequences of being stung by an ovipositor and/or the immunological reaction to the alien substrate influence the behaviour, reproduction, and mortality of the attacked hosts (Ingerslew and Finke, 2017; Abram et al., 2019). This study and previous studies (Abram et al., 2014; Kruitwagen et al., 2021) demonstrate that parasitoid infection also occurs on unsuitable hosts on which parasitoid offspring completely fail to grow. Molecular methods are starting to reveal the prevalence of parasitoids targeting unsuitable hosts in natural settings (Condon et al., 2014; Hepler et al., 2020). In general, parasitoid attack rates in the field are likely to be underestimated (Cebolla et al., 2018; de Bruijn et al., 2022), as is the ecological impact of failed parasitoid attacks (Abram et al., 2019). The hidden interactions between parasitoids and unsuitable hosts affect both host and parasitoid demography (Abram et al., 2016) and their subsequent interactions with suitable hosts (Heimpel et al., 2003; Kaser et al., 2018). Our work is the first to establish, to our knowledge, the functional importance of the non-reproductive effect of parasitism on the tolerance of host populations to other stress (Abram et al., 2019).

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4

Experimental Heatwaves and Warming
Induce Distinctive Community Responses
through Their Interactions with A Novel
Species

Graphical Abstract

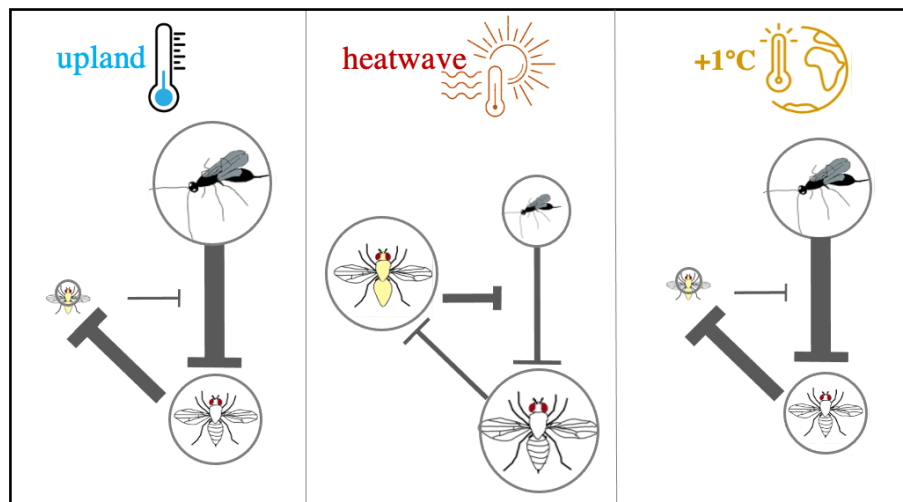


Figure 4.1 A key species interaction motif contributing to the community response to different modes of rising temperatures. The size of circles around a species represents the relative abundance of the adult population. The thickness of the arrows represents the strength of the negative impact.

Abstract

As the climate warms, species shift their distributions at different rates, re-organising ecological communities. The resulting novel interactions will shape the local community's response to ongoing climate change. The distinction between extreme events and a rising mean temperature in driving range expansion of the neighbouring species and the resulting ecological impact propagating through multi-trophic networks have not been examined empirically. In this study, we recreated a high-elevation host-parasitoid community comprising *Drosophila* species and their associated parasitoid species from the Australian Wet Tropics, and subjected them to either heatwaves or warming in combination with the introduction of a species only found at low elevations. Both parasitoid species tested were sensitive to a rise in temperature, while the demographic responses of *Drosophila* species were idiosyncratic, reflecting the combined effects of thermal regimes, parasitism, competition and facilitation. Heatwaves, but not warming, promoted the establishment of lowland species within upland communities. The introduction of the invading species correlated negatively with the abundance of the larval parasitoids, whereas high parasitoid numbers tended to suppress the competitively-dominant *Drosophila* species and its effects on inferior competitors. Our study reveals differing, sometimes contrasting, impacts of heatwaves and warming on community compositions. It also highlights how the scale and direction of climate impacts could be further modified by the indirect effects of a novel species within the trophic network.

4.1 Introduction

Climate change is leading to large-scale shifts in species' distributions (Chen et al., 2011; Parmesan et al., 1999; Parmesan and Yohe, 2003). Differences in the speed (Mamantov et al., 2021), magnitude (Chen et al., 2011) and even direction (Lenoir et al., 2010) of range shifts are causing existing interactions among species to unravel, and new interactions to form.

Despite the widespread occurrence of such novel interactions, and evidence that they can in turn influence community responses to climate change (Alexander et al., 2016; Gilman et al., 2010), the ecological ramifications of climate-driven novel species interactions in multi-trophic communities are poorly understood. The impacts of climate change and novel species interactions on a biological community are mutually dependent (Figure 4.2). Climate change not only affects organisms' performance but also alters the biotic environment (Blois et al., 2013; Descombes et al., 2020; Romero et al., 2018). Climate-driven changes in the demography or behaviour of competitors, predators, and mutualists can modify the direct effects of climate on other species. Such influential species can either be pre-existing components of the community (Barton and Ives, 2014; Harmon et al., 2009; Sala and Gherardi, 2019) or novel species whose arrival is facilitated by climate change (Richman et al., 2020; Schweiger et al., 2010). Furthermore, species interactions, including those between novel species and residents, are themselves climate-dependent (Alexander et al., 2016; LaForgia et al., 2020; Olsen et al., 2016), as two of the dashed lines in Figure 4.2 represent. In summary, predicting biodiversity responses to climate change is challenging because climate change produces new species interactions due to range shifts and new climatic conditions to which species interactions are sensitive (Gilman et al., 2010; HilleRisLambers et al., 2013; Tylianakis et al., 2008).

Experimental approaches have been useful for determining the impact of potential novel species interaction outside the current climatic conditions experienced by communities (Alexander et al., 2016; Descombes et al., 2020; Richman et al., 2020). Nevertheless, previous studies usually focused on a single type of species interaction (e.g., competition, predation). Most biological communities, on the other hand,

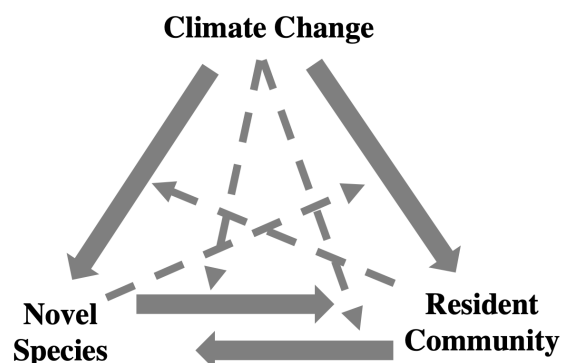


Figure 4.2 The relationships among climate change, novel species and the resident community. The solid arrows represent direct effects. Dashed arrows indicate that a third factor influences the direct effect between the two factors.

comprise at least a few trophic levels and several species within each level, resulting in motifs with a variety of trophic and non-trophic interaction pathways, both antagonistic and facilitative (Barton and Ives, 2014; Losapio et al., 2021; Thierry et al., 2019). Understanding how the impacts of external drivers such as climate and invading species propagate through species interactions and emerge in multi-species, multi-trophic communities remains a key challenge.

While most empirical research on range shifts has focused on the impact of increasing mean temperature (referred to here as “warming”), climate change also manifests as extreme high-temperature events and higher temperature variability, especially in tropical regions (IPCC, 2022; Perkins-Kirkpatrick and Lewis, 2020). Extreme events such as heatwaves also contribute to range dynamics (Diez et al., 2012; Vasseur et al., 2014), prompting interest in the relative importance of warming versus extreme temperature events (Jentsch et al., 2007; Lynch et al., 2014; Ma et al., 2015). A moderate level of warming generally increases interaction intensity (Roslin et al., 2017; Klanderud et al., 2015) or releases physiological constraints (Crozier, 2004). In contrast, heatwaves often induce mortality or infertility (van Heerwaarden and Sgrò, 2021), temporarily releasing the surviving species from antagonistic biotic interactions (Diez et al., 2012). Different mitigation strategies are required for warming and heatwaves as they operate at different timescales.

However, this has seldom been put into practice because knowledge of their differing ecological influences has been lacking (Ma et al., 2021).

To investigate the interactive effects of novel species and different modes of rise in temperature (i.e. warming in mean temperatures versus heatwaves), we focus on an experimental host-parasitoid system. Parasitoids are insects whose larvae develop on or in the bodies of other arthropods and eventually kill them (Hardy et al., 1994). Host-parasitoid networks are often viewed as relatively tractable, strongly-interacting units containing a range of specialist and generalist interactions (Hrček and Godfray, 2015). Parasitoids play a crucial role in regulating the abundance and coexistence of arthropods in natural ecosystems and agriculture (Bonsall and Hassell, 1997; Hardy et al., 1994), but this ecological function is especially vulnerable to global warming (Furlong and Zalucki, 2017; Hance et al., 2007).

Our focal community comprises *Drosophila* hosts and their parasitoid wasps from Australian mountain rainforests (Jeffs et al., 2021). The previous study has shown that the upland community contains heat-sensitive species, and that interspecific competition prevents lowland species from occupying cooler, higher-elevation locations (Chapter 2). Based on this, we predicted that heatwaves, but not warming, would facilitate the invasion of lowland species into the upland community. Second, we predicted that frequent heatwaves would drive heat-intolerant *Drosophila* species to local extinction, while equivalent increases in mean temperature would favour competitively-dominant species. Third, we predicted that heatwave-facilitated establishment of novel species would reduce the abundance of resident *Drosophila* species as a result of interspecific competition. Finally, we predicted that parasitoid wasps would be more sensitive to heatwaves than to warming, and that their reduced abundance under heatwave conditions would have indirect positive effects on the abundance of their *Drosophila* hosts.

4.2 Methods

4.2.1 Study system

We conducted an experiment using *Drosophila* flies and parasitoid wasps in a controlled laboratory environment. Cultures of all species were collected from the Wet Tropics bioregion in Queensland, Australia, which has high levels of endemism associated with cool, moist upland refugia. Most *Drosophila* species and some of the parasitoid wasps from these communities are now in laboratory culture (Chapter

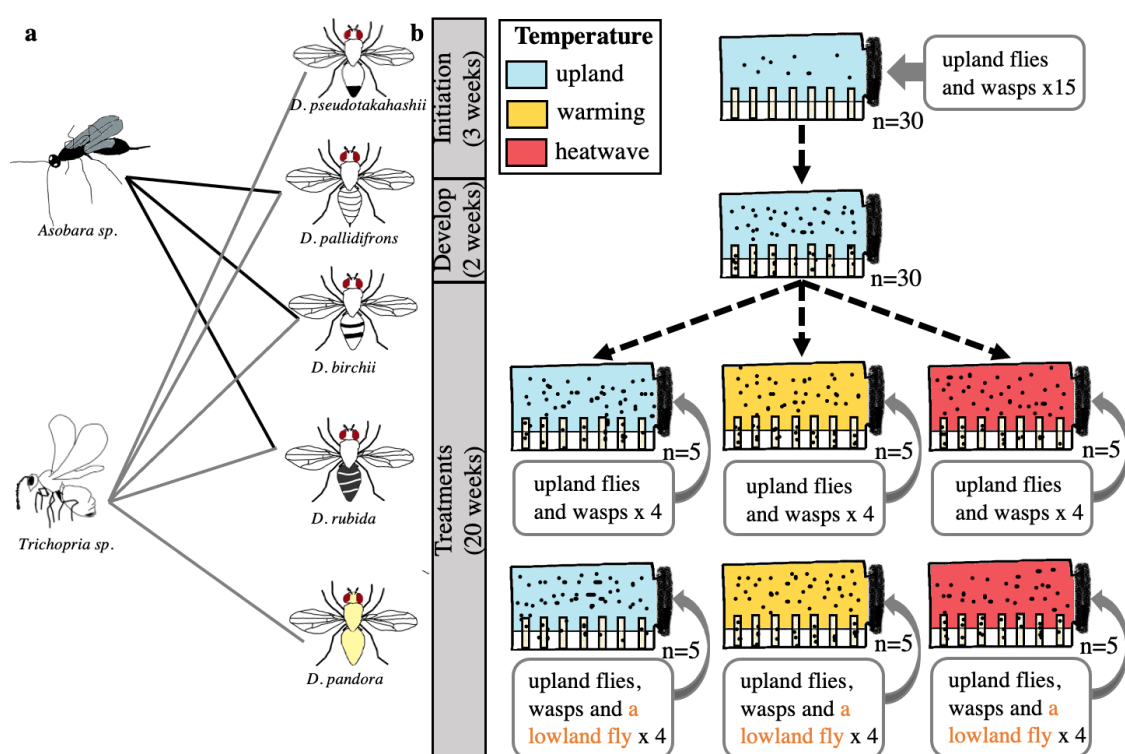


Figure 4.3 Study system and experimental procedures. a) The known trophic interaction network between parasitoid wasps and *Drosophila* flies, based on the observation of successful development of the parasitoids (Chia-Hua Lue, unpublished data) or molecular detection of traces of parasitoids in each host (Jeffs et al. 2021). The lowland species, *D. pandora* was drawn in yellow. b) Experimental communities maintained in sealed cages with regular food provision. 15 pairs of each upland species were added to each cage each week for three weeks, and then experimental communities were allowed to grow for two additional weeks. Following that, a total of 30 cages received combinations of novel species and temperature treatments (5 replicates of each treatment combination; blue: upland temperature; yellow: warming; red: heatwaves). Species and their numbers added weekly are indicated within the rectangular boxes with a grey arrow pointing into each cage.

2), allowing multi-species manipulative experiments in the laboratory.

We assembled an experimental community to reflect species compositions at contemporary high-elevation sites (elevation 730m – 880m), comprising *D. pseudotakahashii*, *D. pallidifrons*, *D. birchii*, *D. rubida*, a specialist larval parasitoid wasp from the genus *Asobara* (lab strain KHB), and a generalist pupal parasitoid wasp from the genus *Trichopria* (lab strain 66LD) (Lue et al., 2021). The species used and the host specificity of the parasitoids are shown in Figure 4.3a. The four *Drosophila* species account for 77% – 95% of *Drosophila* abundance sampled at high-elevation sites. The distribution of the two parasitoid species is unclear because of the small sample sizes, but both occur at high-elevation sites (Jeffs et al., 2021). To represent a lowland species which is a candidate to invade high-elevation communities under climate, we used *D. pandora*, which occurs predominantly at low-elevation (70m) sites (Jeffs et al., 2021).

4.2.2 Community initiation

All experimental communities were initiated with the same species under upland temperature regimes (24°C, light (12h) and 18°C, dark (12h) cycle), mimicking upland conditions. Each community was contained within a sealed 20cm x 5cm x 10cm plastic cage. Card dividers on the floor of the cage could hold up to 24 standard *Drosophila* vials. In each of the first three weeks, 15 females and 15 males of *D. pseudotakahashii*, *D. pallidifrons*, *D. birchii*, *D. rubida*, and 10 females and 10 males of both parasitoid species were added to each cage. The adult flies were of mixed ages, randomly sourced from mass-bred population cages. The adult wasps all emerged within one week. After these first three weeks, we maintained the community cages without adding insects for two more weeks before starting experimental treatments. The schedule is shown in Figure 4.3b.

4.2.3 Treatments and maintenance

After the fifth week of community initiation, a factorial design of three temperature treatments and two introduction treatments was applied to 30 cages (18 cages in

block A and 12 cages in block B, initiated four weeks apart). Cages were randomly assigned to treatments (five replicates per treatment) and maintained for 20 weeks. Three vials of 6ml fresh *Drosophila* food (weight/volume concentration: 8% corn flour, 4% yeast, 5% sugar, 1% agar, and 1.67% methyl-4-hydroxybenzoate) were added to the cage two times a week (three to four days apart). Vials added in the middle of each week were “maintenance vials”, which were removed after 40 days so that most flies and wasps would emerge. Vials added at the start of the week were “sampling vials”, which were removed after ten days, before the emergence of any organisms from the vials.

Temperature treatments. The upland temperature regime fluctuated between 24°C, light (12h) and 18°C, dark (12h). For warming and heatwave treatments, we allocated the same amount of increase in cumulative temperature (+1°C per day) by either an even increase in temperature or by extreme events. The warming regime increased both the day-time and night-time temperatures by 1°C (i.e., 25°C, light (12h) and 19°C, dark (12h)), which is similar to the rise of average global temperature since the last 150 years IPCC (2022). The heatwave regime had the same baseline temperature as the upland regime, but with periodic heatwaves when day-time and night-time temperatures rose by 6°C for five consecutive days (for comparison, the Australian 2017 heatwave raised the highest air temperature at the upland site by 6°C for two consecutive days). Heatwave events happened once per four weeks on average for a total of five heatwaves, with semi-random timing (a two-week minimum interval between any two heatwaves). Temperatures were provided by three incubators of the two similar models (Sanyo MIR-154 & Sanyo MIR-153). To minimise confounding of incubator and thermal treatment effects, the incubators were switched among temperature regimes every week, and the community cages were moved from one incubator to another accordingly.

Introduction treatments. Each week, four females and four males of the lowland species, *D. pandora*, were added to cages in the introduction treatment to simulate the immigration of lowland species into the upland community. To reduce among-cage variation due to any stochastic loss of species, four females and males of each

Drosophila and parasitoid wasps species from the starting community were added to community cages of all treatments at the same frequency as *D. pandora*. In the final two weeks of the experiment, no insects were added into the communities.

4.2.4 Data collection

Regular sampling. The reproductive success of every species was monitored by examining the adults emerging from the regularly sampled vials. Three sampling vials containing 6ml of fresh *Drosophila* food were placed into each cage on Monday of each week and removed 10 days later, before offspring emergence. These vials were kept for 3.5 weeks in the same incubator as the associated cages. In this way, the number of emerging adults from the “sampling vials” would be similar to those “maintenance vials”, reflecting a joint impact by the adult population sizes during sampling, adult fecundity, intra- and inter-specific competition and parasitism. Emerging fly and wasp adults were collected and killed by freezing at -18°C every three or four days. Weekly counts of these adults were recorded, separated by species and sex with exceptions. Females of *D. pseudotakahashii*, *D. pandora*, and *D. birchii* were not separated apart due to their similarity. Therefore, following analyses of any numbers of these three species were the counts of males.

Heatwave-specific sampling. To measure how a single heatwave altered the single-day reproductive success of flies, one day before the final heatwave, three vials containing 6ml of fresh *Drosophila* food were placed into each cage of the heatwave treatment. They were kept in the cage for 24 hours, allowing living adult flies to lay eggs within them. After removal, all vials were kept under the upland temperature regime. Adult flies that emerged in each vial were later identified and counted. There were no parasitoid offspring as the parasitoids were unable to successfully parasitise eggs or first-instar host larvae. The same procedure was applied on the fifth day of the heatwave, and five days after the heatwave ended.

Termination and census. After five weeks of community initiation and 20 weeks of treatments, all living adults in each cage were taken out of cages, killed by freezing, identified and counted. Throughout the maintenance of the community

cages, we did not observe obvious fluctuation in the numbers of *Drosophila* or parasitoids. Therefore, even though the abundance at the termination was only a snapshot of the population dynamics, we think the census data could represent the overall patterns of community composition.

4.2.5 Data analysis

All statistical analyses were performed with R statistical software, version 4.0.3 (R Core Team, 2020).

Temperature effect on introduction success. Adult population sizes (census) of *D. pandora* were modelled as a function of temperature, block, and their interactive effect in a generalized linear model. For regular sampling data, offspring numbers of *D. pandora* were modelled as a function of temperature, block, and their interaction as fixed effects, week of sampling, and cage ID as random effects in a generalized linear mixed-effects model. To test the effects of heatwave on reproduction, the single-day reproduction success (measured by adult offspring number) was modelled as a function of sampling time (relative to heatwave), focal species, introduction of the novel species, block, and the interactive effect between sampling time and focal species in a generalized linear model. All response variables were counts of individuals so models assumed a *Poisson* distribution with a log-link function. Regressions were conducted using the *stat* and *lme4* packages (Bates et al., 2015). If significant main effects are detected, post hoc comparisons were conducted using the *emmeans* package (Lenth, 2022).

Community composition Community composition was analysed separately for hosts and parasitoids. For the four resident upland fly species, compositional differences were visualized using non-metric multidimensional scaling (NMDS) with a dimension of two. For the two parasitoids, abundance values were standardized as z-scores ($\frac{x-\bar{x}}{\sigma}$). Differences in species composition among treatments were tested by non-parametric MANOVA (NPMANOVA), with the distance matrix calculated using the “bray” method and 9999 permutations. Upon detecting overall significant differences among treatment groups, post hoc analyses were performed to test

the differences between pairs of treatments (e.g. “warming + no introduction” vs. “warming + introduction”) using NPMANOVA. NMDS and NPMANOVA analyses were conducted using the *vegan* package (Oksanen et al., 2020). The *multcompView* package was used to summarise significant differences based on the post hoc pairwise comparisons (Graves et al., 2019).

Differences in adult population size (census) of individual species among treatments were analysed using a generalized linear model. The population size of each resident species was modelled as a function of temperature, the introduction of the novel species, block and the interaction between temperature and the introduction of the novel species, assuming a *Poisson* distribution with a log-link function. After detecting significant main effects, post hoc pairwise comparisons were conducted using the *emmeans* package.

Structural equational model (SEM). The direct and indirect effects of the temperature and novel species treatments on the single-generation reproductive success were analyzed by piecewise SEM using the “regular sampling” data. Communities that underwent heatwave treatment were excluded from analyses of the effect of warming, and vice versa. Piecewise SEM (Lefcheck, 2016) decomposes the networks of causal effects into sets of linear regressions for each response, evaluates each set of relationships separately, and then combines them to generate inferences about the entire SEM. The prior model specifies which sets of causal relationships will be tested and the directions of the effects. It also implicitly defines which sets of relationships are not assumed to exist. These assumptions of the causal structure need to be tested by Fisher’s C test, where p-value > 0.05 indicates conditional independence and acceptable goodness-of-fit. SEM requires a large number of observations to test multiple assumptions simultaneously. The “regular sampling” data includes multiple collections at different time points, thus covering more variations in the numbers of different species.

In SEM, we fit a series of univariate linear mixed-effect models in which the square-root-transformed abundance of each species was modelled as a function of introduction of the novel species (exogenous predictor), temperature (exogenous

predictor), and the transformed abundances of other species (endogenous predictors). Temperature and the introduction of the novel species were coded as 0 (upland temperature or no introduction of novel species) or 1 (warming or heatwave treatment, or introduction of novel species) so that the coefficients for temperature and introduction would reflect the change induced by temperature treatment and introduction of the novel species, respectively.

The prior model assumed that each fly species was affected by temperature, introduction, the abundance of single or both parasitoid species (depending on their host ranges), block, and random effects of cage ID and sampling week; and that each parasitoid species is affected by temperature, introduction, block, and random effects of cage ID and sampling week. Parasitoid wasps were assumed not limited by *Drosophila* abundances because the numbers of their hosts were always in saturation (the personal observation that all food surfaces had been populated with pupae). If the prior model was not conditionally independent as judged by Fisher's C test ($p < 0.05$), the prior model was updated by incorporating the significant relationships from the tests of directed separation (e.g. the correlation between *D. pseudotakahashii* and *Asobara*). The directions of the new effects were determined by both expert knowledge and the comparison of AIC scores. The adjusted model was tested for directed separation again and was accepted when $p\text{-value} > 0.05$.

Following SEM modelling, the path coefficients from the univariate regressions were used to calculate direct and indirect effects. Direct effects were defined as coefficients of paths between connected variables (also known as nodes). Indirect effects were defined as those separated by at least one node. They were significant when all the corresponding connecting paths were significant and their values were the product of the coefficients of the connecting paths. The conditional R^2 value for each species was calculated. We fitted regressions using the *lme4* package and used the *piecewiseSEM* (Lefcheck, 2016) package for SEM modelling.

4.3 Results

4.3.1 Effects of warming and heatwaves on the novel species

At the end of the experiment, the abundances of *D. pandora* in upland-temperature and warming treatments were small (estimated means are 15.8 and 14.8 male adults per cage, respectively; coefficient for warming = -0.07, $p = 0.75$; coefficient for warming $\times$ block = 0.001, $p = 0.99$; $df = 11$). In comparison, abundances of *D. pandora* in heatwave treatments was significantly higher (Figure 4.4a; estimated mean 85.1; coefficient for heatwave = 2.0, $p < 0.0001$; coefficient for heatwave $\times$ block = -0.67, $p = 0.008$; $df = 11$). Significantly higher numbers of adult *D. pandora* offspring were produced in the heatwave treatment (Figure 4.4b; coefficient for heatwave = 2.27, $p < 0.0001$). Very few offspring were produced in upland temperature and warming treatments.

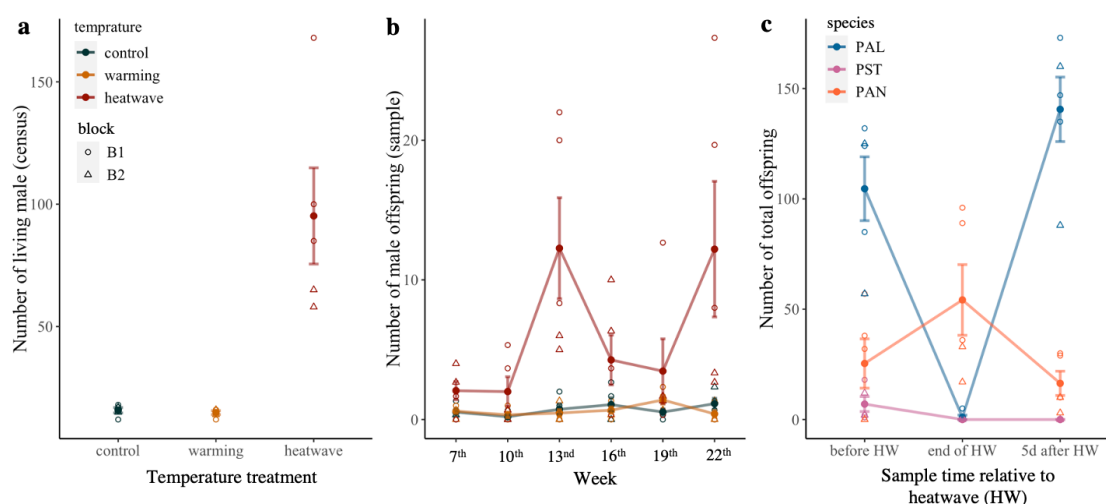


Figure 4.4 Heatwaves promote the establishment of *D. pandora* in upland communities. a) Numbers of male *D. pandora* adults in community cages under different temperature treatments. b) Reproduction of *D. pandora* over time in the upland temperature regime (blue), warming treatment (orange) and heatwave treatment (red). c) Reproductive success on a single day of *D. pallidifrons* (blue), *D. pseudotakahashii* (purple), and *D. pandora* (orange) at different timings relative to a heatwave event. For all graphs, each open symbol represents the number of individuals of the specified species and sex censused or sampled from a community replicate ($n = 5$). Shapes of symbols represent experimental block 1 (circle) or block 2 (triangle). The solid circles represent the means of the treatment groups. Lines connect the mean values of the same temperature (b) or same species (c). The error bar shows ± 1 SD.

To investigate how heatwaves favour *D. pandora*, we compared one-day snapshots of reproductive success at different timings relative to a heatwave event (Figure 4.4c). Heatwaves significantly decreased reproduction success of *D. pallidifrons* (95% confidence interval of mean offspring number before heatwave = 24 – 28; at the end of heatwave = 0.2 – 1), but reproduction recovered to an even higher level five days after the heatwave (confidence interval = 41 – 46). Heatwaves also significantly ($p < 0.0001$) decreased the reproductive success of *D. pseudotakahashii* to zero, and they were unable to recover. In contrast, the heatwave significantly increased the reproductive success of *D. pandora* (confidence interval before HW = 6 – 9; at end of HW = 14 – 18). Its reproductive success then decreased significantly after the heatwave (confidence interval = 3.8 – 6).

4.3.2 Effects of temperature and the novel species on species composition

Temperature treatments and the novel species separately and jointly drive compositional changes in upland communities. Non-parametric MANOVA reveals significant differences in community composition of upland *Drosophila* species and parasitoid species (Figure 4.5; *Drosophila* community: $p = 0.0001$, $r^2 = 0.62$, $df = 5$; parasitoid wasp community: $p = 0.0003$, $r^2 = 0.58$, $df = 5$). Pairs that were significantly different in post hoc comparison were denoted by different letters in Figure 4.5.

Warming did not alter the composition of the resident *Drosophila* community (Figure 4.5a), regardless of the introduction of the novel species. The introduction of the novel species did not induce significant compositional changes in the upland temperature or warming treatments. In contrast, heatwaves alone induced significant shifts in *Drosophila* composition relative to upland temperatures. Furthermore, the introduction of the novel species had a significant effect only under the heatwave treatment. As for parasitoid wasps (Figure 4.5b), warming and heatwaves altered their compositions in similar ways in the absence of *D. pandora*. The introduction of *D. pandora* significantly changed the wasp composition under upland temperature or heatwaves regimes, but had no significant effect under the warming regime.

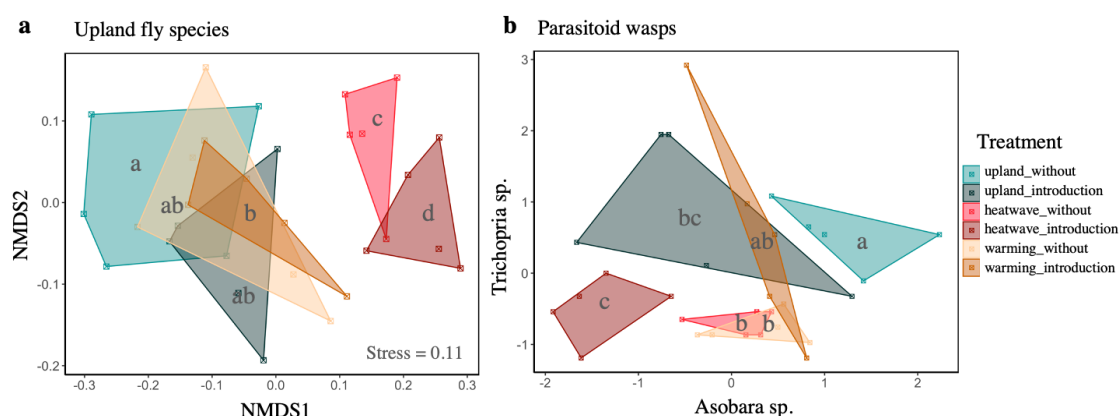


Figure 4.5 Compositional change of hosts (a) and parasitoids (b) under different combinations of temperature and introduction treatments. The host guild includes four *Drosophila* species represented in two dimensions using NMDS. The standardized abundances of the two parasitoid wasp species are shown on the axes. Each small square represents a community, with communities from the same treatment grouped by shaded minimal convex polygons. The treatments are distinguished by the colour of shading as described in the right panel. Letters inside the polygons indicate statistically different groups tested by non-parametric MANOVA.

We further investigated changes in the abundance of individual species within the communities (Figure 4.6). The abundance of *D. pseudotakahashii* was significantly reduced in the warming treatment (coefficient = -0.72, $p < 0.0001$, $df = 23$; df is the same for the following analyses in this paragraph) and to a larger extent in the heatwave treatment (coefficient = -2.27, $p < 0.0001$). The introduction of the novel species significantly reduced the abundance of *D. pseudotakahashii* under upland temperatures (coefficient = -0.35, $p < 0.0001$). The abundance of *D. pallidifrons* decreased in the warming treatment (coefficient = -0.43, $p < 0.0001$), but increased in the heatwave treatment (coefficient = 0.42, $p < 0.0001$). The introduction of the novel species significantly increased *D. pallidifrons* abundance in all three temperature scenarios ($p < 0.0001$). The abundance of *D. birchii* was very low in all cases, with no statistically significant differences between treatments. Warming in the absence of novel species slightly increased the abundance of *D. rubida* (coefficient = 0.32, $p = 0.009$). Numbers of *Asobara* parasitoids were reduced under the warming (coefficient = -0.33, $p < 0.0001$) and heatwave (coefficient = -0.40, $p < 0.0001$) treatments. The introduction of the novel species significantly

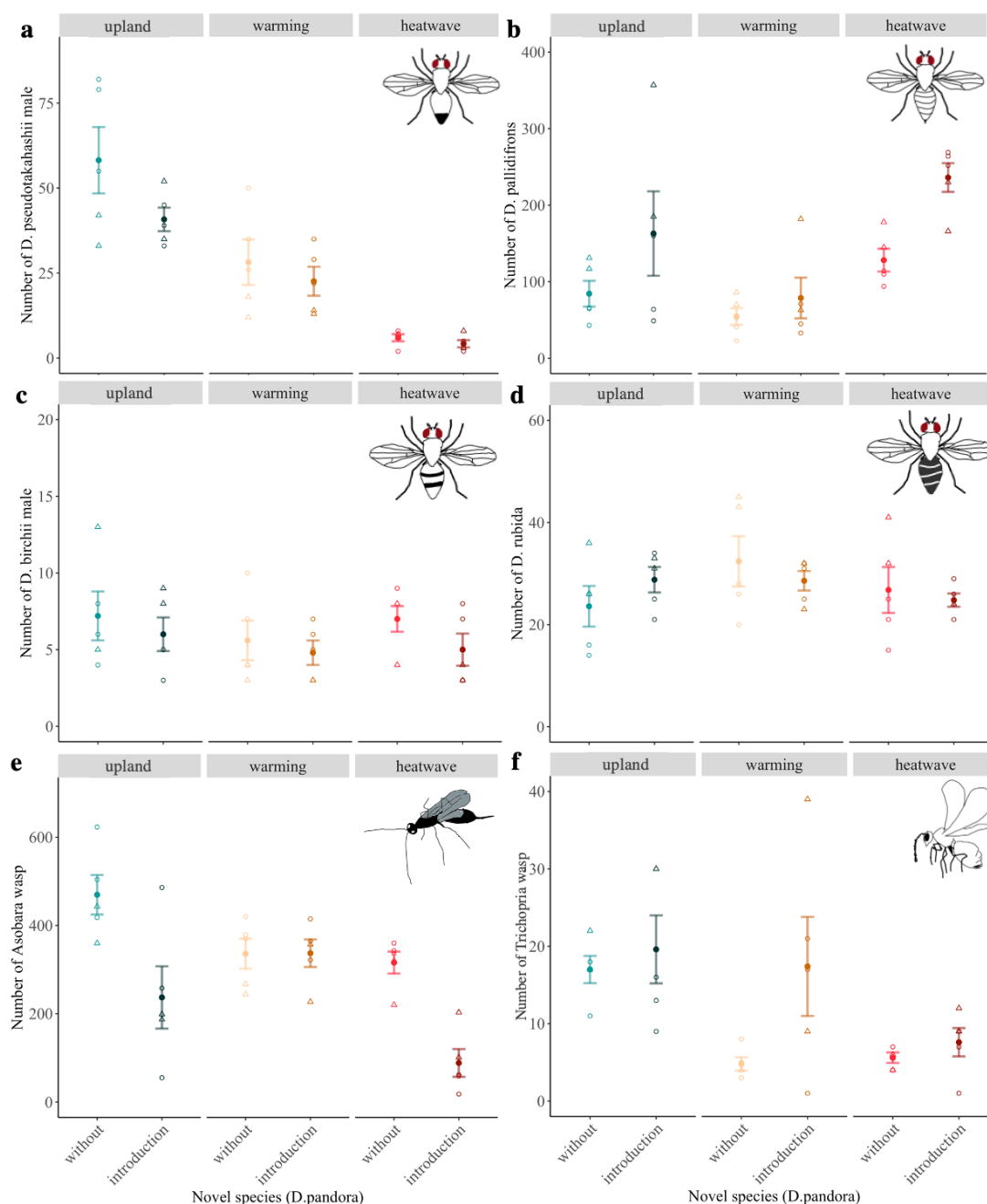


Figure 4.6 Censused abundances of species under temperature and invasion treatments. (a) *D. pseudotakahashii*; (b) *D. pallidifrons*; (c) *D. birchii*; (d) *D. rubida*; (e) *Asobara* species; (f) *Trichopria* species. Each open symbol represents the population size of the indicated species from a community replicate ($n = 5$). Shapes represent experimental block 1 (circle) and block 2 (triangle). The solid circles represent the means of the treatment groups. The error bar shows ± 1 SD.

reduced *Asobara* abundance at upland temperatures (coefficient = -0.68, $p < 0.0001$), and even more so in the heatwave treatment (coefficient = -1.27, $p < 0.0001$), but

not in the warming treatment ($p = 0.91$). *Trichopria* abundance was also sensitive to warming (coefficient = -1.26, $p < 0.0001$) and heatwaves (coefficient = -1.11 $p < 0.0001$). Under the warming scenario, the introduction of additional hosts (the novel species *D. pandora*) increased *Trichopria* abundance (coefficient = 1.29, $p < 0.0001$).

4.3.3 The direct and indirect effects of temperature and the novel species

To explain the patterns of population sizes, we used SEM to evaluate how treatments (i.e. temperature, the novel species) directly influenced the reproductive success of all species and subsequent effects through species interactions (defined as indirect effects). As shown in Figure 4.7a, heatwaves directly decreased reproduction of *D. pseudotakahashii*, *D. pallidifrons* and *Trichopria*. The effect of heatwaves on *Asobara* was also negative, but not significantly so ($p = 0.095$) in our single-generation samples. The SEM reveals that the positive effect of heatwaves on *D. pandora* is indirect, mediated by the response of *D. pallidifrons* to the heatwave. The introduction of *D. pandora* had a direct positive effect on the reproduction of *Trichopria*. Meanwhile, its negative effect on *D. pseudotakahashii* and positive effect on *D. pallidifrons* were both mediated by *Asobara*, whose reproductive success was decreased by the introduction of *D. pandora*. Compared with the heatwave treatment, warming also had direct negative effects on *D. pseudotakahashii*, and *D. pallidifrons* (Figure 4.7b). However, its effect on *D. pallidifrons* did not propagate to affect *D. pandora*. Introduction still had a direct negative effect on *Asobara*, which led to indirect effects on *D. pseudotakahashii* (overall negative) and *D. pallidifrons* (overall positive). The direct effect of the introduction on *Trichopria* disappeared and a new direct positive effect from *Trichopria* to *D. pseudotakahashii* became significant.

4.4 Discussion

Extreme heat events are occurring more frequently and intensively around the globe (IPCC, 2022). In some areas, such as the tropics, heatwaves are the major climatic manifestation of excess atmospheric heat (Perkins-Kirkpatrick and Lewis, 2020).

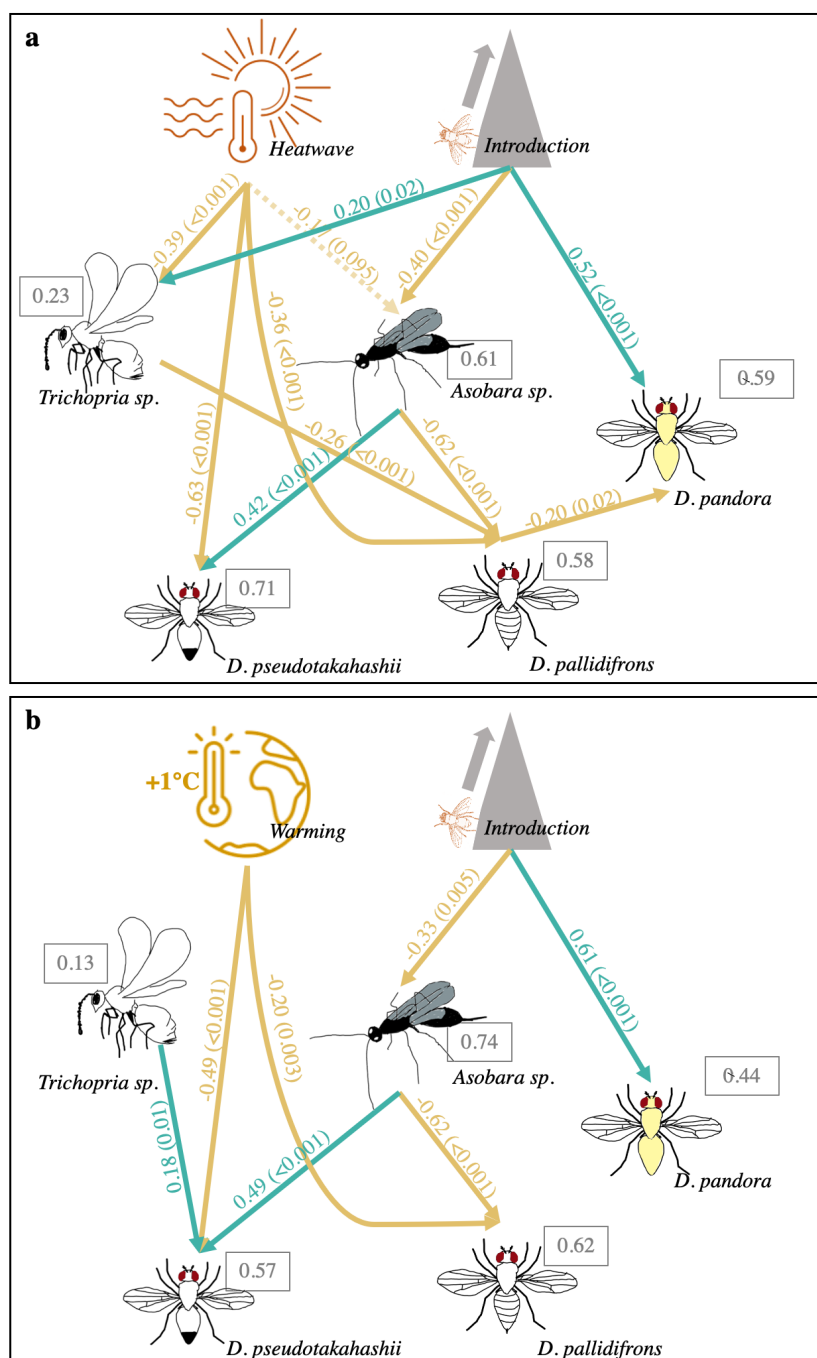


Figure 4.7 Direct and indirect effects of (a) heatwaves and (b) warming on reproductive success in sampling vials, in combination with the introduction of the novel species *D. pandora*. Solid lines linking treatments to species or species to species show significant direct effects. The dashed line shows a direct effect which is close to significance. Arrows indicate the direction of effects and colours distinguish positive (blue) and negative (yellow) effects. Coefficients and p values are shown, and conditional R^2 values of response variables are provided in rectangles next to each species.

Our experimental community corresponds closely to the natural community of closely interacting *Drosophila* and parasitoids in the rainforest of the Wet Tropics, Australia. We show that a relatively simple community can respond in complex and unpredictable ways to different components of climate change via direct and indirect effects within the interaction network. A key outcome was that the effects of heatwaves on communities differed from those associated with an increase in mean temperature (defined as warming): heatwaves but not warming facilitated the establishment of a novel species associated with hotter, low-elevation forests. Invasion of this species additionally altered the composition of the wider community. Our data allow us to unravel some of the mechanisms underlying this shift in community composition and we highlight a key role of indirect effects mediated by parasitoids within this simple bi-trophic network.

4.4.1 Extreme heat events facilitate range expansion

Based on the asymmetry of thermal performance curves, theories predict that the temporal distribution of rising temperatures is essential for biological performance (Dell et al., 2014; Vasseur et al., 2014). We show that with the same rise in mean temperature, heatwave treatment has a stronger direct negative impact on the single-generation reproductive success of most upland *Drosophila* and parasitoid species than warming (Figures 4.7a and b). This large decrease in the reproductive performance of the resident species reduced the biotic resistance of the local community, creating a temporal window when the novel species, *D. pandora*, was able to build up its numbers (Figure 4.4c). Similar demographic changes induced by extremes have been observed in other studies of unmanipulated natural communities, facilitating the population growth of newly colonized species (Jiménez et al., 2011; Sorte et al., 2010; Thibault and Brown, 2008; Wernberg et al., 2013). On the other hand, stable species ranges and community compositions are also sometimes observed despite warming in the environment (Stuart-Smith et al., 2010). Therefore, understanding the mechanisms (e.g. winter temperature, interspecific competition) preventing the establishment of species beyond their

current range boundaries is key to predicting which climate factors will drive biodiversity shifts. High-resolution observational studies (Harley and Paine, 2009; Thibault and Brown, 2008) and experimental studies (Jentsch et al., 2007) like ours can be complementary to reveal the vital distinction between "press" (warming) and "pulse" (heatwave) climatic stresses in structuring biological communities (Easterling et al., 2000; Harris et al., 2018).

4.4.2 Effects of heatwaves and warming on community compositions

In our experimental communities, the effects of heatwaves and warming depend on the species identities. *Drosophila birchii* and *D. rubida* showed no significant responses to any of the treatments. The lack of responses of *D. rubida* was expected, as this species has a relatively even elevational distribution across the temperature and biotic gradients. Numbers of *D. birchii* in our experimental communities were always very low (below ten individuals), which we suspect was maintained by the weekly introduction. The lower single-generation reproductive success and population size of *D. pseudotakahashii* in warming and heatwave treatments are consistent with the fact that *D. pseudotakahashii* is the most sensitive species to high temperatures among the tested *Drosophila* species (Chapter 2). Contrary to our hypothesis, we observed that warming decreased the single-generation reproductive success and population size of *D. pallidifrons*. It might be because warming increased the fertility of *D. pallidifrons*, resulting in over-crowding among larvae and pupae which greatly reduces the survival to adulthood (Ménsua and Moya, 1983). Heatwaves, in contrast, increased the population size of *D. pallidifrons*, likely due to the heatwave-induced reduction of their natural enemies as we predicted.

Both warming and heatwaves reduced the population sizes of the two parasitoid species to a similar level. However, we also observed that the heatwave but not the warming treatment significantly decreased their single-generation reproductive success. Therefore, there might be other processes, such as survival, that were also influenced by the warming treatment, eventually reducing their population size.

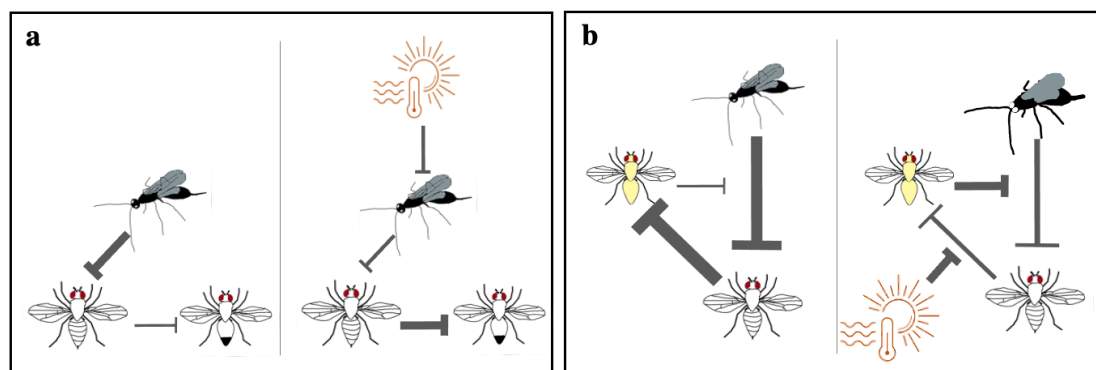


Figure 4.8 Proposed impacts of heatwaves on two species interaction motifs. a) The influence of *Asobara* parasitoids on the dominant and inferior competitors, *D. pallidifrons* and *D. pseudotakahashii*, respectively. b) The relationships between *Asobara* parasitoid, *D. pallidifrons* and the novel species, *D. pandora*. Arrows represent the direct effects from one species to another (species interactions) or from heatwaves to a species or a species interaction. “ —| ” indicates a negative effect. The thicker arrow represents a stronger effect. The left motif in each panel represents the strength of interactions in current upland temperature, and the motif on the right illustrates the influence of heatwaves on species or their interactions.

The impact of the novel species on the resident community also depended on temperature regimes. In our study, the interactive effect between heatwaves and the novel species owes to the successful establishment of the novel species population only under the heatwave regime. The predominant effect of the novel species did not act through direct competition as hypothesized. Instead, a higher abundance of *D. pandora* increased their overall negative effect on the larval parasitoids. This abundance-mediated effect complements the climate-induced changes in the per capita competitiveness shown by other studies (Alexander et al., 2015; LaForgia et al., 2020), resulting in the interactive effect between novel species interactions and climate change.

4.4.3 The importance of indirect effects mediated by parasitism

Our findings highlight the significance of parasitism in modulating the biological impact of global change drivers. Specifically, our system is characterized by two temperature-sensitive interaction motifs, summarised in Figure 4.8. First, the parasitoid *Asobara* was essential for promoting coexistence between the dominant and

inferior competitors. This process can be explained by the differential susceptibility of hosts to the *Asobara* parasitoid found by Chia-Hua Lue (unpublished results). However, as a rising temperature or introduction of the novel species reduced the performance of *Asobara* wasps, there was a corresponding decrease in the population size of *D. pseudotakahashii*. The sensitivity of parasitoids to rising temperatures implies that climate change poses a threat to the biodiversity maintained by their ecological functions.

The second intriguing phenomenon is that the introduction of *D. pandora* facilitated *D. pallidifrons*, reversing the outcome if these two species compete directly in the absence of other species (Chapter 2). The introduction of *D. pandora* reduced the reproductive success of *Asobara* wasps under all temperature regimes. Therefore, we suspect that the facilitation results from the interference of *D. pandora* with the efficiency of *Asobara* wasps parasitizing on *D. pallidifrons* (Figure 4.8b). *Asobara* can attack *D. bipectinata* (another lowland species that is closely related to *D. pandora*) even though *D. bipectinata* is unable to host successful development of the parasitoid (Chapter 3), consistent with the observation that parasitoids often oviposit into the “wrong” hosts (Condon et al., 2014). *Drosophila pandora* may also have a low contribution to the reproductive success of *Asobara* wasps, while its presence dilutes the density or changes the behaviour of suitable hosts (such as *D. pallidifrons*), making suitable hosts more inaccessible to the parasitoids. The facilitation effect was further strengthened by heatwaves as the heatwave treatment increased the abundance of *D. pandora*. Thus, heatwaves resulted in a substantial rise in population size for both species that directly compete against each other. This unexpected result underlines the importance of manipulative research to reveal the dynamic processes a species is involved in structuring the community.

4.4.4 Practical implications for research, conservation and resource management

As we demonstrated in this experiment, extreme events such as heatwaves temporarily reduced the residents’ biotic resistance to invading species to a large extent.

This suggests that during and shortly after extreme climatic events may be the most crucial and effective time to facilitate the full recovery of the resident community (e.g. if endemic species within it are threatened by invasion), for example by manually removing potential invading species. Our study also illustrates that parasitoids are sensitive to both extreme heat events and novel hosts. Parasitoids have been widely introduced to regions outside their natural ranges as biocontrol agents (Hardy et al., 1994). When selecting such biocontrol agents, we may need to consider the interactions between the changing climate conditions and possible invading species. Otherwise, as shown in our experimental communities, their unexpected interactions with novel species might fail parasitism in controlling the target hosts.

4.4.5 Conclusion

Our mountain-top communities or others in fragmented habitats are bounded geographically. As climate change re-organizes biological communities, these communities face combined threats from changing abiotic and biotic conditions (Shah et al., 2020) without the presence of spatial refuges to buffer these threats. The interdependence of abiotic and biotic drivers poses a great challenge to efforts to predict biological responses to climate change. Manipulative experiments that address both abiotic and biotic forces, such as our study, can help identify key species or interaction motifs that drive community responses to climate change. In our study, warming and heatwaves caused very different changes to community composition, with heatwave-facilitated range expansion and its subsequent indirect effect via higher trophic levels playing crucial roles. We found evidence for facilitation between resource-sharing hosts mediated by a parasitoid species, which has rarely been documented before, and highlighted the uncertainty of the ecological consequences of the species interaction between parasitoids and novel hosts.

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5

Natural Enemies Have Inconsistent Impacts on the Coexistence of Competing Species

Graphical Abstract

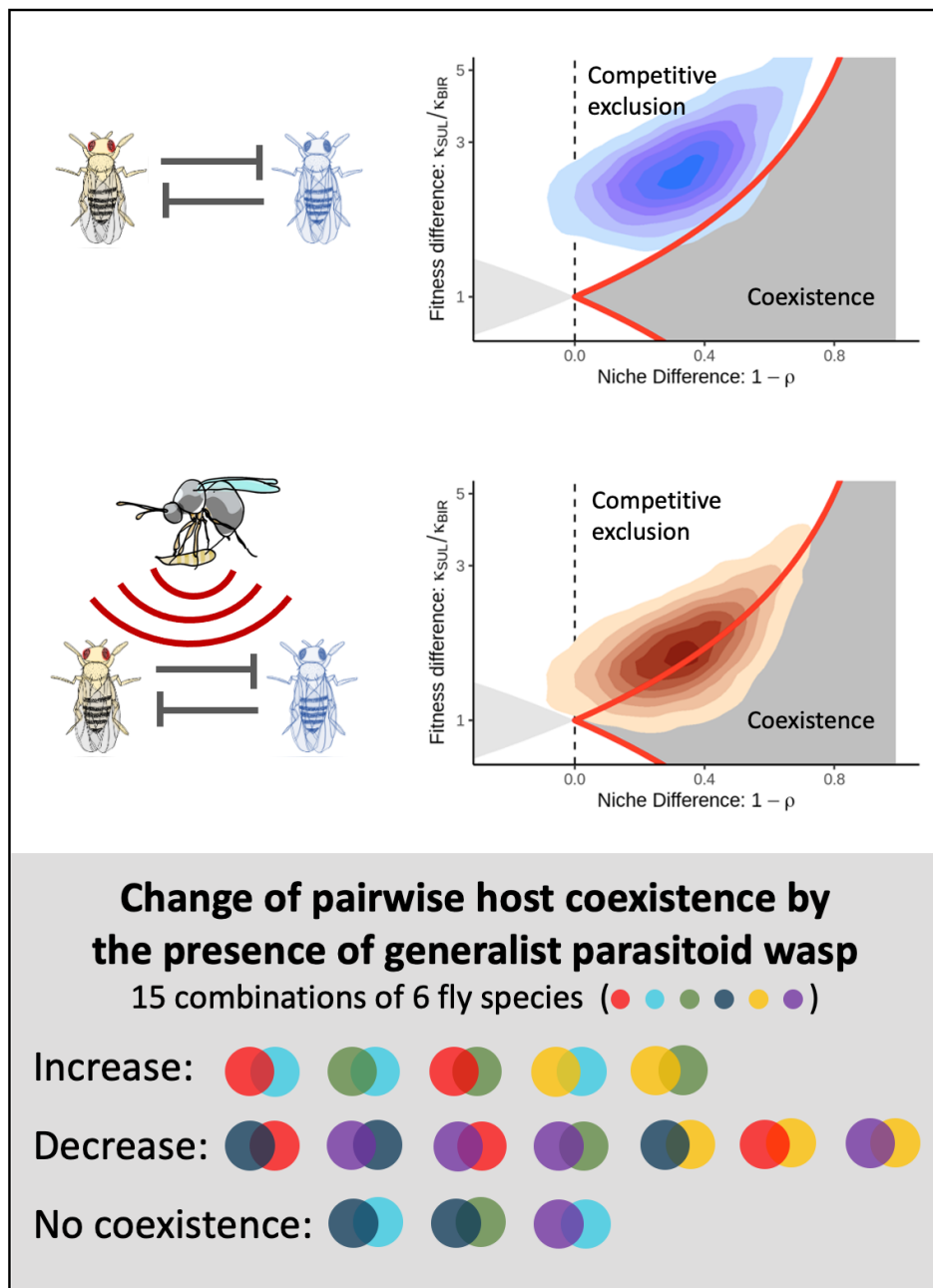


Figure 5.1 The impacts of parasitism on the coexistence between competing host species.

Natural enemies have inconsistent impacts on the coexistence of competing species

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Abstract

1. The role of natural enemies in promoting coexistence of competing species has generated substantial debate. Modern coexistence theory provides a detailed framework to investigate this topic, but there have been remarkably few empirical applications to the impact of natural enemies.
2. We tested experimentally the capacity for a generalist enemy to promote coexistence of competing insect species, and the extent to which any impact can be predicted by trade-offs between reproductive rate and susceptibility to natural enemies.
3. We used experimental mesocosms to conduct a fully factorial pairwise competition experiment for six rainforest *Drosophila* species, with and without a generalist pupal parasitoid. We then parameterised models of competition and examined the coexistence of each pair of *Drosophila* species within the framework of modern coexistence theory.
4. We found idiosyncratic impacts of parasitism on pairwise coexistence, mediated through changes in fitness differences, not niche differences. There was no evidence of an overall reproductive rate–susceptibility trade-off. Pairwise reproductive rate–susceptibility relationships were not useful shortcuts for predicting the impact of parasitism on coexistence.
5. Our results exemplify the value of modern coexistence theory in multi-trophic contexts and the importance of contextualising the impact of generalist natural enemies to determine their impact. In the set of species investigated, competition was affected by the higher trophic level, but the overall impact on coexistence cannot be easily predicted just from knowledge of relative susceptibility. Methodologically, our Bayesian approach highlights issues with the separability of model parameters within modern coexistence theory and shows how using the full posterior parameter distribution improves inferences. This method should be widely applicable for understanding species coexistence in a range of systems.

J. Christopher D. Terry and Jinlin Chen joint first authorship.

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KEYWORDS

Bayesian, competition, *Drosophila*, model fitting, modern coexistence theory, natural enemies, parasitoid

1 | INTRODUCTION

Species compete for limited resources and (in almost all cases) are consumed by other species. Understanding how the impacts of competitors and natural enemies interact to influence species coexistence is a long-standing challenge. Natural enemies are regularly cited as a driver of coexistence, for example by suppressing species that would otherwise be competitively dominant (Paine, 1966), but their impact can be highly variable (Chase et al., 2002).

Analyses investigating the effect of natural enemies often focus on 'reduction in competition' (e.g. Gurevitch et al., 2000), as measured by negative effects between focal species; however, consumers can affect competition and coexistence in convoluted ways (Chase et al., 2002; Pringle et al., 2019). Modern coexistence theory (MCT) focuses on the property of mutual invasibility (Grainger et al., 2019), and it allows the precise framing of questions regarding pairwise species coexistence as a balance between niche differences promoting stabilisation and fitness differences fostering exclusion of one species by another (Chesson, 2000; Letten et al., 2017). Through the lens of modern coexistence theory, interactions mediated through competition for resources and those mediated by natural enemies in the form of 'apparent competition' are conceptually equivalent (Chesson, 2018; Chesson & Kuang, 2008), and a body of theory has developed to integrate consumer effects within the coexistence framework (Chesson & Kuang, 2008; McPeck, 2019). This theoretical work has emphasised that impacts of any process on coexistence can only be determined with information on both intrinsic growth rates and interaction terms. Alongside the theory, empirical studies using the modern coexistence framework to investigate the impact of consumers on coexistence are now emerging (Bartomeus & Godoy, 2018).

Natural enemies can affect both fitness differences and niche differences between species. A frequently invoked mechanism is that they reduce fitness differences, mediated by a trade-off between population growth rate and susceptibility to attack (Kraaijeveld & Godfray, 1997; Paine, 1966). However, such a trade-off is in itself insufficient to enable long-term coexistence, since some kind of stabilising niche difference is required to mitigate any fitness difference (Chesson, 2000). Even with zero fitness difference, stochastic drift would preclude coexistence in the absence of stabilising mechanisms (Adler et al., 2007). Natural enemies can also potentially influence niche differences through modifying the overall competitive pressure exerted between competitors. Enemies may cause competitors to change their behaviour, or natural enemies may change their foraging behaviour as the relative abundances of the competing species change (Abrams, 2010).

Experimental studies of insect communities have considerable potential to further our understanding of the role of natural enemies

in species coexistence. Since parasitoids kill their insect hosts as they develop in or on them, each attack is directly interpretable in terms of host fitness (Hassell, 2000). However, to date, most experimental work within a formal MCT framework has been conducted on plant or microbial systems (Bartomeus & Godoy, 2018; Grainger et al., 2019), including investigation of the effects of seed predation (Nottebrock et al., 2017; Petry et al., 2018) and seed pathogens (Mordecai, 2013). Although ecologists have been competing insect species (especially *Drosophila*) against each other for decades (Davis et al., 1998; Gilpin et al., 1986; Pearl, 1932; Worthen, 1989), very few studies have explicitly tested for mutual invasibility in insect systems (Siepielski et al., 2018), rather than particular conditions necessary for coexistence (Godwin et al., 2020; Spaak & de Laender, 2020).

Here we present an analysis parameterising the impact of generalist consumers on pairwise species coexistence, focusing on a community of six rainforest *Drosophila* species. Our experiment was designed specifically to capture the key parameters of our focal species and their competitive interactions. We examine how niche and fitness differences are influenced by the presence of the parasitoid with a Bayesian approach that propagates uncertainty in model parameters through to niche and fitness differences. We show that the generalist natural enemy can influence the fitness differences between species but has inconsistent impacts on pairwise coexistence that would not be identifiable solely from reproductive rate-susceptibility relationships.

2 | MATERIALS AND METHODS

2.1 | Study species

We investigated a system of six naturally co-occurring *Drosophila* vinegar flies (*D. birchii* (BIR), *D. pallidifrons* (PAL), *D. pandora* (PAN), *D. pseudoananassae* (PSA), *D. simulans* (SIM) and *D. sulfurigaster* (SUL)) from a well-characterised Australian rainforest community (Hangartner et al., 2015; Jeffs et al., 2021; O'Brien et al., 2017), along with a co-occurring generalist parasitoid wasp from the genus *Trichopria* (Hymenoptera: Diapriidae, lab strain 66LD, for taxonomic details see Supporting Information 1 and Lue et al. (2021)). This parasitoid attacks *Drosophila* species at their immobilised pupal stage. Successful development of a parasitoid results in the death of its host. *Drosophila* can mount a variety of physiological defences to parasitoid attack, which can be costly (Fellowes & Godfray, 2000) and similar defence-reproduction trade-offs have been identified in other insects (Schwenke et al., 2016).

Drosophila species have long been used in experimental ecology since they are straightforward to culture under laboratory

conditions, allowing high levels of replication and standardisation. The set of *Drosophila* species used here represents a significant fraction of the diversity of *Drosophila* species co-occurring at rainforest sites in northern Queensland, Australia (Jefferies et al. 2021). The six species span a range of body sizes (0.94–2.64 mg, mean wet mass of female adults) and phylogenetic divergences (Supporting Information 1, Table S1.1) have similar generation times, and can be distinguished visually (at least in the case of males). *Drosophila* are generalist feeders (Valadão et al., 2019) and in natural populations they experience high levels of parasitism, including from a suite of highly generalist natural enemies (Jefferies et al., 2021).

2.2 | Experimental design

We conducted a large, single-generation pairwise competition assay using a response-surface design following the guidelines of Hart et al. (2018), and in the presence or absence of pupal parasitoids. We used 25-mm diameter standard *Drosophila* vials (VWR International) containing 4 ml of cornflour–yeast–agar medium. This creates conditions where *Drosophila* compete for space to lay eggs, for larval food and for pupation sites. Equal numbers of male and female *Drosophila* were introduced to each vial, so we refer to the number of founder pairs. Each two-species combination (15 combinations in total) was tested at different founding densities in a factorial design: (3 pairs of species A, 3 pairs of species B), (6A,3B), (9A,3B), (12A,3B), (3A,6B), (3A,9B) and (3A, 12B), with each combination replicated three times in each parasitism treatment. We also included monocultures of each species, with 12 replicates of a single founding pair, 6 replicates of 3, 9, or 12 pairs within each treatment, making a total of 990 experimental vials (360 single-species, 630 two-species). The entire experiment was split over three blocks, each with identical sets of combinations, but staggered by 2 days. The experiment was conducted in a single large incubator maintained at 26°C, with the

location of trays within the incubator regularly rotated. Further details are given in Supporting Information 1.

2.3 | Initiation of treatments

Founding adults were offspring of adults drawn from mass-bred line cages and were raised in vials at moderate density (approximately 100 adults per vial) at a constant 23°C. These adults emerged 8–10 days before the start of the experiment, and so could be assumed to be sexually mature and mated. On the day preceding the start of the experiment, the founding flies were lightly anaesthetised under CO₂ to be separated by sex. Males were added to the experimental vials immediately. Females were maintained at moderate density (~50 adults per vial) in holding vials with fly medium and were transferred under light CO₂ anaesthesia to experimental vials the following day, minimising the time interval between the addition of each species in multi-species assays. They were able to lay eggs and re-mate for 24 hr before being removed. Figure 1 provides an overview of the experimental protocol.

2.4 | Parasitism

At the onset of pupation, 5 days after the founder flies were removed, the fly vials were moved into Perspex parasitism boxes (35 cm × 20 cm × 14 cm) containing up to 88 vials (Supporting Information 1). Vial positions within each box were randomised. The vial plugs were removed, and 50 female and 50 male mature and mated parasitoid wasps were added. This ratio of parasitoids to *Drosophila* was selected based on prior experience to result in a parasitism rate in line with field-parasitism levels of around 15% or greater (Jefferies et al. 2021). The wasps were free to move between vials within each box. While it is possible that late-pupating *Drosophila* larvae could cross from one

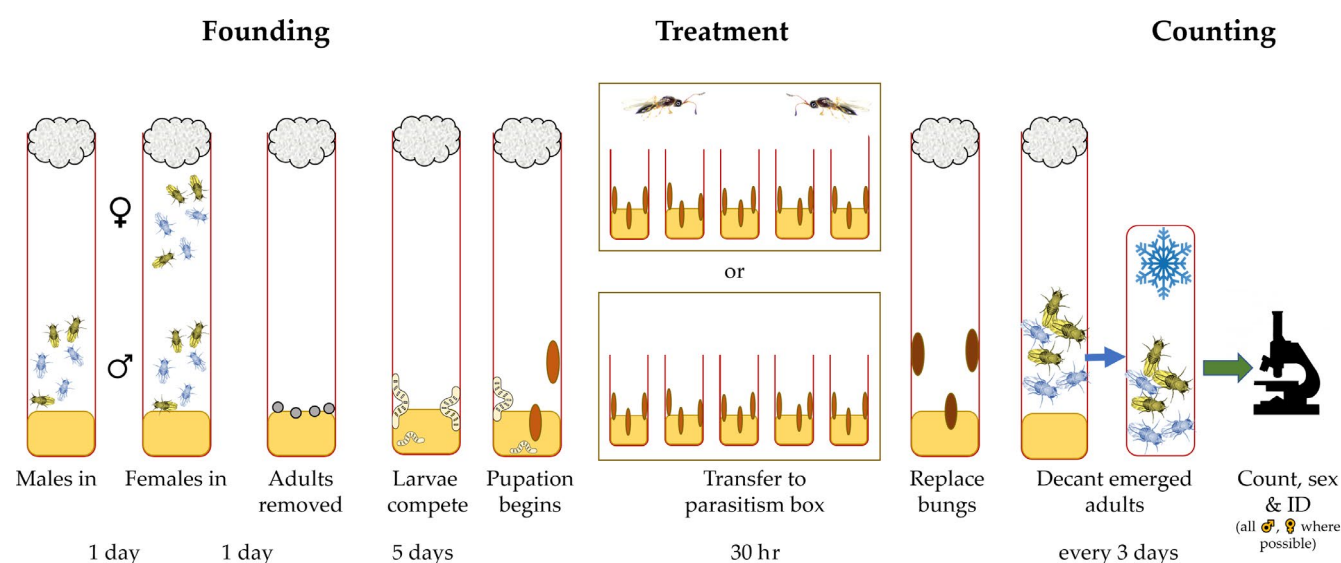


FIGURE 1 Summary of the experimental regime showing the founding process, treatment and data collection procedures described in the main text

vial to another during this period, we only observed a handful of cases where 1–2 individuals of the ‘wrong’ species emerged from a vial, and very few pupae (<5 per box) were developed on the outside of the vials, suggesting that movement was negligible. After 30 hr, before the emergence of the fastest-developing *Drosophila* species, all wasps were removed with an aspirator, vial plugs were replaced, and the vials were returned to their original trays.

2.5 | Counting

We removed adult flies emerging from all vials every 3 days, minimising the risk that emerged adults started a subsequent generation. Flies in single-species vials were sexed and counted at each removal time, while flies in multi-species vials were sexed and identified at the end of the experiment (Figure 1). There was a highly consistent 1:1 sex ratio across the single-species trials and in those two-species trials where females of different species could be reliably distinguished (Supporting Information 2, Figure S2.1). In the five of the combinations (BIR-PAN, BIR-PSA, BIR-SIM, PSA-PAN and PAL-SUL) where it was not possible to identify female flies to species, we counted the males and inferred the total number of flies of each species assuming a 1:1 sex ratio.

2.6 | Modelling

2.6.1 | Determining growth rates

The larval competition of *Drosophila* often occurs within individual fruits that represent a discrete and temporary resource suitable for only a single generation of flies (Atkinson & Shorrocks, 1981; Shorrocks, 1991). We take as our underlying model of growth rates the Beverton–Holt model, without any carry-over between generations:

$$N_{i,t+1} = \frac{R_{0i}N_{i,t}}{1 + \alpha_{ii}N_{i,t} + \alpha_{ij}N_{j,t}}, \quad (1)$$

where $N_{i,t}$ and $N_{i,t+1}$ are the size of the population of species i in the founding and emerging generations, respectively. R_{0i} is a species-specific generational reproduction term, α_{ii} represents the intraspecific competitive effect of species i and α_{ij} are competition coefficients terms representing the effect of species j on i . It is important to emphasise that these α terms capture *all* impacts between species, including those through indirect means. The same function has been used for annual plants that have been the focus of most recent coexistence work (Bartomeus & Godoy, 2018; Levine & HilleRisLambers, 2009; Pérez-Ramos et al., 2019).

The mean number of adults emerging per founder was consistently lower in vials with a solitary founding pair compared to those with three founders (Supporting Information 2, Figure S2.2). *Drosophila* use cues from other females when determining oviposition

sites (Duménil et al., 2016; Wertheim et al., 2002), which could be causing this Allee effect. It is not possible to incorporate such positive density dependence effects directly within the standard MCT framework without additional assumptions (Barabás et al., 2018; Schreiber et al., 2019). Since solitary founders are quite possibly a rather artificial scenario, we excluded data from all singleton pair trials from the model fitting.

2.6.2 | Inferring model parameters

The models were fit within a Bayesian framework and posterior distributions of the parameters were obtained from Hamiltonian Monte-Carlo sampling using STAN (Carpenter et al., 2017). All code and data are archived (Terry et al., 2021). We assumed a negative binomial error distribution and fitted separate overdispersion terms ϕ for each parasitism treatment. Since we counted both species, each two-species experiment contributed two data points. Although this contributes a degree of non-independence, the gain in total sample size is significant and there was no discernible within-vial correlation in residual error (Supporting Information 2, Figure S2.3). Since we excluded the single-founder vials, our effective total ‘ n ’ across both treatments was 1,476, to estimate a maximum of 86 free parameters in our most complex model (including overdispersion terms).

The competition α terms were each fit with an underlying Gaussian prior (mean = 0, σ = 1) and constrained to be positive, implying only competitive interspecific interactions, with no facilitation possible. Growth rate terms R_0 were constrained to be positive, and fit with a weak Gaussian prior (mean = 10, σ = 10). Tests fitting α values with different constraints and priors did not give notably different results (Supporting Information 3).

We fitted and compared a sequence of candidate models of increasing simplicity. In the first, separate R_0 and α terms were fitted for each treatment. In the second, a single set of α terms, but separate R_0 terms were fitted across both treatments (i.e. parasitism affects emergence rate, but not competition coefficients). In the third model, a single set of parameters were fitted across both treatments (i.e. parasitism has no effect). The three models were compared based on expected log pointwise predictive density (ELPD, a measure of out-of-sample predictive capacity), computed using the `loo` R package (Vehtari et al., 2017). This approach allows an assessment of whether there is sufficient evidence in the data to separate the parameters assigned to the treatment groups in a comparable manner to other information criteria such as AIC (Burnham & Anderson, 2002). All STAN model code and data to fit the models are available online. Posterior predictive checks and diagnostics are presented in Supporting Information 4.

2.6.3 | Pairwise coexistence criteria

The invasibility criterion for species coexistence requires each species to have a positive long-term low-density growth rate in the

presence of the rest of the community (Chesson, 2000). Following Godoy and Levine (2014), coexistence of a pair of species is determined to be possible, following Beverton–Holt model dynamics, when:

$$\sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{jj}\alpha_{ii}}} < \frac{R_{0i} - 1}{R_{0j} - 1} \sqrt{\frac{\alpha_{ji}\alpha_{jj}}{\alpha_{ij}\alpha_{ii}}} < \sqrt{\frac{\alpha_{jj}\alpha_{ii}}{\alpha_{ij}\alpha_{ji}}}. \quad (2)$$

Equation 2 expresses the coexistence criterion as a balance between niche overlap (ρ) and fitness differences ($\frac{\kappa_i}{\kappa_j}$) where:

$$\rho = \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{jj}\alpha_{ii}}}, \quad (3)$$

and

$$\frac{\kappa_i}{\kappa_j} = \frac{R_{0i} - 1}{R_{0j} - 1} \sqrt{\frac{\alpha_{ji}\alpha_{jj}}{\alpha_{ij}\alpha_{ii}}}. \quad (4)$$

Following Chesson (2000) and Godoy and Levine (2014), we define the niche difference between two species as $1 - \rho$. Certain α values can result in a niche overlap $\rho > 1$, implying a negative potential for stabilisation through niche differences and highlighting possible cases of priority effects (Ke & Letten, 2018). Although some previous authors have elected to define such niche differences as 0, we present the values as calculated. Under either approach, coexistence is not expected under the MCT framework.

The *Drosophila* species we examined vary in their time to maturation. However, it is important to note that the mathematical expression of the invasibility criteria in a constant environment, Equation 2, is not dependent on generation time. That is to say, the sole criterion for the invader species to expand its population while the resident is at an equilibrium is that its population size in its next generation will be larger, regardless of how long a generation takes.

For each pair of species, we calculated the niche and fitness differences across 3,000 draws from the posterior distributions of parameter estimates. We partitioned the posterior to infer the support given by the data to each of the four outcomes of competition: coexistence, exclusion of either species or a situation of priority effects (see Figure 3a). Note that the dynamics described by our models are fundamentally deterministic—these values express uncertainty in outcome given the data, rather than necessarily stochastic outcomes.

3 | RESULTS

Based on model comparison, there was strong support for the expectation that parasitism reduces the growth rate of each species, but little support for differences in competition coefficients between parasitism and no-parasitism treatments. Our second model

(with different R_0 values for parasitism and no-parasitism treatments but common α values) performed best (ΔELPD versus Model 1 = -43.02 , versus Model 3 = -34.03 ; Supporting Information 4, Table S4.1).

The impact of the parasitoids on coexistence was inconsistent across species pairs (Figure 2c). In three pairs of species, the fitness difference was so large that there was essentially no support for coexistence under either treatment. Of the remaining 12 pairs, 5 had higher support for coexistence under the parasitism treatment while 7 had less support (Table 1). In only two pairs (*D. sulfurgaster* and *D. pallidifrons*, *D. simulans* and *D. pallidifrons*) did the parasitism treatment cause the median estimate of fitness difference to cross the boundary delineating coexistence from competitive exclusion. In both cases, the parasitism was associated with a moderate reduction in support for coexistence (Table 1). In the pair showing the largest change in support for coexistence between parasitism and no-parasitism treatments (+32 percentage points, *D. sulfurgaster* and *D. birchii*; Figure 2b), the median value did not cross the boundary. Strong positive correlations between fitted parameters, led by relationships between R_{0i} and α_{ji} terms, resulted in non-independence of estimates of the fitness and niche differences and non-elliptical posterior distributions (Figure 2b,c).

The parasitism treatment reduced population growth rates, but we did not identify a reproduction rate–susceptibility trade-off across our six species (Figure 3). The pairwise relationship between reproductive rate and susceptibility to parasitism (proportional reduction in R_0) did not provide a useful heuristic in identifying the impact of parasitism on reducing fitness differences. Positive and negative reproduction rate–susceptibility relationships were both associated with increases and decreases in fitness differences.

4 | DISCUSSION

Our results demonstrate an inconsistent impact of natural enemies on the pairwise coexistence of a guild of competing species. *Drosophila* species showed differential susceptibility to parasitism, which manifests in shifts of the fitness differences in the coexistence plane. Natural enemies shift the fitness difference in favour of the species that is less susceptible, but this does not necessarily enhance coexistence. Across our six species, no general reproductive rate–susceptibility trade-off was observable and the presence of parasitoids did not lead to a consistent shift towards (or away from) a state of potential coexistence. At the pairwise level, reproductive rate–susceptibility relationships did not provide a useful guide to the impact of parasitoids on coexistence. This is in line with the results of Petry et al. (2018) where seed predators reshuffled the results of competition and emphasises the insufficiency of intrinsic fecundity as a substitute for actual competitive ability in practice. However, in contrast to Petry et al. (2018), we did not observe any cases of ‘overshooting’ the zone of coexistence and reversing the victor of competition.

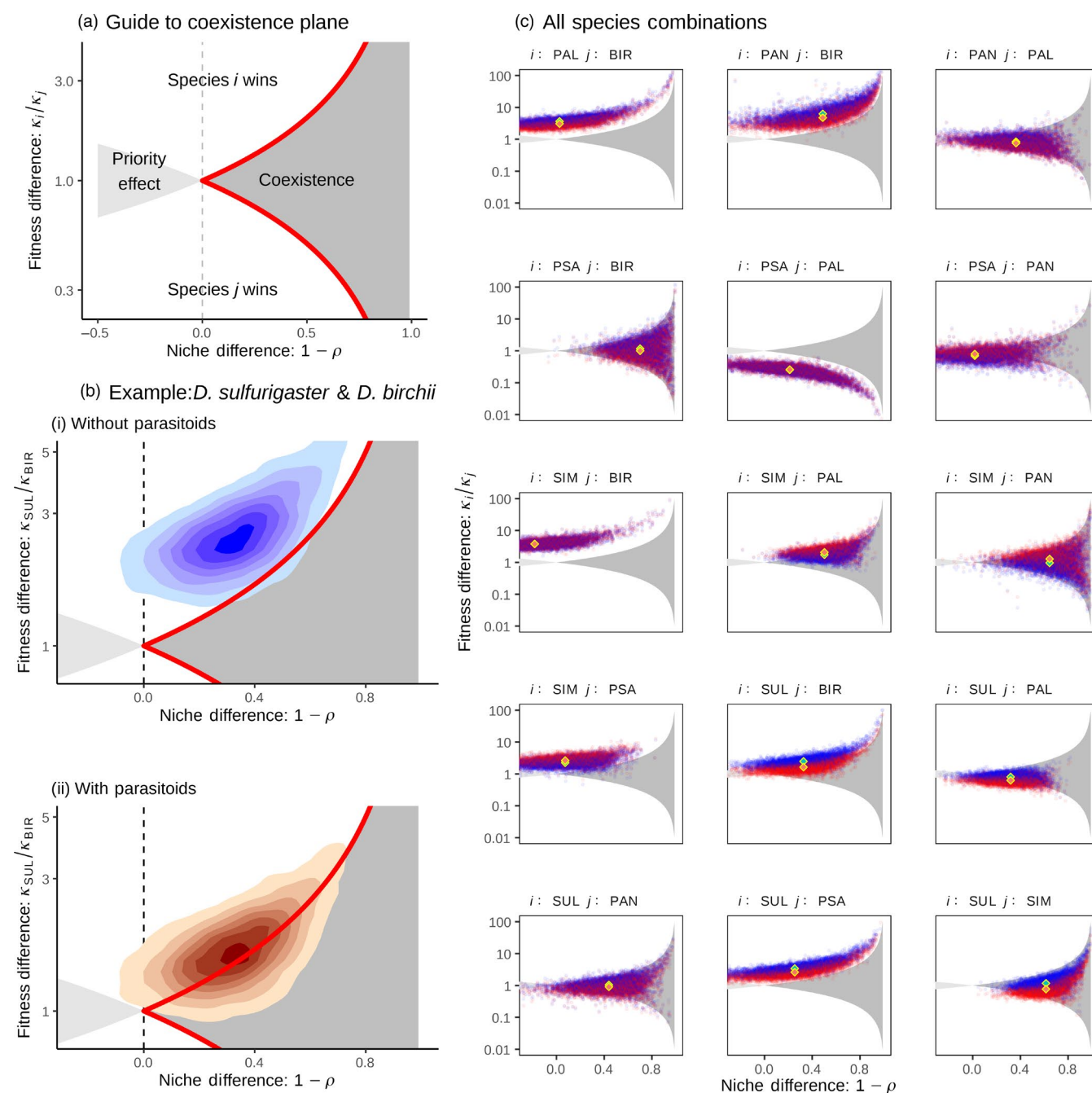


FIGURE 2 The likelihood of coexistence of *Drosophila* species pairs responds idiosyncratically to parasitism. (a) Guide to interpretation of coexistence plane diagrams. The region of coexistence (dark grey, bordered by red line) defines the region where either species could invade from rare. The pale grey area denotes the region of 'priority effects', where neither species could invade the other. The white area is where fitness difference is large enough that the dominant species can competitively exclude the other species despite their niche difference. (b) Full posterior distribution of niche and fitness differences of one example species pair (*D. sulfurigaster* and *D. birchii*) using the best-fitting model. Note the irregular, non-circular, shape ultimately derived from correlations between fitted values in the posterior distribution and alpha terms contributing to both niche and fitness differences. (c) Equivalent contour plots for all 15 species combinations. The median of the posterior distribution of parameters is shown with diamonds. Table 1 details the support assigned to each region for each pair under each treatment. Note that because the best model did not include varying competition coefficients (α) between treatments, only the fitness difference (y-axis) shifts between treatments

Fully quantifying pairwise competitive ability requires considerable effort (Hart et al., 2018) that scales quadratically with the number of species to be assessed. Since an increased fecundity increases competitive ability, taking shortcuts based on direct measured of

fecundity and susceptibility to determine the role of natural enemies is appealing. However, our results provide further evidence that this represents a dangerous trap, and there is no substitute to full characterisation of interspecific competition. Predation can only favour

TABLE 1 Summary of the posterior distribution across the coexistence plane between the two treatments for each species pair. The percentage of posterior draws that fall into the four major regions of the coexistence plane can provide a summary of the confidence in the outcome of a particular competition, given the data

Species <i>i</i>	Species <i>j</i>	Parasitoids present	Median niche difference ($1 - \rho$)	Median fitness difference ($\frac{\lambda_i}{\kappa_i}$)	Coexistence under median estimate	Percentage posterior support for regions of coexistence plane				Change in support for coexistence
						Species <i>i</i> wins	Species <i>j</i> wins	Coexistence	Priority effect	
<i>pallidifrons</i>	<i>birchii</i>	No	0.03	3.64		99.93	0.00	0.00	0.07	
		Yes	0.03	3.07		99.73	0.00	0.00	0.27	0
<i>pandora</i>	<i>birchii</i>	No	0.49	6.27		99.80	0.00	0.18	0.02	
		Yes	0.49	4.75		99.23	0.00	0.70	0.07	0.5
<i>pandora</i>	<i>pallidifrons</i>	No	0.36	0.84	True	9.32	20.97	67.68	2.03	
		Yes	0.36	0.76	True	5.13	30.18	62.62	2.07	-5.1
<i>pseudoananassae</i>	<i>birchii</i>	No	0.70	1.17	True	3.03	0.60	96.37	0.00	
		Yes	0.70	1.00	True	1.35	1.32	97.33	0.00	1
<i>pseudoananassae</i>	<i>pallidifrons</i>	No	0.21	0.25		0.00	99.75	0.03	0.22	
		Yes	0.21	0.26		0.00	99.78	0.05	0.17	0
<i>pseudoananassae</i>	<i>pandora</i>	No	0.02	0.70		6.93	61.57	20.15	11.35	
		Yes	0.02	0.79		10.92	49.73	23.58	15.77	3.4
<i>simulans</i>	<i>birchii</i>	No	-0.18	3.77		99.82	0.00	0.00	0.18	
		Yes	-0.18	3.79		99.88	0.00	0.00	0.12	0
<i>simulans</i>	<i>pallidifrons</i>	No	0.50	1.74	True	33.28	0.00	66.72	0.00	
		Yes	0.50	2.07		53.97	0.00	46.03	0.00	-20.7
<i>simulans</i>	<i>pandora</i>	No	0.64	0.96	True	2.25	3.30	94.45	0.00	
		Yes	0.64	1.28	True	8.35	0.87	90.73	0.05	-3.7
<i>simulans</i>	<i>pseudoananassae</i>	No	0.07	2.25		95.67	0.13	2.62	1.58	
		Yes	0.07	2.63		98.20	0.07	1.05	0.68	-1.6
<i>sulfurgaster</i>	<i>birchii</i>	No	0.33	2.48		95.47	0.00	4.38	0.15	
		Yes	0.33	1.63		61.45	0.73	36.42	1.40	32
<i>sulfurgaster</i>	<i>pallidifrons</i>	No	0.32	0.81	True	1.37	28.87	69.20	0.57	
		Yes	0.32	0.63		0.10	60.47	39.43	0.00	-29.8
<i>sulfurgaster</i>	<i>pandora</i>	No	0.44	1.04	True	6.57	11.27	81.52	0.65	
		Yes	0.44	0.90	True	2.30	17.48	79.92	0.30	-1.6
<i>sulfurgaster</i>	<i>pseudoananassae</i>	No	0.25	3.40		99.80	0.00	0.03	0.17	
		Yes	0.25	2.61		98.58	0.00	0.48	0.93	0.4
<i>sulfurgaster</i>	<i>simulans</i>	No	0.61	1.17	True	0.37	0.62	99.02	0.00	
		Yes	0.61	0.77	True	0.00	7.83	92.17	0.00	-6.9

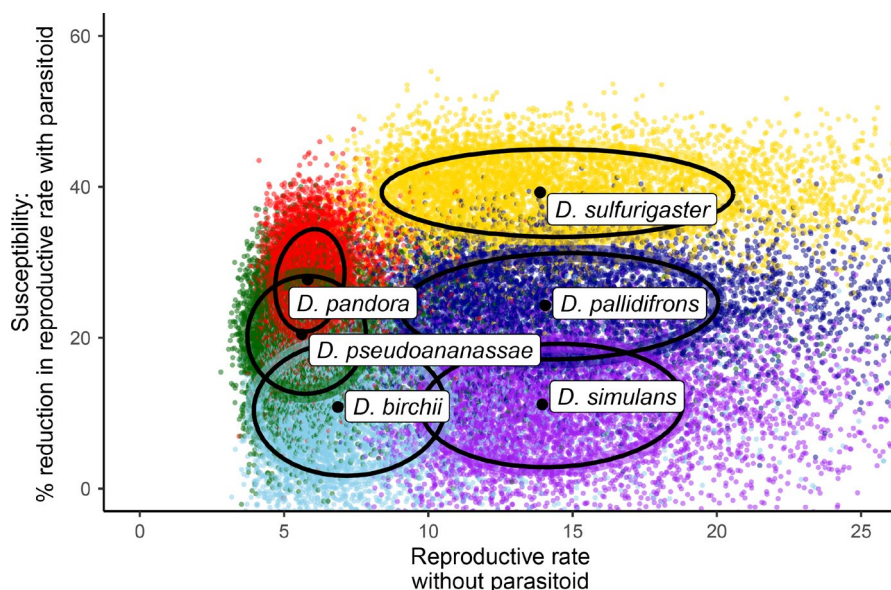


FIGURE 3 No clear reproductive rate–susceptibility trade-off across the six *Drosophila* species. Median values for each species (large black points) are plotted alongside a posterior distribution point cloud and fitted ellipse (60%, fitted assuming a multivariate t distribution) to show the distribution of uncertainty for each species

coexistence when the more susceptible species is otherwise competitively dominant, not just more fecund. This underscores the importance and challenges of accurately measuring competition terms (α) to understand the consequences of any given factor's influence on coexistence.

4.1 | Impact of enemies on competition coefficients

In natural systems, both direct effects of mortality and impacts on the interaction between species will determine the impact of consumers on species coexistence (Pringle et al., 2019). Our study design allowed parasitoids to travel between vials, opening the prospect of either associational susceptibility or resistance mediated through parasitoid foraging behaviour (Abrams, 2010; Holt & Kotler, 1987). If these competitive interaction modification effects were strong enough to be detectable, they would manifest as shifts in the fitted α values and change the niche differences between parasitism and no-parasitism treatments. However, our model selection procedure suggested that modelling changes to the competition coefficients matrix between treatments leads to overfitting, that is, there was no identifiable impact of parasitism on pairwise competition coefficients. Resolving the relative importance of direct effects of predators versus their indirect effects on other species is a long-standing challenge (Werner & Peacor, 2003). Our study suggests that in this case the direct effects are dominant. However, mechanisms that can be modelled as shifts in single-species parameters (e.g. R_0) are inherently more identifiable statistically than changes to multi-species processes (e.g. α_{ij}) as they can be determined more directly from simpler experiments.

Nevertheless, our model selection test is a rather blunt all-or-nothing approach. It may well be that only particular competition coefficients are strongly affected by the parasitoid. Indeed, in our Model 1, while most of the between-treatment α differences

cluster tightly together, several show large divergences (Supporting Information 4, Figure S4.14). There is no clear predictor for the terms that show these large shifts, occurring between different species. A more complex model-fitting approach incorporating regularisation could potentially determine whether such cases are identifiable effects rather than model-fitting noise.

Our single-generation design meant that density-mediated apparent competition derived from an increased total population of natural enemies was not directly detectable. If we had run a multi-generational experiment, the results could be expected to be complex: Sommers and Chesson (2019) show that the relative balance between apparent and resource competition is influenced by prey defensive actions. Methods to compare resource competition and predation effects directly in parameterised models are being developed (Shoemaker et al., 2020) and will be an exciting area for future research.

4.2 | Model fitting considerations within coexistence theory

Recent meta-analyses have emphasised the importance of parameterised models for understanding mechanisms of coexistence (Broekman et al., 2019), particularly since large-scale global environmental changes will create novel communities (Alexander et al., 2016). Modern coexistence theory defines a sharp line between coexistence and the inability to invade from rare. This mathematical clarity and precision represents a major advantage, but poses challenges to the incorporation of inevitable experimental uncertainty, even before comparison to field contexts. It is important to reiterate that the uncertainty of coexistence we discuss should be interpreted as statements of uncertainty in the underlying data, rather than implying a probabilistic outcome. The precision of estimates of parameters can differ between treatments, which can have the effect of changing the support for coexistence, even if the

central estimate does not change. Furthermore, these values should not be directly interpreted as some measure of 'distance from co-existence', although the concepts may be related. The framework of structural stability is better equipped to address such questions (Godoy et al., 2018; Saavedra et al., 2017).

Nonetheless, parameter fitting can have high data demands, and key ecological model parameters are often not fully separable (Bolker, 2008). For example, in a simple model of competition, similar predictions could be made by lowering the growth rate term and increasing the competition term. With a well-designed experiment and sufficient data, uncertainty can be minimised, but in any realistically sized competition experiment, separability of parameters can be a significant but largely underacknowledged issue. This problem is particularly pertinent when working within the framework of modern coexistence theory, where conclusions are drawn from comparisons between ratios composed of multiple parameters from the underlying model (Barabás et al., 2018; Song et al., 2019). Failing to address these issues could dramatically affect the confidence with which conclusions are drawn from experimental data.

Understanding how the interrelationships between parameters affect uncertainty in the derived quantities based upon them is difficult a priori, since the contribution of different correlations will be contingent on both the underlying ecology and the experimental design. In the context of modern coexistence theory, even a moderate confounding of underlying growth rates and intraspecific competition terms may have a significant effect on the conditions for coexistence. In our study, the posteriors were very often curved—posterior draws generating large niche differences were associated with larger relative fitness differences. This has the beneficial effect of often increasing the confidence with which pairs of species are located within regions of the coexistence plane. Although our individual estimates of model parameters may be rather uncertain, we can often be comparatively confident in the result of competition.

This effect can be attributed to indeterminacy, and hence correlation in the posterior, between growth rates and intraspecific competition terms. One consequence of this can be seen clearly when the condition for coexistence (Equation 2) is re-arranged in a simplified form as:

$$\frac{\alpha_{ji}}{\alpha_{ii}} < \frac{R_{0j} - 1}{R_{0i} - 1} < \frac{\alpha_{jj}}{\alpha_{ij}}. \quad (5)$$

In our data, there are strong positive correlations in the posterior draws of α_{ji} and R_{0j} (highlighted). Hence, uncertainty would tend to affect both sides of the inequality simultaneously, increasing the tendency that it maintains its value.

Fitting parameterising models with ecological data is almost always both challenging and illuminating in equal measure. Because the fitted values of the parameters inevitably correlate to some degree, propagating parameter uncertainty from a multi-parameter posterior distribution gives a better understanding of the uncertainty associated with the key quantities (Mordecai, 2013; Uricchio et al., 2019). Multi-dimensional posteriors can pose considerable

challenges both to intuition and to visualisation. However, approaches that directly propagate standard errors associated with individual parameters (e.g. Matías et al., 2018) or estimate uncertainty by bootstrapping observations (e.g. García-Callejas et al., 2020) are unable to capture these effects, and risk misrepresenting the confidence that can be placed in results. Although we took an explicitly Bayesian approach using weak priors, similar methods could equally be followed using multi-dimensional likelihood surfaces computed with a frequentist approach.

4.3 | Limitations of simple laboratory experiments

The modern coexistence theory approach is agnostic with respect to the particular factors involved but will always be limited by the scope of the empirical data available. Experiments such as this one can rarely replicate full natural life cycles of animals or capture the full range of conditions that occur throughout their habitat (Hart et al., 2018). Impacts on one life stage can mitigate differences at another (Moll & Brown, 2008) and our method does not capture differences in reproductive life span. Equally, our experiment uses a highly simplified representation of the consumer pressure experienced by natural communities of *Drosophila*. We included a single pupal parasitoid species; in their natural habitats, these species exist in a complex trophic network (Jefferies et al., 2021) including specialist parasitoids and larval parasitoids (which may directly influence larval competitive performance).

Coexistence may only be possible at particular spatial scales (Hart et al., 2017), or through spatial mechanisms not reflected in our experiment. The laboratory experiments cannot capture environmental heterogeneities hypothesised to aid coexistence (Chesson, 2000; Kuang & Chesson, 2010). Despite these limitations, we believe that our experiment is still instructive in isolating particular aspects for further investigation.

The *Drosophila* species we investigated co-occur in their natural habitat of Australian rainforest (Jefferies et al., 2021), although it is important to note that this is not necessarily evidence that they can coexist in the sense of mutual invasibility (Siepielski & McPeck, 2010). Furthermore, pairwise coexistence is not the same as community-level coexistence, and coexistence or otherwise between pairs does not necessarily translate to the multi-species community (Song et al., 2019), although they are likely to be at least somewhat related (Broekman et al., 2019; Friedman et al., 2017). A diversity of mechanisms can allow multi-species coexistence where pairwise coexistence is not possible (Letten & Stouffer, 2019; Levine et al., 2017; McPeck, 2019).

5 | CONCLUSIONS

Our experiment demonstrates that natural enemies can change the result of competition between species pairs, but that this effect is not consistent or necessarily predictable from pairwise reproduction rate–susceptibility relationships, reiterating the value of

analysing parametrised models (Siepielski & McPeck, 2010). Further work will be needed to investigate whether trait differences impact the niche and fitness differences observed in our system. More experimental studies of the impact of natural enemies on coexistence are needed to form the basis for a more general understanding of their impacts beyond plants (Viola et al., 2010), but we did not find evidence to support the concept that generalist enemies mediate community coexistence. Modern coexistence theory is emerging as a powerful tool to investigate a range of biotic and abiotic impacts on coexistence experimentally (Lanuza et al., 2018; Matías et al., 2018). However, since the precision with which growth rates and competition coefficients can be estimated is limited (even within large studies such as ours), the fundamental inseparability of niche and fitness differences can impact the likelihood assigned to coexistence between any given species pair. Although these challenges are significant, we hope our approach demonstrates that they can be readily and transparently addressed.

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AUTHORS' CONTRIBUTIONS

J.C.D.T. conceptualised the experiment, led the analysis and wrote the initial draft; J.C. led the design and execution of the experimental work. Experimental work was conducted by J.C. and J.C.D.T. All authors contributed to the development of ideas and writing of the manuscript.

DATA AVAILABILITY STATEMENT

All raw observation data and code to replicate the analyses are archived on Zenodo <https://doi.org/10.5281/zenodo.4736655> and at https://github.com/jcdterry/TCL_DrosMCT (Terry et al., 2021).

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SUPPORTING INFORMATION

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

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6

General Discussion

Graphical Abstract

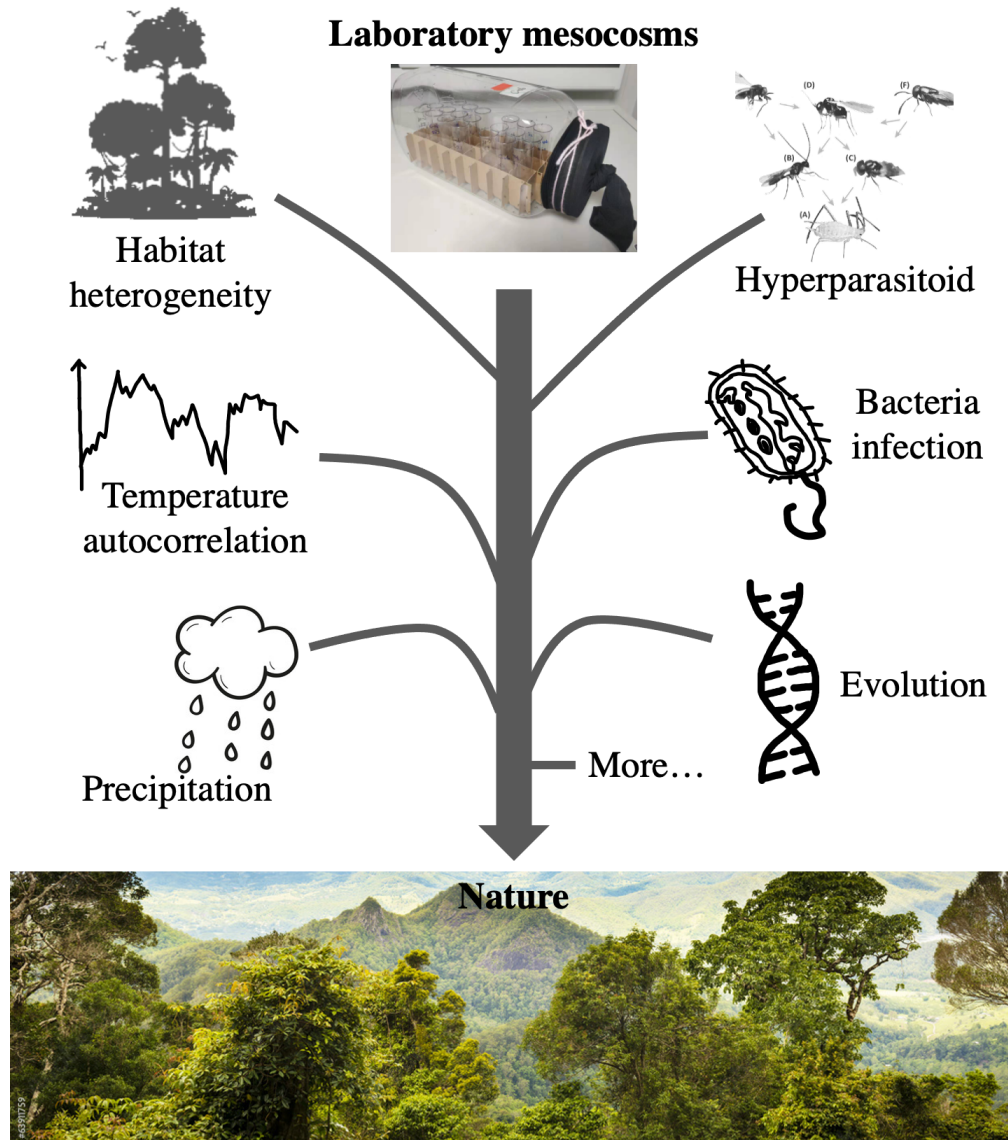


Figure 6.1 Factors and processes to consider when generalizing results from laboratory mesocosms to phenomena in natural systems. Sources of images: habitat heterogeneity and Queensland rainforest (Adobe image stock); hyperparasitoid (Hrček and Godfray, 2015).

This dissertation aims to understand the thermal reactions of individuals, populations and communities, as well as the ecological principles behind the disparities in responses at various biological levels. My research utilised a highly versatile experimental system consisting of rainforest *Drosophila* and their natural enemies, parasitoid wasps. The system enabled detailed examinations of their distribution patterns and interacting networks along an environmental gradient (Jeffs et al., 2021). In addition, the majority of *Drosophila* species and several parasitoid species could be raised in the laboratory and subjected to measurements and manipulations. This bi-trophic system with several hosts and various parasitoid species allows me to delve into the complexities of climate change's direct and indirect biological effects. Here, I will first summarise the key findings and implications of the preceding chapters, followed by a discussion of the limitation and potential directions for enhancing our knowledge of the biodiversity impact of climate change.

6.1 Summary of findings

6.1.1 Mechanistic drivers of distribution

Understanding the mechanistic causes of species distributions is critical for predicting current range and prospective range shifts. In **Chapter 2**, I discovered that, contrary to the popular idea, the warmer boundaries of the *Drosophila* species on the Wet Tropics mountains were set by their reproduction limits in high temperatures, and competitive exclusion by upland *Drosophila* explains why lowland species are not or seldom recorded at high elevations. Based on this knowledge, I expect that rising temperatures at high elevations will put upland species under physiological stress, diminish their competitive ability, and thus allow lowland species to establish populations at higher elevations. In **Chapter 4**, I attempted to investigate which mode of rising temperature that species distribution responds the most to. I demonstrated that, given the same amount of cumulative temperature increase (+1°C per day), high-temperature extremes for a short period (defined as heatwaves) are more likely to promote the establishment of lowland species within upland communities. In contrast, an even but moderate increase in temperature (defined

as warming) would neither assist the range expansion of lowland species nor would it affect the composition of the upland *Drosophila* community, independent of the presence of lowland invaders.

Chapter 2 implies, and **Chapter 4** validates, that the distribution of our tropical *Drosophila* system is susceptible to global warming, particularly to changes in high-temperature extremes. Consistent with our work, it has been demonstrated that extreme events abruptly modify population sizes by affecting movement, recruitment, and the biotic environment (Ma et al., 2021; Smale and Wernberg, 2013; Tanaka et al., 2021; Wethey et al., 2011). The modification may be long-lasting (Smith et al., 2019), particularly when extremes cause a permanent ecological or ecosystem shift (Beaugrand et al., 2008; Thibault and Brown, 2008), such as the establishment of non-native species (Diez et al., 2012; Jiménez et al., 2011; Sorte et al., 2010). The finding suggests a re-examination of predictions and conclusions drawn by comparing species distributions and average temperatures. As heatwaves become more common across the world, management and restoration practices should begin to target the urgent ecological consequences of climatic extremes.

While **Chapter 2** and **Chapter 4** focus on the reasons why some species do not exist in certain abiotic conditions, they did not fully explain how some co-occurring *Drosophila* coexist on a very similar resource. Interactions with natural enemies can influence competitive relationships. In **Chapter 5**, we used experimental mesocosms and modern coexistence theory to quantify the impact of parasitism by a generalist parasitoid on pair-wise competitive coexistence. We demonstrated that a generalist parasitoid altered coexistence patterns in both directions by influencing fitness differences between competing pairs, as opposed to niche differences. Our results reject the idea that generalist natural enemies always promote competitive coexistence by disproportionately affecting species with higher reproductive rates; nor does the reproduction – susceptibility trade-off provide a useful shortcut for determining the effect of parasitoids on coexistence.

6.1.2 Parasitism mediates the impact of high temperatures on populations and communities

In **Chapter 3**, I studied how parasitism modified the demographic effects of a heatwave on host populations. As already demonstrated by previously published research (Abram et al., 2019), parasitoid infection in my study system also caused some hosts to die without resulting in successful development of parasitoids. Moreover, I established that this mortality was dependent on the nutritional (protein resource) state of the hosts and that the most impacted individuals overlapped with those who would die from a heatwave had one occurred. Therefore, exposure to parasitoids mitigated the impact of the heatwave on native host populations, even for *Drosophila* species on which there is no record of parasitoids developing successfully. Characterizing the associated intra-specific variations in defensive traits is a crucial step that links organism-level thermal response (often measured on laboratory-cultured populations with low genetic variations) to the realistic impact of multiple stressors on field populations.

Chapter 4 examined not just the reaction of species distribution to heatwaves and warming, but also the interactive effects of heatwaves, warming, and novel species on the resident community. The investigation revealed two temperature-sensitive interaction motifs, both involving parasitoids, that modulate the structural changes of communities in response to abiotic and biotic treatments. Both modes of rising temperature hampered the ability of parasitoid wasps to promote co-existence between dominant and inferior competitors. Furthermore, the novel species' interference with parasitoid wasps increased as a result of the heatwave-facilitated establishment, leading to an increase in both invaders and resident hosts. This study emphasizes that parasitoids' responses to external stressors (such as high temperature or biological invasion) have a significant influence on the host-parasitoid network.

6.1.3 Missing trade-offs

Trade-offs have been suggested as the major mechanism that promotes diversity and mediates species turnover along environmental gradients (Kraaijeveld and Godfray, 1997; Mauro et al., 2021). Surprisingly, I did not observe any trade-off between key traits studied in this dissertation. We did not find in **Chapter 2** any significant correlation between tolerance to low and high temperatures among nine *Drosophila* species. Comparing six *Drosophila* species, we found no indication of an overall trade-off between reproductive rates and susceptibility to parasitism in **Chapter 5**. In **Chapter 3**, I again found no sign of trade-offs between allocating protein resources for tolerance to heatwaves or defence against parasitoids. Contrary to our expectations, our data indicate that when given abundant dietary protein, individuals with low susceptibility to parasitoids were also likely to die from a heatwave (positive association).

Notably, the sample sizes for testing correlations in **Chapters 2** and **Chapter 5** were small. As a result, any conclusion on the correlations between traits in different species is tentative. Nevertheless, my thesis offers a rare example of studying the variations and associations among various important ecological traits of the vast majority of species found in a real *Drosophila* community. More studies that evaluate multiple traits of a whole guild will be tremendously valuable to reveal the true prevalence of trade-offs in natural systems.

6.2 Future directions

Controlled mesocosm experiments have provided significant insight into the response of ecological networks to climate change (Stewart et al., 2013). Nevertheless, due to logistical constraints, mesocosm experiments typically have a brief duration, simplified habitats and communities, and low initial genetic diversity. As discussed below, these factors contribute to the general limitation of my experimental approaches. In addition, they highlight promising areas of focus for future research.

6.2.1 Heterogeneous habitats and behavioural thermoregulation

Homogeneous culture environments in my laboratory experiments could limit individuals' capability of behavioural thermoregulation. The experimental temperatures were distributed evenly across space, as the temperatures were provided by incubators or water baths. However, in a natural environment, heat sources are directional and temperatures are spatially heterogeneous due to the complex surface structures provided by vegetation (Morecroft et al., 1998). A broad breadth of temperatures, ranging from several to above ten degrees celsius, is often available to individuals within a small spatial coverage (Goller et al., 2014; Porras et al., 2020). These differences often exceed most projected levels of global warming (IPCC, 2022) or the difference in upper thermal limits of all co-existing species (such as Chapter 2 shown). On a heterogeneous landscape, animals are able to actively choose when and where to forage, rest, and reproduce, thereby altering the surrounding temperature in which these activities are conducted (Kearney et al., 2009). Such thermal regulation based on behaviour has been demonstrated to be critical to species distribution (Guo et al., 2020) and to buffer the negative impact of a rising air temperature (Sunday et al., 2014). Therefore, using the breadth of air temperature within the range of species distribution to represent the realised thermal niche of species is problematic (Buckley and Kingsolver, 2021), particularly when comparing species with different capabilities in behavioural thermoregulation (Willott, 1997). In addition, behavioural thermoregulation can modulate the strength of species interactions, allowing spatial or temporal partitioning based on thermal tolerance (Bestelmeyer, 2008; Porras et al., 2020; Verdú et al., 2022). Failure to account for this behavioural process will result in an overemphasis on the direct effect of species interactions.

6.2.2 Plasticity of thermal tolerance

Thermal tolerance is a plastic trait influenced by the thermal environment experienced before (Hoffmann et al., 2003). In our experiments, the temperatures

were regulated in steps, with each step containing a long period of constant temperature (i.e. day, night). However, the field temperature is constantly changing, exhibiting strong autocorrelation patterns across temporal scales (i.e. daily, seasonal, inter-annual) (Dillon et al., 2016). The thermal range of an organism changes significantly if it has been exposed briefly (minutes to hours) to a sublethal high temperature (defined as hardening) or for an extended period (i.e. days, weeks, developmental stage) within the normal viable temperature range (defined as acclimation) (Hoffmann et al., 2003). Therefore, a gradually increasing mean temperature assay will likely result in a higher and more realistic thermal tolerance than a laboratory measurement based on a stepwise increase over a relatively shorter period (Terblanche et al., 2011).

However, the contribution of thermal plasticity to buffer the effects of climate change is still up for debate. (Sørensen et al., 2016) found no systematic patterns between the acclimation capacity and the climatic conditions of the origin, nor does the degree of plasticity predict the current distribution. The basal tolerance – plasticity trade-off, observed in many cases, predicts that species that already adapted to high-temperature environments will not be able to rely on phenotypic plasticity to tolerate more warming (Gerken et al., 2015; van Heerwaarden and Kellermann, 2020). To conclude, what is the limit of plastic thermal responses and what constrains its evolution are still the key questions in predicting whether species can tolerate and/or adapt to global warming (van Heerwaarden and Kellermann, 2020).

6.2.3 The dynamics within the parasitoid community

As Chapter 4 shows, changes in the abundance of parasitoids influence the structure of the community. In addition to demographic changes, the distribution and interactions of multiple parasitoid species, while largely ignored in my dissertation, are also crucial factors affecting the responses of the whole host-parasitoid network to climate change.

The distribution of parasitoids is likely to change when their co-occurring hosts are shifting. However, we lack sufficient information to determine the distribution

patterns of the parasitoids in our system. Due to the large variations in the host range among parasitoids, it is difficult to investigate the contribution of parasitism to the distribution of host communities without characterising the distribution patterns of different parasitoid species. Meanwhile, very little research has revealed the pattern of parasitoid range shift (Delava et al., 2014; Jeffs and Lewis, 2013). Theoretically, higher trophic levels, particularly parasitoids with a more specialised host range, are less likely to track the range set by the changing climate and host range shift (Jeffs and Lewis, 2013; Schweiger et al., 2008; Travis, 2003). Empirical examinations like Menéndez et al. (2008) studying the spatial mismatch of different trophic levels are still rare. Studying the distribution of parasitoids is difficult partly because of the low detection rate of a parasitism event by manual observation. Notably, the rise of molecular methods (e.g. metabarcoding) and its potential for automation can significantly increase the sample size and allow for standardised evaluation of the relative abundances and parasitism rates of various parasitoid species across space (Jeffs et al., 2021).

The effects of parasitoids in my dissertation were mostly examined in isolation from other parasitoid species. However, the strength of host-parasitoid interactions is often influenced by the competition, interference or facilitation between parasitoids (Cusumano et al., 2016). Super-parasitism (more individuals of a parasitoid species occur in the host than can develop to maturity) and multi-parasitism (a host is infected by more than one parasitoid species) are widespread phenomena that influence host mortality and parasitoid reproductive success (Thierry et al., 2022a). Moreover, complementarity and redundancy among parasitoid species are shown to enhance host suppression (Thierry et al., 2022a; Peralta et al., 2014). Nevertheless, the diversity-mediated effects on parasitoid performance are often context-dependent on the identity of co-occurring hosts and parasitoids (Thierry et al., 2022b). The influence of climate-driven reorganisation of parasitoid communities on their ecological functions is worth further investigation.

6.2.4 Additional players and realism

Mesocosms can only account for a limited number of abiotic conditions and interacting species. Inevitably, the species' or communities' responses to temperature in my laboratory experiments left out a variety of co-occurring changes in climate and biotic environment. Humidity, for example, is another important factor influencing *Drosophila* abundance between the rainy and dry seasons in the Wet Tropics. Water-stressed organisms were found to be less tolerant of high temperatures (Bubliy et al., 2012). Consequently, thermal tolerance is likely not static, but driven partly by the high seasonal variations in precipitation at our study sites. In addition, predators, hyperparasitoids, and other competitors that feed on rotten fruits can mediate more indirect effects of climate change on the composition of parasitoid wasps and *Drosophila* flies. Infection by bacteria, viruses, and parasites can also significantly alter the hosts' thermal performance (Hector et al., 2022; Porras et al., 2020). Through our controlled experiments, I was able to investigate the mechanisms by which temperature could influence populations or communities. To generalise an experimental result to the actual response of an ecosystem to climate change, however, requires a deeper understanding of the additional abiotic and biotic players of a particular system.

6.2.5 The potential for adaptive evolution

Evolutionary adaptation can ameliorate the predicted impact of higher temperatures based on species' current upper thermal limits. However, it is debatable whether and how quickly species can evolve in response to a changing climate. Higher individual thermal tolerance (e.g., higher CT_{max}, longer knockdown time by lethal temperatures) evolved under artificial selection for higher survival against lethal temperatures within only a dozen generations (Hangartner and Hoffmann, 2016; Hoffmann et al., 2003; McColl et al., 1996). On the other hand, for populations that naturally evolve in experimental warming, without artificial selection based on a single thermal trait, none of the upper thermal limits increased (van Heerwaarden and Sgrò, 2021). Moreover, few variations in high-temperature tolerance have

been observed in nature, indicating low evolutionary potential (Kellermann et al., 2009; Overgaard et al., 2011).

Species interactions in experimental populations or natural communities may constrain the evolution of higher heat tolerance, as one possible explanation. During population growth in a competitive environment, a trade-off between heat tolerance and competitive ability (intra- and inter-specific), if exists, can impede the adaptive evolution to higher temperatures. However, there is still a dearth of empirical data regarding the relationships between heat tolerance and species interactions. Although there have been numerous evolutionary experiments focusing on thermal traits, the measurements of tolerance and the evolution regimes vary very widely, making it difficult to draw general conclusions (Sinclair et al., 2016). The influence of ecological conditions (e.g. intra-specific competition, inter-specific competition, predation, and parasitism) on the evolutionary trajectory should be considered when designing an evolution experiment.

In addition to thermal tolerance, species interactions such as host-parasitoid interactions also have the potential to evolve within a few generations (Kraaijeveld and Godfray, 1997). As climate change facilitates the reorganization of host-parasitoid networks and creates novel species interactions, the evolution of host immunity, parasitoid virulence and parasitoid foraging behaviours could alter the initial impact of the novel species (Jones et al., 2015; Kruitwagen et al., 2022). Long-term experiments and careful monitoring of trait evolution are important to reveal the contribution of adaptive evolution in the dynamical response of ecological networks to climate change.

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Appendices



Supplementary Materials for Chapter 2

Table A.1: Isofemale lines used to construct mass bred lines of the rainforest *Drosophila* species.

Species	Origin of cultured lines (yes/no)				Lines for mass bred lines
	K low ¹	K high ¹	P low ¹	P high ¹	
<i>D. bunnanda</i>	yes	no	yes	no	KL87, KL134, KL127, PL114
<i>D. pandora</i>	no	no	yes	no	PL17, PL21, PL01 ²
<i>D. bipectinata</i>	yes	no	yes	no	KL84, KL43, PL85, PL20
<i>D. pseudoananassae</i>	yes	yes	yes	no	KL19, KH25, PL30, KH42
<i>D. sulfurigaster</i>	yes	yes	yes	yes	KL08, KH10, PL51, PH18
<i>D. rubida</i>	yes	yes	yes	yes	Construction unfinished ³ .
<i>D. birchii</i>	yes	yes	yes	yes	KL22, KH26, PL122, PH169
<i>D. pallidifrons</i>	no	yes	no	yes	KH20, KH69, PH183, PH184
<i>D. simulans</i>	no	no	yes	no	PL45, PL34, PL42, PL43
<i>D. pseudotakahashii</i>	no	yes	no	yes	Contamination ⁴

Note:

1. “K” is Kirrama, “P” is Paluma. “low” and “L” indicate low elevation sites. “high” and “H” indicate high elevation sites.
2. Only these three isofemale lines were available.
3. Mass bred line construction was not completed by the start of the thermal performance experiment. *Drosophila rubida* grew poorly on the fly medium, and had a significantly longer generation time than other species.
4. Because of contamination within isofemale lines, mass bred lines of *D. pseudotakahashii* were not available at the time of measuring thermal traits. Two isofemale lines were not contaminated and these were used to construct mass bred population for the later competition experiments.

Table A.2: Distribution patterns quantified by *hIndex* and by the effect (coefficient) of elevation on the likelihood of detecting each species. The effects of transect and the elevation X transect interaction are also included except for *D. bunnanda*, *D. pandora*, and *D.pseudotakahashii*, where it was not possible to include the interaction term and/or the transect term because of very skewed distributions (very few observations at one elevation in either or both mountain ranges).

<i>hIndex</i>	Species	Factor	Coefficient	P value
0	<i>D. bunnanda</i>	elevation	-64	0.99
0.07	<i>D. pandora</i>	elevation	-7.8	0.0017
0.12	<i>D. bipectinata</i>	elevation	-3.05	0.02
		transect	1.83	0.001
		elevation*transect	-6.22	0.02
0.40	<i>D. pseudoananassae</i>	elevation	-0.53	0.22
		transect	0.18	0.60
		elevation*transect	-4.82	<0.001
0.43	<i>D. rubida</i>	elevation	-0.77	0.056
		transect	-0.29	0.29
		elevation*transect	0.64	0.22
0.54	<i>D. sulfurigaster</i>	elevation	-0.91	0.54
		transect	0.11	0.90
		elevation*transect	-0.01	0.99
0.64	<i>D. birchii</i>	elevation	1.24	0.27
		transect	0.40	0.62
		elevation*transect	-0.94	0.51
0.76	<i>D. pallidifrons</i>	elevation	2.90	<0.001
		transect	0.44	0.39
		elevation*transect	-0.54	0.51
1	<i>D. pseudotakahashii</i>	elevation	12.5	<0.001

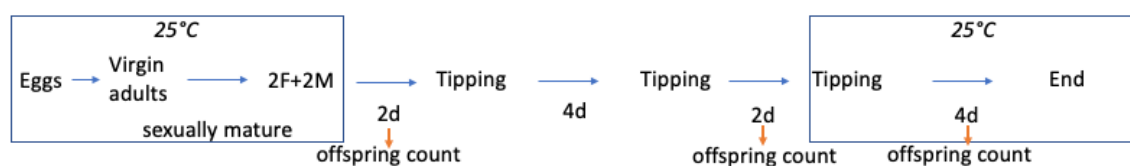
Table A.3: Pairwise comparison (Tukey test) of knockdown times at low temperature for the nine *Drosophila* species.

	Difference	ci_lower	ci_upper	Adjusted p value
BIR-BIP	-0.2857143	-1.2871803	0.71575178	0.99338903
BUN-BIP	-0.1190476	-1.1205137	0.88241844	0.99998982
MEL-BIP	7.28571429	6.28424822	8.28718035	0
PAL-BIP	-0.1428571	-1.1443232	0.85860892	0.99995836
PAN-BIP	0.64285714	-0.3586089	1.6443232	0.54218782
PSA-BIP	-0.3333333	-1.3347994	0.66813273	0.98184092
SIM-BIP	5.07142857	4.06996251	6.07289463	0
SUL-BIP	-0.547619	-1.5490851	0.45384701	0.74241446
BUN-BIR	0.16666667	-0.8347994	1.16813273	0.99986522
MEL-BIR	7.57142857	6.56996251	8.57289463	0
PAL-BIR	0.14285714	-0.8586089	1.1443232	0.99995836
PAN-BIR	0.92857143	-0.0728946	1.93003749	0.09367333
PSA-BIR	-0.047619	-1.0490851	0.95384701	0.99999999
SIM-BIR	5.35714286	4.3556768	6.35860892	0
SUL-BIR	-0.2619048	-1.2633708	0.7395613	0.99635753
MEL-BUN	7.4047619	6.40329584	8.40622797	0
PAL-BUN	-0.0238095	-1.0252756	0.97765654	1
PAN-BUN	0.76190476	-0.2395613	1.76337082	0.30147732
PSA-BUN	-0.2142857	-1.2157518	0.78718035	0.99912764
SIM-BUN	5.19047619	4.18901013	6.19194225	0
SUL-BUN	-0.4285714	-1.4300375	0.57289463	0.92008645
PAL-MEL	-7.4285714	-8.4300375	-6.4271054	0
PAN-MEL	-6.6428571	-7.6443232	-5.6413911	0
PSA-MEL	-7.6190476	-8.6205137	-6.6175816	0
SIM-MEL	-2.2142857	-3.2157518	-1.2128197	8.26E-10
SUL-MEL	-7.8333333	-8.8347994	-6.8318673	0
PAN-PAL	0.78571429	-0.2157518	1.78718035	0.2613271
PSA-PAL	-0.1904762	-1.1919423	0.81098987	0.99963326

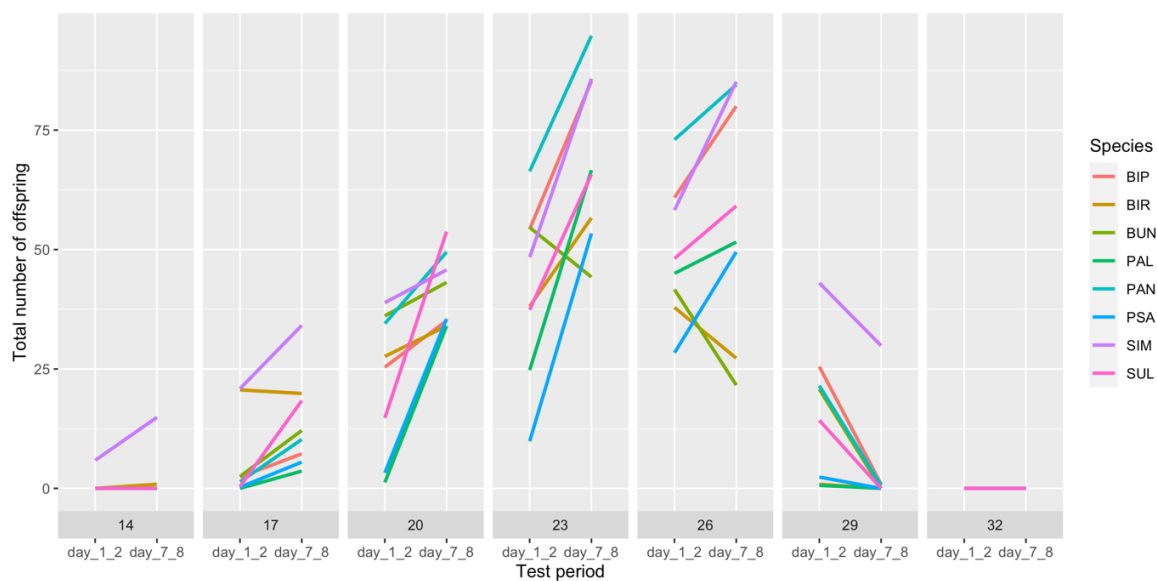
SIM-PAL	5.21428571	4.21281965	6.21575178	0
SUL-PAL	-0.4047619	-1.406228	0.59670416	0.94170483
PSA-PAN	-0.9761905	-1.9776565	0.02527558	0.06263078
SIM-PAN	4.42857143	3.42710537	5.43003749	0
SUL-PAN	-1.1904762	-2.1919423	-0.1890101	0.00732748
SIM-PSA	5.4047619	4.40329584	6.40622797	0
SUL-PSA	-0.2142857	-1.2157518	0.78718035	0.99912764
SUL-SIM	-5.6190476	-6.6205137	-4.6175816	0

Figure A.1: Measuring reproductive thermal performance. A) Procedures and schedule of measurements. B) Change in fecundity from 1st – 2nd day to 7th – 8th day at different temperatures. BIP = *D. bipectinata*, BIR = *D. birchii*, BUN = *D. bunnanda*, PAL = *D. pallidifrons*, PAN = *D. pandora*, PSA = *D. pseudoananassae*, SIM = *D. simulans*, SUL = *D. sulfurigaster*.

A)

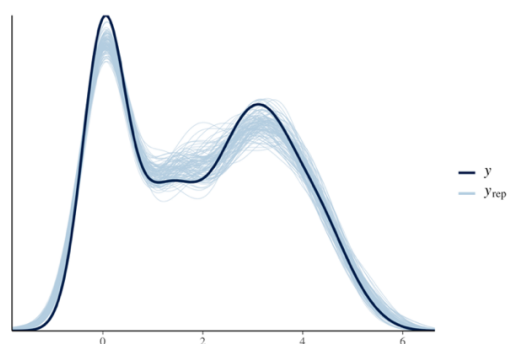


B)

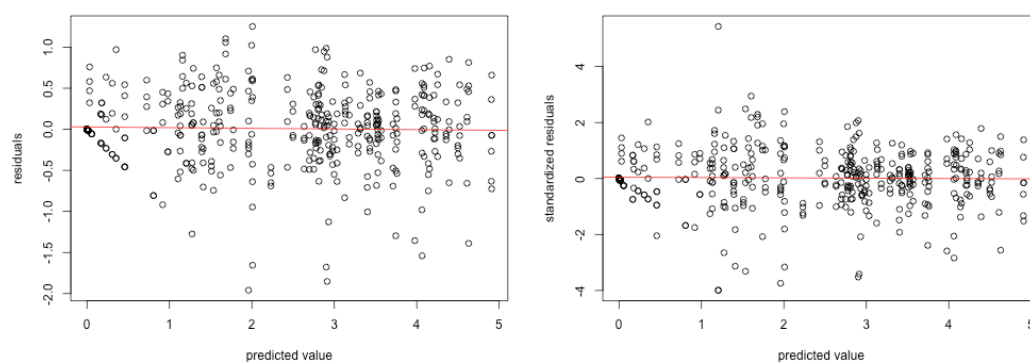


Figures A.2: Diagnostics of fitted thermal performance curves. A) Comparison of observed and fitted values of fecundity. B) Residuals and standardized residuals plot. C) Observed daily fecundity (points) and fitted thermal performance curve (blue line) for each of the nine species.

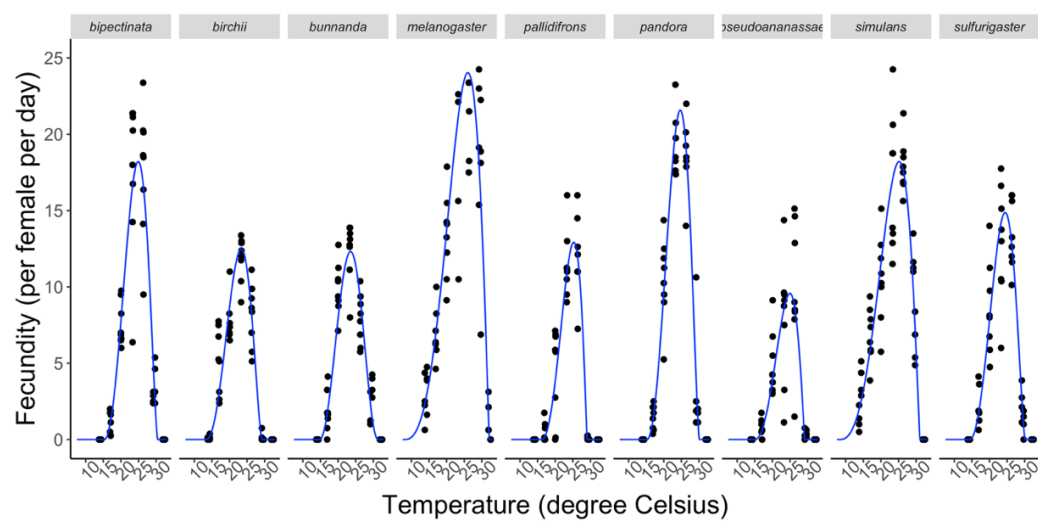
A)



B)



C)



Figures A.3: A) The phylogenetic tree of all *Drosophila* species mentioned in this study. B) The ultrametric phylogenetic tree of the seven species used in the regression analysis of thermal traits. C) The process to create the ultrametric tree from the DrosoPhyla dataset (Finet et al., 2021).

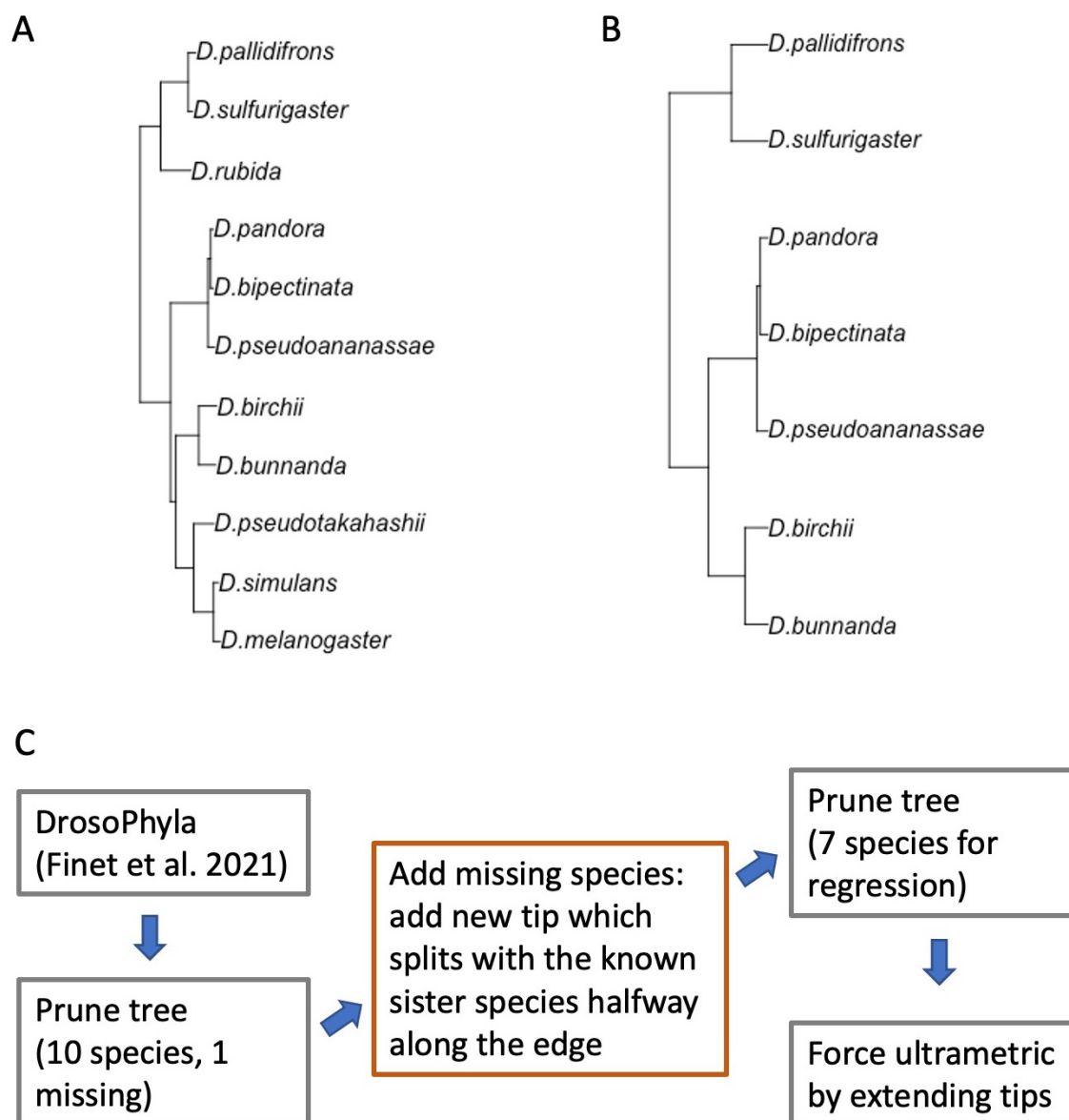


Figure A.4: Field and experimental temperatures of lowland (A) and upland (B) sites at Kirrama and Paluma in February 2017. Each grey line shows the hourly temperature on one day. The blue lines show the average temperature recorded at the time of the day. Temperature regimes used in short-term and long-term competition experiments are indicated by red lines, mimicking the day/night fluctuation of temperatures in the field.

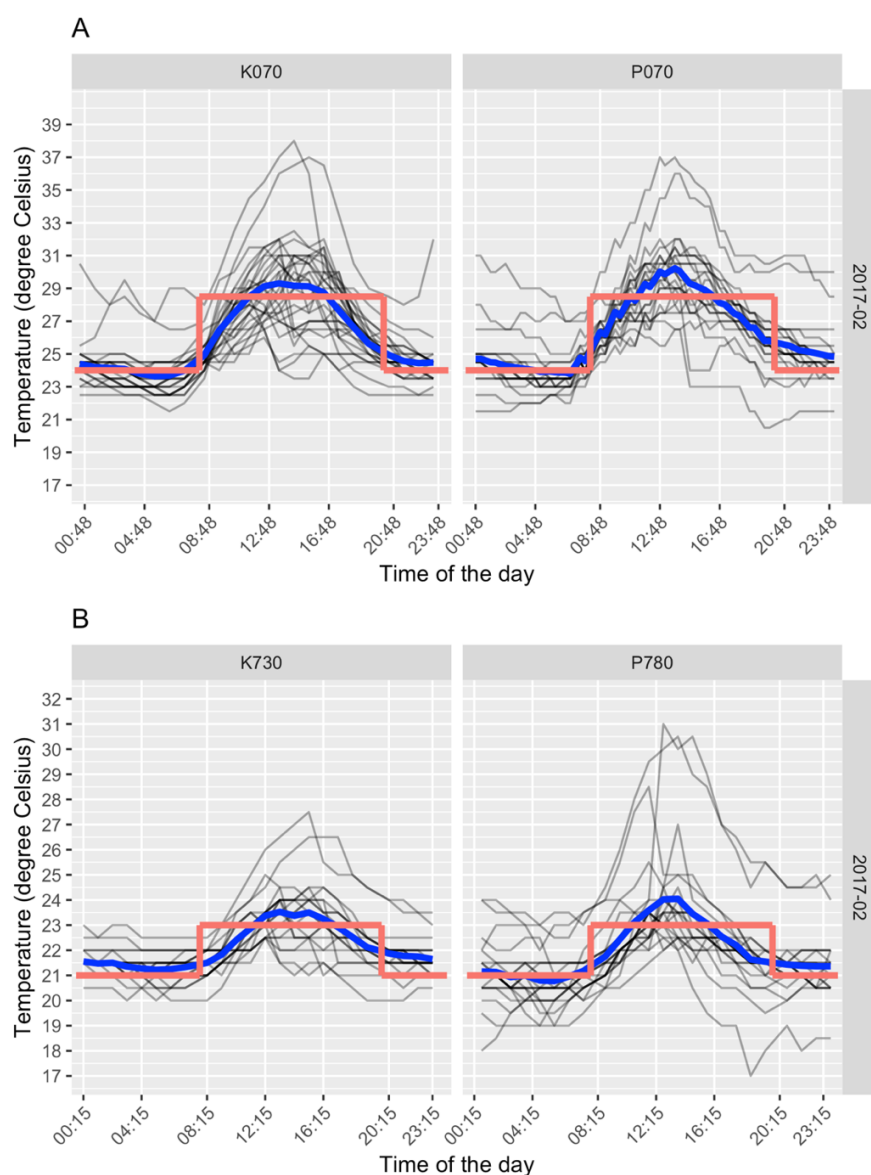
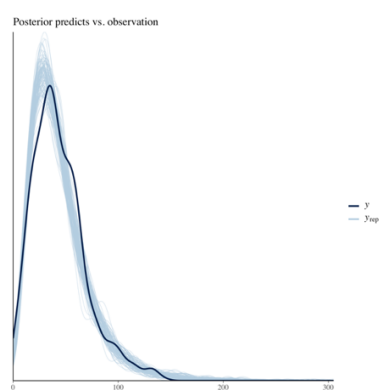
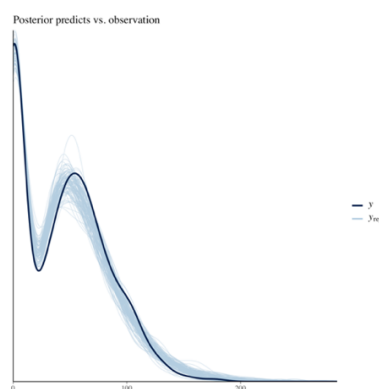


Figure A.5: Diagnostics of the fitted model for short-term competition (Beverton-Holt model) in the cool, upland (A) and warm, lowland (B) treatments, comparing the observed offspring counts and the fitted values. (C) Comparison of the observed population size and the fitted values from the regression for the long-term competition experiment.

A)



B)



C)

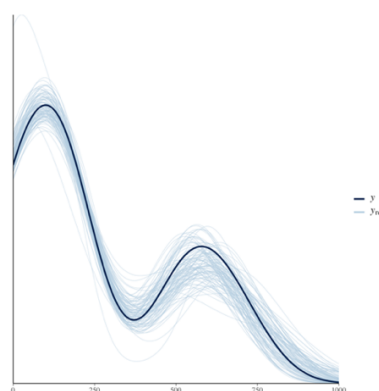
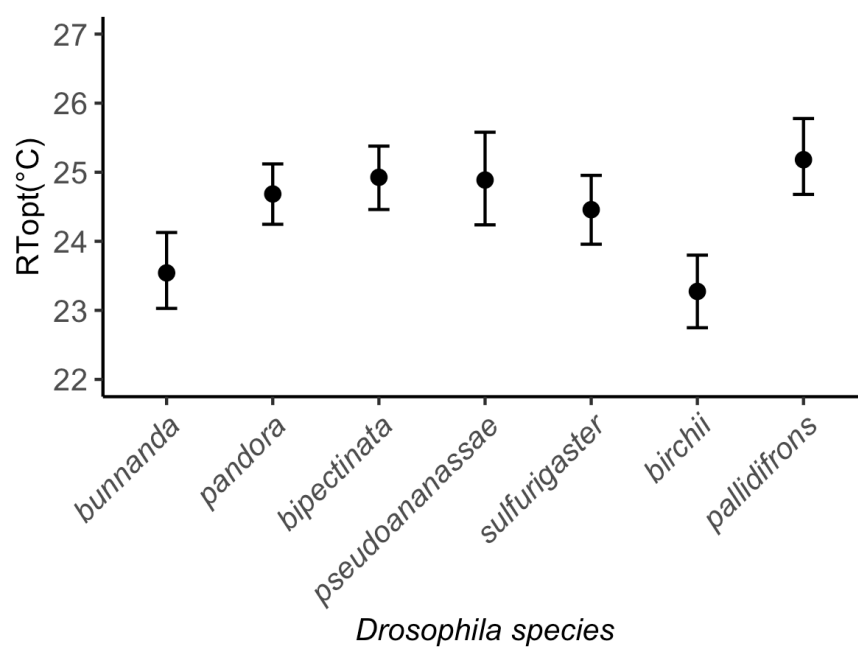


Figure A.6: A) RT_{opt} of each *Drosophila* species ordered by $hIndex$. B) Posterior distribution of RT_{max} and RT_{min} of each *Drosophila* species' thermal performance curve.

A)



B)

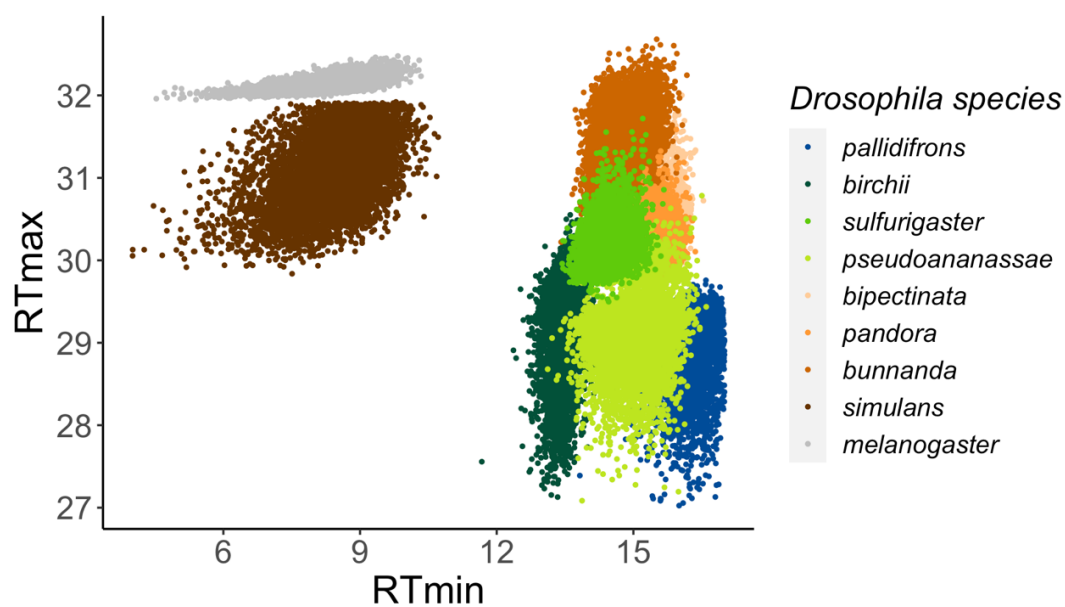
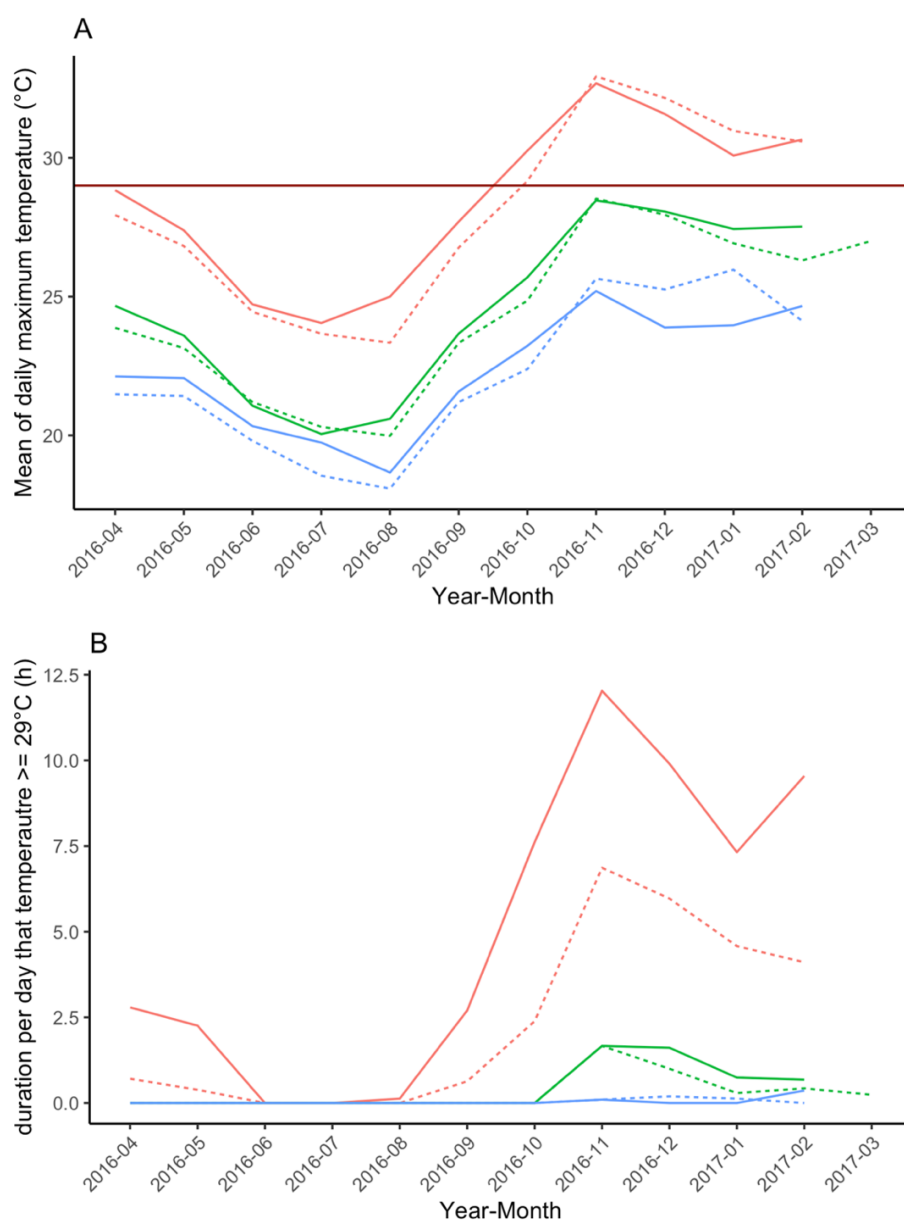


Figure A.7: A) Average of daily maximum temperature in each month. B) Average hours per day that the temperature exceeds 29°C (indicated by the horizontal dark red line), a known stressful temperature for reproduction. Blue, green and red colours indicate sites at high, medium and low elevations, respectively. Solid and dashed lines represent sites at Kirrama and Paluma, respectively.



B

Supplementary Materials for Chapter 5

SI 1 - Supplementary Methods

Chris Terry, Jinlin Chen & Owen Lewis

Species details

Prior to the start of the experiment, all isofemale *Drosophila* lines had been maintained in laboratory monocultures for 20-40 generations. Mass bred lines were constructed from isofemale lines and were maintained in large population cages for half a year. *Drosophila* medium contains 8% corn flour, 4% yeast, 5% sugar, 1% agar, and 1.67% methyl-4-hydroxybenzoate (concentration is measured by weight/volume of water).

Species	Code	Subgroup	Distribution	Wet Weight (mg)
<i>D. birchii</i>	BIR	montium	highland-biased	0.94
<i>D. pallidifrons</i>	PAL	nasuta	highland-biased	2.24
<i>D. pandora</i>	PAN	ananassae	lowland-biased	1.14
<i>D. pseudoananassae</i>	PSA	ananassae	lowland-biased	1.01
<i>D. simulans</i>	SIM	melanogaster	only found at one lowland study site	1.30
<i>D. sulfurigaster</i>	SUL	nasuta	widespread	2.64

Table SI 1. Details of *Drosophila* species used in laboratory experiment. Although all the species co-occur within their Australian rainforest habitat, different species are more abundant at particular elevations.

The parasitoid wasp used is as-yet unnamed (Lue *et al.* 2021), but belongs to the *Trichopria* genus (Hymenoptera: Diapriidae). Its Smithsonian National Museum of Natural History (NMNH) reference vouchers are USNMENT01557121, USNMENT01557273 and USNMENT01557254 (see also http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-AUSPTOID). The parasitoid has been maintained in laboratory monocultures (as lab strain 66LD) for 2 years after collection from the field from the same community as the hosts. During culturing, *Drosophila melanogaster* was used as a host species to reduce specific selection between the parasitoid and the experimental *Drosophila* species used in the laboratory.

Images of experimental set-up.



Figure S1.1 Individually plugged vials in which founder adults laid eggs and larvae competed. Vials were maintained in an incubator at constant temperature.



Figure S1.2 Vertical view of unplugged vials within parasitism boxes. Empty vials containing parasitic wasps were placed in the gaps and the wasps could freely move between the tubes within each box.



Figure S1.3 Parasitism boxes (with lids in place) were maintained in the incubator during the parasitism period.

Location Randomisation Procedure

Within the incubator, vials were arranged in a randomised, but structured arrangement (Fig. S1.4 and S1.5). The structure was necessary to facilitate handling. In all cases the principle that each vial had an equal change of being located at any point was maintained. Within each treatment, within each block, there was one tray for single-species assays, and two for multi-species assays. Species combinations were kept in rows, but the order of the numbers of each species used was randomised.

Unfortunately, a mistake led to parasitoid wasps being released into the a wrong box. Block C, Parasitoid(+) box number 2 did not receive parasitoids as planned, while Block C, Parasitoid(-) box number 1, did get parasitoids when it should not have done. This was corrected by reassigning vials to treatments, but had the result that certain combinations had one greater or fewer data point per treatment than was planned.

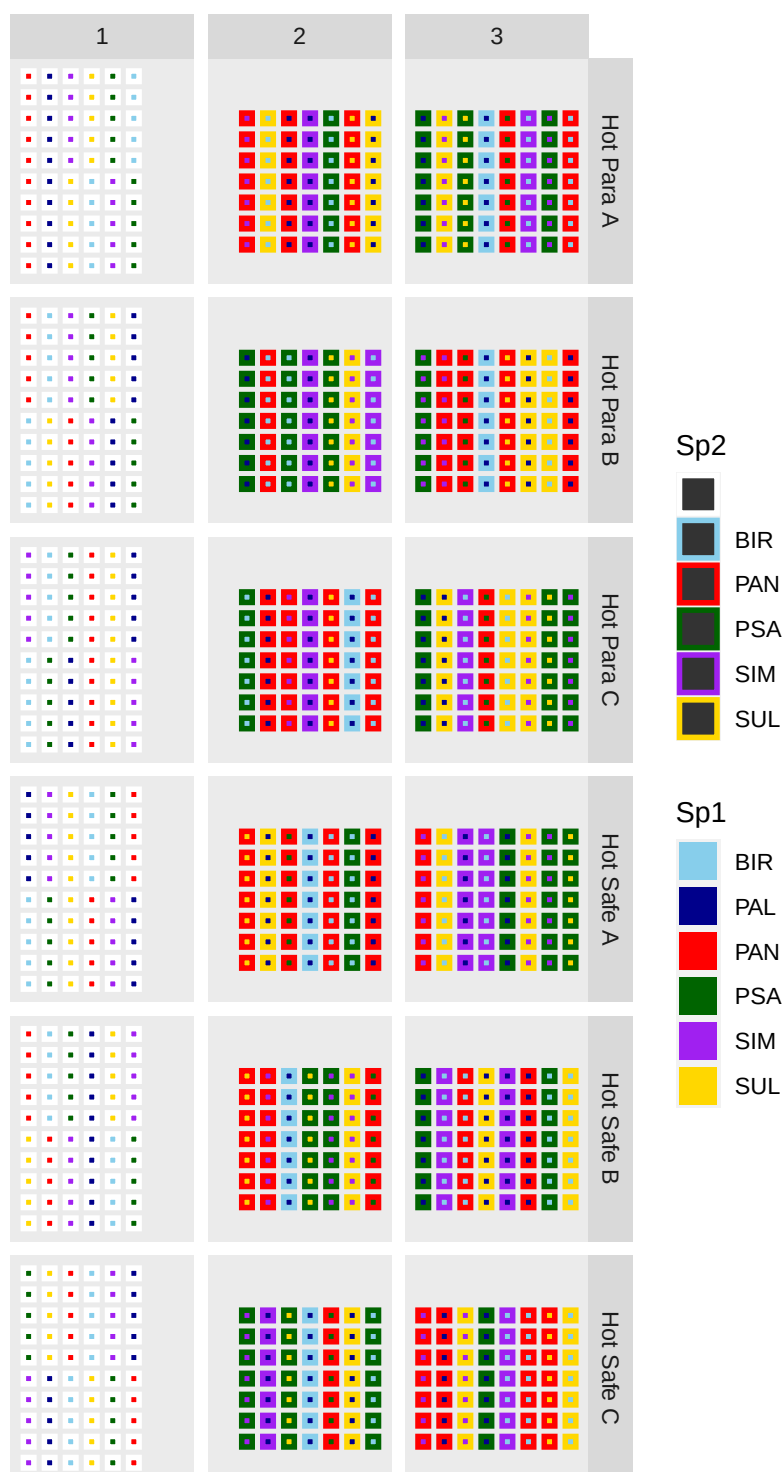


Figure S1.4 Randomisation of vials in trays by species combination for founding and larvae competition. Species combinations were maintained in lines to facilitate rapid handling. Within each sub-block, the order of the founder density levels was randomised. The distinction between species ‘1’ and ‘2’ is arbitrary.

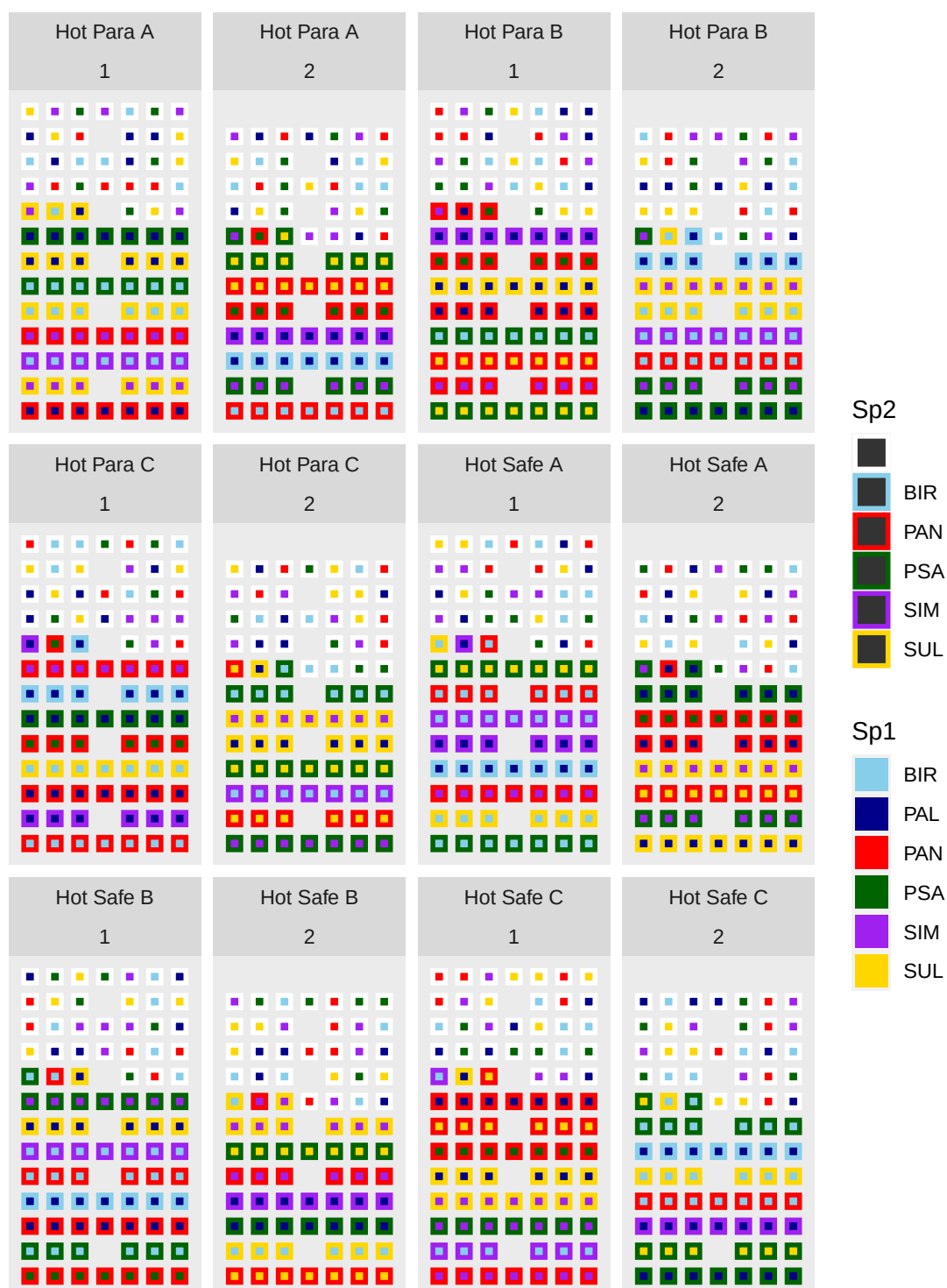


Figure S1.5 Randomisation of vials into parasitoid treatment boxes. For the multi-species vials, the order of the founder density levels was randomised within each line. The distinction between species ‘1’ and ‘2’ is arbitrary.

Temperature Treatment

Our initial experimental design included a low-temperature treatment (20°C). This followed identical procedures, although some incubation times were lengthened to account for slower development times. However, too few adult flies emerged in the 20°C treatment for reasonable identification of competition parameters and we do not consider this cold-temperature treatment further.

References

Lue, C.-H., Buffington, M. L., Scheffer, S., Lewis, M., Elliott, T. A., Lindsey, A. R. I., ... Hreck, J. (2021). DROP: Molecular voucher database for identification of *Drosophila* parasitoids. *BioRxiv*. <https://doi.org/10.1101/2021.02.09.430471>

SI 2 - Model Design - Allee Effect, Sex Ratio & Vial-Level Effects

Chris Terry, Jinlin Chen & Owen Lewis

Sex Ratio

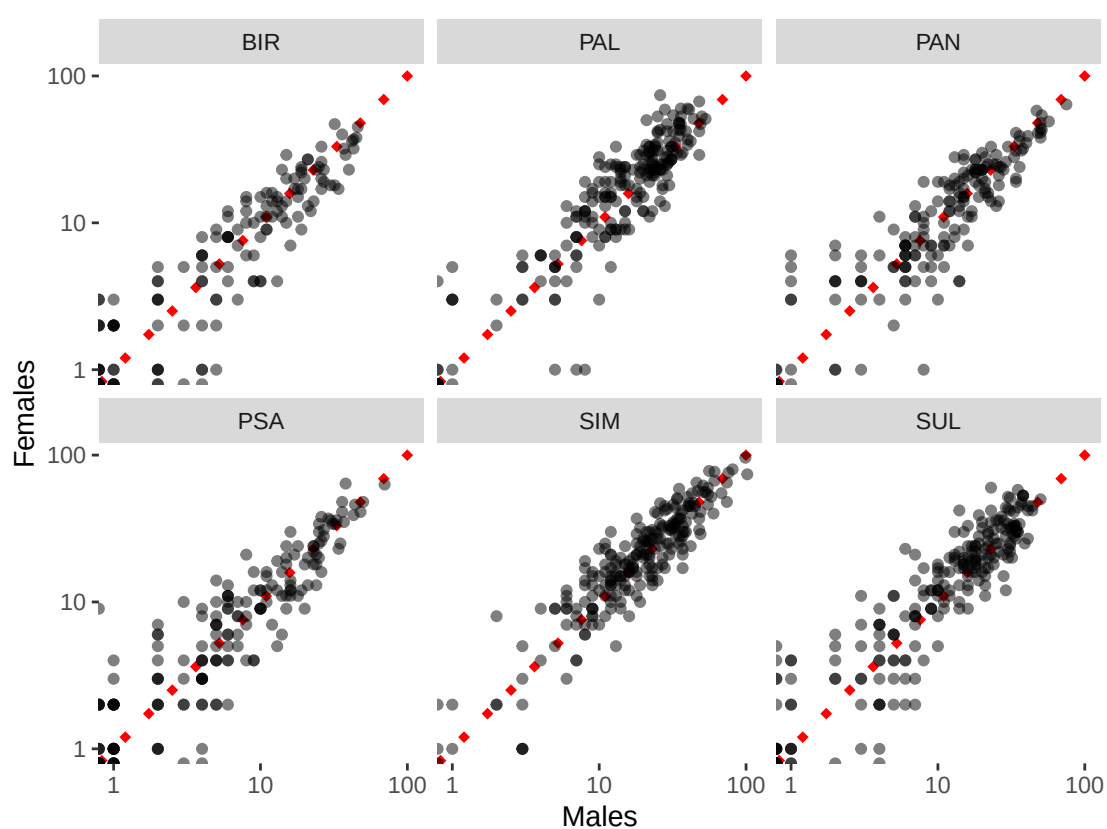


Figure S2.1. Consistent sex-ratio of emergent adults, faceted by species. Across all six species, a binomial GLM estimated only a very slight bias (β coefficient = 0.065) towards females.

Allee Effects

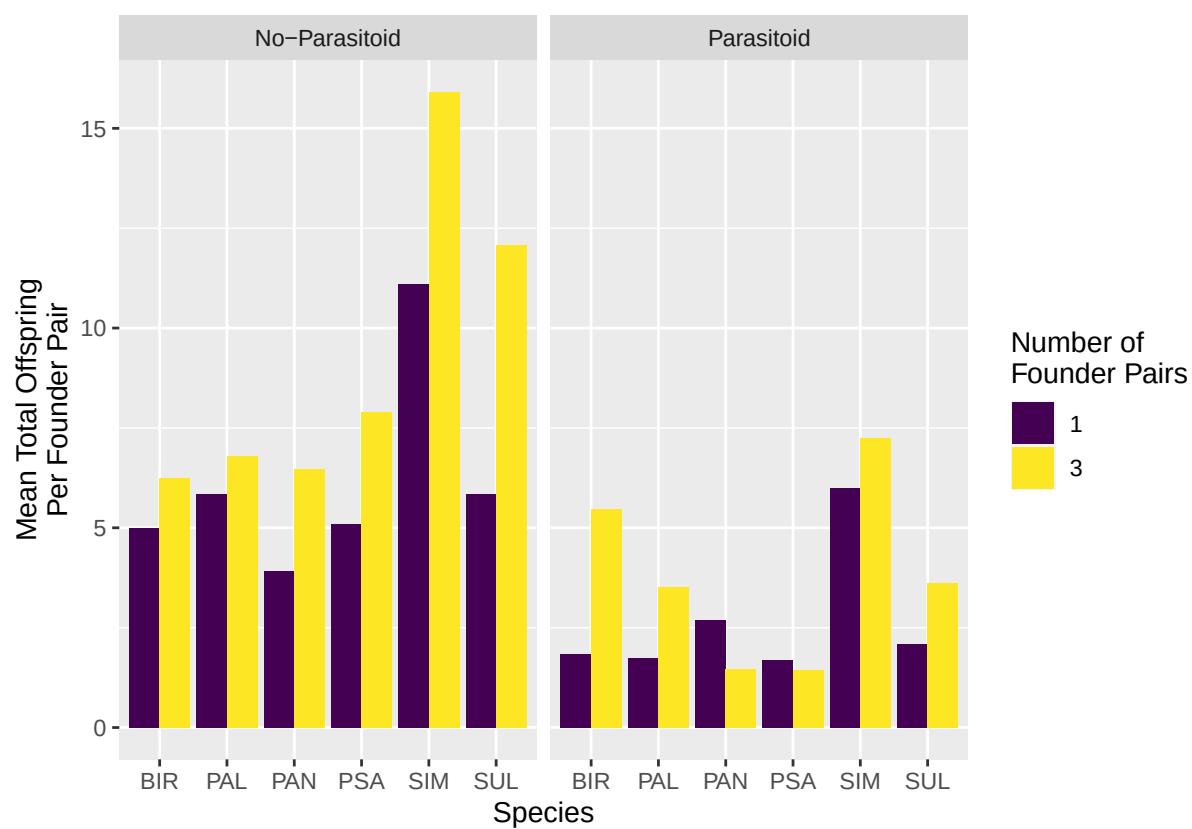


Figure S2.2. Per-female offspring is consistently lower for singleton founder pairs than triplets in the monoculture trials.

Vial level effects

As mentioned in the main text, because we take two measurements from each vial there is a possibility that this would lead to within vial non-independence in residual error of our models. Positive correlations could arise from unaccountable differences in the inherent suitability of vials, or negative within-vial correlations could derive from the competition between species. This would necessitate introducing a more complex error structure including group (vial) level structures. However, an inspection of the residual error of the selected model (Fig. 2.3) shows essentially no correlation in the residual error within the multi-species trial vials, justifying our use of a simpler model.

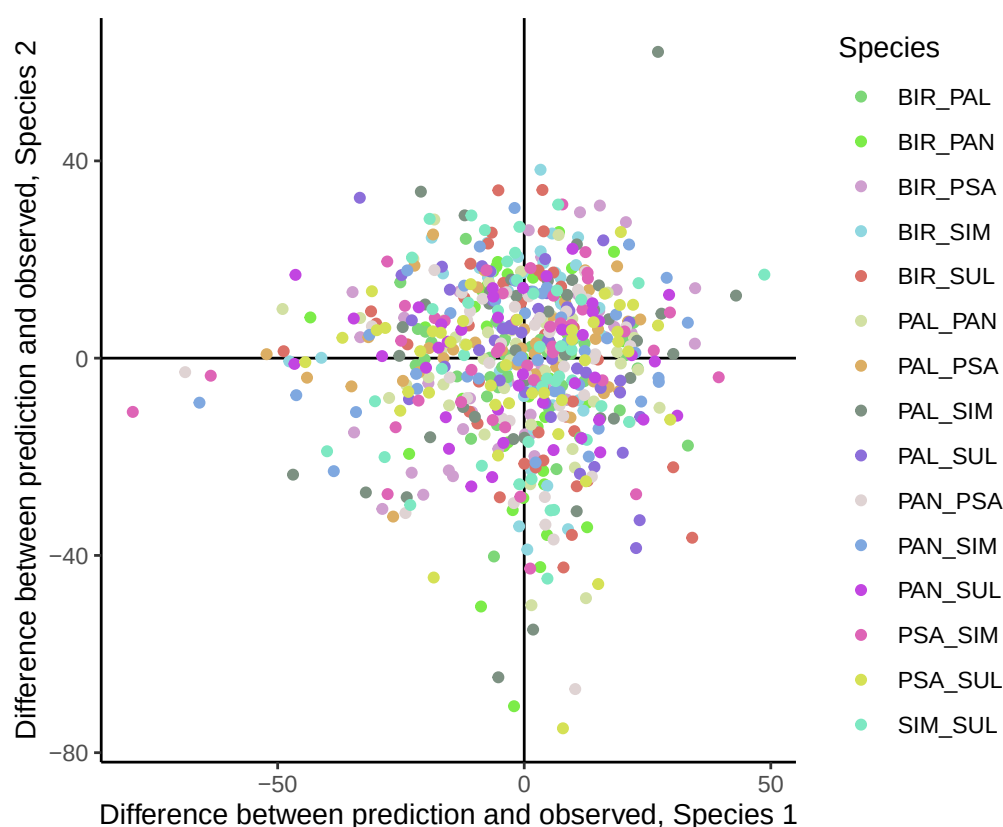


Figure S2.3. No within-vial correlation in residual error in best-fit model (Model 2). Each dot is a single multispecies vial, coloured by the species pair. Assignment to species 1 or 2 was arbitrary. Overall correlation is 0.0012, and facetting by species pair (not shown) also indicates no correlation.

SI 3 - Assessing Sensitivity to Priors

Chris Terry, Jinlin Chen & Owen Lewis

In our model, the R_0 parameters were relatively straightforward to estimate, and there is good reason to be confident that the choice of prior is not particularly influential. However, for the competitive coefficients (α) the fits were considerably more uncertain, and there is a greater potential for the choice of prior to be influential. In the main text, we present results where the prior on each α parameter is constrained to be positive (i.e. exclude the possibility for facilitation) and conditioned on a zero-centered Gaussian distribution with standard deviation 1. To confirm that the choice of prior is not having an outsized effect, we tested tighter ($\sigma = 0.1$) and looser ($\sigma = 10$) priors on the α values, as well as a test where the lower α limit was reduced from 0 to -0.025 (lower values could lead to negative growth rates which are incompatible with the negative binomial error distribution).

Only the very tight prior resulted in markedly different α posterior distributions, indicating that our results are not overly influenced by our choice of priors (Fig S3.1). Only one interaction coefficient had a posterior that notably included facilitatory values ($\alpha_{PAN,BIR}$). We have no particular explanation about why this interaction is negative, and can only assume it derives from data variability. As it is only a single term, we consider it unlikely to have a large influence on our overall results.

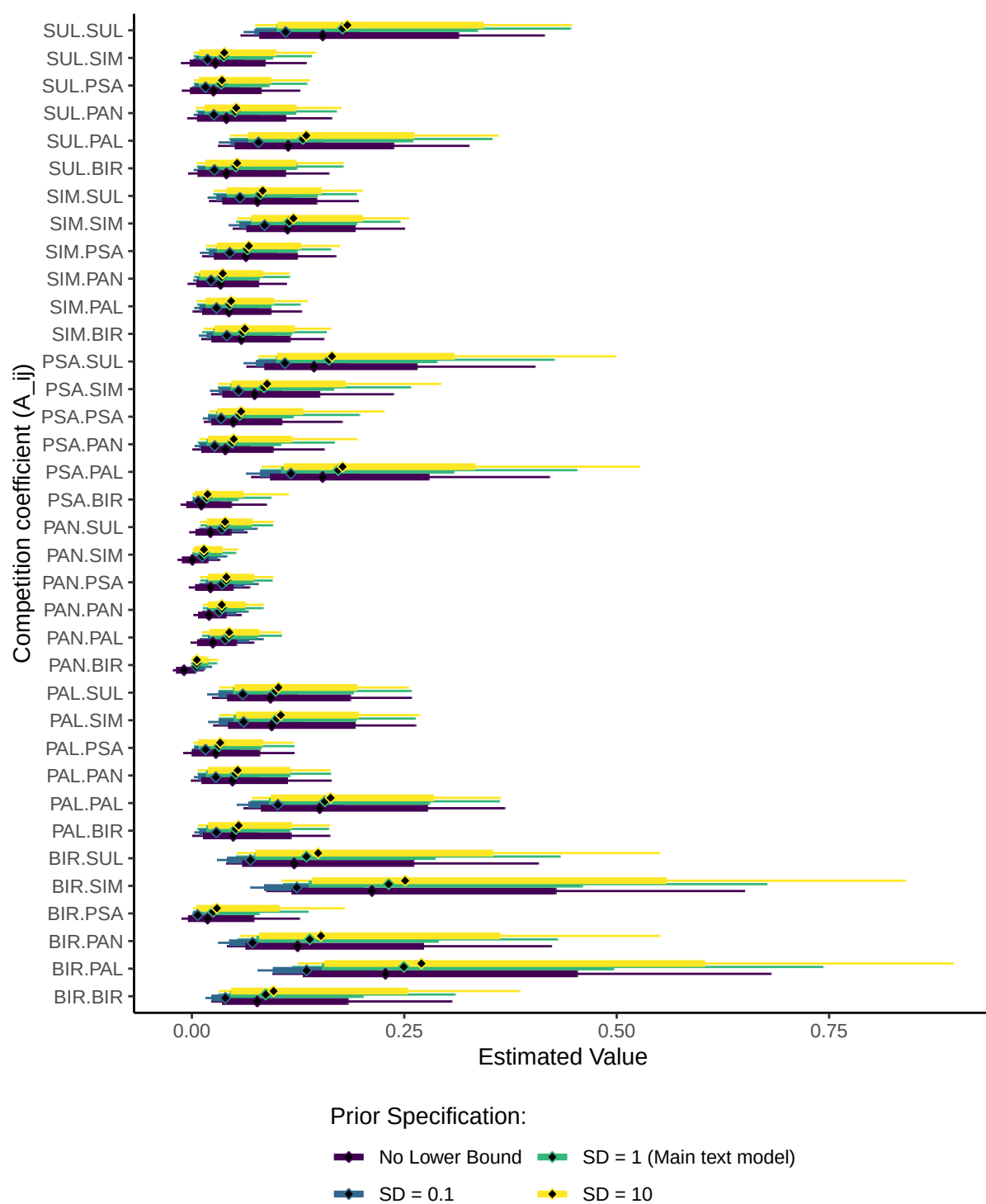


Figure S3.1 Fitted posterior distributions of competitive coefficient terms under different prior assumptions.

To check that the positive bounding of α did not affect the model selection, we refit all three models with the possibility for negative values and repeated the model comparison (Table S3.1). The same result as presented in the main text was refound.

Table S3.1 Model comparison with the possibility of moderate facilitation between species.

	Δ ELPD	Standard Error of Difference	Effective Number of Parameters	Standard Error
model2	0.0000	0.0000	52.7826	2.9515
model3	-33.6173	8.2278	47.9291	2.9894
model1	-43.7014	10.5018	81.2166	5.1718

SI 4 - Additional Results

Chris Terry, Jinlin Chen & Owen Lewis

This appendix details the results of additional analyses to support the results in the main text.

- A. Full results from model comparison
- B. Median estimates for model parameters
- C. Full posterior distribution of model parameters
- D. Diagnostic plots for the model fitting, including posterior predictive plots
- E. Results from maximal model including predation effects on competitive terms

A. Model comparison

	Δ ELPD	Standard Error of Difference	Effective Number of Parameters	Standard Error
model2	0.0000	0.0000	48.5480	2.7322
model3	-34.0281	8.1397	43.6685	2.6892
model1	-43.0215	10.1924	76.6309	5.2007

Table S4.1 Results from model comparison through expected log-pointwise predictive density (ELPD), computed using Pareto-smoothed importance sampling with the loo R package (Vehtari *et al* 2019). Models differ in the degree of divergence in fitted parameters between parasitism treatments. Model 1: separate α and R_0 values (representing the hypothesis that parasitism affects both growth rate and competition terms between species.) Model 2: joint α values, but variable R_0 (the hypothesis that parasitism affects growth rates, but not competition terms), Model 3: joint α and r values (the hypothesis that parasitism has no effect). Differences in ELPD suggest strongest support for Model 2.

B. Median Parameter Values

Despite a major point of the paper being that the full posterior is essential to fully understand the uncertainties in parameter estimates, we appreciate that for some purposes a single point estimate is useful. Table S4.2 and Table S4.3 therefore present median estimates for the key model parameters.

Species	Parasitism	Safe
BIR	6.135	6.865
PAL	10.647	14.053
PAN	4.198	5.822
PSA	4.467	5.612
SIM	12.396	13.951
SUL	8.419	13.875

Table S4.2 Median values for growth rates (R_0) in the two treatments

	BIR	PAL	PAN	PSA	SIM	SUL
BIR	0.0869	0.2495	0.1388	0.0250	0.2316	0.1348
PAL	0.0517	0.1566	0.0516	0.0319	0.1000	0.0987
PAN	0.0058	0.0437	0.0356	0.0406	0.0146	0.0388
PSA	0.0175	0.1723	0.0477	0.0564	0.0850	0.1613
SIM	0.0601	0.0446	0.0352	0.0654	0.1147	0.0806
SUL	0.0518	0.1307	0.0511	0.0346	0.0375	0.1773

Table S4.3 Median values for competition coefficients (α) laid out in standard matrix form ($\alpha_{row,col}$).

C. Parameter posterior distributions (selected model)

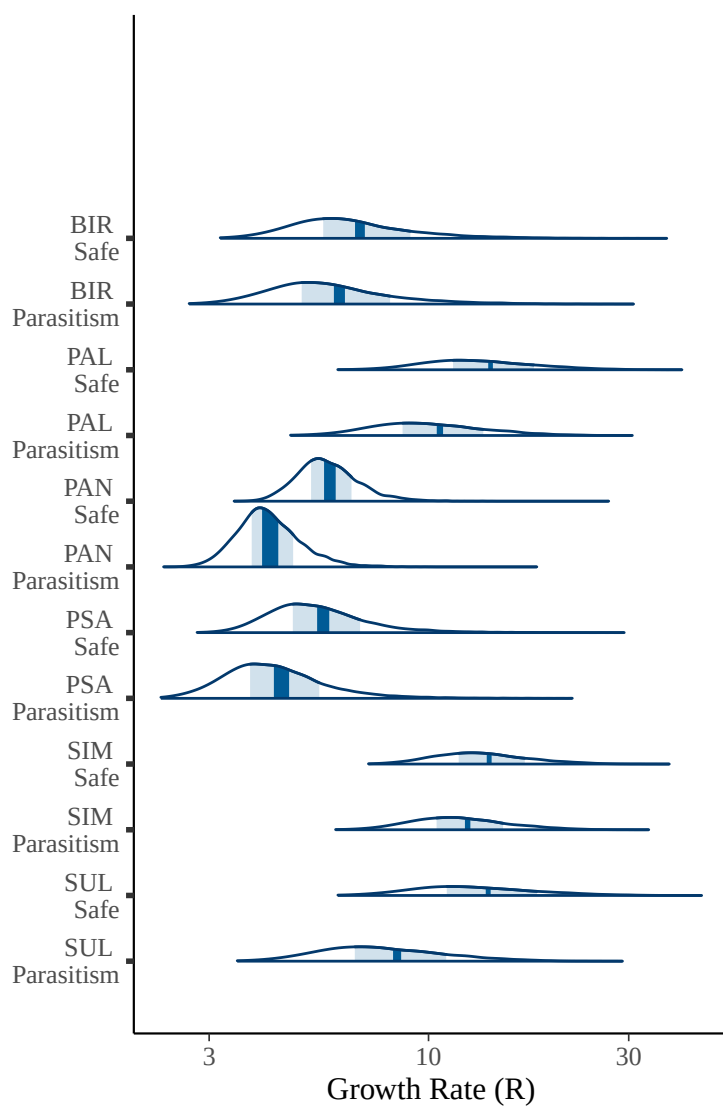


Figure S4.1 Posterior distribution of growth rates under the two treatments. Shaded area shows 50% inner interval. N.B. log-scale.

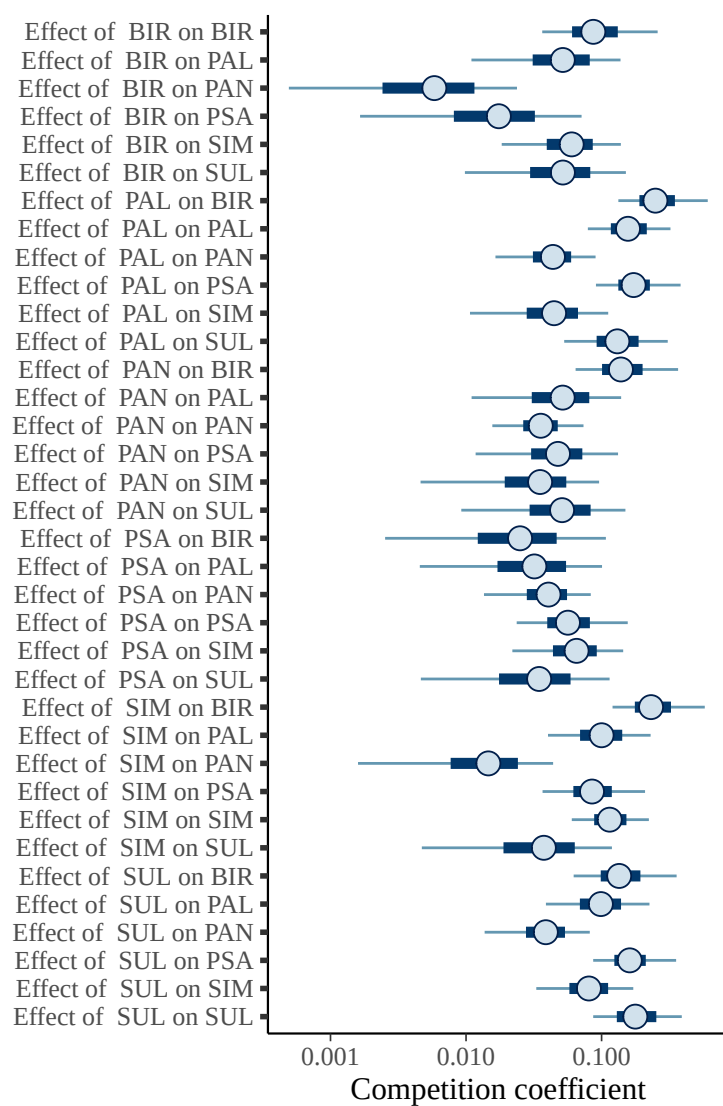


Figure S4.2 Posterior distribution of competition parameters. Wide bar shows 50% inner interval, line shows 90% interval, circle shows median value. N.B. log-scale.

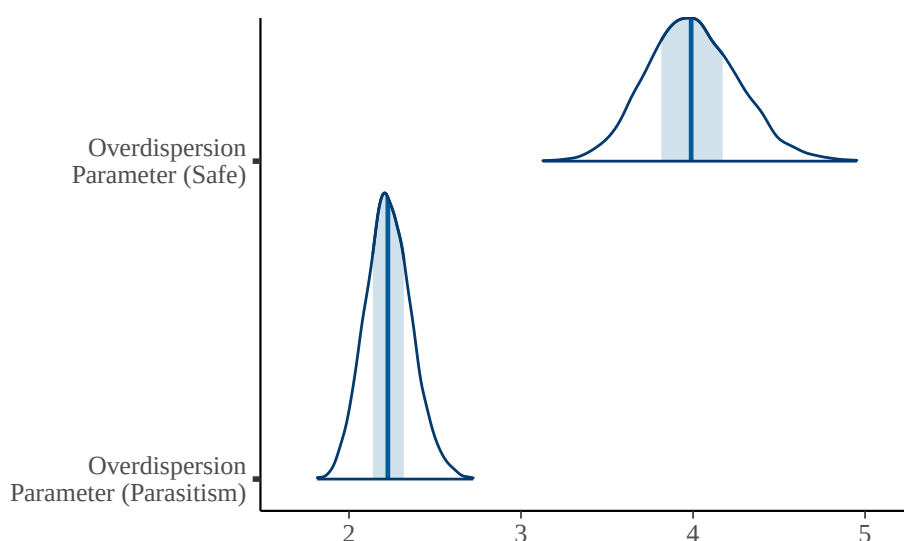


Figure S4.3 Posterior distribution of the ϕ parameters, where the shaded area shows 50% inner interval. Note that in the parameterisation we used (`neg_binomial_2()`), the variance is defined as $\mu + \frac{\mu^2}{\phi}$. Therefore the inverse of ϕ controls the overdispersion, which is scaled by the mean value. ϕ is lower in the parasitoid treatment, suggesting that parasitism increases the variance in the next generation.

Relative uncertainty in components of fitness differences

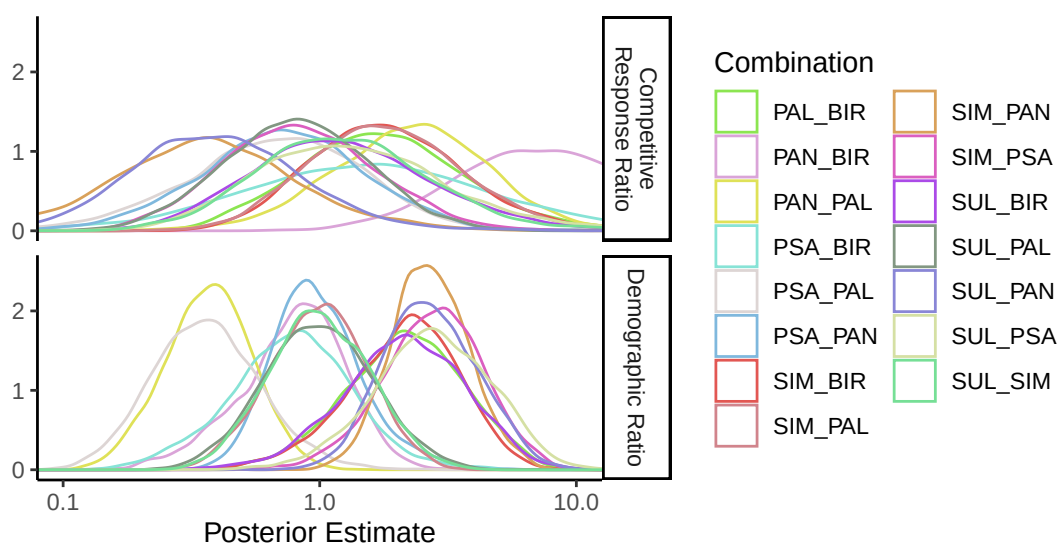


Figure S4.4 Posterior distribution of the two components of the fitness difference. The uncertainty in the ‘competitive response ratio’ $\sqrt{\frac{\alpha_{ij}\alpha_{ii}}{\alpha_{ji}\alpha_{jj}}}$ is much larger than the demographic ratio $\frac{r_j-1}{r_i-1}$ (mean coefficient of variation 32x larger).

D. Model Diagnostics

Overall Predictive Capacity

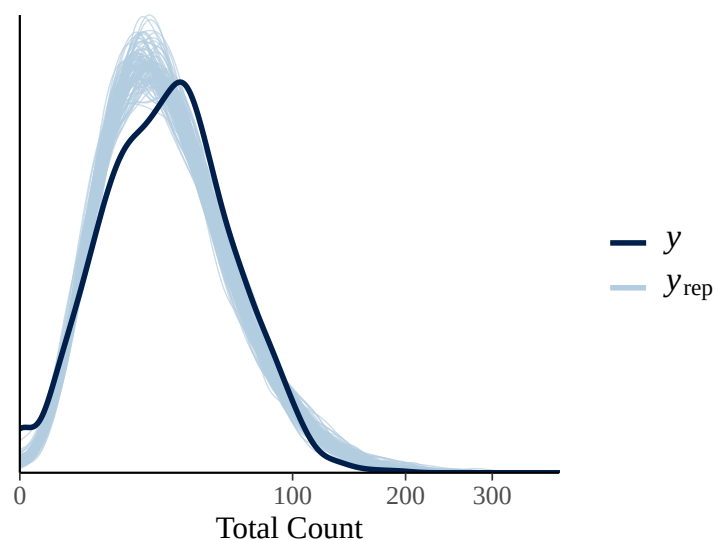


Figure S4.5 Overall predictive capacity of best fit model. 100 sets of predictions from the posterior (blue), are compared to observed values (black). It can be seen that the model captures the overall spread of the data well, but does not pick up the low-growth rate observations (i.e. those cases where no adults were observed to emerge). N.B. the square-root X-scaling to magnify small values.

PSIS diagnostic plot

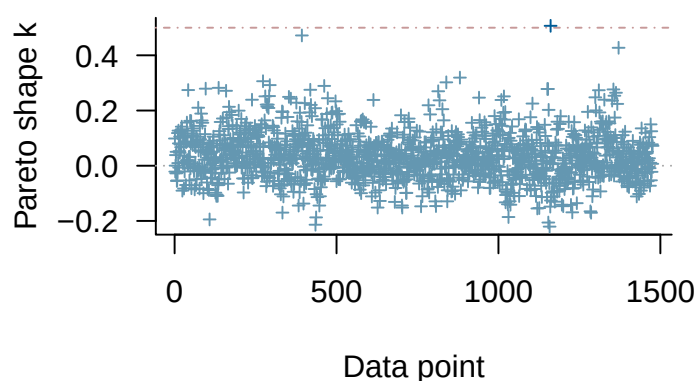
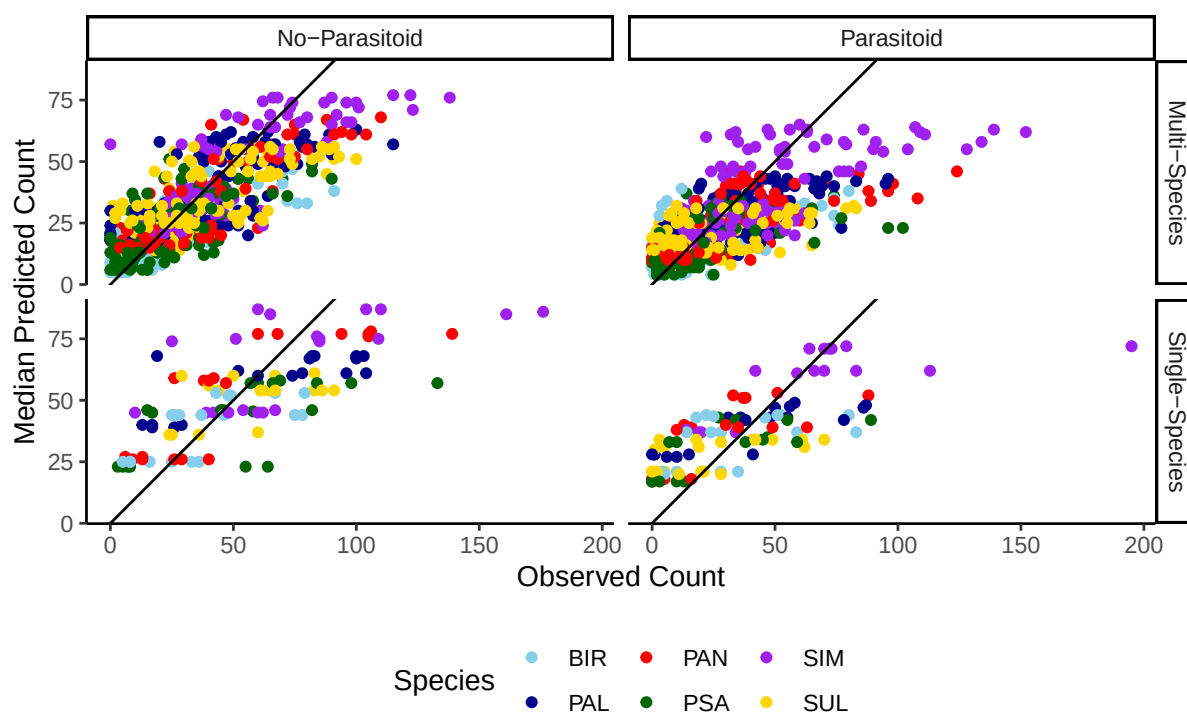


Figure S4.6 Test for model miss-specification, using Pareto-smoothed Importance Sampling to test for strongly outlying data points. No points exceeded the threshold of 0.7 (Gabry *et al.* 2019).

Species-level Predictive Capacity

Figures S4.7 to S4.12 present predictions of the selected model against the raw observations. Because of the multi-dimensional nature of the data, the observations are partitioned by parasitoid treatment, and by the type of competition.



FigS4.7 Overall model predictive accuracy using median posterior values. Plot is faceted by treatment and whether the count was from a single-species or a multi-species trial. Focal species are coloured. In general the model performs well given the inevitable variation, but struggles to capture outlier values across all species.

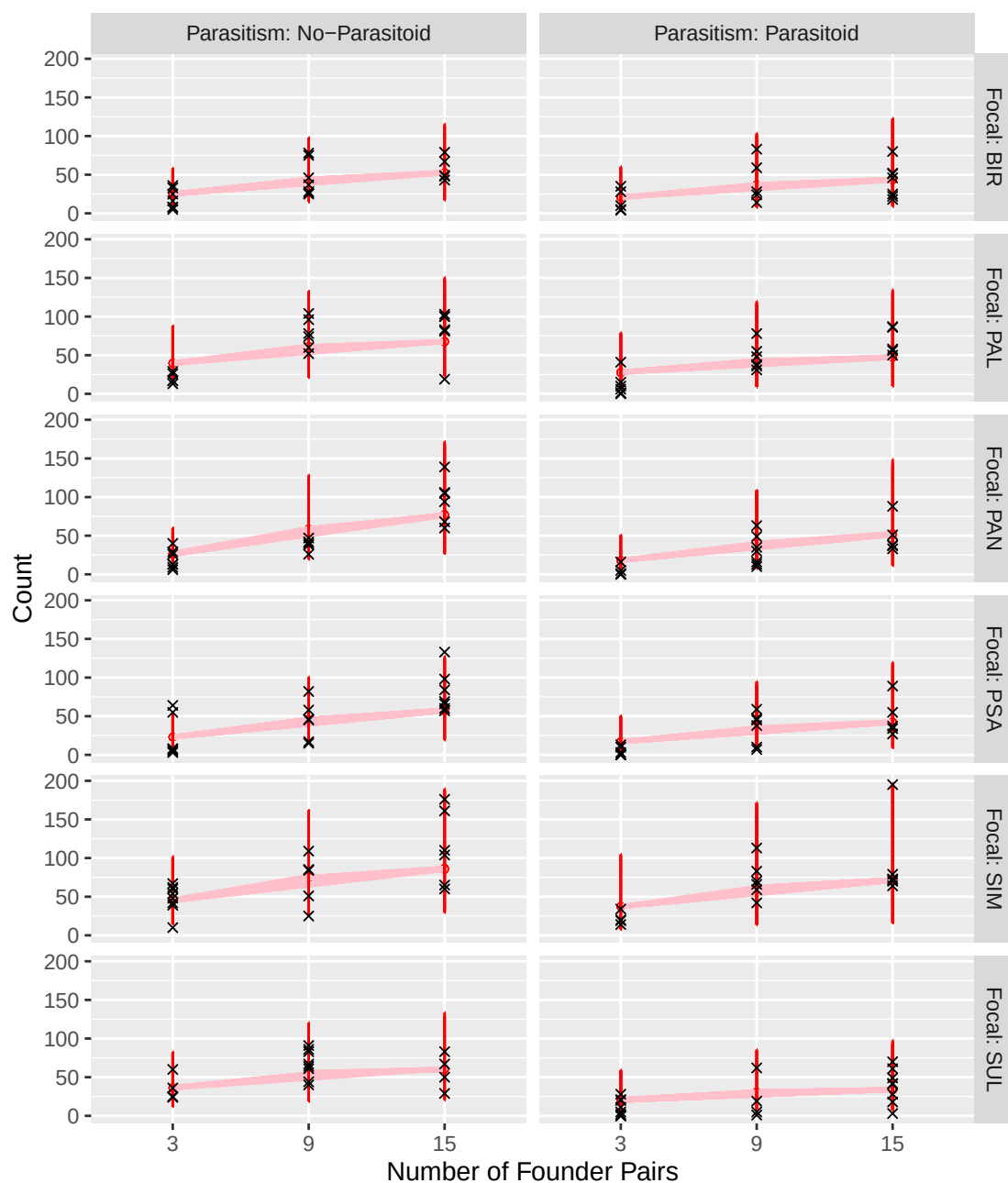


Figure S4.8 Posterior predictions of intraspecific competition. Red dots show median predictions, red lines span central 90% of posterior distribution. Black crosses show observations.

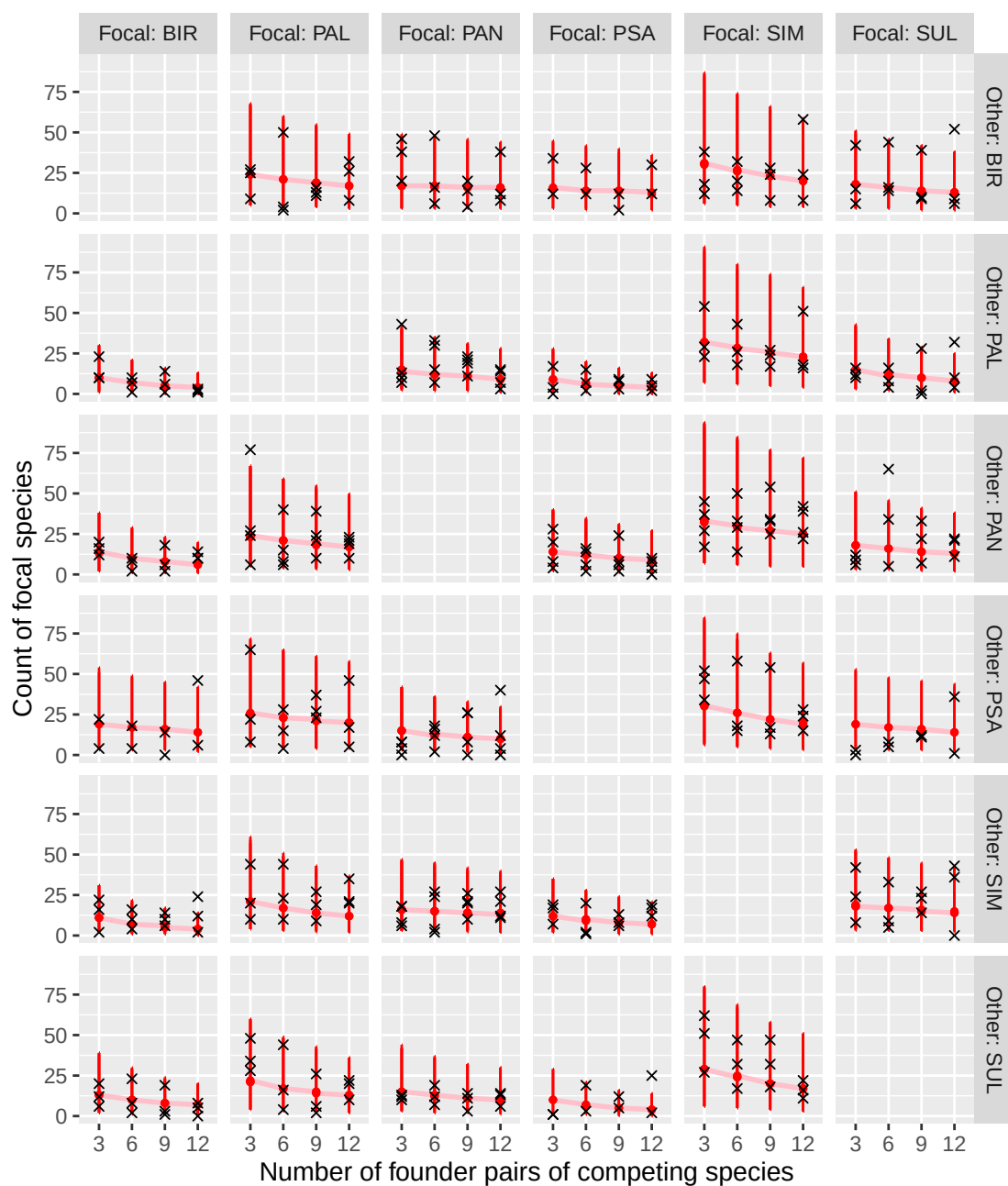
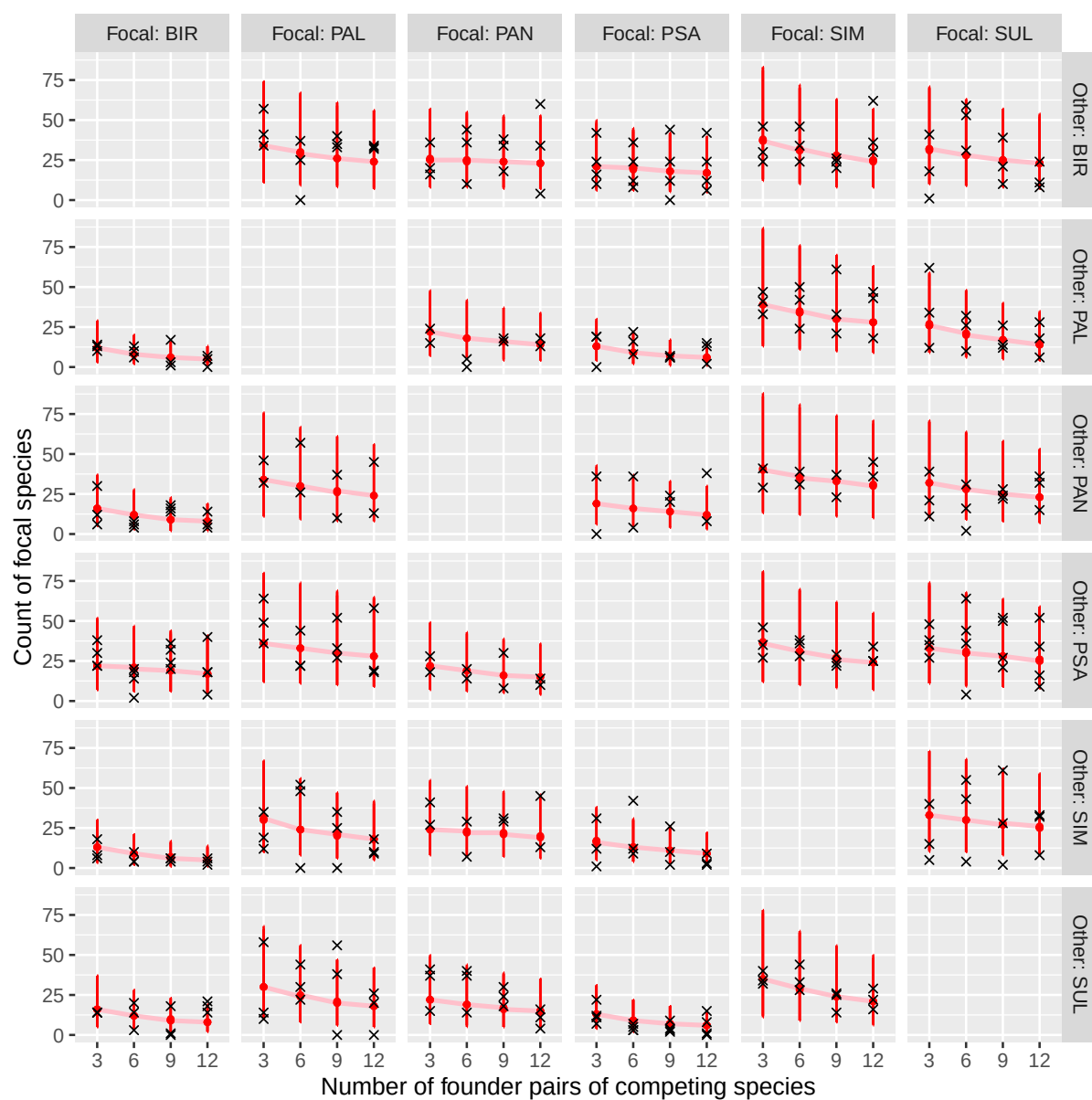
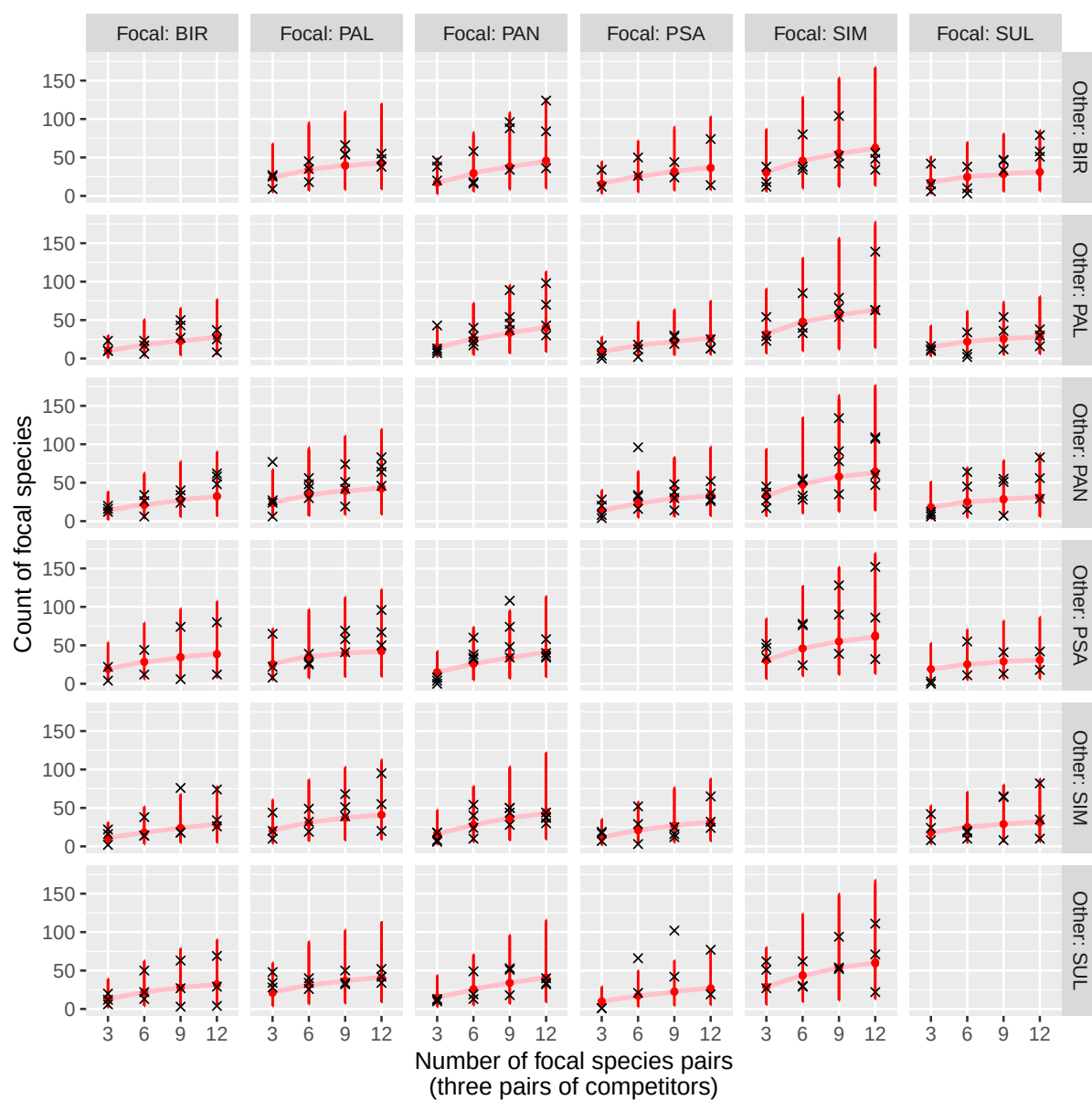


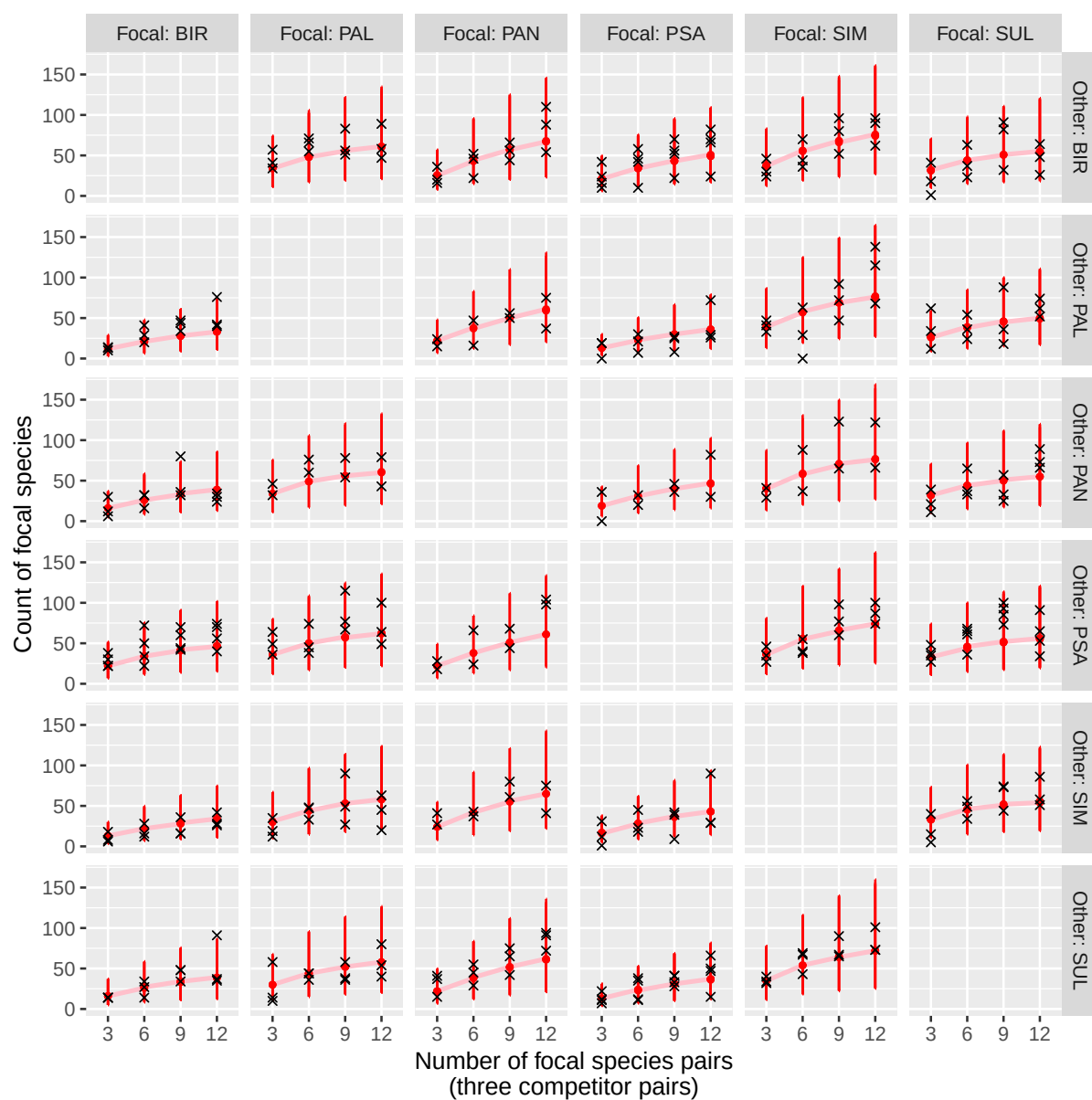
Figure S4.9 Posterior predictions of interspecific competition in the presence of the parasitoid. Red dots show median predictions, red lines span central 90% of posterior distribution. Black crosses show observations.



FigS4.10 Posterior predictions of interspecific competition without the parasitoid. Red dots show median predictions, red lines span central 90% of posterior distribution. Black crosses show observations.



FigS4.11 Posterior predictions covering remaining observations from the parasitoid treatment not easily displayed in previous plots, where the density of the focal species varies and the competing species is represented by 3 founder pairs. Red dots show median predictions, red lines span central 90% of posterior distribution. Black crosses show observations.



FigS4.12 Posterior predictions covering remaining observations from the no-parasitoid treatment not easily displayed in previous plots, where the density of the focal species varies and the competing species is represented by 3 founder pairs. Red dots show median predictions, red lines span central 90% of posterior distribution. Black crosses show observations.

E. Maximal model

Model comparison indicates that there is little support for fitting changes in competitive coefficients due to the parasitism treatment. For completeness, we explore this ‘maximal’ model here. As discussed in the main text, it is possible that an intermediate model, where some competition terms vary, but others are fixed. Fig. S4.13-14 show the divergences in fitted α values between treatments. On average, competition coefficients are larger under parsitism than without.

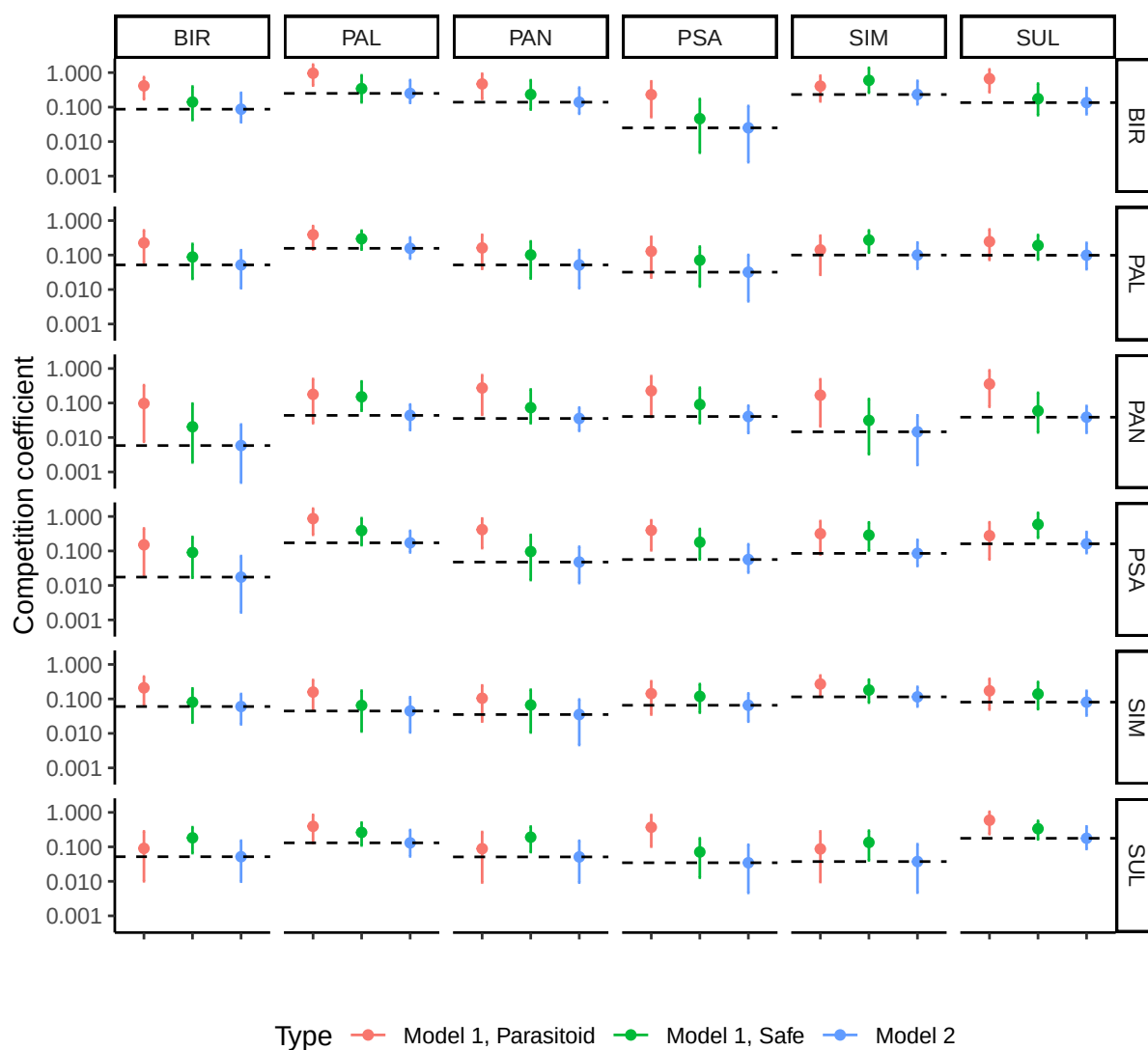


Fig S4.13 Comparison of fitted α values between Model 2 (where a single A matrix is fitted across both treatments) and Model 1 (where a separate A matrix is fitted for each treatment). Dotted line shows median value for Model 2 to allow easier comparison. Error bars show 90% CI.

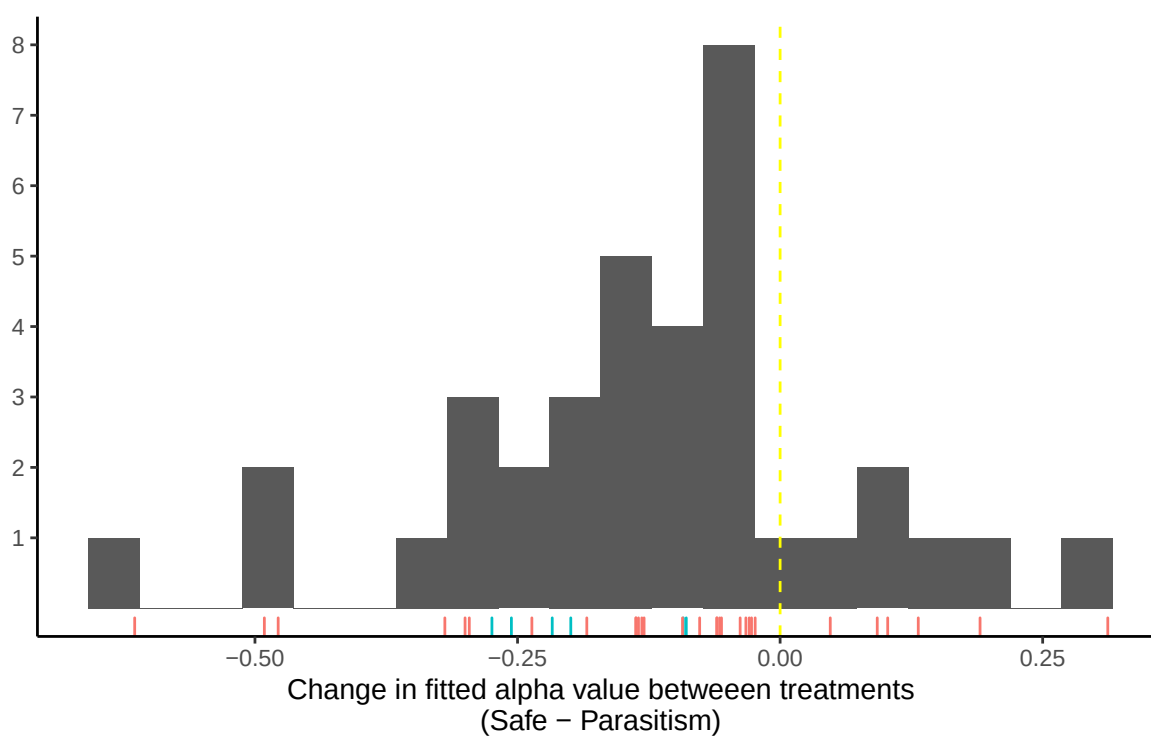


Figure S4.14. Distribution of shifts in median fitted α value between treatments in Model 1. Intraspecific terms are shown in blue, interspecific in red.)

References

1. Gabry, J., Simpson, D., Vehtari, A., Betancourt, M. & Gelman, A. *Visualization in Bayesian workflow*. J. R. Stat. Soc. Ser. A Stat. Soc. 182, 389–402 (2019).