School of Doctoral Studies in Biological Sciences
University of South Bohemia in České Budějovice
Faculty of Science

Mechanisms structuring host-parasitoid communities in a global warming perspective

Ph.D. Thesis

Mélanie Thierry

Supervisor: Mgr. Jan Hrček, Ph.D.
Supervisor-specialist: Prof. Owen T. Lewis, Ph.D.

České Budějovice 2021

This thesis should be cited as:
Thierry, M., 2021: Mechanisms structuring host-parasitoid communities in a global warming perspective. Ph.D. Thesis Series 2021, No. 14. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 190 pp.

Annotation

Ecological communities are composed of a multitude of interacting species, and the outcome of pairwise interactions depends on other co-occurring species within the community. With current global environmental changes, both abiotic and biotic environment are changing, affecting the structure and dynamics of communities. I used a series of laboratory experiments on a set of *Drosophila* species and their parasitic wasps to investigate the effects of biotic and abiotic factors on interactions and communities. I first compared the outcome of host-parasitoid interactions across community modules commonly found in host-parasitoid communities (i.e., pairwise interaction, exploitative competition, apparent competition, and both exploitative and apparent competition). I found generally higher host suppression with multiple parasitoid species, but species-specific effects for parasitoid performance. I then observed that warming impacts host communities through direct effects on species performance rather than altered competitive interactions and parasitism. Finally, I found that temperature strongly influences the effects of multiple parasitoids on host suppression across different parasitoid assemblages, suggesting a general pattern for the environmental dependence of trophic and non-trophic interactions. My thesis emphasizes the importance of considering environmental factors and different interaction types to better predict community dynamics in a rapidly changing world.

Declaration

I hereby declare that I am the author of this dissertation and that I have used only those sources and literature detailed in the list of references.

České Budějovice, 28/06/2021 Mélanie Thierry

This thesis originated from a partnership of Faculty of Science, University of South Bohemia, and Institute of Entomology AS CR, supporting doctoral studies in the Entomology study program.

Přírodovědecká
fakulta
Faculty
of Science

BIOLOGY
CENTRE
CAS

Financial support

The projects in this thesis were supported by the Grant Agency of Czech Republic (17-27184Y) and (20-30690S), the National Environment Research Council (NE/N010221/1), and the Grant Agency of the University of South Bohemia (04-134/2019/P) and (038/2019/P).

Acknowledgements

I would like to thank all the marvelous people that accompanied me during these 4 and a half years of PhD. It has not been always easy, but they made this important chapter in my life a lot more enjoyable in their own way.

First, I would like to thank my supervisor Jan Hrček. He trusted me to be one of the first members of the Laboratory of Experimental Ecology with no prior experience in working with live insects nor designing experiments. Taking part in establishing a new system was a rich experience, and I definitely learnt a lot thanks to the space and liberty I had for trial and error, which allowed me to grow as a scientist. I am grateful for his guidance throughout my thesis, and the care and support he is always giving to all the team members.

Second, my thanks go to Owen Lewis, my co-supervisor. His wisdom and knowledge greatly contributed to improve my work. I particularly enjoyed the few occasions we had to discuss in person, first during fieldwork in Australia, then in Oxford and during his visit in our lab.

Many thanks to all my teammates that helped me in so many aspects of my PhD: in the lab, with scientific discussions, and outside work as friends. To Martin, Anna J., Nick, Chia-Hua, and Joel who were there in the early days and helped me a lot settling in this new job. To all the ones that came after that, Miguel, Anna M., Andrea, Vincent, Terka, Jeni, and Vid, and keep making this lab such a pleasant environment to work in. I would like to thank particularly Nick, who help improving all parts of this thesis at all stages, and has been like a mentor to me throughout my PhD. To the Miracles Ladies who helped me on so many occasions. And to Maria, who brightens our days in the office by feeding us with so many delicious cakes.

I am deeply grateful to all my other colleagues in the Department of Ecology that contribute to creating such a kind work environment. Thanks to the ones that were there when I arrived and made me feel welcome right from the start, and all the ones that have arrived since and keep bringing good energy. Thanks also to the rest of the amazing people at BC that I had the pleasure to meet. There are too many to be named here. I feel lucky to be able to consider many of them as my friends. A particular thank goes to the ones with whom I shared so many beers with, my playfellows, and my little climbing community. If you recognize yourself in those lines, thank you. Special thanks to Vincent who supported me during the last months of this journey.

Thanks to my Australian friends that made me discover beautiful North Queensland. To Dahe and Dave, probably the coolest landlords that I will ever have. To Luen who brought me on crazy adventures in his big backyard, and forced me back into climbing. To my housemates with whom I enjoyed trips and chill evenings in our garden. To the people at JCU that helped me during field and lab work. And to the rest of the amazing people I have met during my 7 months out there, especially those that almost made me a snake lover.

Thanks to Ulrich Brose and his group who made me feel at home during my stay in Germany, especially my officemates Georg and Angelos, and my collaborators Benjamin Rosenbaum and Benoit Gauzens. Special thanks to Alexei for helping me explore Leipzig surroundings, and to Malena, Kate, Kevin, Franzi, and Juliana for many delicious dinners and boardgame nights.

Thanks to my friends back home in Montpellier and Toulouse who were always there to help me unwind and forget about science for a while.

And last but not least, a big MERCI to my family, especially my parents and Jeanne & Jean, who always supported me no matter the path I chose. I am beyond gratefully for all the love and care they gave me for nearly 30 years now. I would not have made it all this way without them.

Cover by Chia-Hua Lue.

List of papers and author’s contribution

The thesis is based on the following papers (listed chronologically):

I. **Thierry, M.**, Hrček, J., & Lewis, O. T. (2019). Mechanisms structuring host–parasitoid networks in a global warming context: a review. *Ecological Entomology*, *44*(5), 581-592. (IF = 2.465).

MT conceived the idea and wrote the manuscript (90 %).

II. **Thierry, M.**, Pardikes, N. A., Lue, C. H., Lewis, O. T., & Hrček, J. (2021). Experimental warming influences species abundances in a *Drosophila* host community through direct effects on species performance rather than altered competition and parasitism. *PLOS one*, *16*(2), e0245029. (IF = 2.740).

MT planned and conducted the laboratory experiment (90%), analyzed the data (100%), and wrote the manuscript (95%).

III. **Thierry, M.**, Pardikes, N. A., Ximénez-Embún, M.G., Proudhom, G., & Hrček, J. Multiple parasitoid species enhance top-down control, but parasitoid performance is context-dependent. (submitted).

MT planned and conducted the laboratory experiment (90%), analyzed the data (100%), and wrote the manuscript (95%).

IV. **Thierry, M.**, Pardikes, N. A., Rosenbaum, B., Ximénez-Embún, M.G., & Hrček, J. Warming decreases host survival with multiple parasitoids, but parasitoid performance also decreases. (manuscript).

MT planned and conducted the laboratory experiment (90%), analyzed the data (95%), and wrote the manuscript (95%).

Co-author agreement

Jan Hrček, the supervisor of this Ph.D. thesis and co-author of the presented papers, fully acknowledges the stated contribution of Mélanie Thierry to these manuscripts.

Jan Hrček

Mgr. Jan Hrček, Ph.D.

Table of Contents

Introduction

CONTEXT

In the context of human induced global changes, the environment in which species live is shifting at an unprecedented rate. In the past few decades, we have seen a drastic increase in habitat loss and fragmentation, pollution and use of pesticides, atmospheric CO_2 levels, and profound changes in climate (Pachauri et al. 2014). Current warming trends are expected to have direct effects on species through their sensitivity to temperatures, but also on their biotic interactions, ultimately impacting individuals, populations, communities, and ecosystems (Sinclair et al. 2016, Boukal et al. 2019). In response to climate change, many species have already shifted their ranges and phenology (Parmesan 2006), leading to novel communities of species that did not co-occur or interact before. To forecast ecological consequences of further changes in the environment, it is thus important to investigate the effects of both the abiotic and the biotic contexts that organisms experience, and the interplay between these factors (Agrawal et al. 2007).

In my thesis, I focus on the effects of abiotic and biotic environments on communities of parasitoids and their *Drosophila* hosts. Insect arthropods are ectothermic, thus particularly vulnerable to climate change. A study surveying flying insects in natural reserves in Germany revealed a decline in biomass of 76% in 27 years (Hallmann et al. 2017). Although no obvious fingerprint of climate change *per se* was detected in this study, this alarming result caused many to recognize the ongoing insect declines, now sometimes referred to by the media and public as “insectageddon”, with concern that this is happening worldwide and across taxa (Eggleton 2020, Wagner et al. 2021). Half of the animal biomass and a majority of the species in the animal kingdom are insects (Bar-On et al. 2018), where they represent two thirds of the world’s terrestrial species (Sánchez-Bayo and Wyckhuys 2019). Many insect species remain to be discovered (Hamilton et al. 2010), but many will probably disappear before being described. They are functionally diverse, link aquatic and terrestrial ecosystems, and consequently are responsible for many essential ecosystem functions such as pollination, nutrient cycle, and top-down control. By eroding insect biomass and biodiversity, global changes are threatening those important ecosystem functions and services.

Thus, understanding the mechanisms underlying insect community response to environmental changes is of key importance to better preserve ecosystem integrity.

A. Biotic and abiotic environments interact to shape ecological communities

A.1. Effects of warming on organisms and interactions

Insects are poikilotherms (i.e., no physiological means to generate heat), and generally ectotherms (i.e., unable to produce and conserve adequate metabolic heat to maintain a body temperature that is above their habitat), thus rely on ambient temperature for their fitness and performance (Sinclair et al. 2016). Any increase in temperature that approaches the thermal optimum (T_{opt}) increases insect metabolism and respiration, and therefore activity and performance (Neven 2000). However, thermal performance curves (TPC) have a characteristic asymmetric shape (Figure 1a; Huey and Stevenson 1979), and once the thermal optimum is reached a further increase in temperature can result in a rapid decline in performance until its upper critical threshold (T_{max}). Above this level, the rate of mortality exceeds that of reproduction and development (Dell et al. 2011). The potential for acclimatation (either through phenotypic plasticity, or evolution) to higher temperatures is limited compared to low temperatures (Addo-Bediako et al. 2000, Overgaard et al. 2011, Kellermann et al. 2012), suggesting that climate warming will be detrimental for most species.

Species from the tropics are particularly at risk in the face of global warming. Indeed, metabolic rates increase with temperature below the thermal optima. The narrower the thermal breadth, the steeper the curve (Figure 1b; Gillooly et al. 2001), and species from the tropics typically have a narrower thermal breadth than temperate species (Deutsch et al. 2008). Moreover, tropical species already experience high mean temperatures, and their metabolic rates would therefore increase more than those of ectotherms in temperate regions with lower mean temperature, despite a smaller increase in temperature in the tropics compare to temperate regions (Dillon et al. 2010). Tropical species can also be closer to their T_{max}, and thus have a small margin of thermal refuge. However, under global climate change, both mean temperatures and variability are expected to increase. Depending on the shape of the TPC, an increase in temperature fluctuations would decrease ectotherms' performance due to the nonlinearity of the TPCs (i.e., Jensen's inequality), while increasing the chance to experience lethal temperatures (Ruel and Ayres 1999). Due to this phenomenon, Kingsolver et al. (2013) and Vasseur et al. (2014) argued that temperate species could be more vulnerable to climate warming than tropical species because of higher thermal variability in temperate regions, which increases the chances of those detrimental temperatures despite lower mean temperatures. Moreover, insect species in temperate regions are not active all year long, and their thermal safety margins do not differ much from the ones in the tropics when temperatures during only the months insects are active are considered (Johansson et al. 2020). In any case, signs of species decline and biomass loss are visible at all latitudes worldwide (Walther et al. 2002, Janzen and Hallwachs 2021).

In addition to the direct effects on organisms discuss above, abiotic factors, such as temperature, can influence species interactions (Chamberlain et al. 2014). Temperature is a main factor in the strength of predator-prey interactions (Archer et al. 2019), and influences predator metabolic rates (Rall et al. 2010). High temperatures can impact an individual's ability to either find food and/or resist predators (Le Lann et al. 2014, Sentis et al. 2017c). Predator attack rates show a concave response curve with temperature, while handling times vary in a convex manner, producing a maximum feeding rate at intermediate temperatures (Englund et al. 2011). Such warming induced changes in feeding rates have important implications for population and food web stability (Binzer et al. 2012, Gilbert et al. 2014, Sentis et al. 2017a). Temperature can also determine the outcome of competitive interactions (Davis et al. 1998b, Fleury et al. 2004), and of host-parasite interactions (Thomas and Blanford 2003). Understanding effects of warming on species performance, their interactions, and how it scales up to communities and ecosystems is an ongoing endeavor.

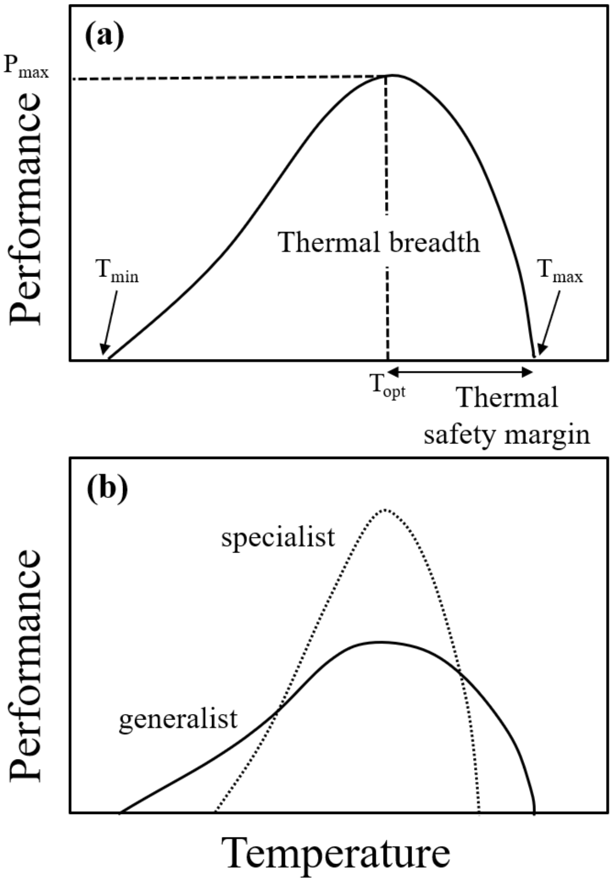


Figure 1. Typical shape of thermal performance curves showing the relationship between ectotherm performance (e.g., fecundity, growth rate, etc.) and temperature. (a) Performance increases with temperature from the lower thermal limit (T_{min}) until the thermal optimum (T_{opt}), then decreases until the upper thermal limit (T_{max}). (b) Temperate species (i.e., generalists) can perform over a wider range of temperatures, but perform more poorly than tropical species (i.e., specialists) at the optimal temperature.

A.2. Biotic environment effects on organisms and interactions

An organism's performance depends not only on its abiotic environment, as discussed previously, but also on the other organisms present in its environment (i.e., its biotic environment or community context). All pairwise interactions are entangled in complex networks (Kéfi et al. 2015), and indirect and high-order interactions with other co-occurring species affect how two focal species interact (Bairey et al. 2016, Abdala-Roberts et al. 2019, Terry et al. 2020). The loss of a keystone predator can lead to community-wide extinction cascade via an increase in competition at the prey level (Sanders et al. 2015, Donohue et al. 2017). This phenomenon, referred to as "trophic cascade", was first termed by Paine (1980). Though, Darwin described how domestic cats were beneficial for plants by controlling mice populations, which allowed their pollination by bumblebees in *The Origin of Species* (Ripple et al. 2016). One famous example of a trophic cascade caused by behavioral changes is the reintroduction of gray wolfs (*Canis lupus*) in Yellowstone National Park (USA) in 1995 (Ripple and Beschta 2012). This reintroduction led to fear-induced avoidance behavior in elk (*Cervus elaphus*), that were thus spending less time foraging, resulting in lower herbivory pressure on woody plant species. This trait-mediated indirect interaction (TMII) caused an increase in woody plant, thus decreasing competitive pressure on beavers (*Caster canadensis*) and bison (*Bison bison*). This textbook example shows how much species are interconnected and how changes into a single species can cascade throughout an entire ecosystem. Such changes can even cascade across ecosystems. For example, the presence of fish in ponds alters aquatic insect populations that have an aquatic larval stage, but whose adults are terrestrial and pollinators, thus affecting pollination of terrestrial plants (Knight et al. 2005).

Community modules are a useful tool to investigate how complex communities are structured by isolating specific patterns of interactions between a small number of species (Holt 1997). Some common community modules in food webs are tri-trophic interactions (e.g., plant-herbivore-predator), exploitative competition (e.g., two herbivores sharing the same plant resource), apparent competition (e.g., two herbivores sharing a natural enemy), and intra-guild predation (e.g., a top predator attacking both an intermediate predator and a common prey). Those community modules, also called "motifs", are the building blocks of most ecological networks (Milo et al. 2002). Studying how species behave in such motifs can thus inform us about the effects of co-occurring species, and help identify the underlying mechanism that structure their communities.

A.3. How biotic and abiotic environments interact to influence species interactions is uncertain

Abiotic factors shape the biotic environment, and together the abiotic and biotic factors act to structure ecological communities. Indeed, climate change can indirectly affect insect foraging behavior through changes in host plant availability and distribution, and changes in natural enemy abundance and distribution (Lister and Garcia 2018). Warming might alter consumer-resource interactions in the tropics due to species losses, while in temperate regions it might be due to extreme fluctuations in species' abundances (Amarasekare 2019). Davis et al.'s (1998) experimental study on a community of *Drosophila* showed the importance of community context in order to predict shifts in species ranges with warming accurately. They found that species temperature ranges were constrained by the presence of competitors and natural enemies. Barton and Schmitz (2009) showed that predator species shifted their habitat use with warming, resulting in a decrease in niche differentiation, lowering the suppression of herbivores (i.e., altered multiple predator effects). Direct effects of warming on species interactions depend on other species present in the community, and thus taking into account the biotic environment and different types of interactions is important (Sentis et al. 2017b).

The biggest effects of climate change might not be on the focal species *per se*, but due to the changes in the biotic environment it induces because of asynchronous responses among species (Alexander et al. 2015). Indeed, to adapt to climate change, species are shifting their ranges and phenology (Hällfors et al. 2021). Species are generally moving upward along elevational gradients and poleward in latitude to escape warmer temperatures and track their thermal niches (Parmesan and Yohe 2003). With shifts in latitude, species experience new daylength regimes. As photoperiod drives many aspects of an organisms life history (Beck 2012), species may shift their phenology with daylength changes, in addition to shifts due to changes in temperature. But species show differences in sensitivity and responsiveness to these changes (Abarca and Spahn 2021, Freeman et al. 2021), and species will thus experience new environments, with new co-occurring species (biotic environment). Cascading effects add on to the erosion of biodiversity with global changes, and is an important driver of insect decline (Kehoe et al. 2020a). It is therefore important to investigate effects of warming on organisms and species interactions, as well as indirect effects of temperature on organisms through changes in their biotic environment.

B. The case of host-parasitoid communities

Parasitoids use arthropod hosts as food for their offspring, laying one or several eggs inside (i.e., endoparasitoids) or on (i.e., ectoparasitoids) the hosts. Koinobionts allow the hosts to continue development, which is often the case for endoparasitoids attacking egg or larval stages, while idiobionts stop host development after oviposition, often the case for ectoparasitoids attacking pupal or adult stages. Parasitoids differ from parasites in that parasitoids will always kill their host as part of their life cycle, and they differ from predators in that only one individual is necessary for them to complete their development. Some parasitoids can also feed upon other parasitoids, either as obligatory hyperparasitoids or facultatively depending on host availability. Their lifestyle makes them particularly dependent upon their host, and their interactions can be easily observed and quantified (van Veen et al. 2006). Moreover, parasitoids have short generation times, making their responses to changes in the environment relatively fast. For these reasons, insect host-parasitoid systems are useful and important to study the response of multi-species communities in a changing world.

B.1. Parasitoid diversity and value

Insect parasitism was first described by Lu Dian in 1096 based on his observations of the tachinid fly’s life cycle in what is now modern day China (Cai et al. 2005). Interestingly, the term “parasitoid” first appeared in 1916 in a book on insect habits and life histories by the German entomologist Odo Morannal Reuter (Reuter 1913). However, before being named, parasitoids were already recognized for their potential as biological control agents. In the U.S., the ecosystem service that parasitoids provide to the agriculture industry is estimated at $20 billion per year (Pennisi 2010). Research on parasitoids keeps growing, for applied purposes, but also because they provide excellent model organisms to explore questions in ecology and evolution. Furthermore, parasitoids play an essential role in natural ecosystems (Lafferty et al. 2008), and are ubiquitous worldwide. Almost all insect arthropods are parasitized by parasitoids. Currently, there are about

77,000 described parasitoids species (80% are Hymenopterans, the rest are mainly Dipterans, and few are from the orders Neuroptera, Trichoptera, Lepidoptera, Strepsiptera, and Coleoptera). It is clear from the numerous undescribed and cryptic species uncovered during field studies, such as the study on the system I am using for my thesis (Jeffs et al. 2021), that we are a long way from documenting the true parasitoid biodiversity. The most conservative calculations estimate the number of parasitoid species worldwide at 680,000 (Heraty 2017). New molecular tools developed, such as DNA barcoding, represent a great avenue to better assess their biodiversity and interactions (Wirta et al. 2014, Hrček and Godfray 2015).

B.2. Host-parasitoid interactions and coevolution

Parasitoids and their insect hosts share a long evolutionary history, and are thus highly specialized. Hosts have evolved immune resistance to parasitoids. Eggs of endoparasitoids in the host's hemocoel are recognized as non-self by the host's immune system, and hemocytes quickly proliferate and differentiate to help kill the foreign parasitoid egg. In *Drosophila,* hemocytes differentiate into plasmatocytes and lamellocytes under the control of the gene *collier,* expressed in the posterior region of the lymph gland. Lamellocytes can account for up to 50% of the circulating blood cells in parasitized hosts. They attach to the surface of the parasitoid egg and form a multilayered hemocytic capsule. Lamellocytes also play a role in the phenol oxidase (PO)-mediated melanogenesis activated by the Toll pathway and are responsible for the melanin deposition over the hemocytes surrounding parasitoid eggs. During melanogenesis, cytotoxic molecules are also generated. The combined actions of encapsulation, melanization, and production of cytotoxic molecules constitute an effective mechanism for host resistance to parasitoids (Carton et al. 2008). Some other host defenses come from their mutualistic interactions with symbiotic bacteria. Three main mechanisms are at the source of symbiont-conferred protection against parasites: 1) activation of the host immune response, 2) interference competition by producing toxins, and 3) exploitative competition for the host lipids (Vorburger and Perlman 2018). However, there is a trade-off between defenses against parasitoids and other important processes. Host resistance conferred by symbionts comes at a cost for fitness and competitive ability, and will therefore be selected or not depending on the abiotic and biotic environment (Kraaijeveld and Godfray 1997, Oliver et al. 2014).

Parasitoids have evolved a large array of mechanisms to manipulate host physiology, biochemistry, and behavior in order to make them more suitable for development and avoid the host immune response (Beckage and Gelman 2004). Venoms, symbiotic polydnaviruses (PDVs), or other virus-like particles (VLPs) injected by the parasitoid during oviposition inhibit the host immune response. Braconids, of the genus *Asobara,* that attack *Drosophila* larvae do no use venoms, but have eggs with sticky surfaces that are embedded among host fat body tissue and escape detection by the host immune system. In some cases, superparasitism (i.e., several parasitoid eggs laid within the same host) may be voluntary to overwhelm the host's physiological defenses (Carton et al. 2008).

Parasitoids, especially endoparasitoid koinobionts, have a tight interaction with their host, and have thus coevolved with their host species (Kraaijeveld and Godfray 2009). The strongest determinant of host suitability for the parasitoid is therefore its phylogeny (Henri and Van Veen 2011). However, host-parasitoid interactions are embedded in multitrophic networks, and how they interact depends on their biotic environment.

B.3. Non-trophic interactions in host-parasitoid networks

Mechanisms describing host-parasitoid interactions and parasitoid coexistence have been extensively studied, especially in biological control contexts (e.g., Mills and Getz 1996, Pedersen and Mills 2004). Effects of multiple parasitoids, or more generally multiple predator effects (MPEs), on host suppression are of particular interest for biological control purposes, and for ecosystem functioning. It is not clear whether one or several parasitoid species are needed to control host populations. In some cases, if parasitoids present some degree of niche separation, several parasitoid species would be preferable; for example, if co-occurring parasitoid species have different phenologies or attack different host stages. However, some studies argue that one efficient parasitoid species is enough to efficiently control an arthropod population (Pedersen and Mills 2004). Moreover, parasitoids often compete, either at the adult stage for territory and oviposition, or at the larval stage within a host (Harvey et al. 2013). Indeed, multiparasitism (i.e., multiple parasitoid species ovipositing in the same host individual) and superparasitism (i.e., multiple parasitoid eggs from the same species in a same host individual) are common in nature (e.g., Ortiz-Martínez et al. 2019), but only one species can successfully emerge from a host. Parasitoids can chemically suppress their competitor or attack them in physical combat. On some occasions, multiparasitism events could result in facilitation when the initial parasitoid lowers the host defenses, providing a better opportunity for the subsequent parasitoid species to escape host immune defenses and successfully develop. However, such facilitation between parasitoid species has only been reported once (Cusumano et al. 2016). Parasitoids can also interact with other natural enemies in diverse ways. Intraguild predation often occurs, for example when the ladybird *Harmonia axyridis* predates a parasitized aphid with larvae of the parasitoid *A. ervi* inside (Snyder and Ives 2003). Thus, other natural enemies can be both competitors and predators, increasing the complexity of such systems.

Host species sometimes share the same habitat, and can therefore compete for space or resources, but they most often compete indirectly through a shared parasitoid (i.e., apparent competition; Holt and Lawton 1994). However, indirect interactions among hosts can also result in positive outcomes (i.e., apparent mutualism). A diverse community of host and non-host species prevent the host being overexploited by parasitoids (Kehoe et al. 2016). It is therefore important to consider co-occurring species in the environment to correctly assess a parasitoid's efficiency at suppressing a targeted host, and potential negative impacts on non-targeted species (Wajnberg et al. 2001).

B.4. Our *Drosophila*-parasitoid system

Drosophila flies (Diptera: Drosophilidae) are well known as a model system in genetics, cellular biology, and developmental biology. *Drosophila melanogaster* is one of the most emblematic biological models, with the first documentation of the use of *Drosophila* in the laboratory from 1901 by William Castle's group. However, it is the use of *D. melanogaster* by Thomas Hunt Morgan to define the role played by the chromosome in heredity that made this model famous (Morgan 1910). This model organism is of great importance for medical advances because 75% of the known human disease genes match those in the *Drosophila* genome (Reiter et al. 2001), and the whole genome has been sequenced since March 2000 (Adams et al. 2000) and is freely accessible via “Flybase” (Tweedie et al. 2009). *Drosophila* flies are easy and inexpensive to culture in the lab, have a short life cycle, produce large numbers of offspring, and can be genetically modified in numerous ways, making them an ideal biological model. However, less is known about their utility to study community ecology, and network response to environmental changes.

Parasitoids of *Drosophila* are all Hymenopterans. *Drosophila* larval parasitoids are koinobionts and belong to two families: Braconidae (including the genera *Asobara, Aphaereta, Phaenocarpa, Tanycarpa, Aspilota, Opius*) and Figitidae (*Leptopilina, Ganaspis, Leptolamina, Kleidotoma*). *Drosophila* pupal parasitoids are idiobionts and belong to three families: Diapriidae (*Trichopria, Spilomicrus*), Pteromalidae (*Pachycrepoideus, Spalangia, Trichomalopsis, Toxomorpha*) and Encytidae (*Tachinaephagus*) (Lue et al. 2021). Host-specificity across the *Drosophila* parasitoids is poorly characterized. Some can parasitize other families of Diptera, but most are thought to be limited to *Drosophila* hosts (Carton et al. 1986).

Most of my thesis is based on a new model system of *Drosophila* and their parasitoids from tropical Australian rainforest. An important part of my doctoral research was to help establish this novel system in the Hrček lab and develop the methodology to use this system in ecological studies such as those presented in this thesis. I spent seven months at the beginning of my doctoral research collecting live *Drosophila* and parasitoid lines for transport to the Czech Republic. Most of the parasitoid species used for this thesis have yet to be taxonomically described, but vouchers are available (Lue et al. 2021). Not all species have been successfully established in cultures, but most of them have been listed in Jeffs et al. (2021). A detailed description of the species used for the thesis is given in the methods section of each chapter. This novel system of species co-occurring in nature allowed me to design laboratory experiments manipulating both the abiotic and biotic environments in a fully factorial design to investigate the emergent properties of those factors in combination on interactions and communities.

C. Host-parasitoid communities in a warming world

There is a growing body of evidence on the potential effects of global changes on host-parasitoid networks. Tylianakis et al. (2007) were among the first to provide empirical evidence on the negative effects of human activity on large natural host-parasitoid networks. Subsequently, several studies have demonstrated altered host-parasitoid network structure due to deforestation (Laliberté and Tylianakis 2010), habitat fragmentation (Grass et al. 2018), habitat loss (Liao et al. 2020), decreases in plant quality (Bukovinszky et al. 2008), and climate change (Derocles et al. 2018).

With warming, host resistance to parasitoids and other natural enemy attacks change (Stacey and Fellowes 2002). Heat shock can reduce host resistance to a parasitoid, but a moderate increase in temperature tends to increase the probability that the host will successfully defend itself against its parasitoid (Fellowes et al. 1999, Thomas and Blanford 2003). The timing at which high temperatures are experienced is also important for the outcome of host-parasitoid interaction (Valls et al. 2020, Pardikes et al. 2021). Moreover, effects of climate change on host-parasitoid interactions are likely to depend on species identity, and both parasitoid and host temperature sensitivity. Parasitoids generally exhibit lower thermal tolerances than their hosts, and have to withstand the cumulative effects of warming on them and on lower trophic levels (Chidawanyika et al. 2019), making parasitoids particularly vulnerable to changes in temperature.

Shifts in ranges and phenology with climate change also have important effects on host-parasitoid interactions. Because many organisms are shifting their latitudinal ranges poleward with warming (Parmesan and Yohe 2003), they experience changes in daily light regimes, with longer summer days. Longer daylength means longer activity periods for diurnal consumer species such as many parasitoids, which can lead to higher parasitism rates, therefore impacting host-parasitoid interaction strength, and ultimately population dynamics (Kehoe et al. 2020b). Additionally, changes in daylength can change the competitive interaction strength between host species (Kehoe et al. 2018), thus changing the structure of host communities. Phenological mismatch is an important effect of warming on host-parasitoid dynamics (Abarca and Spahn 2021), and might be particularly detrimental for monophagous parasitoid species that rely on a single host species and host stage. It could also alter the strength of non-trophic interactions in host and parasitoid communities. For example, an earlier emergence of a host species at the beginning of the season allows parasitoid populations to growth early and can thus prevent the establishment of other host species that would emerge later through apparent competition. On the other hand, a synchronized arrival could lead to the exclusion of some host species through resource competition (Jones et al. 2009).

As discussed above, the strength of host-parasitoid interactions depends on both abiotic and biotic environments. As the environment is changing at an unprecedented pace, with changes in abiotic conditions and community composition, the structure and dynamics of host-parasitoid communities will be altered. How exactly communities will change with modifications in their abiotic and biotic environments, and emergent effects of the two in combinations, remains largely unknown.

AIMS AND SCOPE OF THIS THESIS

The main aim of my thesis is to investigate how the combination of abiotic and biotic environments influences the structure of host-parasitoid communities.

Chapter 1 provides an overview of the wide variety of trophic and non-trophic interactions occurring within host-parasitoid networks and discusses how they are likely to be impacted by global warming. We discuss published evidence for altered rates of parasitism with increasing temperature, and the mechanisms which have long been considered important in structuring host-parasitoid networks (e.g., apparent competition), but which have enhanced relevance in the context of global warming. We particularly highlight the role of symbiotic bacteria as an important factor structuring interactions between hosts and parasitoids.

In **Chapter 2** we investigated the direct and indirect interactions that structure host-parasitoid communities. We examined how the outcome of host-parasitoid interactions is altered by the co-occurrence of different host and parasitoid species, using different community modules of different species assemblages. The experimental design allowed us to identify which aspects of species interactions were primarily driven by community structure, and which aspects were driven by species identity.

In **Chapter 3** we investigated how warming affects a host community, either directly through species performance, or indirectly through the effect of temperatures on their biotic interactions (competition among hosts and parasitism by parasitoids).

In **Chapter 4** we investigated the effect of warming on multiple parasitoid effects for top-down control. We used an experimental approach coupled with mathematical modelling to compare estimated versus observed host suppression when two parasitoid conspecifics or heterospecifics are present, whether warming temperature affects the emergent effects of multiple parasitoids, and the mechanisms (changes in super- and multiparasitism rates, and/or changes in melanization rate) behind these effects.

References

Abarca, M., and R. Spahn. 2021. Direct and indirect effects of altered temperature regimes and phenological mismatches on insect populations. Current Opinion in Insect Science.

Abdala-Roberts, L., A. Puentes, D. L. Finke, R. J. Marquis, M. Montserrat, E. H. Poelman, S. Rasmann, A. Sentis, N. M. van Dam, G. Wimp, K. Mooney, and C. Björkman. 2019. Tri-trophic interactions: bridging species, communities and ecosystems. Ecology Letters 22:2151–2167.

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne, P. G. Amanatides, ... and F. Scheeler. 2000. The genome sequence of Drosophila melanogaster. Science 287:2185–2195.

Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2000. Thermal tolerance, climatic variability and latitude. Proceedings of the Royal Society B: Biological Sciences 267:739–745.

Agrawal, A. A., D. D. Ackerly, F. Adler, A. E. Arnold, C. Cáceres, D. F. Doak, E. Post, P. J. Hudson, J. Maron, K. A. Mooney, M. Power, D. Schemske, J. Stachowicz, S. Strauss, M. G. Turner, and E. Werner. 2007. Filling key gaps in population and community ecology. Frontiers in Ecology and the Environment 5:145–152.

Alexander, J. M., J. M. Diez, and J. M. Levine. 2015. Novel competitors shape species' responses to climate change. Nature 525:515–518.

Amarasekare, P. 2019. Effects of Climate Warming on Consumer-Resource Interactions: A Latitudinal Perspective. Frontiers in Ecology and Evolution 7:146.

Archer, L. C., E. H. Sohlström, B. Gallo, M. Jochum, G. Woodward, R. L. Kordas, B. C. Rall, and E. J. O'Gorman. 2019. Consistent temperature dependence of functional response parameters and their use in predicting population abundance. Journal of Animal Ecology 88:1670–1683.

Bairey, E., E. D. Kelsic, and R. Kishony. 2016. High-order species interactions shape ecosystem diversity. Nature Communications 7:12285.

Bar-On, Y. M., R. Phillips, and R. Milo. 2018. The biomass distribution on Earth. Proceedings of the National Academy of Sciences of the United States of America 115:6506–6511.

Barton, B. T., and O. J. Schmitz. 2009. Experimental warming transforms multiple predator effects in a grassland food web. Ecology Letters 12:1317–1325.

Beck, S. D. 2012. Insect Photoperiodism. Academic Press Inc.

Beckage, N. E., and D. B. Gelman. 2004. Wasp parasitoid disruption of host development: Implications for new biologically based strategies for insect control. Annual Review of Entomology 49:299–330.

Binzer, A., C. Guill, U. Brose, and B. C. Rall. 2012. The dynamics of food chains under climate change and nutrient enrichment. Philosophical Transactions of the Royal Society B: Biological Sciences 367:2935–2944.

Boukal, D. S., A. Bideault, B. M. Carreira, and A. Sentis. 2019. Species interactions under climate change: connecting kinetic effects of temperature on individuals to community dynamics. Current Opinion in Insect Science 35:88–95.

Bukovinszky, T., F. J. F. van Veen, Y. Jongema, and M. Dicke. 2008. Direct and indirect effects of resource quality on food web structure. Science 319:804–807.

Cai, W., Y. Yan, and L. Li. 2005. The earliest records of insect parasitoids in China. Biological Control 32:8–11.

Carton, Y., M. Boulétreau, J. J. M. van Alphen, and J. C. van Lenteren. 1986. The *Drosophila* parasitic wasps. Pages 347–394 *in* M. Ashburner, J. L. Carson, and J. N. Thompson, editors. The genetic and biology of *Drosophila*. Academic press, London.

Carton, Y., M. Poirié, and A. J. Nappi. 2008. Insect immune resistance to parasitoids. Insect Science 15:67–87.

Chamberlain, S. A., J. L. Bronstein, and J. A. Rudgers. 2014. How context dependent are species interactions? Ecology Letters 17:881–890.

Chidawanyika, F., P. Mudavanhu, and C. Nyamukondiwa. 2019. Global climate change as a driver of bottom-up and top-down factors in agricultural landscapes and the fate of host-parasitoid interactions. Frontiers in Ecology and Evolution 7:80.

Cusumano, A., E. Peri, and S. Colazza. 2016. Interspecific competition/facilitation among insect parasitoids. Elsevier Inc.

Davis, A. J., L. S. Jenkinson, J. H. Lawton, B. Shorrocks, and S. Wood. 1998a. Making mistakes when predicting shifts in species range in response to global warming. Nature 391:783–786.

Davis, A. J., J. H. Lawton, B. Shorrocks, and L. S. Jenkinson. 1998b. Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. Journal of Animal Ecology 67:600–612.

Dell, A. I., S. Pawar, and V. M. Savage. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. Proceedings of the National Academy of Sciences of the United States of America 108:10591–10596.

Derocles, S. A. P., D. H. Lunt, S. C. F. Berthe, P. C. Nichols, E. D. Moss, and D. M. Evans. 2018. Climate warming alters the structure of farmland tritrophic ecological networks and reduces crop yield. Molecular Ecology 27:4931–4946.

Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the

National Academy of Sciences of the United States of America 105:6668–6672.

Dillon, M. E., G. Wang, and R. B. Huey. 2010. Global metabolic impacts of recent climate warming. Nature 467:704–706.

Donohue, I., O. L. Petchey, S. Kéfi, A. Génin, A. L. Jackson, Q. Yang, and N. E. O'Connor. 2017. Loss of predator species, not intermediate consumers, triggers rapid and dramatic extinction cascades. Global Change Biology 23:2962–2972.

Eggleton, P. 2020. The state of the world's insects. Annual Review of Environment and Resources 45:61–82.

Englund, G., G. Öhlund, C. L. Hein, and S. Diehl. 2011. Temperature dependence of the functional response. Ecology Letters 14:914–921.

Fellowes, M. D. E., A. R. Kraaijeveld, and H. C. J. Godfray. 1999. Cross-resistance following artificial selection for increased defense against parasitoids in *Drosophila melanogaster*. Evolution 53:966–972.

Fleury, F., N. Ris, R. Allemand, P. Fouillet, Y. Carton, and M. Boulétreau. 2004. Ecological and genetic interactions in Drosophila-parasitoids communities: a case study with *D. melanogaster, D. simulans* and their common *Leptopilina* parasitoids in south-eastern France. Pages 181–194 *in* P. Capy, P. Gibert, and I. Boussy, editors. Drosophila melanogaster, Drosophila simulans: So Similar, So Different. Springer Netherlands.

Freeman, B. G., Y. Song, K. J. Feeley, and K. Zhu. 2021. Montane species track rising temperatures better in the tropics than in the temperate zone. Ecology Letters:ele.13762.

Gilbert, B., T. D. Tunney, K. S. Mccann, J. P. Delong, D. A. Vasseur, V. Savage, J. B. Shurin, A. I. Dell, B. T. Barton, C. D. G. Harley, H. M. Kharouba, P. Kratina, J. L. Blanchard, C. Clements, M. Winder, H. S. Greig, and M. I. O'Connor. 2014. A bioenergetic framework for the temperature dependence of trophic interactions. Ecology Letters 17:902–914.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293:2248–2251.

Grass, I., B. Jauker, I. Steffan-Dewenter, T. Tscharntke, and F. Jauker. 2018. Past and potential future effects of habitat fragmentation on structure and stability of plant–pollinator and host–parasitoid networks. Nature Ecology & Evolution 2:1408–1417.

Hällfors, M. H., J. Pöyry, J. Heliölä, I. Kohonen, M. Kuussaari, R. Leinonen, R. Schmucki, P. Sihvonen, and M. Saastamoinen. 2021. Combining range and phenology shifts offers a winning strategy for boreal Lepidoptera. Ecology Letters:ele.13774.

Hallmann, C. A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson, and H. De

Kroon. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS ONE 12:e0185809.

Hamilton, A. J., Y. Basset, K. K. Benke, P. S. Grimbacher, S. E. Miller, V. Novotný, G. A. Samuelson, N. E. Stork, G. D. Weiblen, and J. D. L. Yen. 2010. Quantifying uncertainty in estimation of tropical arthropod species richness. The American Naturalist 176:90–95.

Harvey, J. A., E. H. Poelman, and T. Tanaka. 2013. Intrinsic inter- and intraspecific competition in parasitoid wasps. Annual Review of Entomology 58:333–351.

Henri, D. C., and F. J. F. Van Veen. 2011. Body size, life history and the structure of host–parasitoid networks. Pages 135–180 *in* A. Belgrano, editor. Advances in Ecological Research. Academic Press.

Heraty, J. 2017. Parasitoid Biodiversity and Insect Pest Management. Pages 603–625 Insect Biodiversity. John Wiley & Sons, Ltd.

Holt, R. D. 1997. Community modules. Page *in* A. C. Gange and V. K. Brown, editors. Multitrophic Interactions in Terrestrial Ecosystems. Blackwell Science, Oxford.

Holt, R. D., and J. H. Lawton. 1994. The ecological consequences of shared natural enemies. Annual Review of Ecology and Systematics 25:495–520.

Hrček, J., and H. C. J. Godfray. 2015. What do molecular methods bring to host–parasitoid food webs? Trends in Parasitology 31:30–35.

Huey, R. B., and R. D. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. Integrative and Comparative Biology 19:357–366.

Janzen, D. H., and W. Hallwachs. 2021. To us insectometers, it is clear that insect decline in our Costa Rican tropics is real, so let’s be kind to the survivors. Proceedings of the National Academy of Sciences 118:e2002546117.

Jeffs, C. T., J. C. D. Terry, M. Higgie, A. Jandová, H. Konvičková, J. J. Brown, C. H. Lue, M. Schiffer, E. K. O’Brien, J. Bridle, J. Hrček, and O. T. Lewis. 2021. Molecular analyses reveal consistent food web structure with elevation in rainforest *Drosophila* - parasitoid communities. Ecography 44:403–413.

Johansson, F., G. Orizaola, and V. Nilsson-Örtman. 2020. Temperate insects with narrow seasonal activity periods can be as vulnerable to climate change as tropical insect species. Scientific Reports 10:1–8.

Jones, T. S., H. C. J. Godfray, and F. J. F. van Veen. 2009. Resource competition and shared natural enemies in experimental insect communities. Oecologia 159:627–635.

Kéfi, S., E. L. Berlow, E. A. Wieters, L. N. Joppa, S. A. Wood, U. Brose, and S. A. Navarrete. 2015. Network structure beyond food webs: Mapping non-trophic and trophic interactions on Chilean rocky shores. Ecology 96:291–303.

Kehoe, R. C., D. Cruse, D. Sanders, K. J. Gaston, and F. J. F. van Veen. 2018. Shifting daylength regimes associated with range shifts alter aphid-parasitoid community dynamics. Ecology and Evolution 8:8761–8769.

Kehoe, R., E. Frago, C. Barten, F. Jecker, F. van Veen, and D. Sanders. 2016. Nonhost diversity and density reduce the strength of parasitoid–host interactions. Ecology and Evolution 6:4041–4049.

Kehoe, R., E. Frago, and D. Sanders. 2020a. Cascading extinctions as a hidden driver of insect decline. Ecological Entomology:een.12985.

Kehoe, R., D. Sanders, D. Cruse, M. Silk, K. J. Gaston, J. R. Bridle, and F. van Veen. 2020b. Longer photoperiods through range shifts and artificial light lead to a destabilizing increase in host–parasitoid interaction strength. Journal of Animal Ecology 89:2508–2516.

Kellermann, V., J. Overgaard, A. A. Hoffmann, C. Fløjgaard, J.-C. Svenning, and V. Loeschcke. 2012. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. Proceedings of the National Academy of Sciences 109:16228–16233.

Kingsolver, J. G., S. E. Diamond, and L. B. Buckley. 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Functional Ecology 27:1415–1423.

Knight, T. M., M. W. McCoy, J. M. Chase, K. A. McCoy, and R. D. Holt. 2005. Trophic cascades across ecosystems. Nature 437:880–883.

Kraaijeveld, A. R., and H. C. J. Godfray. 1997. Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. Nature 389:278–280.

Kraaijeveld, A. R., and H. C. J. Godfray. 2009. Evolution of host resistance and parasitoid counter-resistance. Pages 257–280 Advances in Parasitology.

Lafferty, K. D., S. Allesina, M. Arim, C. J. Briggs, G. De Leo, A. P. Dobson, J. A. Dunne, P. T. J. Johnson, A. M. Kuris, D. J. Marcogliese, N. D. Martinez, J. Memmott, P. A. Marquet, J. P. McLaughlin, E. A. Mordecai, M. Pascual, R. Poulin, and D. W. Thieltges. 2008. Parasites in food webs: the ultimate missing links. Ecology Letters 11:533–546.

Laliberté, E., and J. M. Tylianakis. 2010. Deforestation homogenizes tropical parasitoid–host networks. Ecology 91:1740–1747.

Le Lann, C., M. Lodi, and J. Ellers. 2014. Thermal change alters the outcome of behavioural interactions between antagonistic partners. Ecological Entomology 39:578–588.

Liao, J., X. Xi, D. Bearup, and S. Sun. 2020. Metacommunity robustness of plant–fly–wasp tripartite networks with specialization to habitat loss. Ecology 101.

Lister, B. C., and A. Garcia. 2018. Climate-driven declines in arthropod abundance restructure a rainforest food web. Proceedings of the National Academy of Sciences of the United States of America

115:E10397–E10406.

Lue, C.-H., M. L. Buffington, S. Scheffer, M. Lewis, T. A. Elliott, A. R. I. Lindsey, A. Driskell, A. Jandova, M. T. Kimura, Y. Carton, R. R. Kula, T. A. Schlenke, M. Mateos, S. Govind, J. Varaldi, E. Guerrier, M. Giorgini, X. Wang, K. Hoelmer, K. M. Daane, P. K. Abram, N. A. Pardikes, J. J. Brown, M. Thierry, M. Poirie, P. Goldstein, S. E. Miller, D. Tracey, J. S. Davis, F. M. Jiggins, B. Wertheim, O. Lewis, J. Leips, P. P. A. Staniczenko, and J. Hrcek. 2021. DROP: Molecular voucher database for identification of *Drosophila* parasitoids. Molecular Ecology Resources.

Mills, N. J., and W. M. Getz. 1996. Modelling the biological control of insect pests: A review of host- parasitoid models. Ecological Modelling 92:121–143.

Milo, R., S. Shen-Orr, S. Itzkovitz, N. Kashtan, D. Chklovskii, and U. Alon. 2002. Network motifs: simple building blocks of complex networks. Science 298:824–827.

Morgan, T. H. 1910. Chromosomes and heredity. The American Naturalist 44:449–496.

Neven, L. G. 2000. Physiological responses of insects to heat. Postharvest Biology and Technology 21:103–111.

Oliver, K. M., A. H. Smith, and J. A. Russell. 2014. Defensive symbiosis in the real world – advancing ecological studies of heritable, protective bacteria in aphids and beyond. Functional Ecology 28:341–355.

Ortiz-Martínez, S., J. S. Pierre, J. van Baaren, C. Le Lann, F. Zepeda-Paulo, and B. Lavandero. 2019. Interspecific competition among aphid parasitoids: molecular approaches reveal preferential exploitation of parasitized hosts. Scientific Reports 9.

Overgaard, J., T. N. Kristensen, K. A. Mitchell, and A. A. Hoffmann. 2011. Thermal tolerance in widespread and tropical *Drosophila* species: Does phenotypic plasticity increase with latitude? American Naturalist 178.

Pachauri, R. K., L. Meyer, S. Hallegatte France, W. Bank, G. Hegerl, S. Brinkman, L. van Kesteren, N. Leprince-Ringuet, and F. van Boxmeer. 2014. IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change e [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. Page IPCC. IPCC, Geneva, Switzerland.

Paine, R. T. 1980. Food webs: Linkage, interaction strength and community infrastructure. The Journal of Animal Ecology 49:666.

Pardikes, N., T. Revilla, C.-H. Lue, Thierry Mélanie, D. Soutos-Villaros, and J. Hrcek. 2021. Community context modifies response of host-parasitoid interactions to phenological mismatch under warming. Authorea.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.

Pedersen, B. S., and N. J. Mills. 2004. Single vs. multiple introduction in biological control: The roles of parasitoid efficiency, antagonism and niche overlap. Journal of Applied Ecology 41:973–984.

Pennisi, E. 2010. The little wasps that could. Science 327:260–262.

Rall, B. Ö. C., O. Vucic-Pestic, R. B. Ehnes, M. EmmersoN, and U. Brose. 2010. Temperature, predator-prey interaction strength and population stability. Global Change Biology 16:2145–2157.

Reiter, L. T., L. Potocki, S. Chien, M. Gribskov, and E. Bier. 2001. A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. Genome Research 11:1114–1125.

Reuter, O. M. 1913. Lebensgewohnheiten und Instinkte der Insekten. Friendlander, Berlin.

Ripple, W. J., and R. L. Beschta. 2012. Trophic cascades in Yellowstone: The first 15 years after wolf reintroduction. Biological Conservation 145:205–213.

Ripple, W. J., J. A. Estes, O. J. Schmitz, V. Constant, M. J. Kaylor, A. Lenz, J. L. Motley, K. E. Self, D. S. Taylor, and C. Wolf. 2016. What is a trophic cascade? Trends in Ecology & Evolution 31:842–849.

Ruel, J. J., and M. P. Ayres. 1999. Jensen's inequality predicts effects of environmental variation. Trends in Ecology and Evolution 14:361–366.

Sánchez-Bayo, F., and K. A. G. Wyckhuys. 2019. Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation 232:8–27.

Sanders, D., R. Kehoe, and F. J. F. van Veen. 2015. Experimental evidence for the population-dynamic mechanisms underlying extinction cascades of carnivores. Current Biology 25:3106–3109.

Sentis, A., A. Binzer, and D. S. Boukal. 2017a. Temperature-size responses alter food chain persistence across environmental gradients. Ecology Letters 20:852–862.

Sentis, A., C. Gémard, B. Jaugeon, and D. S. Boukal. 2017b. Predator diversity and environmental change modify the strengths of trophic and nontrophic interactions. Global Change Biology 23:2629–2640.

Sentis, A., J. L. Hemptinne, and J. Brodeur. 2017c. Non-additive effects of simulated heat waves and predators on prey phenotype and transgenerational phenotypic plasticity. Global Change Biology 23:4598–4608.

Sinclair, B. J., K. E. Marshall, M. A. Sewell, D. L. Levesque, C. S. Willett, S. Slotsbo, Y. Dong, C. D. G. Harley, D. J. Marshall, B. S. Helmuth, and R. B. Huey. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures?

Ecology Letters 19:1372–1385.

Snyder, W. E., and A. R. Ives. 2003. Interactions between specialist and generalist natural enemies: parasitoids, predators, and pea aphid biocontrol. Ecology 84:91–107.

Stacey, D. A., and M. D. E. Fellowes. 2002. Influence of temperature on pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae) resistance to natural enemy attack. Bulletin of Entomological Research 92:351–357.

Terry, J. C. D., M. B. Bonsall, and R. J. Morris. 2020. Identifying important interaction modifications in ecological systems. Oikos 129:147–157.

Thomas, M. B., and S. Blanford. 2003. Thermal biology in insect-parasite interactions. Trends in Ecology & Evolution 18:344–350.

Tweedie, S., M. Ashburner, K. Falls, P. Leyland, ... and R. Wilson. 2009. FlyBase: Enhancing *Drosophila* Gene Ontology annotations. Nucleic Acids Research 37:555–559.

Tylianakis, J. M., T. Tscharntke, and O. T. Lewis. 2007. Habitat modification alters the structure of tropical host–parasitoid food webs. Nature 445:202–205.

Valls, A., K. Kral-O'Brien, J. Kopco, and J. P. Harmon. 2020. Timing alters how a heat shock affects a host-parasitoid interaction. Journal of Thermal Biology 90:102596.

Vasseur, D. A., J. P. DeLong, B. Gilbert, H. S. Greig, C. D. G. Harley, K. S. McCann, V. Savage, T. D. Tunney, and M. I. O'Connor. 2014. Increased temperature variation poses a greater risk to species than climate warming. Proceedings of the Royal Society B: Biological Sciences 281.

van Veen, F. J. F., R. J. Morris, and H. C. J. Godfray. 2006. Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. Annual Review of Entomology 51:187–208.

Vorburger, C., and S. J. Perlman. 2018. The role of defensive symbionts in host–parasite coevolution. Biological Reviews 93:1747–1764.

Wagner, D. L., E. M. Grames, M. L. Forister, M. R. Berenbaum C, and D. Stopak. 2021. Insect decline in the Anthropocene: Death by a thousand cuts. Proceedings of the National Academy of Sciences 118.

Wajnberg, E., J. K. Scott, and P. C. Quimby. 2001. Evaluating Indirect Ecological Effects of Biological Control. CABI.

Walther, G.-R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J.-M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature 416:389–395.

Wirta, H. K., P. D. N. Hebert, R. Kaartinen, S. W. Prosser, G. Várkonyi, and T. Roslin. 2014. Complementary molecular information changes our perception of food web structure. Proceedings of the National Academy of Sciences 111:1885–1890.

Chapter I

Mechanisms structuring host-parasitoid networks in a global warming context

(In Ecological Entomology, 2019)

Mechanisms structuring host-parasitoid networks in a global warming context: a review

Mélanie Thierry[*][1, 2]**, Jan Hrček** [1, 2] **& Owen T. Lewis** [3]

[1]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, [2]Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice, Czech Republic and [3]Department of Zoology, University of Oxford, Oxford, U.K.

*Correspondence: Melanie Thierry, University of South Bohemia, Faculty of Science, Branisovska 31, Ceske Budejovice 37005, Czech Republic. E-mail: m.thierry@entu.cas.cz

Abstract. 1. In natural communities, multiple host and parasitoid species are linked to form complex networks of trophic and non-trophic interactions. Understanding how these networks will respond to global warming is of wide relevance for agriculture and conservation.

2. We synthesize the emerging evidence surrounding host-parasitoid networks in the context of global warming. We summarize the suite of direct and indirect interaction types within host-parasitoid networks, and their sensitivity to temperature changes; and we compile and review studies investigating the responses of whole host-parasitoid networks to increasing temperatures or proxy variables. We find limited evidence overall for the prediction that parasitism will be reduced under global warming: approximately equal numbers of studies show elevated and reduced parasitism.

3. Increasingly, endosymbiotic bacteria are recognized as influential mediators of host-parasitoid interactions. These endosymbionts can change how individual species respond to global warming, and their effects can cascade to affect whole host-parasitoid networks. We review the evidence that symbiotic bacteria are likely to affect the response of host-parasitoid networks to global warming. Symbionts can protect hosts from their parasitoids or influence thermal

tolerance of their host species. Furthermore, the symbionts themselves can be impacted by global warming.

4. We conclude by considering the most promising avenues for future research into the mechanisms structuring host-parasitoid networks in the context of global warming. Alongside the increasing availability of modern molecular methods to document the structure of real, species-rich host-parasitoid networks, we highlight the utility of manipulative experiments and mathematical models.

Key words. Climate change, endosymbiont, host–parasite networks, indirect interactions, interaction networks, non-trophic interactions.

Introduction

In the last century, climate warming has become a major concern for ecologists (Walther *et al.*, 2002; Parmesan & Yohe, 2003; Root *et al.*, 2003; Walther, 2010), mainly because it threatens biodiversity and ecosystem functioning (Pecl *et al.*, 2017). Species' responses to global warming, such as shifts in phenology, physiological changes and range shifts, have been widely studied (Walther *et al.*, 2002). However, responses of individual species to global warming can have cascading effects via their interactions with other species (Valiente-Banuet *et al.*, 2015), and influence the structure of ecological networks linking the wider community (Grass *et al.*, 2018). Despite this, most previous studies on the effect of global warming on terrestrial ecosystems have focused on individual species in isolation, or at most have considered pairwise interactions between species (Tylianakis *et al.*, 2008; Walther, 2010), overlooking important effects on ecological network structure (Tylianakis *et al.*, 2007) and ecosystem functioning (Goudard & Loreau, 2008; Miele *et al.*, 2018).

Ecological networks linking insect hosts to their parasitoids may be particularly sensitive to global warming (Hance *et al.*, 2007). Most parasitoids are wasps (Hymenoptera) or flies (Diptera); their larvae live as parasites on or within the bodies of their arthropod hosts, eventually killing them (Godfray, 1994). Host-parasitoid networks are defined by the direct trophic interactions between host species and parasitoid species, but a variety of non-trophic interactions, both direct (e.g., exploitative competition) and indirect (e.g., apparent competition), also occur. Changes in ecological network structure under global warming can result from existing interactions becoming uncoupled and new ones being established, or from shifting interaction frequencies (Davis *et al.*, 1998b; Blois *et al.*, 2013; Péré *et al.*, 2013; Maunsell *et al.*, 2015; Pellissier *et al.*, 2017). Since each host-parasitoid association may respond differently to environmental changes (Hance *et al.*, 2007), predictions about network structure changes under global warming based on individual and pairwise species responses are likely to be highly misleading (Davis *et al.*, 1998a; McCann, 2007; Gilman *et al.*, 2010; Tylianakis & Romo, 2010; Harvey *et al.*, 2017). Moreover, the interactions within these network (‘edges’) might be more sensitive to global warming than the species (‘nodes’), because interactions require that multiple species are present at the same place at the same time (Valiente-Banuet *et al.*, 2015; Jordano, 2016a; Poisot *et al.*, 2017). Considering how whole networks of interacting host and parasitoid species respond to global warming could give us a better understanding of its impact on associated ecosystem functions and services such as biological control (Costanza *et al.*, 1997).

When the hosts are herbivores, ecological networks of insect hosts and their parasitoids are linked to a third level: their host plants. Understanding the mechanisms involved in plant-herbivore-parasitoid tri-tropic interactions (see Kaplan *et al.*, 2016 for a review), and how global warming could impact them is particularly relevant for predicting herbivore pest outbreaks in agricultural systems. Moreover, plant community response to global warming can have bottom-up effect on both insect herbivores and their parasitoids (Gillespie *et al.*, 2012; but see Flores-Mejia *et al.*, 2017; Dong *et al.*, 2018). Here, however, we restrict our focus to the more general case of bipartite interactions between hosts and parasitoids. Understanding these interactions is an essential precursor to understanding even more complex tri-trophic networks.

Over the last decade a newly-identified mechanism has emerged as a potentially important force structuring host-parasitoid networks: the influence of endosymbiotic bacteria (Duron & Hurst, 2013; Hrček *et al.*, 2016; McLean *et al.*, 2016). These microorganisms live inside host cells or hemolymph and display strong co-evolutionary dynamics with their hosts

(Henry *et al.*, 2015). They can be obligate or primary endosymbionts, which are present in all individuals of the host species, or facultative endosymbionts (also known as secondary endosymbionts), which are not present in all individuals. Endosymbionts can improve host thermal tolerance (Russell & Moran, 2005; Brumin *et al.*, 2011), mediate host-parasitoid interactions (Oliver *et al.*, 2003, 2005; Xie *et al.*, 2010, 2014), influence competitive ability of their hosts (Oliver *et al.*, 2008) and shape host-parasitoid food webs (Ye *et al.*, 2018; Monticelli *et al.*, 2019). Thus, the effects of endosymbionts on host-parasitoid network structure might be critical to predicting how host-parasitoid network structure would respond to global warming.

In this review we first describe the different trophic and non-trophic interactions that occur within host-parasitoid networks. We then discuss the impacts of global warming on species, interactions, and entire host-parasitoid networks. Finally, we review the role endosymbiotic bacteria play in modifying the responses of host-parasitoid networks to global warming and discuss future research priorities. We focus on the impacts of global warming, as the effects of other effects of global environmental change (e.g. CO_2 concentrations and extreme weather) on ecological networks have been recently reviewed (Tylianakis & Binzer, 2014; Tylianakis & Morris, 2017). Our goal is to highlight the diversity of mechanisms structuring host-parasitoid networks and how they are expected to change with global warming.

Interactions within host-parasitoid networks

Interactions between species can be classified as direct or indirect (i.e., involving one or more other species) and as trophic or non-trophic. These different interaction types have typically been studied separately. However, all interactions work in concert to shape network structure (Eubanks & Finke, 2014), so considering them simultaneously should be informative about the patterns and processes within host-parasitoid networks (Fontaine et al., 2011). Furthermore, the stability of networks depends on how those different types of interaction function together (Bastolla *et al.*, 2009). In Figure 1, we illustrate the variety of interactions that may operate within host-parasitoid networks. Below, we discuss each of these in turn, providing the biological context needed to understand how they are likely to be affected, individually and in combination, by global warming.

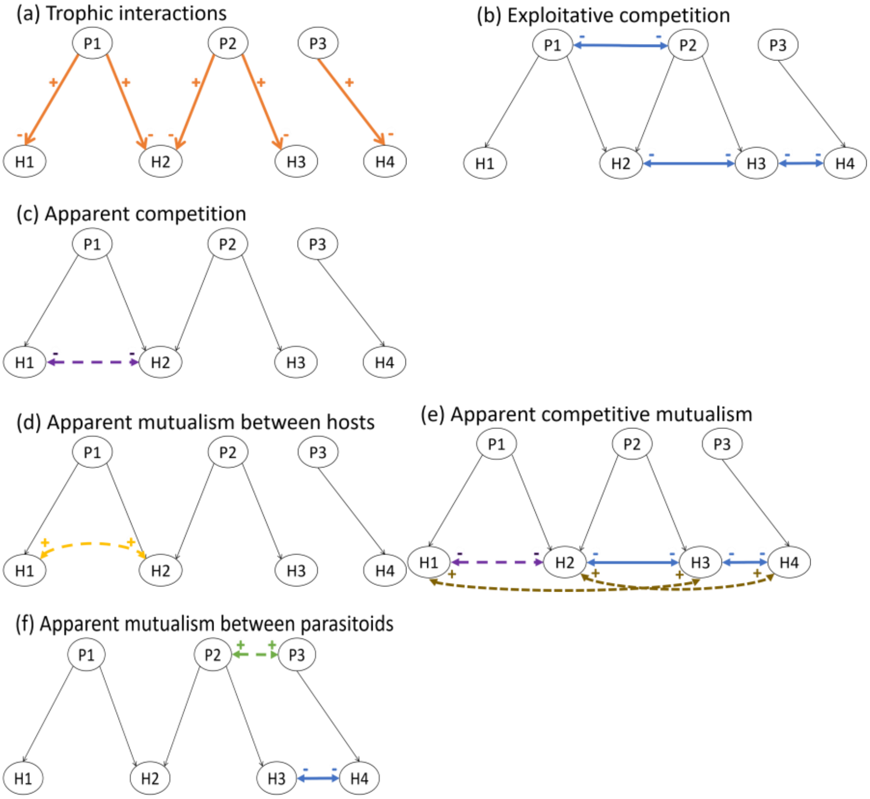


Figure 1. Interaction types in host-parasitoid networks, adapted from the framework of Abrams (1995). Nodes represent species of parasitoid (P1, P2 and P3) or hosts (H1, H2, H3 and H4). Edges are shown using single-headed arrows (trophic links) and double-headed arrows (non-trophic links). Solid arrows represent direct interactions and dotted arrows indirect interactions. The plus or minus signs at the end of the arrows represent a positive or a negative effect respectively for the population close to that end. These hypothetical examples are unweighted, i.e., they do not represent differences in abundances of the interacting species and the frequencies of the interactions among them. (a) Trophic interactions for a hypothetical food web of three parasitoids (P1, P2 and P3) attacking four hosts (H1, H2, H3 and H4); (b) Exploitative competition (blue arrows): P1 and P2 share the host species H2 and compete for it when this resource is limited. H2 and H3 and H3 and H4 also compete for resources in this hypothetical example; (c) Apparent competition (purple arrows): H1 and H2 share a parasitoid P1.

Their dynamics are linked in a way that parallels competition: if either prey population increases, their shared parasitoid also increases which has a negative impact on the other host population. (d) Apparent mutualism between hosts (yellow arrows): H1 and H2 share a parasitoid P1. If the presence of either host species serves to decrease attack rates of P1 on the other species, their populations growth rates will be positively correlated, and they would appear to be mutualists. (e) Apparent competitive mutualism (brown arrows): H3 and H4 compete for resources and H2 and H3 are apparent competitors. H2 and H4 are thus indirect mutualists because they both have a negative impact on their shared competitor H3. The same applies for H1 and H3 because both are competitors of H2; (f) Apparent mutualism between parasitoids (green arrow): P2 and P3 do not interact directly or share a host species, but attack different hosts that are competitors. If the population of P2 increases, its host population H3 decreases. This allows the other population of H4 to increase, providing more food for, and increased populations of, E3.

Direct trophic interactions

The most obvious interactions within host-parasitoid networks, and the ones that are most simply documented and quantified, are the trophic interactions between host species and their parasitoids (Fig. 1a). A key difference from many other trophic networks is that all parasitoids have obligate associations with their hosts. Parasitoids vary from those that are specialized on a single host species, to those that are generalists, able to exploit multiple host species (Hassell & Waage, 1984). Hosts have a variety of defense mechanisms against parasitoids, such as hemocyte encapsulation of parasitoid eggs and larvae (Carton *et al.*, 2008). In turn, parasitoids have evolved counter-strategies (Godfray, 1994): *Leptopilina* species inject virus-like particles into the *Drosophila* host to express proteins that disable the host immune system, while *Asobara tabida* produces sticky eggs that embed within host tissue, escaping the encapsulation process (Kraaijeveld & Godfray, 2009). Not all parasitoids are equally virulent in a given host (Schlenke *et al.*, 2007), and both host resistance and parasitoid virulence control host range and susceptibility of a given parasitoid species (Lee *et al.*, 2009). This in turn influences trophic interactions and the structure of host-parasitoid networks.

Interspecific competition

One of the most prevalent non-trophic interactions is interspecific competition for resources (i.e., exploitative competition; Fig. 1b). Superior competitors are those that can persist at the lowest level of a shared limiting resource, leading to the exclusion of inferior competitors (Tilman, 1982). Exploitative competition can occur among host species when they share resources (Jones *et al.*, 2009) influencing their relative abundances. Exploitative competition can also occur among parasitoid species during the host-selection process and during larval development in or on the host (Harvey *et al.*, 2013). Parasitoids use a range of mechanisms to compete within hosts, including physical attack and physiological suppression (Harvey *et al.*, 2009). To reduce competition, some parasitoids have evolved to specialize on one host species or to exploit different host stages. Because of this high specialization, host-parasitoid networks are often highly modular (i.e. the network is divided into relatively discrete compartments) (Thébault & Fontaine, 2010). However, if a parasitoid that is a superior competitor is excluded from the community, other parasitoid species might extend their diet breadth, modifying the structure of the host-parasitoid network.

Parasitism as a modulator of competition

The interaction between parasitism and competition is essential to explain the structure of species-rich host-parasitoid networks. Parasitoids can shape host communities and ecological networks by preventing competitive exclusion (Holt & Lawton, 1993; LeBrun & Feener, 2002; van Veen *et al.*, 2005), or by modifying the relative strength of intraspecific and interspecific competition (Grover, 1994). Release from top-down control by parasitoids can also have cascading effects, for example causing extinction of parasitoid and host species via indirect interactions (Sanders & van Veen, 2012; Sanders *et al.*, 2013, 2015).

Apparent competition

Whether or not they are competing directly for resources, species at the same trophic level in ecological networks can also have negative effects on each other through shared enemies. This process is called apparent competition (Holt, 1977; Morris *et al.*, 2004) (Fig. 1c), and can result from either short-term aggregation of parasitoids or from a long-term demographic response (Holt and Kotler 1987). Where host populations are spatially subdivided and function as metapopulations, alternative host species can suffer apparent competition even if they are not in the same patch as the most competitive host species (Holt & Lawton, 1993, 1994; Davis *et al.*, 1998a). Apparent competition can even occur among species across the landscape if ecological networks in multiple habitats function as a single meta-network (Frost *et al.*, 2016). Both short-term effects on parasitoid density and long-term effect on parasitoid demographic growth rates could impact the relative abundances of host species and thus the structure of their communities and ecological networks. Moreover, parasitism can also interact with apparent competition and allow coexistence of apparent competitors through the effects of parasitoid aggregation (Bonsall & Hassell, 1999).

Apparent mutualism

Apparent mutualism occurs where populations of host species increase in concert because of indirect effects on a predator (Abrams & Matsuda, 1996; Abrams *et al.*, 1998) (Fig. 1d). These effects can be mediated by the density of alternative hosts or non-hosts (density-mediated indirect interactions) or by behavior (trait-mediated indirect interaction; see Werner & Peacor, 2003 for a review). Species can also appear to have a mutualistic relationships if they have a negative effect on a common competitor (Abrams & Matsuda, 1996) (Fig. 1e). Indeed, if a species decreases the population of another species through exploitative or apparent competition, this species will have a positive indirect effect on other species sharing the same competitor.

In a similar way, two parasitoid species could also be apparent mutualists if they attack different host species that compete (Fig. 1f). The negative effect of a parasitoid on one host species is beneficial for the host species with which it competes. Thus, the parasitoid indirectly facilitates the competitor of its host species, which in turn will be beneficial for the parasitoid species attacking that host.

The wide variety and complexity of the possible direct and indirect interactions within host-parasitoid networks, summarized above, means that the loss of any species, or a change in its abundance, may have widespread and seemingly unpredictable cascading effects on other species (Hammill *et al.*, 2015). Sampling ecological interactions in nature is difficult as they can't always be observed (Jordano, 2016b). However, a clear understanding of the diversity of interaction types and how they might interact is an important step for understand how perturbations might reconfigure these ecological networks. In the next section, we address the likely pathways by which global warming is most likely to impinge on host-parasitoid networks.

Effect of global warming on host-parasitoid network structure

We first discuss the effects of global warming on immunological aspects of host-parasitoid interactions (i.e., immunological contingencies, or the combination of host defenses and parasitoid virulence) and on host-parasitoid trophic interactions. We then address how global warming impacts host-parasitoid spatio-temporal synchrony and non-trophic interactions. Finally, we explore how the likely effect of global warming on species and their interactions could change the structure of entire networks.

Effect of global warming on host immunity and host-parasitoid trophic interactions

One mechanism through which increased temperatures can alter the strength and frequency of host-parasitoid interactions is by changing host immune response functions (host resistance) (Thomas & Blanford, 2003). For example, at elevated temperatures pea aphids are more resistant to fungal pathogens (Stacey & Fellowes, 2002) but more susceptible to parasitoids (Bensadia *et al.*, 2006). This suggests that the effects of temperature on host immune responses also depends on a parasite’s identity. The response of host immune function against parasitoids to global warming is poorly known and is likely to vary among and within species.

Temperature can also affect parasitoid attack rates, and their ability to develop in a host and to circumvent host defenses (parasitoid virulence) (Ris *et al.*, 2004; Le Lann *et al.*, 2014; Delava *et al.*, 2016). Romo & Tylianakis (2013) found that attack rates by parasitoids on aphids increased with temperature, both in the field and in the laboratory; however, the effect was reversed when increasing temperatures were combined with drought treatments, as expected under global change for most parts of the world (Dai, 2013). However, prolonged heat stress can induce high mortality rates in adult parasitoids (Roux *et al.*, 2010). These studies suggest that global warming might decrease rates of parasitism (see Table 1), but more studies, focusing on a wider variety of systems, are needed to establish how general these patterns are likely to be.

In addition to effects on immunology, global warming can also alter existing host-parasitoid trophic interactions by changing the quality and quantity of hosts. Heatwaves can be particularly detrimental for insect growth rates (Roitberg & Mangel, 2016). With elevated temperatures, generalist parasitoids have been found to favor large-bodied or abundant host species (de Sassi *et al.*, 2012). This change in preference could decrease the strength of interactions with smaller or less abundant host species. In a field experiment, elevated temperatures led to a doubling of the biomass of herbivorous insects without altering parasitoid biomass significantly (de Sassi & Tylianakis, 2012). As parasitoids can be limited in the number of eggs they can lay (Heimpel & Rosenheim, 1998), they could fail to regulate host populations under global warming. Another field experiment simulating the effects of increasing temperature on plant-aphid-parasitoid network structure in wheat crops observed an aphid outbreak without change in parasitism rates (Derocles *et al.*, 2018). The long-term effects remain unknown.

Together, these effects of temperature on immunology and trophic interactions suggest that global warming might result in long-term changes to host-parasitoid networks with fewer and weaker trophic links. While this could have profound impacts on ecosystem functioning and ecosystem services, evidence remains sparse.

Effect of global warming on host and parasitoid synchrony

Global warming could modify host and parasitoid population dynamics (Jeffs & Lewis, 2013) by altering life-history traits such as developmental time, lifespan and winter diapause, for both hosts and parasitoids (Hance, 2007; Schreven *et al.*, 2017; Tougeron *et al.*, 2018). As host-parasitoid trophic interactions are typically specialized, and hosts and parasitoids often respond differently, this could disrupt phenological synchrony between hosts and parasitoids (Visser & Both, 2005; Klapwijk *et al.*, 2010; Dyer *et al.*, 2013). One possible outcome is release of hosts from top-down control (Godfray, 1994; Schweiger *et al.*, 2008; Lavergne *et al.*, 2010; Furlong & Zalucki, 2017) with consequent changes to host-parasitoid network structure.

Table 1. Studies investigating the effect of global warming on parasitism rate, together with the study system, the direction and strength of the effect, the type of evidence, the nature of treatment and the proposed mechanism.

Study system	**Direction of the effect**	**Strength of the effect**	**Type of evidence**	**Nature of treatment**	**Proposed mechanism**	**Reference**
Drosophila-parasitoids	Depends on the species	Depends on the species	Laboratory experiment	Simulated global warming	Indirect interactions	Davis *et al.* (1998a)
Caterpillar-parasitoids	Less parasitism	Strong	Laboratory experiment	Simulated global warming	Caterpillar development time decreases	Dyer *et al.* (2013)
Aphid-parasitoids	Aphid outbreak but no effect on parasitism rate	No effect	Field experiment	Simulated global warming	Experimental design does not allow to assess long-term effects	Derocles *et al.* (2018)
Leaf miners-parasitoids	More parasitism	Weak (no evidence of increase in parasitism rate when translocated to lower elevations)	Field experiment	Elevational gradient	Host and parasitoids respond differently to environmental changes	Maunsell *et al.* (2015)

Aphids-parasitoids	More parasitism	Strong (but hyperparasitism also increases with temperature)	Field experiment	Natural temperature gradient	Higher rates of parasited population	Romo & Tylianakis (2013)
Cavity-nesting Hymenoptera-parasitoids	More parasitism	Weak	Field observations	Elevational gradient	Increase in diversity with elevation	Morris *et al.* (2015)
Insects	Less parasitism	Strong	Meta-analysis	Elevational gradient	Decreases in species-specific rates of parasitism	Péré *et al.* (2013)
Caterpillar-parasitoids	Less parasitism	Strong	Field observations	Spectrum of climatic regimes	Climatic variability alters parasitoid ability to track host populations	Stireman *et al.* (2005)

Shifts in the geographical distribution of species' ranges are a common response to global warming: populations colonize new localities and habitats as these become thermally suitable, while retreating from regions and habitats that become too hot (Jeffs & Lewis, 2013; Nicholls *et al.*, 2018). For a *Drosophila*-parasitoid system, Davis and collaborators (1998b) demonstrated using microcosm experiments that range shifts depend not only on temperatures but also on species interactions and the effect of temperature on them. Studies on the effect of temperature on interactions, and not only species, are thus important for predicting the consequences of global warming for host-parasitoid networks.

Overall, reduced overlap in activity period between host and parasitoid and host species shifting range induced by global warming could completely change current host-parasitoid network structure and could lead to marked increases in pest outbreaks (Johnson & Jones, 2017).

Effects of global warming on host-parasitoid non-trophic interactions

Most work on global warming has focused on its effects on individual species and pairwise trophic interactions (reviewed in Tylianakis *et al.*, 2008), but non-trophic interactions can also be impacted by global warming. If one host is more sensitive to global warming, other host species might benefit through competitive release (Jones & Lawton, 2012). Moreover, the outcome of competition depends on environmental conditions (Holt & Lawton, 1993; Davis *et al.*, 1998b). An example of environment-mediated interactions has been documented in *Drosophila* (Fleury *et al.*, 2004). When parasitoids are absent, *D. melanogaster* always eliminates *D. simulans* when they compete for limited resources in any thermal regime. But the outcome of competition is modified by the presence of parasitoids and varies according to temperature. Indeed, at 28°C, *D. melanogaster* still eliminates *D. simulans* through resource competition. At 25°C, both species coexist and at 22°C, *D. simulans* increases until *D. melanogaster* nearly goes extinct. Hence, global warming could change the equilibrium between host species mediated by parasitoids.

Consequences of global warming for entire host-parasitoid networks

The structure of a host-parasitoid network depends not only on interactions among species, but also on how those interactions (either trophic or non-trophic) respond to environmental conditions. Moreover, effect of global warming on populations and communities depends on the structure of the networks of interactions (Bascompte & Stouffer, 2009). Network modularity (i.e. the degree of compartmentalization of a network) should increase host-parasitoid robustness to coextinction (Thébault & Fontaine, 2010; Grass *et al.*, 2018). However, evidence on the effect of global warming on host-parasitoid networks remain sparse. We give an overview of published evidence in Table . Among all the studies looking at the effect of temperature on parasitism, either directly with laboratory experiments (Davis *et al.*, 1998b; Dyer *et al.*, 2013) and recently with field experiments (Derocles *et al.*, 2018), or more commonly using proxy such as altitudinal gradients (Péré *et al.*, 2013; Maunsell *et al.*, 2015; Morris *et al.*, 2015), approximately half of the studies finds increase in parasitism, while the other half finds a decrease or no changes at all. Evidence for reduced parasitism under global warming thus remains ambiguous.

Most of the potential mechanisms discussed here affect either host or parasitoid communities, or links between host and parasitoid species. Trøjelsgaard & Olesen (2016) argue that these small-scale properties are necessary to understand how the whole network changes. However, Dallas & Poisot (2018) suggest that changes in host and parasite community composition do not imply necessary changes in host and parasite interaction patterns. Hence ecological networks could remain stable if the communities that compose them experience compositional shifts but with functionally redundant species. Despite this, the body-size structure of host communities, which is expected to change under global warming scenarios, might impact host-parasitoid interaction networks (Henri *et al.*, 2012).

Role of facultative endosymbionts

Facultative endosymbionts are endosymbionts that can be removed from their hosts without killing them. They have until recently been studied only under laboratory conditions, and have been largely ignored by field ecologists. Their role on communities and ecological network structure through effects on both trophic and non-trophic interactions is now emerging (Hrček *et al.*, 2016; Rothacher *et al.*, 2016; Sanders *et al.*, 2016). Further, global warming can impact endosymbionts directly and change their effects on host-parasitoid interactions. We describe below how facultative endosymbionts mediate host-parasitoid interactions and review the effects that global warming can have on endosymbionts and their interactions with their host.

Endosymbionts mediate host-parasitoid trophic interactions

Facultative endosymbionts infecting host species mediate interactions between their hosts and parasitoids, and can thus affect host-parasitoid network structure though effects on trophic links (McLean *et al.*, 2016; Corbin *et al.*, 2017; Ye *et al.*, 2018). We list the main effects which endosymbionts have been found to confer in Table 2. Some facultative endosymbionts protect their host against parasitoids, pathogens, and nematodes. For example, the bacterium *Spiroplasma* increases *Drosophila hydei* resistance to parasitoids (Xie *et al.*, 2010, 2014) and *Drosophila neotestacea* resistance to nematode worm parasites (Jaenike *et al.*, 2010). In *D. melanogaster* and *Drosophila suzukii*, *Wolbachia* protects its host from a common viral pathogen (Hedges *et al.*, 2008; Cattel *et al.*, 2016). The protection against parasitoids that endosymbionts confer on their hosts modulates host-parasitoid interactions, which could in turn explain host-parasitoid network structure. Facultative endosymbionts can also infect parasitoids which can affect host-parasitoid interactions for the same reasons. For example, *Wolbachia*-infected *Leptopilina heterotoma* experience higher encapsulation rates in *Drosophila simulans* than symbiont-free parasitoids (Fytrou *et al.*, 2006). Aphids, *Drosophila,* and mosquitoes are the best studied insects carrying symbionts. We expect to discover more mutualistic relationship between insects and endosymbionts with future studies (McLean *et al.*, 2016).

Table 2. Examples of host-parasite interactions mediated by endosymbionts

Role	Endosymbiont	Host	References
Reduce success of parasitoid wasp larval development	*Hamiltonella defensa* and *Serratia symbiotica*	Aphids	Oliver *et al.* (2003, 2005); Hrček *et al.* (2016); Rothacher *et al.* (2016)
Increases host resistance to some fungal pathogens	*Regiella insecticola*, *Rickettsia* and *Rickettsiella*	Pea aphids	Ferrari *et al.* (2004); Łukasik Piotr *et al.* (2012); Parker *et al.* (2013)
Increases host resistance to parasitoids	*Regiella insecticola*	Aphids	Vorburger *et al.* (2010), but see Oliver *et al.* (2003)
Increases host resistance to parasitoids	*Spiroplasma* species	*Drosophila hydei*	Xie et al. (2010, 2014)
Increases host resistance to nematode parasites	*Spiroplasma* species	*Drosophila neotestacea*	Jaenike et al. (2010)
Increases host resistance to a common viral pathogen	*Wolbachia*	*Drosophila melanogaster* and *Drosophila suzukii*	Hedges et al. (2008); Cattel *et al.* (2016)
Higher encapsulation rates by the host *D. simulans*	*Wolbachia*	*Leptopilina heterotoma*	Fytrou et al. (2006)

Endosymbionts mediate host-parasitoid non-trophic interactions

Facultative endosymbionts can also influence non-trophic interactions within host-parasitoid networks. Defensive endosymbionts that protect their host from parasitism can modify competitive relationships among parasitoid species (McLean & Godfray, 2017). Hosts that carry protective endosymbionts can still attract ovipositing parasitoids, but parasitoid success is reduced; this could lower local parasitoid density, which would be beneficial for all host species, resulting in a form of apparent mutualism (van Veen *et al.*, 2006). Frago *et al.* (2017) recently found that plants attacked by pea aphids carrying the endosymbiont *Hamiltonella defensa* released lower quantities of volatiles, hence recruiting fewer parasitic wasps, and thus increasing aphid fitness. However, parasitoids can detect whether or not a potential aphid host carries a protective symbiont (Oliver *et al.*, 2012); this could lead to the parasitoid switching to its alternative host, leading to apparent competition. By modifying one link in a host-parasitoid network, protective endosymbionts can completely change the structure of the network through cascading effects (Sanders *et al.*, 2016; Ye *et al.*, 2018). Endosymbionts can also alter the competitive ability of their hosts, allowing host species coexistence, especially when parasitoids are present (Hertäg & Vorburger, 2018). Because of the protection facultative endosymbionts give to their hosts and the changes that it induces in parasitoid behavior, parasitoid density and host competitive ability, the mutualistic relationship between endosymbionts and host species could be a key to understanding how host-parasitoid networks are structured.

Endosymbionts and global warming

Endosymbionts can be very sensitive to temperature changes (Corbin *et al.*, 2017), and the benefits they afford to their hosts are also sensitive to environmental conditions (Ross *et al.*, 2017). However, facultative endosymbionts can buffer their hosts against the negative consequences of global warming. We first give several examples of the protective role endosymbionts provide under predicted temperature changes. We then discuss how global warming is expected to impact endosymbionts, with an emphasis on immunological contingencies.

Facultative endosymbionts can protect their host under heat stress, thus playing an important role in the adaptation of insects to their abiotic environment. For example, aphids infected with *Serratia symbiotica* (Russell & Moran, 2006) and whiteflies infected by *Rickettsia* (Brumin *et al.*, 2011) are more resistant to heat shock than uninfected individuals.

Insects exposed to high temperatures can lose their endosymbionts (Thomas & Blanford, 2003). Vertical transmission (i.e. from a mother to its offspring) is reduced at high temperatures for *Wolbachia* (Hurst *et al.*, 2000). High temperatures also reduce *Spiroplasma* vertical transmission, but not symbiont proliferation (Anbutsu *et al.*, 2008). Thus, variation in natural infection rates and symbiont prevalence in a focal species depend on interactions between the endosymbiont and the prevailing thermal conditions (Watts *et al.*, 2009; Corbin *et al.*, 2017). Indeed, although endosymbionts can protect their hosts against natural enemies, most remain facultative in natural populations because carrying symbionts has a cost for host individuals (Oliver *et al.*, 2008; Vorburger & Gouskov, 2011; Kriesner *et al.*, 2016). The trade-off between the advantages and costs conferred by endosymbionts depends on environmental conditions and parasitoid attack rate (Sasaki & Godfray, 1999), and is expected to be altered by global warming.

The protective role played by facultative endosymbionts can also be altered by global warming. For example, *Hamiltonella defensa* fails to protect its aphid host against parasitoids under heat stress (Bensadia *et al.*, 2006). However, pea aphids carrying PAXS (pea aphid X-type symbiont) in association with *H. defensa* are more resistant to parasitoid development than aphids carrying the *H. defensa* symbiont only, and its protection holds under heat stress (Guay *et al.*, 2009). We expect that further work on additional insect and endosymbiont species will reveal further examples of temperature affecting interactions between endosymbionts and their hosts.

Thus, endosymbionts can either protect their hosts from global warming or be lost because of it. Nevertheless, these studies suggest that facultative endosymbionts would mainly enhance host resistance to extreme temperatures rather than making the hosts more vulnerable. This will add to the other effect of global warming that tend to decrease parasitism, unbalancing the equilibrium between hosts and parasitoids in favor of host populations. However, evidence of the role of endosymbionts within entire host-parasitoid networks is lacking.

Future directions

Ecological networks are a valuable tool for improving our understanding of the processes taking place within ecological communities (Poisot *et al.*, 2016). However, most of the research on host-parasitoid interactions summarized here focuses on interactions between a single parasitoid species and one or two host species. Studies on more complex system are lacking, but necessary to fully understand the mechanisms that structure entire host-parasitoid networks and how they may respond to global warming (McCann, 2007; Poisot *et al.*, 2015; Evans *et al.*, 2017). Here we highlight three main approaches that might prove fruitful: manipulative experiments, mathematical models, and new molecular methods.

Researchers have only recently started to conduct experimental work on the mechanisms operating within ecological networks (Sanders *et al.*, 2015, 2016). Habitat filters and dispersal can confound the effect of ecological interactions in the field, making it difficult to identify the processes structuring ecological networks (Barner *et al.*, 2018); experimental approaches bring the potential to isolate mechanisms. Laboratory experiments that manipulate community composition (individual host species, multiple-host species communities, with or without one or several parasitoid species) under different temperature treatments should clarify the contribution of each interaction type to network structure, and clarify the likely response to global warming.

Models aimed at predicting how networks of interactions will respond to environmental changes and global warming are being developed (Staniczenko *et al.*, 2017). Multiple types of interactions (e.g. competition and parasitism) can be explicitly considered in these models using multilayer networks (Kéfi *et al.*, 2016). The strength of trophic and non-trophic interactions can be inferred using a combination of experimental data and mathematical models (Terry *et al.*, 2017). For example, Sentis and collaborators (2017) used a factorial experiment involving different temperatures, prey densities, and predator assemblages to parameterize mathematical models. The models allowed estimations of the strength of both trophic interactions (i.e., the total flux from resource to consumers) and non-trophic interactions (estimated by comparing the results of models that included or excluded non-trophic interactions). Future studies on host-parasitoid network structure, whether experiments or models, should include realistic diversity of host and parasitoid species and consider the different interaction types that compose the ecological network along with the evolutionary responses. In parameterizing these models it is important to consider that the incidence of thermal extremes is likely to be higher in a future, warmer world, with implications for species interactions and ecological networks (Hance, 2007). One likely outcome is that the strength and even the direction of interactions in ecological networks and the effect of endosymbionts on host fitness may become much more variable than would be predicted under increased mean temperatures alone.

Finally, ecological entomologists will need to capitalize on the availability of new molecular methods such as DNA metabarcoding as well as proven methods like multiplex PCR, to study complex ecological networks (Hrček & Godfray, 2015; Evans *et al.*, 2016; Ye *et al.*, 2017; Derocles *et al.*, 2018; Kitson *et al.*, 2018). Such approaches can reveal new host-parasitoid associations in complex communities (Condon *et al.* 2014), and are also vital to identify facultative endosymbionts – and hence their effects - within these networks. Presence or absence of endosymbionts could be a useful variable to predict host-parasitoid network structure, especially under different experimental temperature regimes.

Conclusions

Both trophic and non-trophic interactions play an important role in structuring host-parasitoid networks. However, global warming can impact all of these interactions, and thus markedly change host-parasitoid network structure. The long-term effects of such changes on ecosystem functioning are still unknown, but will be particularly important in agriculture, where parasitoids are used as biocontrol agents against insect pests. Evidence for reduced parasitism under global warming remains limited. Research on endosymbionts could be key to understanding host-parasitoid network structure and its response to global warming. Symbionts interact with both host-parasitoid interactions and environmental conditions, and many patterns observed in host-parasitoid network structure could be explained by the presence of endosymbionts. More work is needed on the level of whole host-parasitoid networks, both in the laboratory and in the field, to obtain a clear picture of the mechanisms structuring them, and to help predict how they will be affected by global warming.

Acknowledgements

We are grateful for the insightful comments of Chia-Hua Lue, Nicholas Pardikes, Moria Robinson and two anonymous reviewers for their contributions to the improvement of the manuscript. We also acknowledge funding support from the Czech Science Foundation (GACR) grant no. 17-27184Y and UK National Environment Research Council (NE/N010221/1 to OTL). The authors declare there are no conflicts of interest.

Author contributions

MT and JH conceived the review; MT produced the first draft of the article; and JH and OTL critically revised the article.

References

Abrams, P.A. (1995) Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. *The American Naturalist*, **146**, 112–134.

Abrams, P.A., Holt, R.D. & Roth, J.D. (1998) Apparent competition or apparent mutualism? Shared predation when populations cycle. *Ecology*, **79**, 201–212.

Abrams, P.A. & Matsuda, H. (1996) Positive indirect effects between prey species that share predators. *Ecology*, **77**, 610–616.

Anbutsu, H., Goto, S. & Fukatsu, T. (2008) High and low temperatures differently affect infection density and vertical transmission of male-killing *Spiroplasma* symbionts in *Drosophila* hosts. *Applied and Environmental Microbiology*, **74**, 6053–6059.

Barner, A.K., Coblentz, K.E., Hacker, S.D. & Menge, B.A. (2018) Fundamental contradictions among observational and experimental estimates of non-trophic species interactions. *Ecology*, **99**, 557–566.

Bascompte, J. & Stouffer, D.B. (2009) The assembly and disassembly of ecological networks. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 1781–1787.

Bastolla, U., Fortuna, M.A., Pascual-García, A., Ferrera, A., Luque, B. & Bascompte, J. (2009) The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature*, **458**, 1018–1020.

Bensadia, F., Boudreault, S., Guay, J.-F., Michaud, D. & Cloutier, C. (2006) Aphid clonal resistance to a parasitoid fails under heat stress. *Journal of Insect Physiology*, **52**, 146–157.

Blois, J.L., Zarnetske, P.L., Fitzpatrick, M.C. & Finnegan, S. (2013) Climate change and the past, present, and future of biotic interactions. *Science*, **341**, 499–504.

Bonsall, M.B. & Hassell, M.P. (1999) Parasitoid-mediated effects: apparent competition and the persistence of host–parasitoid assemblages. *Researches on Population Ecology*, **41**, 59–68.

Brumin, M., Kontsedalov, S. & Ghanim, M. (2011) *Rickettsia* influences thermotolerance in the whitefly *Bemisia tabaci* B biotype. *Insect Science*, **18**, 57–66.

Carton, Y., Poirié, M. & Nappi, A.J. (2008) Insect immune resistance to parasitoids. *Insect Science*, **15**, 67–87.

Cattel, J., Martinez, J., Jiggins, F., Mouton, L. & Gibert, P. (2016) *Wolbachia*-mediated protection against viruses in the invasive pest *Drosophila suzukii*. *Insect Molecular Biology*, **25**, 595–603.

Condon, M.A., Scheffer, S.J., Lewis, M.L., Wharton, R., Adams, D.C. & Forbes, A.A. (2014) Lethal interactions between parasites and prey increase niche diversity in a tropical community. *Science*, **343**, 1240–1244.

Corbin, C., Heyworth, E.R., Ferrari, J. & Hurst, G.D.D. (2017) Heritable symbionts in a world of varying temperature. *Heredity*, **118**, 10–20.

Costanza, R., Arge, R. d', Groot, R. de, Farber, S., Grasso, M., Hannon, B., *et al.* (1997) The value of the world's ecosystem services and natural capital. *Nature*, **387**, 253–260.

Dai, A. (2013) Increasing drought under global warming in observations and models. *Nature Climate Change*, **3**, 52–58.

Dallas, T. & Poisot, T. (2018) Compositional turnover in host and parasite communities does not change network structure. *Ecography*, **41**, 1534–1542.

Davis, A.J., Jenkinson, L.S., Lawton, J.H., Shorrocks, B. & Wood, S. (1998a) Making mistakes when predicting shifts in species range in response to global warming. *Nature*, **391**, 783–786.

Davis, A.J., Lawton, J.H., Shorrocks, B. & Jenkinson, L.S. (1998b) Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. *Journal of Animal Ecology*, **67**, 600–612.

Delava, E., Fleury, F. & Gibert, P. (2016) Effects of daily fluctuating temperatures on the *Drosophila–Leptopilina boulardi* parasitoid association. *Journal of Thermal Biology*, **60**, 95–102.

Derocles, S.A.P., Lunt, D.H., Berthe, S.C.F., Nichols, P.C., Moss, E.D. & Evans, D.M. (2018) Climate warming alters the structure of farmland tritrophic ecological networks and reduces crop yield. *Molecular Ecology*, **27**, 4931–4946.

Dong, Y.-C., Han, P., Niu, C.-Y., Zappalà, L., Amiens-Desneux, E., Bearez, P., *et al.* (2018) Nitrogen and water inputs to tomato plant do not trigger bottom-up effects on a leafminer parasitoid through host and non-host exposures. *Pest Management Science*, **74**, 516–522.

Duron, O. & Hurst, G.D. (2013) Arthropods and inherited bacteria: from counting the symbionts to understanding how symbionts count. *BMC Biology*, **11**, 45.

Dyer, L.A., Richards, L.A., Short, S.A. & Dodson, C.D. (2013) Effects of CO2 and temperature on tritrophic interactions. *PLoS One*, **8**, e62528.

Eubanks, M.D. & Finke, D.L. (2014) Interaction webs in agroecosystems: beyond who eats whom. *Current Opinion in Insect Science*, Ecology, **2**, 1–6.

Evans, D.M., Gilbert, J.D.J. & Port, G.R. (2017) Everything is connected: network thinking in entomology. *Ecological Entomology*, **42**, 1–3.

Evans, D.M., Kitson, J.J.N., Lunt, D.H., Straw, N.A. & Pocock, M.J.O. (2016) Merging DNA metabarcoding and ecological network analysis to understand and build resilient terrestrial ecosystems. *Functional Ecology*, **30**, 1904–1916.

Ferrari, J., Darby, A.C., Daniell, T.J., Godfray, H.C.J. & Douglas, A.E. (2004) Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. *Ecological Entomology*, **29**, 60–65.

Fleury, F., Ris, N., Allemand, R., Fouillet, P., Carton, Y. & Boulétreau, M. (2004) Ecological and genetic interactions in *Drosophila*-parasitoids communities: a case study with *D. melanogaster, D. simulans* and their common *Leptopilina* parasitoids in south-eastern France. In *Drosophila melanogaster, Drosophila simulans: So Similar, So Different*, Contemporary Issues in Genetics and Evolution (ed. by Capy, P., Gibert, P. & Boussy, I.). Springer Netherlands, pp. 181–194.

Flores-Mejia, S., Fournier, V. & Cloutier, C. (2017) Performance of a tri-trophic food web under different climate change scenarios. *Food Webs*, **11**, 1–12.

Fontaine, C., Guimaraes Jr, P.R., Kefi, S. *et al.* (2011). The ecological and evolutionary implications of merging different types of networks. *Ecology letters*, **14**, 1170–1181.

Frago, E., Mala, M., Weldegergis, B.T., Yang, C., McLean, A., Godfray, H.C.J., *et al.* (2017) Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles. *Nature Communications*, **8**, 1860.

Frost, C.M., Peralta, G., Rand, T.A., Didham, R.K., Varsani, A. & Tylianakis, J.M. (2016) Apparent competition drives community-wide parasitism rates and changes in host abundance across ecosystem boundaries. *Nature Communications*, **7**, 12644.

Furlong, M.J. & Zalucki, M.P. (2017) Climate change and biological control: the consequences of increasing temperatures on host–parasitoid interactions. *Current Opinion in Insect Science*, **20**, 39–44.

Fytrou, A., Schofield, P.G., Kraaijeveld, A.R. & Hubbard, S.F. (2006) *Wolbachia* infection suppresses both host defense and parasitoid counter-defense. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 791–796.

Gillespie, D.R., Nasreen, A., Moffat, C.E., Clarke, P. & Roitberg, B.D. (2012) Effects of simulated heat waves on an experimental community of pepper plants, green peach aphids and two parasitoid species. *Oikos*, **121**, 149–159.

Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W. & Holt, R.D. (2010) A framework for community interactions under climate change. *Trends in Ecology & Evolution*, **25**, 325–331.

Godfray, H.C.J. (1994) *Parasitoids: behavioral and evolutionary ecology*. Princeton University Press, Princeton, New Jersey.

Goudard, A. & Loreau, M. (2008) Non-trophic interactions, biodiversity, and ecosystem functioning: an interaction web model. *The American Naturalist*, **171**, 91–106.

Grass, I., Jauker, B., Steffan-Dewenter, I., Tscharntke, T. & Jauker, F. (2018) Past and potential future effects of habitat fragmentation on structure and stability of plant–pollinator and host–parasitoid networks. *Nature Ecology & Evolution*, **2**, 1408–1417.

Grover, J.P. (1994) Assembly rules for communities of nutrient-limited plants and specialist herbivores. *The American Naturalist*, **143**, 258–282.

Guay, J.-F., Boudreault, S., Michaud, D. & Cloutier, C. (2009) Impact of environmental stress on aphid clonal resistance to parasitoids: Role of *Hamiltonella defensa* bacterial symbiosis in association with a new facultative symbiont of the pea aphid. *Journal of Insect Physiology*, **55**, 919–926.

Hammill, E., Kratina, P., Vos, M., Petchey, O.L. & Anholt, B.R. (2015) Food web persistence is enhanced by non-trophic interactions. *Oecologia*, **178**, 549–556.

Hance, T. (2007) Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology*, **52**, 107–126.

Hance, T., van Baaren, J, Vernon, P. & Boivin, G. (2007) Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology*, **52**, 107–126.

Harvey, E., Gounand, I., Ward, C.L. & Altermatt, F. (2017) Bridging ecology and conservation: from ecological networks to ecosystem function. *Journal of Applied Ecology*, **54**, 371–379.

Harvey, J.A., Gols, R. & Strand, M.R. (2009) Intrinsic competition and its effects on the survival and development of three species of endoparasitoid wasps. *Entomologia Experimentalis et Applicata*, **130**, 238–248.

Harvey, J.A., Poelman, E.H. & Tanaka, T. (2013) Intrinsic inter- and intraspecific competition in parasitoid wasps. *Annual Review of Entomology*, **58**, 333–351.

Hassell, M.P. & Waage, J.K. (1984) Host-parasitoid population interactions. *Annual Review of Entomology*, **29**, 89–114.

Hedges, L.M., Brownlie, J.C., O'Neill, S.L. & Johnson, K.N. (2008) *Wolbachia* and virus protection in insects. *Science*, **322**, 702–702.

Heimpel, G.E. & Rosenheim, J.A. (1998) Egg limitation in parasitoids: a review of the evidence and a case study. *Biological Control*, **11**, 160–168.

Henri, D.C., Seager, D., Weller, T. & van Veen, F.J.F. (2012) Potential for climate effects on the size-structure of host–parasitoid indirect interaction networks. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **367**, 3018–3024.

Henry, L.M., Maiden, M.C.J., Ferrari, J. & Godfray, H.C.J. (2015) Insect life history and the evolution of bacterial mutualism. *Ecology Letters*, **18**, 516–525.

Hertäg, C. & Vorburger, C. (2018) Defensive symbionts mediate species coexistence in phytophagous insects. *Functional Ecology*, **32**, 1057–1064.

Holt, R.D. (1977) Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology*, **12**, 197–229.

Holt, R.D. & Kotler, B.P. (1987) Short-term apparent competition. *The American Naturalist*, **130**, 412–430.

Holt, R.D. & Lawton, J.H. (1993) Apparent competition and enemy-free space in insect host-parasitoid communities. *The American Naturalist*, **142**, 623–645.

Holt, R.D. & Lawton, J.H. (1994) The ecological consequences of shared natural enemies. *Annual Review of Ecology and Systematics*, **25**, 495–520.

Hrček, J. & Godfray, H.C.J. (2015) What do molecular methods bring to host–parasitoid food webs? *Trends in Parasitology*, **31**, 30–35.

Hrček, J., McLean, A.H.C. & Godfray, H.C.J. (2016) Symbionts modify interactions between insects and natural enemies in the field. *Journal of Animal Ecology*, **85**, 1605–1612.

Hurst, G.D.D., Johnson, A.P., Schulenburg, J.H.G. v d & Fuyama, Y. (2000) Male-killing *Wolbachia* in *Drosophila*: a temperature-sensitive trait with a threshold bacterial density. *Genetics*, **156**, 699–709.

Jaenike, J., Unckless, R., Cockburn, S.N., Boelio, L.M. & Perlman, S.J. (2010) Adaptation via symbiosis: recent spread of a *Drosophila* defensive symbiont. *Science*, **329**, 212–215.

Jeffs, C.T. & Lewis, O.T. (2013) Effects of climate warming on host–parasitoid interactions. *Ecological Entomology*, **38**, 209–218.

Johnson, S.N. & Jones, T.H. (2017) *Global climate change and terrestrial invertebrates*. John Wiley & Sons, Chichester, U.K.

Jones, T.S., Godfray, H.C.J. & van Veen, F.J.F. (2009) Resource competition and shared natural enemies in experimental insect communities. *Oecologia*, **159**, 627–635.

Jordano, P. (2016a) Chasing ecological interactions. *PLoS Biology*, **14**, e1002559.

Jordano, P. (2016b) Sampling networks of ecological interactions. *Functional Ecology*, **30**, 1883–1893.

Kaplan, I., Carrillo, J., Garvey, M. & Ode, P.J. (2016) Indirect plant–parasitoid interactions mediated by changes in herbivore physiology. *Current Opinion in Insect Science*, Ecology * Parasites/Parasitoids/Biological control, **14**, 112–119.

Kéfi, S., Miele, V., Wieters, E.A., Navarrete, S.A. & Berlow, E.L. (2016) How structured is the entangled bank? The surprisingly simple organization of multiplex ecological networks leads to increased persistence and resilience. *PLoS Biology*, **14**, e1002527.

Kitson, J.J.., Hahn, C., Sands Richard J., Straw Nigel A., Evans Darren M. & Lunt David H. (2018) Detecting host–parasitoid interactions in an invasive Lepidopteran using nested tagging DNA metabarcoding. *Molecular Ecology*, **28**, 471–483.

Klapwijk, M.J., Gröbler, B.C., Ward, K., Wheeler, D. & Lewis, O.T. (2010) Influence of experimental warming and shading on host–parasitoid synchrony. *Global Change Biology*, **16**, 102–112.

Kraaijeveld, A.R. & Godfray, H.C.J. (2009) Evolution of host resistance and parasitoid counter-resistance. In *Advances in Parasitology*, Academic Press, New York, NY, pp. 257–280.

Kriesner, P., Conner, W.R., Weeks, A.R., Turelli, M. & Hoffmann, A.A. (2016) Persistence of a *Wolbachia* infection frequency cline in *Drosophila melanogaster* and the possible role of reproductive dormancy. *Evolution*, **70**, 979–997.

Lavergne, S., Mouquet, N., Thuiller, W. & Ronce, O. (2010) Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. *Annual Review of Ecology, Evolution, and Systematics*, **41**, 321–350.

Le Lann, C., Lodi, M. & Ellers, J. (2014) Thermal change alters the outcome of behavioural interactions between antagonistic partners. *Ecological Entomology*, **39**, 578–588.

LeBrun, E.G. & Feener, D.H. (2002) Linked indirect effects in ant-phorid interactions: impacts on ant assemblage structure. *Oecologia*, **133**, 599–607.

Lee, M.J., Kalamarz, M.E., Paddibhatla, I., Small, C., Rajwani, R. & Govind, S. (2009) Virulence Factors and Strategies of *Leptopilina* spp.: Selective Responses in *Drosophila* Hosts. In *Advances in Parasitology*, Academic Press, New York, NY, pp. 123–145.

Łukasik, P., Asch, M., Guo, H., Ferrari, J., Godfray, H.C.J. & van der. Putten, W. (2012) Unrelated facultative endosymbionts protect aphids against a fungal pathogen. *Ecology Letters*, **16**, 214–218.

Maunsell, S.C., Kitching, R.L., Burwell, C.J. & Morris, R.J. (2015) Changes in host–parasitoid food web structure with elevation. *Journal of Animal Ecology*, **84**, 353–363.

McCann, K. (2007) Protecting biostructure. *Nature*, **446**, 29–29.

McLean, A.H.C. & Godfray, H.C.J. (2017) The outcome of competition between two parasitoid species is influenced by a facultative symbiont of their aphid host. *Functional Ecology*, **31**, 927–933.

McLean, A.H.C., Parker, B.J., Hrček, J., Henry, L.M. & Godfray, H.C.J. (2016) Insect symbionts in food webs. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **371**, 20150325.

Miele, V., Guill, C., Ramos-Jiliberto, R. & Kéfi, S. (2018) The diversity of interaction types drives the functioning of ecological communities. *bioRxiv*, 411249.

Monticelli, L.S., Outreman, Y., Frago, E. & Desneux, N. (2019) Impact of host endosymbionts on parasitoid host range - from mechanisms to communities. *Current Opinion in Insect Science*, **32**, 77–82.

Morris, R.J., Lewis, O.T. & Godfray, H.C.J. (2004) Experimental evidence for apparent competition in a tropical forest food web. *Nature*, **428**, 310–313.

Morris, R.J., Sinclair, F.H. & Burwell, C.J. (2015) Food web structure changes with elevation but not rainforest stratum. *Ecography*, **38**, 792–802.

Nicholls, J.A., Schönrogge, K., Preuss, S. & Stone, G.N. (2018) Partitioning of herbivore hosts across time and food plants promotes diversification in the *Megastigmus dorsalis* oak gall parasitoid complex. *Ecology and Evolution*, **8**, 1300–1315.

Oliver, K.M., Campos, J., Moran, N.A. & Hunter, M.S. (2008) Population dynamics of defensive symbionts in aphids. *Proceedings of the Royal Society of London B: Biological Sciences*, **275**, 293–299.

Oliver, K.M., Moran, N.A. & Hunter, M.S. (2005) Variation in resistance to parasitism in aphids is due to symbionts not host genotype. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 12795–12800.

Oliver, K.M., Noge, K., Huang, E.M., Campos, J.M., Becerra, J.X. & Hunter, M.S. (2012) Parasitic wasp responses to symbiont-based defense in aphids. *BMC Biology*, **10**, 11.

Oliver, K.M., Russell, J.A., Moran, N.A. & Hunter, M.S. (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 1803–1807.

Parker, B.J., Spragg, C.J., Altincicek, B. & Gerardo, N.M. (2013) Symbiont-mediated protection against fungal pathogens in pea aphids: a role for pathogen specificity? *Applied and Environmental Microbiology*, **79**, 2455–2458.

Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37–42.

Pecl, G.T., Araújo, M.B., Bell, J.D., Blanchard, J., Bonebrake, T.C., Chen, I.-C., *et al.* (2017) Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science*, **355**, eaai9214.

Pellissier, L., Albouy, C., Bascompte, J., Farwig, N., Graham, C., Loreau, M., *et al.* (2017) Comparing species interaction networks along environmental gradients. *Biological Reviews*, **93**, 785–800.

Péré, C., Jactel, H. & Kenis, M. (2013) Response of insect parasitism to elevation depends on host and parasitoid life-history strategies. *Biology Letters*, **9**, 20130028.

Poisot, T., Guéveneux-Julien, C., Fortin, M.-J., Gravel, D. & Legendre, P. (2017) Hosts, parasites and their interactions respond to different climatic variables. *Global Ecology and Biogeography*, **26**, 942–951.

Poisot, T., Stouffer, D.B. & Gravel, D. (2015) Beyond species: why ecological interaction networks vary through space and time. *Oikos*, **124**, 243–251.

Poisot, T., Stouffer, D.B. & Kéfi, S. (2016) Describe, understand and predict: why do we need networks in ecology? *Functional Ecology*, **30**, 1878–1882.

Ris, N., Allemand, R., Fouillet, P. & Fleury, F. (2004) The joint effect of temperature and host species induce complex genotype-by-environment interactions in the larval parasitoid of *Drosophila*, *Leptopilina heterotoma* (Hymenoptera: Figitidae). *Oikos*, **106**, 451–456.

Roitberg, B.D. & Mangel, M. (2016) Cold snaps, heatwaves, and arthropod growth. *Ecological entomology*, **41**, 653–659.

Romo, C.M. & Tylianakis, J.M. (2013) Elevated temperature and drought interact to reduce parasitoid effectiveness in suppressing hosts. *PLoS One*, **8**, e58136.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. (2003) Fingerprints of global warming on wild animals and plants. *Nature*, **421**, 57–60.

Ross, P.A., Wiwatanaratanabutr, I., Axford, J.K., White, V.L., Endersby-Harshman, N.M. & Hoffmann, A.A. (2017) *Wolbachia* infections in *Aedes aegypti* differ markedly in their response to cyclical heat stress. *PLoS Pathogens*, **13**, e1006006.

Rothacher, L., Ferrer-Suay, M. & Vorburger, C. (2016) Bacterial endosymbionts protect aphids in the field and alter parasitoid community composition. *Ecology*, **97**, 1712–1723.

Roux, O., Lann, C.L., van Alphen, J.J.M. & van Baaren, J. (2010) How does heat shock affect the life history traits of adults and progeny of the aphid parasitoid *Aphidius avenae* (Hymenoptera: Aphidiidae)? *Bulletin of Entomological Research*, **100**, 543–549.

Russell, J.A. & Moran, N.A. (2005) Horizontal transfer of bacterial symbionts: heritability and fitness effects in a novel aphid host. *Applied and Environmental Microbiology*, **71**, 7987–7994.

Russell, J.A. & Moran, N.A. (2006) Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proceedings of the Royal Society of London B: Biological Sciences*, **273**, 603–610.

Sanders, D., Kehoe, R. & van Veen, F.J.F. (2015) Experimental evidence for the population-dynamic mechanisms underlying extinction cascades of carnivores. *Current Biology*, **25**, 3106–3109.

Sanders, D., Kehoe, R., van Veen, F.J.F., McLean, A., Godfray, H.C.J., Dicke, M., *et al.* (2016) Defensive insect symbiont leads to cascading extinctions and community collapse. *Ecology Letters*, **19**, 789–799.

Sanders, D., Sutter, L. & van Veen, F.J.F. (2013) The loss of indirect interactions leads to cascading extinctions of carnivores. *Ecology Letters*, **16**, 664–669.

Sanders, D. & van Veen, F.J.F. (2012) Indirect commensalism promotes persistence of secondary consumer species. *Biology Letters*, **8**, 960–963.

Sasaki, A. & Godfray, H.C.J. (1999) A model for the coevolution of resistance and virulence in coupled host–parasitoid interactions. *Proceedings of the Royal Society of London B: Biological Sciences*, **266**, 455–463.

Sassi, C. de, Staniczenko, P.P.A. & Tylianakis, J.M. (2012) Warming and nitrogen affect size structuring and density dependence in a host–

parasitoid food web. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **367**, 3033–3041.

Sassi, C. de & Tylianakis, J.M. (2012) Climate change disproportionately increases herbivore over plant or parasitoid biomass. *PLoS One*, **7**, e40557.

Schlenke, T.A., Morales, J., Govind, S. & Clark, A.G. (2007) Contrasting infection strategies in generalist and specialist wasp parasitoids of *Drosophila melanogaster*. *PLoS Pathogens*, **3**, e158.

Schreven, S.J.J., Frago, E., Stens, A., Jong, P.W. de & van Loon, J.J.A. (2017) Contrasting effects of heat pulses on different trophic levels, an experiment with a herbivore-parasitoid model system. *PLoS One*, **12**, e0176704.

Schweiger, O., Settele, J., Kudrna, O., Klotz, S. & Kühn, I. (2008) Climate change can cause spatial mismatch of trophically interacting species. *Ecology*, **89**, 3472–3479.

Sentis, A., Gémard, C., Jaugeon, B. & Boukal, D.S. (2017) Predator diversity and environmental change modify the strengths of trophic and nontrophic interactions. *Global Change Biology*, **23**, 2629–2640.

Stacey, D.A. & Fellowes, M.D.E. (2002) Influence of temperature on pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae) resistance to natural enemy attack. *Bulletin of Entomological Research*, **92**, 351–357.

Staniczenko, P.P.A., Lewis, O.T., Tylianakis, J.M., Albrecht, M., Coudrain, V., Klein, A.-M., *et al.* (2017) Predicting the effect of habitat modification on networks of interacting species. *Nature Communications*, **8**, 792.

Stireman, J.O., Dyer, L.A., Janzen, D.H., Singer, M.S., Lill, J.T., Marquis, R.J., *et al.* (2005) Climatic unpredictability and parasitism of caterpillars: implications of global warming. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 17384–17387.

Terry, J.C.D., Morris, R.J. & Bonsall, M.B. (2017) Trophic interaction modifications: an empirical and theoretical framework. *Ecology Letters*, **20**, 1219–1230.

Thébault, E. & Fontaine, C. (2010) Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science*, **329**, 853–856.

Thomas, M.B. & Blanford, S. (2003) Thermal biology in insect-parasite interactions. *Trends in Ecology & Evolution*, **18**, 344–350.

Tilman, D. (1982) *Resource competition and community structure*. Princeton University Press, Princeton, New Jersey.

Tougeron, K., Damien, M., Lann, C.L., Brodeur, J. & van Baaren, J. (2018) Rapid responses of winter aphid-parasitoid communities to climate warming. *bioRxiv*, 411975.

Trøjelsgaard, K. & Olesen, J.M. (2016) Ecological networks in motion: micro- and macroscopic variability across scales. *Functional Ecology*, **30**, 1926–1935.

Tylianakis, J.M. & Binzer, A. (2014) Effects of global environmental changes on parasitoid–host food webs and biological control. *Biological Control*, **75**, 77–86.

Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, **11**, 1351–1363.

Tylianakis, J.M. & Morris, R.J. (2017) Ecological networks across environmental gradients. *Annual Review of Ecology, Evolution, and Systematics*, **48**, 25–48.

Tylianakis, J.M. & Romo, C.M. (2010) Natural enemy diversity and biological control: making sense of the context-dependency. *Basic and Applied Ecology*, **11**, 657–668.

Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007) Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature*, **445**, 202–205.

Valiente-Banuet, A., Aizen, M.A., Alcántara, J.M., Arroyo, J., Cocucci, A., Galetti, M., *et al.* (2015) Beyond species loss: the extinction of ecological interactions in a changing world. *Functional Ecology*, **29**, 299–307.

van Veen, F.J.F., van Holland, P.D. & Godfray, H.C.J. (2005) Stable coexistence in insect communities due to density- and trait-mediated indirect effects. *Ecology*, **86**, 3182–3189.

van Veen, F.J.F., Morris, R.J. & Godfray, H.C.J. (2006) Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. *Annual Review of Entomology*, **51**, 187–208.

Visser, M.E. & Both, C. (2005) Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London B: Biological Sciences*, **272**, 2561–2569.

Vorburger, C., Gehrer, L. & Rodriguez, P. (2010) A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. *Biology Letters*, **6**, 109–111.

Vorburger, C. & Gouskov, A. (2011) Only helpful when required: a longevity cost of harbouring defensive symbionts. *Journal of Evolutionary Biology*, **24**, 1611–1617.

Walther, G.-R. (2010) Community and ecosystem responses to recent climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 2019–2024.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., *et al.* (2002) Ecological responses to recent climate change. *Nature*, **416**, 389–395.

Watts, T., Haselkorn, T.S., Moran, N.A. & Markow, T.A. (2009) Variable incidence of *Spiroplasma* infections in natural populations of *Drosophila* species. *PLoS One*, **4**, e5703.

Werner, E.E. & Peacor, S.D. (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology*, **84**, 1083–1100.

Xie, J., Butler, S., Sanchez, G. & Mateos, M. (2014) Male killing *Spiroplasma* protects *Drosophila melanogaster* against two parasitoid wasps. *Heredity*, **112**, 399–408.

Xie, J., Vilchez, I. & Mateos, M. (2010) *Spiroplasma* bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp *Leptopilina heterotoma*. *PLoS One*, **5**, e12149.

Ye, Z., Vollhardt, I.M.G., Girtler, S., Wallinger, C., Tomanovic, Z. & Traugott, M. (2017) An effective molecular approach for assessing cereal aphid-parasitoid-endosymbiont networks. *Scientific Reports*, **7**, 3138.

Ye, Z., Vollhardt, I.M.G., Parth, N., Rubbmark, O. & Traugott, M. (2018) Facultative bacterial endosymbionts shape parasitoid food webs in natural host populations: a correlative analysis. *Journal of Animal Ecology*, **87**, 1440–1451.

Accepted 2 May 2019

Associate Editor: Alex Cordoba-Aguilar

Chapter II

Multiple parasitoid species enhance top-down control, but parasitoid performance is context-dependent

(submitted)

Multiple parasitoid species enhance top-down control, but parasitoid performance is context-dependent

Mélanie Thierry*[1,2], Nicholas A. Pardikes[2], Miguel G. Ximénez-Embún[2], Grégoire Proudhom[1,2] & Jan Hrček[1, 2]

[1]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic and [2]Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice, Czech Republic.

*Correspondence: Melanie Thierry, University of South Bohemia, Faculty of Science, Branisovska 31, Ceske Budejovice 37005, Czech Republic. E-mail: m.thierry@entu.cas.cz

Abstract. Ecological communities are composed of a multitude of interacting species, forming complex networks of interactions. Current global changes are altering community composition and we thus need to understand if the mechanisms structuring species interactions are consistent across different species compositions. However, it is challenging to explore which aspects of species interactions are primarily driven by community structure and which by species identity. Here we compared the outcome of host-parasitoid interactions across four community modules that are common in host-parasitoid communities with a laboratory experiment using a pool of three *Drosophila* host and three larval parasitoid species, resulting in nine different species assemblages. Our results show general patterns of community structure for host-parasitoid interactions. Multiple parasitoid species enhanced host suppression without general antagonistic effects between parasitoid species. Presence of an alternative host species had no general effects on host suppression nor on parasitoid performance, therefore showing no evidence of indirect interactions between host species nor any host switching behavior. However, effects of community structure for parasitoid performance were species-specific and dependent on the identity of co-occurring species. Consequently, our findings highlight the importance of both the structure of the community and its species composition for the outcome of interactions.

Key words. community modules, multiple predator effects, community composition, interaction modification, *Drosophila*

Introduction

In nature, species interact in a variety of ways, forming complex ecological networks (Fontaine et al. 2011, Kéfi et al. 2012, 2015, Pilosof et al. 2017, García-Callejas et al. 2018, Miele et al. 2019). How species interact depends on the structure of the community, but also on identity of species in the assemblage (Bográn et al. 2002). With environmental changes such as climate warming, species are shifting their ranges and phenology (Parmesan and Yohe 2003). But species show differences in their sensitivity and responsiveness to these changes, disrupting historical patterns of interactions and co-occurrences, with communities of new species composition (Alexander et al. 2015). We thus need to determine which aspects of species interactions are primarily driven by community structure, and which are driven by species identity to accurately forecast the ecological consequences of changes in the biotic environment induced by global changes.

Together, trophic and non-trophic interactions, and their modifications by other co-occurring species act in combinations to shape communities (Thierry et al. 2019), and their dynamics (Kawatsu et al. 2021). A predator-prey interaction can be weakened by the presence of another predator via exploitative competition, interference or intraguild predation, enhanced via facilitation, or unchanged if predators have additive effects on prey suppression (Sih et al. 1998). Top-down control might also be driven by a single influential predator species independently of predator diversity (Letourneau et al. 2009), or be enhanced if predators show some degree of niche differentiation (Bográn et al. 2002, Pedersen and Mills 2004, Snyder et al. 2006, 2008). A predator might switch prey species with the presence of a competing predator, or with change in relative prey abundances (Siddon and Witman 2004, Randa et al. 2009). In addition, two competing species are able to coexist in nature, in part, because of indirect interactions through a shared natural enemy, which reduces the frequency of the dominant competitor that would otherwise exclude less competitive species (i.e., apparent competition) (Bonsall and Hassell 1999, Singh and Baruah 2020). Most studies looking at complex networks are observational (e.g., Tylianakis et al. 2007, Jeffs et al. 2021), and typically unable to disentangle the potential mechanisms driving species interactions described above. Experimental systems are thus needed to disentangle the mechanisms structuring networks of interacting species. For this purpose, community modules (i.e., a small number of species interacting in a specified pattern; Holt 1997, also referred to as “motifs” in the literature; Milo et al. 2002) represent a powerful tool to isolate certain key interactions that structure complex networks. They are the building blocks of natural communities (Gilman et al. 2010), and thus allow us to disentangle the mechanisms structuring them. Common community modules in food webs are pair of predator-prey, two prey species sharing a common natural enemy (i.e., apparent competition or mutualism; hereafter apparent competition module), or two predator species attacking the same prey (i.e., exploitative competition, interference, or facilitation; hereafter exploitative competition module). But experimental studies investigating the mechanisms structuring interactions with community modules rarely consider potential variations due to species-specific effects (but see Bográn et al. 2002, Snyder et al. 2006). Thus, it is unclear whether the mechanisms structuring interactions are consistent when looking at community modules of different species compositions (Cusumano et al. 2016).

Experiments manipulating interactions in different community contexts with different species assemblages are still rare, and usually manipulate species assemblage of one trophic level at a time. For instance, Bográn et al. (2002) revealed competitive interactions among predator species in some, but not all the predator assemblages studied. However, the study used only one prey species. Snyder et al. (2006) found varying strength in the effect of predator species diversity on aphid suppression depending on the aphid species considered, but did not vary species composition in multiple predator treatment. Understanding how the identity of co-occurring species at both trophic levels affects the outcome of consumer-resource interactions is of particular importance in the context of current global changes.

Here, we investigated the mechanisms structuring consumer-resource interactions using a host-parasitoid system. Parasitoids are a diverse group of insects that use other arthropods as a nursery for their offspring, killing their host to complete development (Godfray 2004). Parasitoids are important for top-down control in agricultural and natural ecosystems, and widely used as biological control agents. Interactions between hosts and parasitoids are easily observed, and host-parasitoid communities thus represent a good model system to study how the structure and composition of communities influence species interactions. We used a set of three *Drosophila* species and three of their larval parasitoids from a natural tropical community in Australia (Jeffs et al. 2021) in a laboratory experiment to isolate direct and indirect interactions within host-parasitoid communities. We aimed to uncover general effects of community modules in our *Drosophila*-parasitoid system, and detect any species-specific effects depending on the co-occurring species identity (using 9 species assemblages for each of the four common community modules in host-parasitoid networks: host-parasitoid pair, exploitative competition module, apparent competition module, and both exploitative and apparent competition module). Specifically, we tested the following hypotheses: (i) host suppression will be higher with the presence of multiple parasitoid species (i.e., exploitative competition module) because of increased chances to have an efficient parasitoid species (Pedersen and Mills 2004, Letourneau et al. 2009), (ii) despite potential multiparasitism events and therefore a decrease in parasitoid performance (Harvey et al. 2013). (iii) Pairwise interaction between a focal host-parasitoid pair will weaken with the presence of an alternative host in the apparent competition module because of trait- and density- mediated indirect effects (Werner and Peacor 2003). (iv) Combined effects of exploitative competition among parasitoids and apparent competition among hosts in the four-species module will differ from three-species modules depending on the identity of the co-occurring species because of species-specific effects (Bográn et al. 2002, Sentis et al. 2017).

Methods

Study system

The experiment used cultures of *Drosophila* species and their associated parasitoids collected from two tropical rainforest locations in North Queensland Australia: Paluma (S18° 59.031' E146° 14.096') and Kirrama Range (S18° 12.134' E145° 53.102') (< 100 m above sea level) (Jeffs et al. 2021). *Drosophila* and parasitoid cultures were established between 2017 and 2018, identified using both morphology and DNA barcoding, and shipped to the Czech Republic under permit no. PWS2016-AU-002018 from Australian Government, Department of the Environment. All cultures were maintained at 23°C on a 12:12 hour light and dark cycle at Biology Centre, Czech Academy of Sciences. Three host species (*Drosophila birchii*, *D. simulans* and *D. pallidifrons*), and three larval parasitoid species *Asobara sp.* (Braconidae: Alysiinae; strain KHB, reference voucher no. USNMENT01557097, reference sequence BOLD process ID:DROP043-21), *Leptopilina sp.* (Figitidae: Eucolinae; strain 111F, reference voucher no. USNMENT01557117, reference sequence BOLD process ID:DROP053-21), and *Ganaspis sp.* (Figitidae: Eucolinae; strain 84BC, reference voucher no. USNMENT01557102 and USNMENT01557297, reference sequence BOLD process ID:DROP164-21) were used (for more details on the parasitoid strains used see Lue et al. 2021). *Drosophila* isofemale lines were kept on standard *Drosophila* medium (corn flour, yeast, sugar, agar and methyl-4-hydroxybenzoate) for approximately 45 to 70 non-overlapping generations. To revive genetic variation, four to seven lines from each host species were combined to establish two population cages per species of mass-bred lines prior the start of the experiment. Single parasitoid isofemale lines were used, and maintained for approximately 25 to 40 non-overlapping generations prior to the start of the experiment by providing them every week with 2-days-old larvae of *Drosophila melanogaster*. This host species was not used in the experiment, thus avoiding potential bias due to maternal effects.

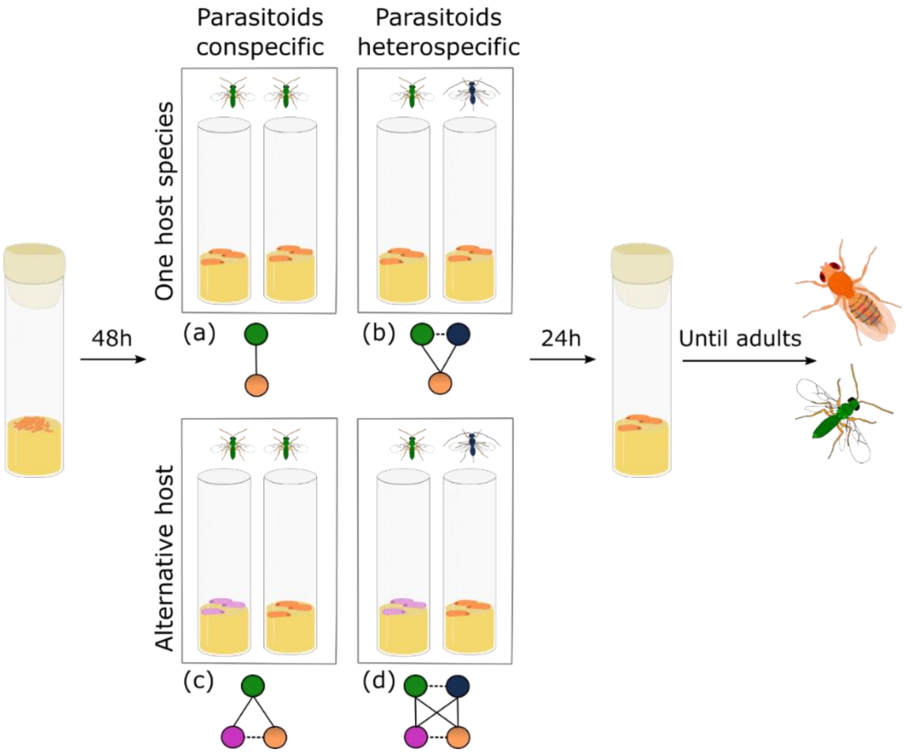


Figure 1. Schematic representation of the experimental treatments with the potential direct and indirect interactions in each community module. Orange and pink nodes and larvae represent different *Drosophila* host species, and green and blue nodes and wasps represent different parasitoid species, assembled in a fully factorial design in four different community modules represented schematically below their corresponding experimental box: a) host-parasitoid pair (one host and one parasitoid species), b) exploitative competition module (one host and two parasitoid species), c) apparent competition module (two host and one parasitoid species) and, d) both exploitative and apparent competition module (two host and two parasitoid species). In the community module schemas, solid lines represent trophic interactions, and dashed lines represent non-trophic interactions (in b) either exploitative competition, interference, or facilitation between parasitoids, c) either apparent competition or mutualism between hosts, and d) potential for all the above). Direct interaction between host species were not allowed. See Thierry et al. (2019) for a detailed description of each interaction type.

Experimental design

To investigate the effects of community structure and species composition on host-parasitoid interactions, we used four community modules, and 9 different species assemblages each (6 host and 6 parasitoid assemblages, from the pool of three host species and three parasitoid species) replicated 6 times (Figure 1). Each replicate was represented by a set of two vials in one box, for a total of 216 boxes. Either conspecific (Figure 1a and c) or heterospecific (Figure 1b and d) parasitoids were used. The two vials contained *Drosophila* larvae from either the same host species (Figure 1a and b) or different host species (Figure 1c and d). We also included control treatments for each host species to acquire baseline levels of survival in the absence of parasitoids (replicated 8 times).

To initiate the experiment, twenty-five eggs of each host species were placed in a single glass vial with 10mL of food media. To collect *Drosophila* eggs, an egg-wash protocol was developed based on Nouhaud et al. (2018). The day before the egg-washed protocol was conducted, two egg-laying mediums (petri dishes with agar gel topped with yeast paste) were introduced in each population cage for flies to laying eggs overnight. We used a #3 round paintbrush and distilled water to rinse out the yeast paste and collect the eggs on a fine black mesh that allowed only yeast and water to filter through, leaving the eggs on the surface. Eggs were transferred into petri dishes containing PBS (1 mM Calcium chloride dihydrate, 0.5 mM Magnesium Chloride Hexahydrate, pH = 7.4), and collected under microscope using a pipette to transfer eggs from the petri dish with PBS to the experimental vials.

After 48 hours, two vials with *Drosophila* second instar larvae (initially eggs) were placed in a hermetically sealed plastic box (15x11x19 cm) with four 3-to-5-days-old parasitoids (1:1 sex ratio). Twenty-four hours later, parasitoids were removed, and vials were removed from the boxes and plugged for rearing (Figure S1). Every vial was checked daily for emerges until the last individual emerged (up to 41 days for the species with the longest developmental time). We stopped collecting host emerges after 5 consecutive days without emerges to avoid collecting the second generation. All emerges were collected, identified, sexed, and stored in 95% ethanol. A total of 11,400 host eggs were collected across 456 experimental vials, of which 7,494 (65.7%) successfully emerged as adults (3,702 hosts and 3,792 parasitoids).

Data analysis

We characterized the outcome of host-parasitoid interactions by a combination of degree of infestation (DI) for each host species (i.e., the probability of a larvae to be parasitized and die), and successful parasitism rate (SP) for each host-parasitoid pair representing parasitoid performance (i.e., the probability of a parasitized host to give rise to an adult parasitoid) (Carton and Kitano 1981, Boulétreau and Wajnberg 1986). Degree of infestation (DI) was calculated as the proportion of host attacked (the difference between adult hosts emerging from the controls without parasitoids and from the experiment) among the total of hosts (set to 0 if the number of hosts emerging from the experiment was greater than the controls). Successful parasitism rate (SP) was calculated as the proportion of parasitoid adults emerging among the number of hosts attacked (Carton and Kitano 1981, Boulétreau and Wajnberg 1986). If no parasitoid emerged or if the number of hosts attacked was estimated to be zero, SP was set to 0. If the number of parasitoids that emerged was greater than the estimated number of hosts attacked, SP was set to 1. For treatments with one parasitoid species, we assumed that each of the two parasitoid individuals were attacking hosts equally, therefore the number of parasitoid adults emerging was divided by two to allow comparison of parasitism rates between single and multiple parasitoid species.

Data were analyzed using generalized linear mixed-effects models (GLMMs). Model assumptions were verified with the *DHARMa* package (Hartig 2019). To correct for overdispersion of the residuals and zero inflation, data were modeled using zero-inflation models with a beta binomial error distribution and a logit link function using the *glmmTMB* function from the *TMB* package (Lüdecke et al. 2019). Three model types were used to investigate general effects of community modules, species-specific responses, and effects of community composition for each focal species. (i) "Community module models" used two explanatory variables and their two-way interaction to account for the fully-factorial design of the experiment that resulted in four community modules (exploitative competition treatment with two levels: presence or not of a parasitoid heterospecific, and apparent competition treatment with two levels: presence or not of an alternative host species). Box ID (214 levels) was included as a random factor to remove the variation between the different species assemblages and thus extract general effects of community modules. Host species (three levels) for DI, and host-parasitoid pairs for SP were also included as random factors to remove the variation between different species. (ii) “Species-specific community module models” used the same explanatory variables than previously described, and Box ID as a random factor, but host species and host-parasitoid pairs were included as fixed factors to test if effects varied depending on the focal species. All three and two-way interactions between treatments (exploitative and apparent competition), host species, and host-parasitoid pairs were tested and kept in our models if found to be significant based on backward model selection using Likelihood-ratio tests. Models for SP were also run for each host-parasitoid pair separately to quantify differences in the sign and magnitude of the effects of community structure on pairwise interaction depending on the focal species. (iii) “Community composition models” used species assemblages rather than community modules as explanatory variables (host species assemblage: 6 levels, and parasitoid species assemblage: 6 levels). The two-way interaction between host and parasitoid assemblages was always kept in the models to account for the fully-factorial design of the experiment. Models for DI were run for each host species, and models for SP were run for each host-parasitoid pair separately. Blocks (6 levels) were included in all models as a random effect. Significance of the effects was tested using Wald type III analysis of deviance with Likelihood-ratio tests. Factor levels of community modules and species assemblages were compared to the reference module and species assemblages of the host-parasitoid pair in isolation by Tukey's HSD *post hoc* comparisons of all means, using the *emmeans* package (Lenth 2018). All analyses were performed using R 4.0.2 (Team 2017).

Results

Effects of community structure on host suppression

The presence of multiple parasitoid species in the module significantly increased the probability of host being infested (DI) by 48% (CI 26-70%) (community module model: $\chi2_{(1)} = 7.08$, $P = 0.008$; Post Hoc Odds Ratio

(OR) exploitative competition module/pairwise interaction = 1.58, P = 0.076; OR exploitative and apparent competition module/pairwise interaction = 1.32, P = 0.376). However, DI did not significantly change with the presence of an alternative host species ($\chi2_{(1)}$ = 0.56, P = 0.452; OR apparent competition module/pairwise interaction = 0.96, P = 0.984), and the two-way interaction between apparent and exploitative competition treatments had no significant effect (community module model: $\chi2_{(1)}$ = 0.22, P = 0.638) (Figure 2a).

Effects of host species and community composition on host suppression

Host DI did not differ significantly across host species (species-specific community module model: $\chi2_{(2)}$ = 0.07, P = 0.965). The directionality of the effect of parasitoid diversity did not vary depending on species assemblages (community composition models; Figure S2 and Supplement Material S2).

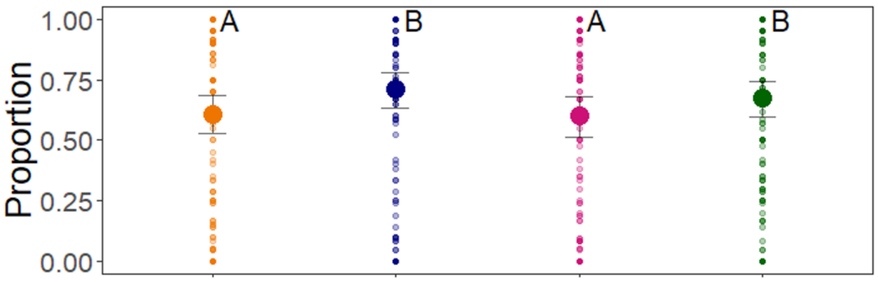
Effects of community structure on parasitoid performance

Community modules had no general effects of successful parasitism rates (SP) (community module model; Figure 2b), but the effects significantly varied across host-parasitoid pairs (species-specific community module model; three-way interaction: $\chi2(8)$ = 36.81, P < 0.0001; Table 1). The interaction between exploitative and apparent competition treatments had a significant effect on SP for one out of the nine host-parasitoid pairs (*Ganaspis sp*. on *D. simulans*). SP of two other host-parasitoid pairs significantly decreased with exploitative competition between parasitoid species (*Ganaspis sp*. on *D. birchii* and on *D. pallidifrons*). SP of one host-parasitoid pair significantly increased with apparent competition (*Asobara sp*. on *D. simulans*). SP for the rest of the host-parasitoid pairs did not significantly changed between community modules when compared to the host-parasitoid pair in isolation (Table 1).

Effects of community composition on parasitoid performance

Effects of an alternative host and a parasitoid competitor on parasitoid performance varied depending on co-occurring species identity, both in term of magnitude and direction of their response (community composition models). The interaction between host and parasitoid species assemblages had a significant effect on SP for four out of the nine host-parasitoid pairs: *Asobara sp*. on *D. simulans*, *Leptopilina sp*. on *D. birchii*, and *Ganaspis sp*. on *D. birchii* and on *D. simulans*. Effects of species assemblages on SP for each host-parasitoid pair are summarized in Table 2 and Figure 3, and presented in Supplement material S3.

(a) Degree of Infestation



(b) Succesful Parasitism rate

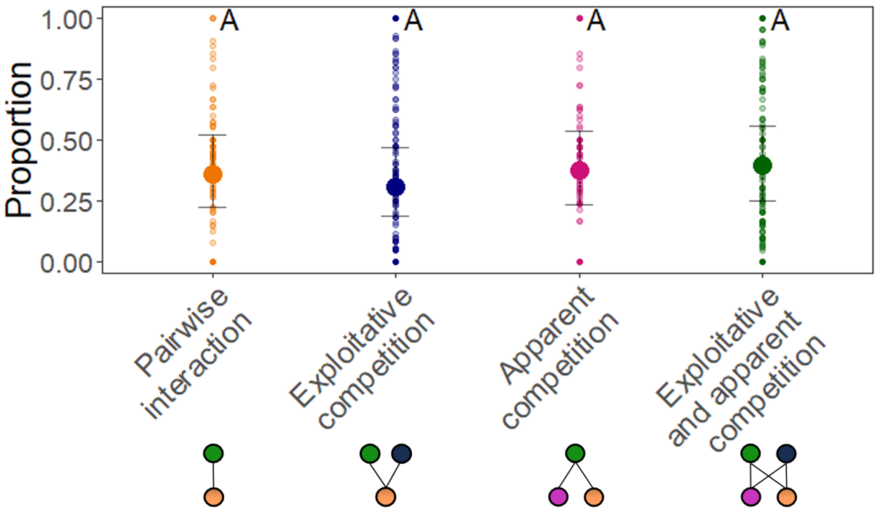


Figure 2. Effects of community structure (represented by the community module bellow each treatment) on (a) degree of infestation and on (b) successful parasitism rate. Different capital letters denote significant differences between community structure from the community module models. The small points represent the observed values, and the large points represent the predicted values with their 95% confidence intervals

Table 1. Odds ratios of having a successful parasitism event in each community module (exploitative competition, apparent competition, and both exploitative and apparent competition) compared to the host-parasitoid pair in isolation for each pair (host abbreviations: b: *D. birchii*, p: *D. pallidifrons*, s: *D. simulans*, and parasitoid abbreviations: A: *Asobara sp.*, L: *Leptopilina sp.*, G: *Ganaspis sp*.). Odds Ratios superior or inferior to 1 translate an increased or a decreased probability of having successful parasitism, respectively. Results come from the species-specific community module models run for each host-parasitoid pair separately. Significant Odds Ratios are highlighted in bold.

Module	b-A		p-A		s-A		b-L		p-L		s-L		b-G		p-G		s-G	
Expl. Comp.	1.49	(ns)	0.63	(ns)	1.56	(ns)	0.85	(ns)	1.36	(ns)	0.00	(ns)	**0.30**	*****	**0.19**	*****	0.67	(ns)
App. Comp.	0.95	(ns)	1.03	(ns)	**4.29**	*******	1.16	(ns)	1.65	(ns)	0.00	(ns)	0.94	(ns)	0.92	(ns)	0.83	(ns)
Expl. + app. Comp.	0.65	(ns)	038	(ns)	0.76	(ns)	1.44	(ns)	1.82	(ns)	0.00	(ns)	0.73	(ns)	0.45	(ns)	**4.12**	*******
Df residuals	65		65		65		65		65		65		64		64		65	

Table 2. Effects of community composition on the probability to have a successful parasitism event for each host-parasitoid pair. Effects are shown by the summary of Likelihood-ratio chi-square tests on the community composition models with the effects of host and parasitoid species assemblages (3 levels each) (host abbreviations: b: *D. birchii*, p: *D. pallidifrons*, s: *D. simulans*, and parasitoid abbreviations: A: *Asobara sp.*, L: *Leptopilina sp*., G: *Ganaspis sp*.). For p-A and s-L models contain only host species assemblage as a fixed effect due to convergence problem with the full model. Degrees or freedom (Df) are given for each factor and for the residuals. χ2 values are presented with the significance of the effect: (***) P < 0.001, (**) P < 0.01, (*) P < 0.05, (ns) P > 0.05.

Effects	**Df**	**b-A**		**p-A**		**s-A**		**b-L**		**p-L**		**s-L**		**b-G**		**p-G**		**s-G**	
Host sp.	2	**11.14**	**	1.12	(ns)	**15.56**	***	4.80	(ns)	4.83	(ns)	**10.08**	**	**34.14**	***	2.23	(ns)	**19.71**	***
Para sp.	2	**6.63**	*	-		0.11	(ns)	**38.32**	***	4.36	(ns)	-		2.57	(ns)	1.73	(ns)	**13.81**	**
Host x para	4	7.80	(ns)	-		**24.19**	***	**40.57**	***	7.81	(ns)	-		**25.22**	***	9.01	(ns)	**20.51**	***
Df res		60		66		60		60		60		66		59		59		60	

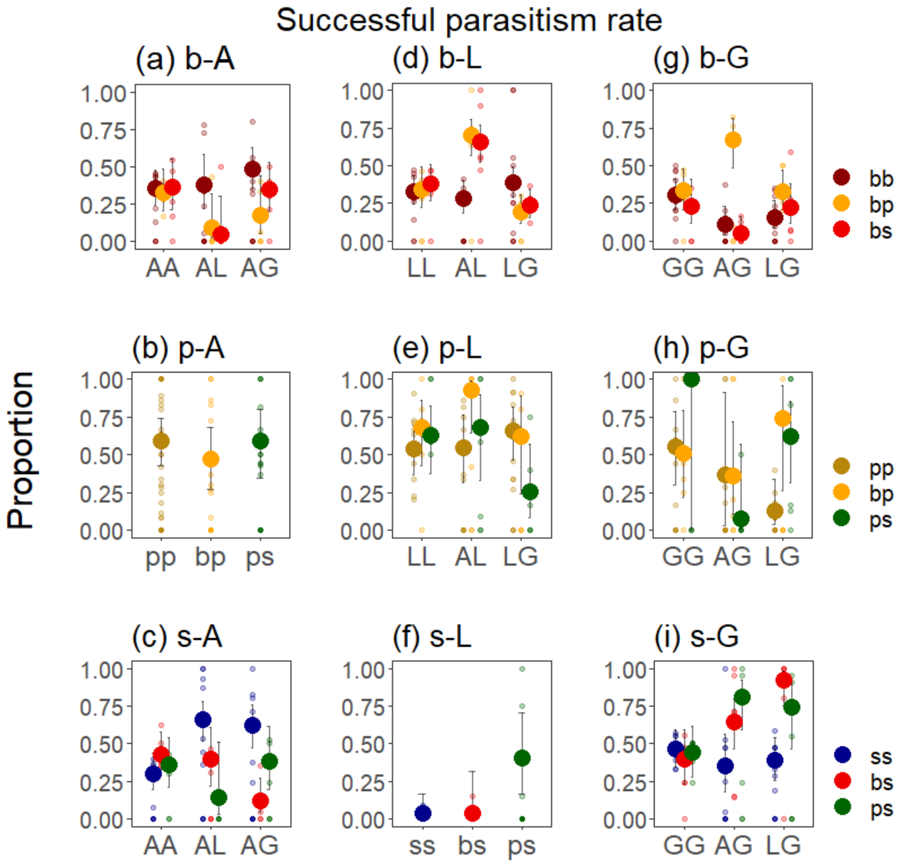


Figure 3. Effects of community composition (identity of the alternative host and the parasitoid heterospecific) on the successful parasitism rate of each parasitoid species on each host species [rows are host species (host abbreviations: b: *D. birchii*, p: *D. pallidifrons*, s: *D. simulans*), and columns are parasitoid species (parasitoid abbreviations: A: *Asobara sp.*, L: *Leptopilina sp.*, G: *Ganaspis sp.*)]. Host assemblages are represented by the different colors, and parasitoid assemblages are on the x axis. For SP p-A and SP s-L, only effect of host assemblages was analyses due to convergence problem with the full model, and are represented for all parasitoid assemblages combined. The small points represent the observed values, and the large points represent the predicted values with their 95% confidence intervals from the community composition models

Discussion

Our results confirm some general effects of community structure on consumer-resource interactions over a number of species combinations, but also reveal important species-specific effects linked to the identity of species composing the community. Specifically: (i) the presence of multiple parasitoid species consistently increased host suppression, showing the prevalence of synergistic effects between consumer species in our system. On the contrary, (ii) the presence of an alternative host had no general effect on host suppression, but increased or decreased successful parasitism rate depending on host-parasitoid pairs and co-occurring species identity.

Positive effects of consumer diversity on top-down control

The presence of multiple parasitoid species generally increased host suppression. An increase in top-down control compared to single-species treatments with one consumer species is predicted when the different natural enemies present a certain degree of niche differentiation, therefore complementing each other (Pedersen and Mills 2004), which have been reported in several experimental studies (reviewed in Letourneau et al. 2009). Here, we observed a general positive effect of consumer diversity on top-down control independently of the number of host species present in the community. No general effects were detected on parasitoid performance, suggesting no general difference between intra- and interspecific competition between consumers. Moreover, the positive effects of parasitoid diversity on host suppression were mainly driven by the presence of the most efficient parasitoid species for the focal host (e.g., presence of *Ganaspis sp*. for *D. simulans*). These results match the sampling effect model suggesting that an increase in top-down control with an increase in consumer diversity is explained because of an increasing probability that a superior natural enemy species will be present in the community (Myers et al. 1989).

No indirect interactions detected among prey species

Contrary to the presence of an additional parasitoid species, we did not detect any effect of an additional host species on host suppression, reveling no host switching behavior nor any indirect interactions between host species over this one generation experiment. Another empirical study failed to uncover any evidence of indirect interactions in natural host-parasitoid food webs (Kaartinen and Roslin 2013). Indirect interactions between prey, mediated by a shared natural enemy, is supposedly common in nature (e.g., Morris et al. 2001), and an important mechanism for prey species coexistence via density- and trait-mediated effects (Holt 1977, Fleury et al. 2004, van Veen et al. 2005, McPeek 2019). But direct exploitative competition between host species, which was not allowed in our experiment, might have a stronger effect on host population and community structure than apparent competition (Jones et al. 2009). Furthermore, frequency-dependent attack rate, in which a predator switches between two prey species depending on their relative densities, through aggregative behavior (Bonsall and Hassell 1999), and through learning (Ishii and Shimada 2012), is another important mechanism determining the strength of predator-prey interactions (van Veen et al. 2005), but was not tested in the present study. Varying host and non-host densities could also change parasitoid foraging behavior (Kehoe et al. 2016). In our study, potential effects of an alternative host (i.e., apparent competition module and both exploitative and apparent competition module) on host-parasitoid interactions were tested over a single generation and with constant host density. Therefore, experimental studies manipulating alternative prey density over several generations might be needed to detect indirect interactions between host species.

Importance of community composition for consumer-resource interactions

Our study was based on a particular set of interacting species, yet even the relatively small number of species used in our experiment allowed us to uncover species-specific response within a given community module. Community modules have been extensively used as a tool to study the mechanisms structuring and stabilizing complex natural communities (Bascompte and Melián 2005, Rip et al. 2010), yet the effects of species identity in such studies is often ignored. Our results highlight the variation in directionality and magnitude of the effects of a particular community module on host-parasitoid interactions depending on the species assemblage considered.

Successful parasitism rate increased in modules with a parasitoid competitor compared to the pairwise interaction in 6 species combinations (out of the 67 species combinations representing host-parasitoid pairs in modules with either or both exploitative and apparent competition), suggesting that some parasitoid species benefit from presence of an heterospecific. According to a recent review on interspecific interactions among parasitoids (Cusumano et al. 2016), and to the best of our knowledge, only one study showed facilitation between two parasitoid species on cabbage white caterpillars (Poelman et al. 2014). Our case seems to be different because successful parasitism rates did not increase for both parties. Here, modules with the pairwise interaction in isolation had two parasitoid conspecifics, and our results therefore suggest that in these 6 cases, interspecific competition between parasitoids was weaker than intraspecific competition. Parasitoids can compete both as adults for oviposition and as larvae within an host (Harvey et al. 2013). Extrinsic competition would have negative effects on parasitoid attack rates, linked to search efficiency and handling time, leading to a potential decrease in host mortality (Xu et al. 2016), which seems to not be the case in our study. Intrinsic competition is the result of a super- or multiparasitism event when two parasitoids (conspecifics or heterospecific, respectively) parasitize the same host individual. It is usually detrimental for the host survival, and therefore the most likely interaction between parasitoids happening in our experiment that would explain an increase in host suppression. Furthermore, parasitoids can inflict non-reproductive effects on their hosts (i.e., ovipositor probing and egg laying without successful parasitism) that can lead to host death (reviewed in Abram et al. 2019). This could also explain that host suppression increased with multiple parasitoids while successful parasitism rate did not generally increase. Our contrasting results on successful parasitism rate depending on the host-parasitoid pair and the other parasitoid species present in the community are likely due to differences in traits (e.g., immune response of the hosts, and oviposition behavior and virulence of the parasitoids; Carton et al. 2008). The different trait combinations and trade-offs across host and parasitoid species are likely an important mechanism driving species interactions and co-occurrences in natural communities (Wong et al. 2019).

Concluding remarks

Our study is the first to our knowledge to investigate the effects of community module of different species assemblages on consumer-resource interactions at both trophic levels. Studies looking at interaction between predator species on single prey (e.g., Valente et al. 2019, Ortiz-Martínez et al. 2019), or studies looking at the effect of an alternative prey with single predator species (e.g., Ishii and Shimada 2012, De Rijk et al. 2016), will overlook important mechanisms present in nature where a number of species co-occur. With current global changes such as climate warming, the structure and composition of communities is changing, either via direct effects on species performance (Thierry et al. 2021), via effects on interactions (Hance et al. 2007, Thierry et al. 2019), or due to shift in ranges and phenology, and an increase in invasive species. It is therefore imperative to take into account the context in which species interact, both abiotic and biotic, as all those factors are likely to act together in influencing the outcome of the interaction between focal species (Gilman et al. 2010).

Acknowledgements

We thank Anna Mácová, Andrea Weberova, Petr Lavička, and Joel Brown for their help to set up the experiment, Chia-Hua Lue for fruitful discussions on the results, and Owen Lewis for comments on the manuscript. The drawings used for Figure 1 was made by Tereza Holicová. We acknowledge funding support from the Czech Science Foundation grant no. 20-30690S.

Author contributions

MT conceived the project; NP and JH contributed to the experimental design; MT, NP, MG, and GP collected the data; MT analyzed the data. All authors contributed critically to the draft and gave final approval for publication.

Competing interests

The author(s) declare no competing interests.

Data availability

All raw data used for this study are available from the Zenodo database: 10.5281/zenodo.5106122.

References

Abram, P. K., J. Brodeur, A. Urbaneja, and A. Tena. 2019. Nonreproductive effects of insect parasitoids on their hosts. Annual Review of Entomology 64:259–276.

Alexander, J. M., J. M. Diez, and J. M. Levine. 2015. Novel competitors shape species' responses to climate change. Nature 525:515–518.

Bascompte, J., and C. J. Melián. 2005. Simple trophic modules for complex food webs. Ecology 86:2868–2873.

Bográn, C. E., K. M. Heinz, and M. A. Ciomperlik. 2002. Interspecific competition among insect parasitoids: Field experiments with whiteflies as hosts in cotton. Ecology 83:653–668.

Bonsall, M. B., and M. P. Hassell. 1999. Parasitoid-mediated effects: apparent competition and the persistence of host–parasitoid assemblages. Researches on Population Ecology 41:59–68.

Boulétreau, M., and E. Wajnberg. 1986. Comparative responses of two sympatric parasitoid cynipids to the genetic and epigenetic variations of the larvae of their host, *Drosophila melanogaster*. Entomologia Experimentalis et Applicata 41:107–114.

Carton, Y., and H. Kitano. 1981. Evolutionary relationships to parasitism by seven species of the *Drosophila melanogaster* subgroup. Biological Journal of the Linnean Society 16:227–241.

Carton, Y., M. Poirié, and A. J. Nappi. 2008. Insect immune resistance to parasitoids. Insect Science 15:67–87.

Cusumano, A., E. Peri, and S. Colazza. 2016. Interspecific competition/facilitation among insect parasitoids. Elsevier Inc.

Fleury, F., N. Ris, R. Allemand, P. Fouillet, Y. Carton, and M. Boulétreau. 2004. Ecological and genetic interactions in Drosophila-parasitoids communities: a case study with D. melanogaster, D. simulans and their common Leptopilina parasitoids in south-eastern France. Pages 181–194 *in* P. Capy, P. Gibert, and I. Boussy, editors. *Drosophila melanogaster, Drosophila simulans*: So Similar, So Different. Springer Netherlands.

Fontaine, C., P. R. Guimarães, S. Kéfi, N. Loeuille, J. Memmott, W. H. van der Putten, F. J. F. van Veen, and E. Thébault. 2011. The ecological and evolutionary implications of merging different types of networks. Ecology Letters 14:1170–1181.

García-Callejas, D., R. Molowny-Horas, and M. B. Araújo. 2018. Multiple interactions networks: towards more realistic descriptions of the web of life. Oikos 127:5–22.

Gilman, S. E., M. C. Urban, J. Tewksbury, G. W. Gilchrist, and R. D. Holt. 2010. A framework for community interactions under climate change. Trends in Ecology & Evolution 25:325–331.

Godfray, H. C. J. 2004. Parasitoids. Current Biology 14:R456.

Hance, T., J. van Baaren, P. Vernon, and G. Boivin. 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. Annual Review of Entomology 52:107–126.

Hartig, F. 2019. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.

Harvey, J. A., E. H. Poelman, and T. Tanaka. 2013. Intrinsic inter- and intraspecific competition in parasitoid wasps. Annual Review of Entomology 58:333–351.

Henry, L. M., B. O. Ma, and B. D. Roitberg. 2009. Size-mediated adaptive foraging: A host-selection strategy for insect parasitoids. Oecologia 161:433–445.

Holt, R. D. 1977. Predation, apparent competition, and the structure of prey communities. Theoretical Population Biology 12:197–229.

Holt, R. D. 1997. Community modules. Page *in* A. C. Gange and V. K. Brown, editors. Multitrophic Interactions in Terrestrial Ecosystems. Blackwell Science, Oxford.

Ishii, Y., and M. Shimada. 2012. Learning predator promotes coexistence of prey species in host–parasitoid systems. Proceedings of the National Academy of Sciences 109:5116–5120.

Jeffs, C. T., J. C. D. Terry, M. Higgie, A. Jandová, H. Konvičková, J. J. Brown, C. H. Lue, M. Schiffer, E. K. O'Brien, J. Bridle, J. Hrček, and O. T. Lewis. 2021. Molecular analyses reveal consistent food web structure with elevation in rainforest *Drosophila* - parasitoid communities. Ecography 44:403–413.

Jones, T. S., H. C. J. Godfray, and F. J. F. van Veen. 2009. Resource competition and shared natural enemies in experimental insect communities. Oecologia 159:627–635.

Kaartinen, R., and T. Roslin. 2013. Apparent competition leaves no detectable imprint on patterns of community composition: observations from a natural experiment. Ecological Entomology 38:522–530.

Kawatsu, K., M. Ushio, F. J. F. van Veen, and M. Kondoh. 2021. Are networks of trophic interactions sufficient for understanding the dynamics of multi-trophic communities? Analysis of a tri-trophic insect food-web time-series. Ecology Letters 24:543–552.

Kéfi, S., E. L. Berlow, E. A. Wieters, L. N. Joppa, S. A. Wood, U. Brose, and S. A. Navarrete. 2015. Network structure beyond food webs: Mapping non-trophic and trophic interactions on Chilean rocky shores. Ecology 96:291–303.

Kéfi, S., E. L. Berlow, E. A. Wieters, S. A. Navarrete, O. L. Petchey, S. A.

Wood, A. Boit, L. N. Joppa, K. D. Lafferty, R. J. Williams, N. D. Martinez, B. A. Menge, C. A. Blanchette, A. C. Iles, and U. Brose. 2012. More than a meal… integrating non-feeding interactions into food webs. Ecology Letters 15:291–300.

Kehoe, R., E. Frago, C. Barten, F. Jecker, F. van Veen, and D. Sanders. 2016. Nonhost diversity and density reduce the strength of parasitoid–host interactions. Ecology and Evolution 6:4041–4049.

Lenth, R. V. 2018. Emmeans: Estimated marginal means, aka least-squares means. R package version.

Letourneau, D. K., J. A. Jedlicka, S. G. Bothwell, and C. R. Moreno. 2009. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 40:573–592.

Lüdecke, D., D. Makowski, and P. Waggoner. 2019. Performance: Assessment of regression models performance.

Lue, C.-H., M. L. Buffington, S. Scheffer, M. Lewis, T. A. Elliott, A. R. I. Lindsey, A. Driskell, A. Jandova, M. T. Kimura, Y. Carton, R. R. Kula, T. A. Schlenke, M. Mateos, S. Govind, J. Varaldi, E. Guerrier, M. Giorgini, X. Wang, K. Hoelmer, K. M. Daane, P. K. Abram, N. A. Pardikes, J. J. Brown, M. Thierry, M. Poirie, P. Goldstein, S. E. Miller, D. Tracey, J. S. Davis, F. M. Jiggins, B. Wertheim, O. Lewis, J. Leips, P. P. A. Staniczenko, and J. Hrcek. 2021. DROP: Molecular voucher database for identification of *Drosophila* parasitoids. Molecular Ecology Resources.

McPeek, M. A. 2019. Mechanisms influencing the coexistence of multiple consumers and multiple resources: resource and apparent competition. Ecological Monographs 89:e01328.

Miele, V., C. Guill, R. Ramos-Jiliberto, and S. Kéfi. 2019. Non-trophic interactions strengthen the diversity—functioning relationship in an ecological bioenergetic network model. PLoS Computational Biology 15:e1007269.

Milo, R., S. Shen-Orr, S. Itzkovitz, N. Kashtan, D. Chklovskii, and U. Alon. 2002. Network motifs: simple building blocks of complex networks. Science 298:824–827.

Morris, R. J., C. B. Müller, and H. C. J. Godfray. 2001. Field experiments testing for apparent competition between primary parasitoids mediated by secondary parasitoids. Journal of Animal Ecology 70:301–309.

Myers, J. H., C. Higgins, and E. Kovacs. 1989. How many insect species are necessary for the biological control of insects? Environmental Entomology 18:541–547.

Nouhaud, P., F. Mallard, R. Poupardin, N. Barghi, and C. Schlötterer. 2018. High-throughput fecundity measurements in *Drosophila*. Scientific Reports 8:4469.

Ortiz-Martínez, S., J. S. Pierre, J. van Baaren, C. Le Lann, F. Zepeda-Paulo, and B. Lavandero. 2019. Interspecific competition among aphid parasitoids: molecular approaches reveal preferential exploitation of parasitized hosts. Scientific Reports 9.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.

Pedersen, B. S., and N. J. Mills. 2004. Single vs. multiple introduction in biological control: The roles of parasitoid efficiency, antagonism and niche overlap. Journal of Applied Ecology 41:973–984.

Pilosof, S., M. A. Porter, M. Pascual, and S. Kéfi. 2017. The multilayer nature of ecological networks. Nature Ecology & Evolution 1:101.

Poelman, E. H., R. Gols, A. V. Gumovsky, A. M. Cortesero, M. Dicke, and J. A. Harvey. 2014. Food plant and herbivore host species affect the outcome of intrinsic competition among parasitoid larvae. Ecological Entomology 39:693–702.

Randa, L. A., D. M. Cooper, P. L. Meserve, and J. A. Yunger. 2009. Prey switching of sympatric canids in response to variable prey abundance. Journal of Mammalogy 90:594–603.

De Rijk, M., X. Zhang, J. a. H. Van Der Loo, B. Engel, M. Dicke, and E. H. Poelman. 2016. Density-mediated indirect interactions alter host foraging behaviour of parasitoids without altering foraging efficiency. Ecological Entomology 41:562–571.

Rip, J. M. K., K. S. McCann, D. H. Lynn, and S. Fawcett. 2010. An experimental test of a fundamental food web motif. Pages 1743–1749 Proceedings of the Royal Society B: Biological Sciences. Royal Society.

Sentis, A., C. Gémard, B. Jaugeon, and D. S. Boukal. 2017. Predator diversity and environmental change modify the strengths of trophic and nontrophic interactions. Global Change Biology 23:2629–2640.

Siddon, C. E., and J. D. Witman. 2004. Behavioral indirect interactions: Multiple predator effects and prey switching in the rocky subtidal. Ecology 85:2938–2945.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends in Ecology and Evolution 13:350–355.

Singh, P., and G. Baruah. 2020. Higher order interactions and species coexistence. Theoretical Ecology:1–13.

Snyder, G. B., D. L. Finke, and W. E. Snyder. 2008. Predator biodiversity strengthens aphid suppression across single- and multiple-species prey communities. Biological Control 44:52–60.

Snyder, W. E., G. B. Snyder, D. L. Finke, and C. S. Straub. 2006. Predator biodiversity strengthens herbivore suppression. Ecology Letters 9:789–796.

Team, R. C. 2017, January 29. R: The R Project for statistical computing.

Thierry, M., J. Hrček, and O. T. Lewis. 2019. Mechanisms structuring host–parasitoid networks in a global warming context: a review. Ecological

Entomology:een.12750.

Thierry, M., N. A. Pardikes, C. H. Lue, O. T. Lewis, and J. Hrček. 2021. Experimental warming influences species abundances in a Drosophila host community through direct effects on species performance rather than altered competition and parasitism. PLoS One 16:e0245029.

Tylianakis, J. M., T. Tscharntke, and O. T. Lewis. 2007. Habitat modification alters the structure of tropical host–parasitoid food webs. Nature 445:202–205.

Valente, C., C. Afonso, C. I. Gonçalves, and M. Branco. 2019. Assessing the competitive interactions between two egg parasitoids of the Eucalyptus snout beetle, Gonipterus platensis, and their implications for biological control. Biological Control 130:80–87.

van Veen, F. J. F., P. D. van Holland, and H. C. J. Godfray. 2005. Stable coexistence in insect communities due to density- and trait-mediated indirect effects. Ecology 86:3182–3189.

Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84:1083–1100.

Wong, M. K. L., B. Guénard, and O. T. Lewis. 2019. Trait-based ecology of terrestrial arthropods. Biological Reviews 94:999–1022.

Xu, H. Y., N. W. Yang, M. Duan, and F. H. Wan. 2016. Functional response, host stage preference and interference of two whitefly parasitoids. Insect Science 23:134–144.

Supplement Material

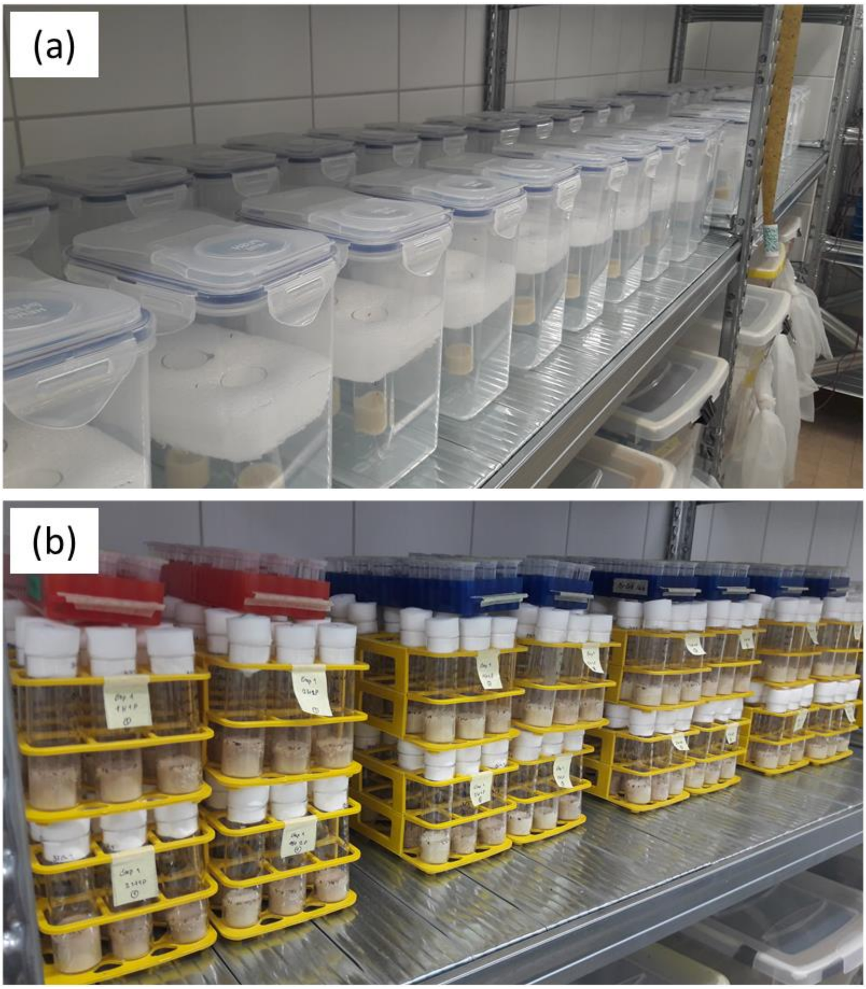


Figure S1. Experimental set up. (a) Boxes contained two vials with 25 two-days-old Drosophila larvae. Four three-to-five days old parasitoids (1:1 sex ratio) were placed in each box for 24 hours. The experiment counts a total of 216 boxes. (b) After 24 hours, parasitoids were removed, and vials were plugged for rearing. Emerges were collected daily and kept in 95% ethanol.

Supplement material S2: Effects of community composition on host suppression

Probability of host infestation responded differently depending on parasitoid species assemblage (community composition model: $\chi2(5) = 32.70$, $P < 0.0001$), and the interaction between parasitoid and host assemblages ($\chi2(25) = 68.21$, $P < 0.0001$).

Neither parasitoid assemblage ($\chi2(5) = 7.08$, $P = 0.214$), host species assemblage ($\chi2(2) = 0.58$, $P = 0.748$), nor their interaction ($\chi2(25) = 4.12$, $P = 0.942$) had a significant effect on *D. birchii* DI (Figure S2a). *Drosophila pallidifrons* DI increased with presence of multiple parasitoids, but only significantly when *Ganaspis sp.* was associated with *Leptopilina sp.* (Post Hoc Odds Ratio (OR) LG/GG = 3.77, $P = 0.039$, but OR AG/GG = 1.17, $P = 0.999$; Figure S2b). *Drosophila simulans* DI only significantly increased with presence of multiple parasitoids when either *Leptopilina sp.* or *Asobara sp.* was associated with *Ganaspis sp.* (OR LG/LL = 33.07, $P < 0.0001$, but OR AL/LL = 4.51, $P = 0.105$; OR AG/AA = 9.20, $P = 0.0007$, but OR AL/AA = 0.39, $P = 0.318$; Figure S2c).

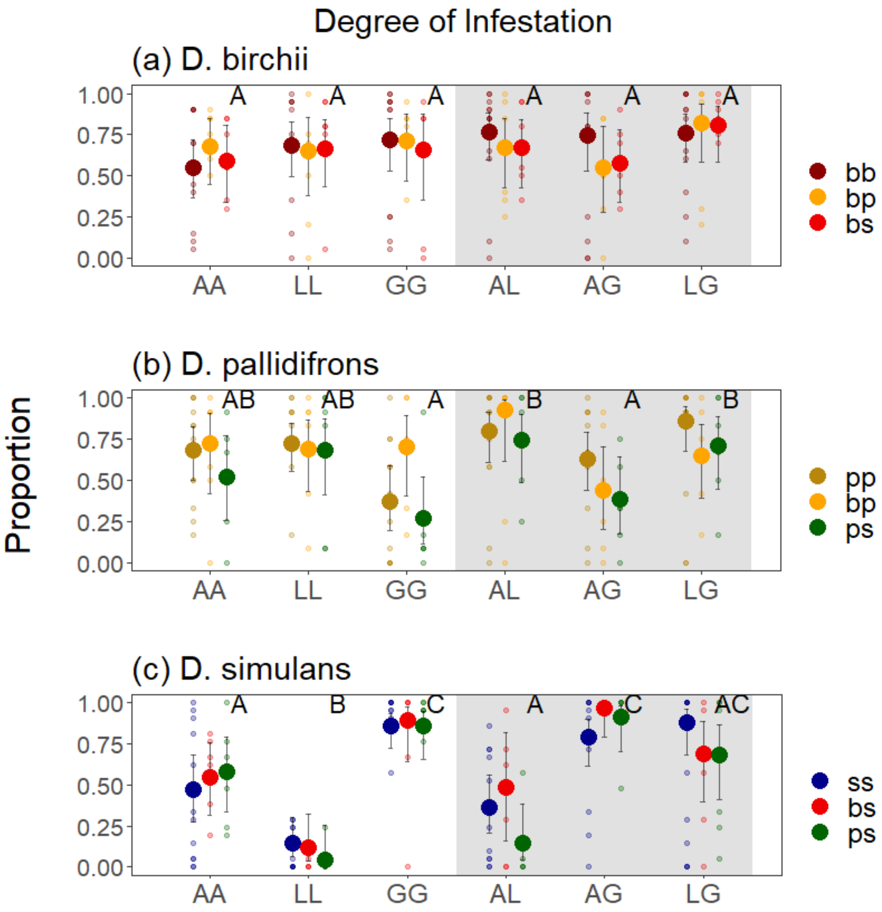


Figure S2. Effects of community composition on the degree of infestation (DI) of each host species depending on host assemblage (represented by different colors; host abbreviations: b: *D. birchii*, p: *D. pallidifrons*, s: *D. simulans*) and parasitoid assemblage (on the x axis; parasitoid abbreviations: A: *Asobara sp*., L: *Leptopilina sp*., G: *Ganaspis sp*.). Different capital letters denote significant differences between parasitoid assemblage (from community composition models). White/grey panel: without/with interspecific exploitative competition between parasitoid species. The small points represent the observed values, and the large points represent the predicted values with their 95% confidence intervals

Supplement material S3: Effects of community composition on parasitoid performance

Successful parasitism rates significantly increased in modules with a parasitoid competitor for *Asobara sp*. on *D. simulans* with the presence of *Leptopilina sp*. (Post Hoc Odds Ratio (OR) = 4.53, P = 0.003) and *Ganaspis sp*. (OR = 3.88, P = 0.008) in the exploitative competition modules compared to the host-parasitoid pair in isolation, but not in modules with both exploitative and apparent competition (Figure 4c). The increase in successful parasitism rate of *Asobara sp*. on *D. simulans* in the exploitative competition modules could be due to the suppression of the *D. simulans* immune response by *Leptopilina sp*. and *Ganaspis sp*., and the contrasting results when an alternative host was present could be due to differences in oviposition behavior (some parasitoid randomly lay eggs in many hosts while Braconid species are more specialized in certain groups of hosts), although those mechanisms were not tested in the present study.

SP of *Ganaspis sp*. on *D. simulans* also significantly increased in modules with a parasitoid competitor compared to the host-parasitoid pair in isolation, but only in both exploitative and apparent competition modules, and for certain species assemblages. It increased with the presence of *Leptopilina sp*. only when *D. simulans* was associated with *D. birchii* (OR = 13.69, P = 0.004), and marginally with the presence of *Asobara sp*. only when *D. simulans* was associated with *D. pallidifrons* (OR = 4.70, P = 0.053) (Figure 4i).

SP of *Leptopilina sp*. on *D. birchii* significantly increased with presence of *Asobara sp*., but only in modules with both exploitative and apparent competition (when *D. birchii* was associated with *D. pallidifrons* OR = 4.83, P = 0.0001, and when *D. birchii* was associated with *D. simulans* OR = 3.86, P = 0.0003), and was not significantly affected by the presence of *Ganaspis sp*. for any of the host combinations (Figure 4d).

SP of *Ganaspis sp*. on *D. birchii* was significantly affected by the presence of *Asobara sp*., but only in modules with both exploitative and apparent competition, and the direction of the effect depended on the alternative host species. It increased when *D. birchii* was associated with *D. pallidifrons* (OR = 4.69, P = 0.004), but decreased with presence of *Asobara* *sp*., when *D. birchii* was associated with *D. simulans* (OR = 0.13, P = 0.016), suggesting antagonistic interactions between those species with that host assemblage (Figure 4g).

SP of the other eight host-parasitoid pair did not significantly differ with either or both the presence of a parasitoid heterospecific (exploitative competition modules) and of an alternative host (apparent competition modules) compared to the module with the host-parasitoid pair in isolation (Figure 4).

Chapter III

Experimental warming influences species abundances in a *Drosophila* host community through direct effects on species performance rather than altered competition and parasitism

(In PLOS One, 2021)

Experimental warming influences species abundances in a *Drosophila* host community through direct effects on species performance rather than altered competition and parasitism

Mélanie Thierry*[1, 2], Nicholas A. Pardikes [2], Chia-Hua Lue [2], Owen T. Lewis [3] & Jan Hrček [1, 2]

[1]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, [2]Department of Ecology, Institute of Entomology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic, [3]Department of Zoology, University of Oxford, Oxford, United Kingdom

* m.thierry@entu.cas.cz

Abstract. Global warming is expected to have direct effects on species through their sensitivity to temperature, and also via their biotic interactions, with cascading indirect effects on species, communities, and entire ecosystems. To predict the community-level consequences of global climate change we need to understand the relative roles of both the direct and indirect effects of warming. We used a laboratory experiment to investigate how warming affects a tropical community of three species of *Drosophila* hosts interacting with two species of parasitoids over a single generation. Our experimental design allowed us to distinguish between the direct effects of temperature on host species performance, and indirect effects through altered biotic interactions (competition among hosts and parasitism by parasitoid wasps). Although experimental warming significantly decreased parasitism for all host-parasitoid pairs, the effects of parasitism and competition on host abundances and host frequencies did not vary across temperatures. Instead, effects on host relative abundances were species-specific, with one host species dominating the community at warmer temperatures, irrespective of parasitism and competition treatments. Our results show that temperature shaped a *Drosophila* host community directly through differences in species' thermal performance, and not via its influences on biotic interactions.

Key words. Parasitoid, temperature, interactions, thermal performance, climate change

Introduction

It is becoming evident that many species are declining as the climate changes [1,2], and increasing numbers of extinctions are expected as a result in the coming decades [3]. Animals are directly impacted by warming temperatures through changes in their fecundity, mortality, metabolic rates, body growth rate, and phenology [4–7]. Species in the tropics are likely to be more sensitive to global warming because they are closer to their upper thermal limits [3,8], and the predicted increase in temperatures by a few degrees would exceed their thermal maxima. Ectotherms, such as insects, have particularly narrow thermal limits and are facing severe declines in abundances with rising temperature [9]. Warming temperatures directly affect physiology and demography depending on species' thermal tolerances (i.e., their ability to survive exposure to extreme temperatures) and their thermal performance (i.e., their fitness-related traits over a range of temperatures). Both thermal tolerance and thermal performance are expected to influence population sizes and community structure with ongoing global warming [5].

However, ecological communities are not defined solely by the species that compose them, but also by the way those species interact with one another, via both trophic and non-trophic interactions [10,11]. Trophic interactions, such as predation, herbivory, or parasitism have strong effects on community composition and evenness [12,13]. Non-trophic interactions such as competition and pollination are also ubiquitous and can alter community composition in many ways (e.g. if some species are competitively excluded, or if species coexistence is enhanced) [14–16]. Trophic and non-trophic interactions act together to structure ecological communities [17–19], and a theoretical understanding is emerging of how these different types of interactions shape the structure and dynamics of more complex ecological networks [20]. However, empirical evidence on the combined effects of trophic and non-trophic interactions on the structure of terrestrial species-rich communities remain sparse. Moreover, global warming may modify such mechanisms structuring ecological communities, since warming temperatures are expected to have direct effects on both component species and their interactions [21,22]. Temperature can alter resource-consumer interactions via its effects on metabolic processes such as growth and reproduction, and change in behaviors [23–25]. The main mechanisms behind species interactions response to climate change are the differences in effects among interacting species, such as asymmetrical responses in their phenology [26], growth rate [27], and body mass [28]. Furthermore, changes in the outcome of species interaction with warming temperatures can have cascading effects on individual fitness, populations and communities [25,29,30]. Despite calls for more investigations of how species interactions respond to global climate change [31,32], most such studies focus either on aquatic systems [21,33], on a single interaction type [34], or on a small number of species [35]. We urgently need more data to predict how environmental changes modify different types of interactions (both trophic and non-trophic) in more complex ecological networks [36,37].

Insect host-parasitoid communities are excellent model systems to investigate how species and their interactions respond to warming temperatures [14]. Parasitoids are insects which develop in or on the bodies of arthropod hosts, killing the host as they mature, and playing an important role in regulating host populations in both natural and agricultural ecosystems [38]. As ectotherms, many parasitoid traits involved in species interactions are sensitive to changes in temperature [39,40]. Empirical studies suggest that global warming could weaken top-down control by parasitoids by increasing parasitoid mortality, by decreasing parasitoid virulence and/or increasing host immune response, and by increasing host-parasitoid asynchrony, thus increasing the frequency of pest outbreaks [41–43]. However, most studies of host-parasitoid interactions are limited to a pair of interacting species, and it is unclear how host-parasitoid communities respond to warming temperatures when more complex systems are considered [14,44]. Community level responses to global warming may depend on how species interact, and the effect of species interactions on community structure might change depending on environmental conditions.

For instance, parasitoids can mediate host coexistence, but the outcome may depend on temperature [45]. Furthermore, competitive interactions among hosts can affect the responses of species and communities to environmental changes [30], but such responses may differ for intraspecific and interspecific competition [46]. Thus, to help forecast the impacts of global warming on host-parasitoid communities, it will be critical to examine the combined responses of species and their interactions under simulated warming conditions [47].

In this study, we use a laboratory experiment with intra vs. inter specific competition between hosts and parasitism in a fully factorial design to investigate how temperature affects host communities directly through difference in species responses, and indirectly through effects on parasitism and competition with other host species. We used host abundances and their relative frequencies to describe the host community. We also measured host body mass as a proxy for host fitness under the different treatments, and because an increase in temperature generally produces smaller individuals, which could influence the outcome of competition [28]. We focus on a set of three *Drosophila* species which are members of a natural *Drosophila*-parasitoid community in Australian tropical rainforests [48]. We test the predictions that elevated temperature will affect the relative abundance of the hosts directly through the thermal performance of individual species, and indirectly through effects on their interactions with other species. Elevated temperatures could alter the competitive abilities of the hosts (linked to species' thermal performance) and the extent to which they are parasitized (linked to effects of temperature on parasitoid attack rates and virulence) [39], with consequences for the relative abundance of hosts in the community [14]. An interactive effect of trophic and non-trophic interactions on host relative abundances is expected due to a trade-off between resistance to parasitoids and larval competitive abilities [49]. This study aims to disentangle the direct and indirect effects of warming on structuring our focal tropical *Drosophila* community, and provides an important step forward in our understanding of the potential mechanisms driving tropical insect community responses to global warming.

Materials and Methods

Study system

The experiment was established from cultures of *Drosophila* species and their associated parasitoids collected from two tropical rainforest locations in North Queensland, Australia: Paluma (S18° 59.031' E146° 14.096') and Kirrama Ranges (S18° 12.134' E145° 53.102') (<100 m above sea level). *Drosophila* and parasitoid cultures were established from 2017 to 2018, identified using both morphology and DNA barcoding, and shipped to the Czech Republic under permit no. PWS2016-AU-002018 from Australian Government, Department of the Environment. Three host species (*Drosophila birchii*, *D. pseudoananassae* and *D. sulfurigaster*, together accounting for ~ 48% of the host abundances sampled at the study sites [48]) and two of their natural larval parasitoid species *Asobara* sp.1 (Hymenoptera: Braconidae; Smithsonian National Museum of Natural History (NMNH) reference vouchers USNMENT01557096 [BOLD sequence accession: DROP042-21] and USNMENT01557097 [BOLD sequence accession: DROP043-21] and *Leptopilina* sp.1 (Hymenoptera: Figitidae; NMNH reference vouchers USNMENT01557104 [BOLD sequence accession: DROP050-21] and USNMENT01557117 [BOLD sequence accession: DROP053-21]) able to parasitize all three host species were used in this experiment. The parasitoid species are new undescribed species unambiguously identified by the above vouchers and sequences in order for this paper to be linked to them once they will be formally described. Data on thermal performance of the three host species have been previously measured by MacLean, Overgaard, and collaborators [50,51] (Table 1). All cultures were maintained at 23°C on a 12:12 hour light and dark cycle at Biology Centre, Czech Academy of Sciences. *Drosophila* isofemale lines were maintained on standard *Drosophila* medium (corn flour, yeast, sugar, agar and methyl-4-hydroxybenzoate) for approximately 15 to 30 non-overlapping generations. To ensure genetic variation, five lines from each host species were combined to establish mass-bred lines immediately before the start of the experiment. Isofemale lines of parasitoid lines were maintained for approximately 10 to 20 non-overlapping generations prior to the start of the experiment by providing them every week with 2-day-old larvae of *Drosophila melanogaster*. This host species is not present naturally at the field locations where hosts and parasitoids originated, and was not used in the experiment, thus avoiding bias of host preferences. Single parasitoid isofemale lines were used.

Table 1. **Host species thermal tolerance upper limit (T_{max}) and thermal performances: optimal temperature (T_{opt}) and thermal breadth (T_{breath} defined here as the range where performance is above 80% of optimal) for overall species fitness (product of fecundity, developmental success and developmental speed) and fecundity measured as egg-laying capacity ± SD.** Data are from [51].

Host species	***D. birchii***	***D. pseudoananassae***	***D. sulfurigaster***
T_{max}	38.51 ± 0.32	39.02 ± 0.32	36.55 ± 0.11
Fitness T_{opt}	25.33 ± 1.05	24.00 ± 0.45	24.72 ± 0.73
Fitness T_{breath}	4.27 ± 0.57	5.15 ± 0.36	4.51 ± 0.31
Fecundity T_{opt}	26.18 ± 0.62	24.62 ± 1.52	24.84 ± 0.72
Fecundity T_{breath}	5.37 ± 1.16	9.31 ± 1.11	5.26 ± 0.44

Experimental design

To disentangle the effects of warming temperatures on host species and their interactions, we manipulated the presence of parasitoids and interspecific competition between host species in a fully factorial design (Fig 1) at ambient and elevated temperatures. We aimed to study the independent and combined effects of parasitism and host competition when both forms of antagonistic interaction occur at strong (but realistic) levels. As the focus of the experiment was to compare the direct and indirect effects of warming temperatures on host communities, competitive interactions between parasitoids were not assessed nor manipulated, but potentially present in all treatments with parasitoids. Parasitoid preferences were not quantified, but the two parasitoid species used were able to parasitize all three hosts species during trials.

Transparent plastic boxes (47cm x 30cm x 27.5cm) with three ventilation holes (15 cm in diameter) covered with insect-proof nylon mesh served as the experimental units (S1 Fig). Each box contained three 90 mm high and 28 mm diameter glass vials containing 2.5 mL of *Drosophila* food medium.

Interactions were manipulated by establishing vials containing a single host (Fig 1a and 1c) or multiple host species (Fig 1b and 1d), and by including (Fig 1c and 1d) or excluding (Fig 1a and 1b) parasitoids. A total of 60 three-day-old virgin adult hosts, with 1:1 sex ratio, were placed in each vial to allow mating and oviposition (i.e., a total of 180 adults per box) and removed after 48 hours. In the multi-host treatment, the 60 hosts were split evenly across the three species (i.e., 20 adults for each species). The density of adult hosts was selected based on preliminary observations to achieve a high level of resource competition (i.e., the density at which strong intraspecific competition was observed for all host species; S1 Table) while keeping the number of adults for each of the three host species and the total number of adult hosts consistent across treatments and species. The treatment allowed competition both at the adult stage for oviposition space, and at the larval stage of their offspring for food resources [52,53], but we did not aim to identify which was the primary source of competition. All results relate to the host offspring (their abundances and frequencies).

For treatments that included parasitoids (Fig 1c and 1d), ten parasitoids (3-7 days old, 1:1 sex ratio) from each species (n = 2, i.e., 20 parasitoids per box), corresponding to 9 % of the total number of adult hosts, were placed in a box immediately after the hosts were removed (at 48h) and remained in each box for 72 hours, creating high but realistic parasitoid pressure (within the range of parasitism rate observed in this system in nature: 8-42% [48]). Vials were removed from the boxes simultaneously with the parasitoids (72 hours after parasitoid introduction), and individually sealed. Each treatment combination was replicated once across four time-blocks, and each treatment and replicate were therefore represented by three vials. The duration of the experiment corresponded to a single generation of both the hosts and the parasitoids (i.e., about 30 days for the species with the longest developmental time to emerge).

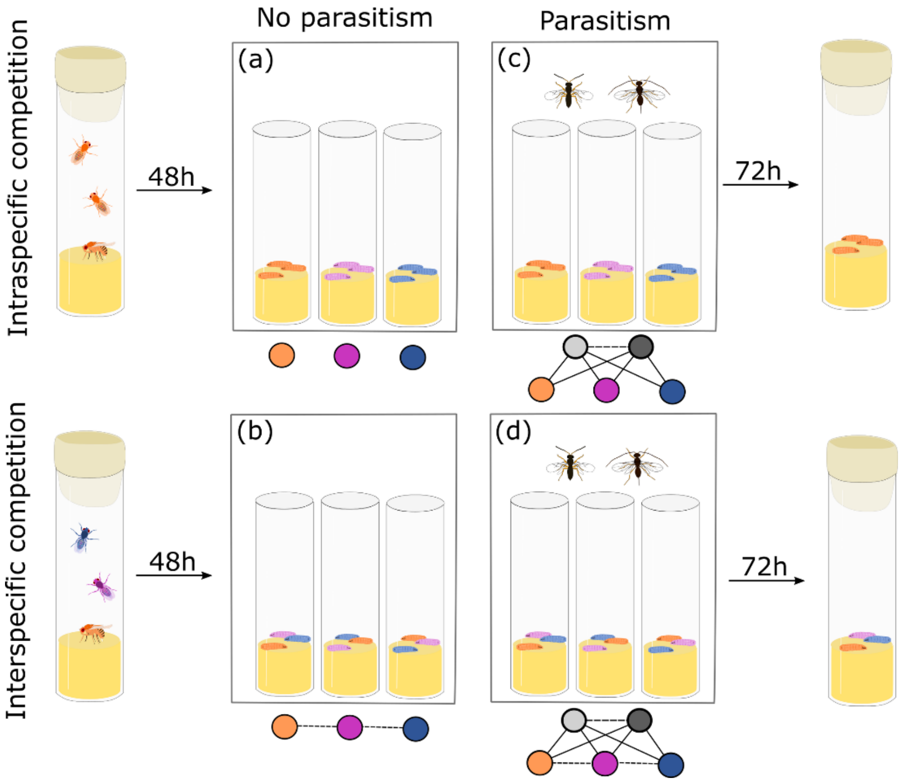


Fig 3. Schematic representation of the steps of the protocol and the experimental treatments. Orange, pink, and blue nodes represent the three host species, and white and grey nodes represent the two parasitoid species. Solid arrows show possible trophic interactions, and dashed arrows show possible competitive interactions in each treatment. The type of competition between host species (intraspecific/interspecific) and presence or absence of parasitoids in the cages were manipulated in a fully factorial design: a) intraspecific competition, b) interspecific competition, c) intraspecific competition with parasitism, and d) all interactions.

The experimental temperatures were chosen to simulate current mean yearly temperature at the two study sites [48]: 23.2 ± 0.4°C (65.9 ± 2.8% humidity), and projected temperatures representing a plausible future scenario under climate change: 26.7 ± 1.0°C (65.1 ± 2.8% humidity). The simulated difference was therefore 3.5°C (projected change in global mean surface temperature for the late 21st century is 3.7°C for the IPCC RCP8.5

baseline scenario [54]). Vials were placed at their corresponding temperature treatment from the first day the adult hosts were introduced for mating and oviposition to the last emergence (up to 40 days). All four blocks included both ambient and warming temperature treatments.

To calculate parasitism rates for each host-parasitoid species pair, pupae from the three vials of each box were randomly sampled 12 days after the initiation of the experiment. All sampled pupae were transferred into 96-well PCR plates (on average 169 ± 30 SD pupae sampled per box) and kept at their corresponding temperature treatment until adult insects emerged (up to 40 days for the slowest-developing parasitoid species). Sampled pupae were identified to their corresponding host species on the basis of pupal morphology (S2 Fig), and the outcome was recorded as either a host, a parasitoid, an empty pupal case, or an unhatched pupa. We assumed that any pupae which were empty at the time of sampling resulted in adult hosts because this period was too short for parasitoids to complete development and emerge. We calculated parasitism rates from the pupae sampled in plates only. Parasitism rates for each host-parasitoid pair were calculated as the proportion of each parasitoid species that emerged from the total number of sampled pupae of each host species.

All hosts that emerged (from both vials and sampling plates) were used to quantify the following aspects of host community structure: abundances of each host species, and their relative frequencies (i.e., the fraction of all host individuals belonging to each host species). All hosts and parasitoids that emerged from vials before and after subsampling for parasitism rates were collected, identified, and stored in 95% ethanol until four consecutive days of no adult emergences. Individual dry body mass of hosts was measured with 1 µg accuracy using a Sartorius Cubis ™ micro-balance. Only fully-eclosed and intact individuals were included in body mass measurements.

Statistical analysis

All vials with fewer than ten total emergences or pupae were removed from analyses of host abundances, frequencies, and parasitism rates (S2 Table, deleted observation due to *D. sulfurigaster*), as these outcomes were associated with low success during the mating process and not with experimental treatments (results with the whole dataset can be found in S3

Table). We used 3-day-old hosts and allowed them to mate and lay eggs for 48 hours. *Drosophila sulfurigaster* females generally take 4 days to mature compared to 3-4 days for *D. birchii* females and 3 days *for D. pseudoananassae*, which could explain the low abundances sometimes observed for *D. sulfurigaster* compared with the two other host species.

Data were analyzed with generalized linear models (GLMs). After testing for overdispersion of the residuals, abundance data were modeled using a negative binomial error distribution, host body mass using a gaussian error distribution, and frequencies of host species and parasitism rates using a quasibinomial error distribution. Parasitism (two levels), type of competition (two levels), host species (three levels), parasitoid species (two levels), and temperature (two levels) were included as categorical predictor variables within each model. Blocks were included in the models as a fixed effect. Each two-way interaction was tested and kept in our models if judged to be statistically significant on the basis of backward selection using Likelihood-ratio tests. Interaction between temperature and parasitism, temperature and competition, and parasitism and competition were systematically kept in our models as the experiment was designed to test for the significance of these interactions. The three-way interaction between temperature, parasitism, and competition was tested for host abundances, host frequencies, and host body mass, but was not significant. Significance of the effects was tested using type III analysis of deviance with F-tests.

Post-hoc multiple comparisons were performed using the *emmeans* package, and P-values were adjusted using the Tukey method. Model assumptions were verified with the *DHARMa* package. All analyses were performed using R 3.5.2 [55] with the packages *stats*, *MASS* [56], *car* [57], *performance* [58], *DHARMa* [59], and *emmeans* [60].

Results

In total, 7627 individuals (7063 hosts and 564 parasitoids) were reared across all treatments and replicates (238.3 ± 13.3 SD on average per box). Across all treatments and replicates, a total of 2717 pupae were sampled to estimate parasitism rates, of which 2227 (82%) produced an adult host or parasitoid. Mean host abundances, host body mass, and parasitism rates are presented for each treatment in S4 Table. We focused on the effects of temperature, parasitism, competition and their interactions on host abundances, host frequencies, and host body mass (Table 2).

Direct effect of warming on the host community

The effect of temperature on host relative abundances varied significantly across host species (Table 2, Fig 2). At 23°C, *D. birchii* and *D. pseudoananassae* had similar relative abundances across treatments (mean frequency of *D. birchii* = 0.426 ± 0.05; mean frequency of *D. pseudoananassae* = 0.471 ± 0.05 for all treatments combined at 23°C). At 27°C, *Drosophila pseudoananassae* relative abundances increased by 12.8% (Post Hoc odd ratio (OR) = 0.336, P < 0.0001) while *D. birchii* relative abundances decreased by 56.1% (Post Hoc OR = 3.190, P < 0.0001) (mean frequency of *D. birchii* = 0.187 ± 0.02; mean frequency of *D. pseudoananassae* = 0.743 ± 0.02 for all treatments combined at 27°C). The change in frequency of *D. sulfurigaster* with temperature was not significant (at 23°C: 0.178 ± 0.03, at 27°C: 0.118 ± 0.02; Post Hoc OR = 1.361, P = 0.440). Elevated temperature had no effect on host body mass ($F_{1,65} = 1.88$, P = 0.175, S3 Fig).

Effect of biotic interactions on the host community

Parasitism significantly reduced mean abundances of all three host species by 50 ± 0.22 (SEM) hosts on average across species ($\beta = -0.339$, $F_{1,68} = 21.80$, P < 0.0001; Fig 3a), and the negative effect of parasitism was consistent across host species (Table 2). Competition type did not significantly impact host abundances or relative host frequencies. Effects of competition on host body mass depended both on host identity ($F_{2,65} = 27.80$, P < 0.0001), and on presence or absence of parasitoids ($F_{1,65} = 4.87$, P = 0.038). *D. pseudoananassae* was the host species that varied the most in body mass with treatments (S3 Fig). Its body mass decreased with interspecific competition in the absence of parasitoids but increased with interspecific competition with presence of parasitoids. Changes in body mass for the other two host species were less pronounced.

Table 2. Table showing the effect of temperature (23°C or 27°C), parasitism (present or absent), competition between host species (intraspecific or interspecific), host species (n = 3), parasitoid species (n = 2), interactions between terms, and block (n = 4) on host abundances, host frequencies, host body mass, and parasitism rate.

	Df	Host abundances		Host frequencies		Host body mass		Parasitism rate	
Temperature	1	1.41	(ns)	0.47	(ns)	1.88	(ns)	4.89	*
Parasitism	1	21.80	***	0.03	(ns)	2.98	(ns)	-	-
Competition	1	0.15	(ns)	0.06	(ns)	10.76	**	1.14	(ns)
Host species	2	27.07	***	64.7	***	426.64	***	2.47	(ns)
Parasitoid species	1	-	-	-	-	-	-	2.29	(ns)
Temperature x Parasitism	1	0.26	(ns)	0.05	(ns)	0.60	(ns)	-	-
Temperature x Competition	1	0.00	(ns)	0.07	(ns)	1.32	(ns)	0.04	(ns)
Temperature x Host species	2	7.90	***	24.12	***	-	-	-	-
Parasitism x Competition	1	1.58	(ns)	0.00	(ns)	4.49	*	-	-
Competition x Host species	2	-	-	-	-	27.80	***	-	-
Host x parasitoid species	2	-	-	-	-	-	-	20.23	***
Block	3	1.02	(ns)	0.47	(ns)	4.53	**	1.49	(ns)
Df error		68		68		65		70	
R^2		0.87		0.05		0.93		0.10	

Degrees of freedom (Df) for each F-ratio are given for each factor and for the error. F values are presented with the significance of the effect: (***) P < 0.001, (**) P < 0.01, (*) P < 0.05, (ns) P > 0.05.

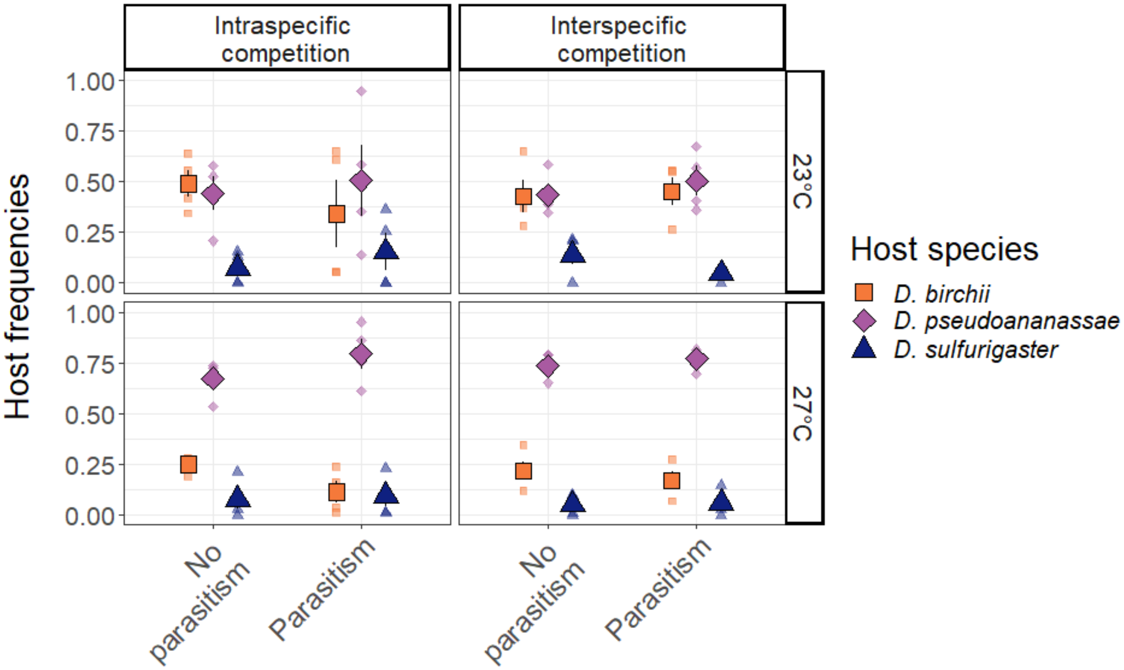


Fig 2. Effect of experimental treatments on host frequencies. Experimental warming changed the frequencies of hosts for all treatments. See Figure 1 for detailed description of the treatments. The small points represent the values from each block, the large points represent the grand mean, and the bars represent standard errors of the means.

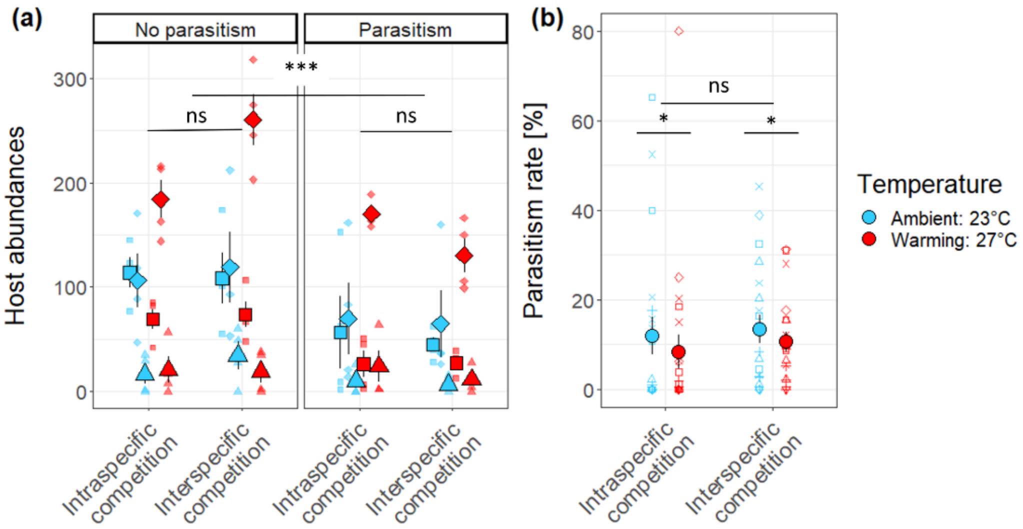


Fig 3. Effect of experimental treatments on host community and host-parasitoid interactions. (a) Host abundances (■: *D. birchii*, ♦: *D. pseudoananassae*, ▲: *D. sulfurigaster*) were significantly reduced by parasitism across treatments. (b) Parasitism rates were reduced at higher temperature (□: *Asobara* sp. - *D. pseudoananassae*, ∧: *Asobara* sp. - *D. birchii*, +: *Asobara* sp. - *D. sulfurigaster*, × *Leptopilina* sp. - *D. pseudoananassae*, ◊: *Leptopilina* sp. - *D. birchii*, ∨: *Leptopilina* sp. - *D. sulfurigaster*). See Fig 1 for detailed description of the treatments. The small points represent the values from each block, the large points represent the grand mean, and the bars represent standard errors of the means. Significance of treatment effects is indicated as follows: (***) P < 0.001, (**) P < 0.01, (*) P < 0.05, (ns) P > 0.05

Indirect effect of warming on host community structure through parasitism and interspecific competition

Experimental warming significantly decreased parasitism rates for all host-parasitoid pairs ($\beta = -0.29$, $F_{1,70} = 4.89$, $P = 0.030$, Table 2, Fig 3b). However, the effects of parasitism and competition did not vary with temperature in affecting any of our measures of community structure ($P > 0.05$, Table 2).

Discussion

Our results revealed that experimental warming directly affected *Drosophila* host community structure through differences in thermal performance among species, and decreased parasitism rates, without effects on host competition. However, warming did not impact the effect of parasitism on host community structure over the timescale investigated. The type of competition (intraspecific or interspecific) among hosts did not change host community structure.

Our results suggests that ongoing rises in global temperatures could directly alter arthropod host community structure through differences in thermal performance across species, as has been shown for communities of fish [61], plants [62], and insects [63]. Changes in host frequencies in warmer temperatures was primarily due to a dramatic increase in the relative abundance of a single host species, *D. pseudoananassae*, the species with the largest thermal performance breath [51], and our main conclusions should thus not be impacted by the low abundances sometimes observed for *D. sulfurigaster* due to mating problems. This increase occurred across all combinations of parasitism and competition treatments, and without a change in *Drosophila* body mass, suggesting a direct effect of temperature on host fecundity due to the preferred temperature of the adults for egg-laying and/or offspring egg-to-adult viability related to their thermal preference [64]. In our system, *D. pseudoananassae* distribution is limited to low elevation sites [48], and this species has a higher thermal tolerance and a bigger thermal breadth than either of the other two species considered in this study [51]. In nature, *Drosophila* species distributions are driven by differences in innate thermal tolerance limits, with low phenotypic plasticity for thermal tolerance limits in both widespread and tropical species [65]. This suggests that warming temperatures, in the context of global climate change, will have a strong effect on community composition through direct effect on fitness.

Our data also revealed a significant decrease in parasitism rates with warming. Reviews suggest that parasitism rates would decrease under global warming scenarios due to an increase in parasitoid mortality, and host-parasitoid spatial and temporal asynchrony [14,44]. However, the presence of parasitoids significantly decreased abundances of the three host species independently of the temperature regime, suggesting that warming treatments did not decrease attack rate, but decreased successful parasitism rate [66,67]. The decrease in parasitism rates at higher temperatures could also result from improved host immune response, decreasing the vulnerability of hosts to parasitoid attacks [68]. Therefore, host immune function responses to temperature should be considered alongside host thermal performance and tolerance to predict the effects of increasing temperatures on host communities [14]. This experiment was performed over a single generation, so long-term consequences of decreased parasitism rates with elevated temperatures for host-parasitoid dynamic cannot be assessed, but a decrease in parasitism rates could lead to the release of hosts from top-down control. However, in the case of a simple linear tritrophic interaction, the results of Flores-Mejia *et al.* [69] suggest that parasitoid top-down control might be less sensitive to temperature than previously thought. Nevertheless, with warming temperatures, stronger host and parasitoid genotype congruence has been observed, which could decrease parasitoid diet breadth and thus decrease parasitism rates [70]. In our experiment, the role of parasitoids in lowering insect abundance was not reduced under experimental warming. However, parasitism rates were reduced, suggesting that an indirect effect of warming temperatures on the structure of the host community, mediated by parasitoids, might emerge over multiple generations.

Our results demonstrate that differences in thermal performance across host species may be a stronger determinant of how host communities respond to warming temperatures than shifts in the strength of biotic interactions in arthropod host communities. We used high, but realistic levels of competition and parasitism that would have allowed us to detect their effects on host species relative abundances if there were any. We did not find an interactive effect of parasitism and competition treatments on host abundances and frequencies. This result is in line with results from another laboratory experiment performed on the same system [71] showing that parasitism did not significantly affect host competitive coefficients. Furthermore, the type of competition between hosts did not significantly affect total host abundance, suggesting that the amount of food included was only able to support a certain number of hosts that did not vary with the type of competition. Aspects of our results contrast with those from a field transplant experiment on two species drawn from the same Australian *Drosophila*-parasitoid community [72]. Investigating fitness of *D. birchii* and *D. bunnanda* along an elevation gradient, the authors found an interacting effect between the abiotic environment and interspecific competition. However, the field experiment excluded parasitoids, and the elevational gradient studied is likely to include variations such as humidity as well as temperature, which might influence the outcome [73]. Our results also contrast with the conclusions from a systematic review on the mechanisms underpinning natural populations response to climate [47]. They found greater support for indirect effects of climate on populations through altered species interactions than direct effects. However, this review included drought in addition to temperature in the climatic variables, and the relative importance of biotic and abiotic mechanisms varied with trophic level. Moreover, the authors brought out a bias in the published studies toward temperate ecosystems and mammals, highlighting the need for more studies investigating the mechanisms driving tropical arthropod community responses to global climate changes.

Our study serves as example of the mechanisms that can be expected to drive community responses to global warming, but general conclusions on the potential impact of warming temperature on host-parasitoid networks will require replication with different species compositions and different systems. Especially, most host-parasitoid systems are tri-trophic (plants-arthropods-parasitoids), and climate warming is likely to impact host-parasitoid networks through bottom-up effects [74]. Few such experiments have been undertaken, despite the need to better disentangle direct and indirect effects of warming temperature on species communities. Ideally, future studies will also need to investigate the longer-term dynamics of such systems. Moreover, as temperatures continue to increase, species from diverse taxa are shifting their distribution worldwide to higher latitudes and elevations [75], changing their biotic environment with novel species interactions and different community assemblages [76]. Dispersal was not permitted in this study, but is likely to mediate some of the effects of warming temperature on species and their interactions [30,77]. Understanding the mechanisms driving community responses to warming scenarios is particularly important for tropical communities, which face more severe impacts of climate warming than temperate communities [8], and contain most threatened species of global concern [78]. Here, we demonstrate that warming had a direct effect on our focal tropical *Drosophila* host community through differences in thermal performance, without affecting the relative strength of parasitism and competition.

Acknowledgements

We thank Inga Freiberga, Anna Jandová, Martin Libra and Joel Brown for their help during the collection of the data, Petr Šmilauer for his advices on the statistical analysis, and Jinlin Chen and J. Chris D. Terry for useful comments on the results. The drawings used for Fig 1 was made by Tereza Holicová.

Author contributions

Conceptualization: Mélanie Thierry, Owen T. Lewis, Jan Hrček.
Data curation: Mélanie Thierry.
Formal analysis: Mélanie Thierry.
Funding acquisition: Mélanie Thierry, Owen T. Lewis, Jan Hrček.
Investigation: Mélanie Thierry, Nicholas A. Pardikes, Chia-Hua Lue.
Methodology: Mélanie Thierry.
Resources: Jan Hrček.
Visualization: Mélanie Thierry.
Writing – original draft: Mélanie Thierry.
Writing – review & editing: Mélanie Thierry, Nicholas A. Pardikes, Chia-Hua Lue, Owen T. Lewis, Jan Hrček.

Editor: Lucas D. B. Faria, Universidade Federal de Lavras, BRAZIL

Received: December 19, 2020
Accepted: January 28, 2021
Published: February 11, 2021

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pone.0245029

Copyright: © 2021 Thierry et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All raw data used for this study are available from the Zenodo database: https://doi.org/10.5281/zenodo.4362189

Funding: We acknowledge funding support of JH from the Grantova Agentura České Republiky no. 17-27184Y. MT was further supported by Jihočeska Univerzita v Českych Budějovice (GAJU no. 038/2019/P), and OTL by the UK Natural Environment Research Council (NE/N010221/1).

Competing interests: The authors have declared that no competing interests exist.

References

1. Sage RF. Global change biology: A primer. Glob Chang Biol. 2020;26: 3–30. doi:10.1111/gcb.14893
2. Wagner DL, Grames EM, Forister ML, Berenbaum C MR, Stopak D. Insect decline in the Anthropocene: Death by a thousand cuts. Proc Natl Acad Sci. 2021;118. doi:10.1073/pnas.2023989118

3. Román-Palacios C, Wiens JJ. Recent responses to climate change reveal the drivers of species extinction and survival. Proc Natl Acad Sci U S A. 2020;117: 4211–4217. doi:10.1073/pnas.1913007117
4. Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, et al. Ecological responses to recent climate change. Nature. 2002;416: 389–395. doi:10.1038/416389a
5. Ohlberger J. Climate warming and ectotherm body size - from individual physiology to community ecology. Funct Ecol. 2013;27: 991–1001. doi:10.1111/1365-2435.12098
6. Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL. Effects of size and temperature on metabolic rate. Science (80-). 2001;293: 2248–2251. doi:10.1126/science.1061967
7. Benard MF. Warmer winters reduce frog fecundity and shift breeding phenology, which consequently alters larval development and metamorphic timing. Glob Chang Biol. 2015;21: 1058–1065. doi:10.1111/gcb.12720
8. Tewksbury JJ, Huey RB, Deutsch CA. Ecology: Putting the heat on tropical animals. Science (80-). 2008;320: 1296–1297. doi:10.1126/science.1159328
9. García-Robledo C, Kuprewicz EK, Staines CL, Erwin TL, Kress WJ. Limited tolerance by insects to high temperatures across tropical elevational gradients and the implications of global warming for extinction. Proc Natl Acad Sci U S A. 2016;113: 680–685. doi:10.1073/pnas.1507681113
10. Kéfi S, Berlow EL, Wieters EA, Joppa LN, Wood SA, Brose U, et al. Network structure beyond food webs: Mapping non-trophic and trophic interactions on Chilean rocky shores. Ecology. 2015;96: 291–303. doi:10.1890/13-1424.1
11. Godoy O, Bartomeus I, Rohr RP, Saavedra S. Towards the integration of niche and network theories. Trends Ecol Evol. 2018;33: 287–300. doi:10.1016/j.tree.2018.01.007
12. Woodman RL, Price PW. Differential larval predation by ants can influence willow sawfly community structure. Ecology. 1992;73: 1028–1037. doi:10.2307/1940177
13. Palkovacs EP, Post DM. Experimental evidence that phenotypic divergence in predators drives community divergence in prey. Ecology. 2009;90: 300–305. doi:10.1890/08-1673.1
14. Thierry M, Hrček J, Lewis OT. Mechanisms structuring host–parasitoid networks in a global warming context: a review. Ecol Entomol. 2019; een.12750. doi:10.1111/een.12750
15. Kéfi S, Berlow EL, Wieters EA, Navarrete SA, Petchey OL, Wood SA, et al. More than a meal… integrating non-feeding interactions into food webs. Ecol Lett. 2012;15: 291–300. doi:10.1111/j.1461-0248.2011.01732.x

16. Melián CJ, Bascompte J, Jordano P, Krivan V. Diversity in a complex ecological network with two interaction types. Oikos. 2009;118: 122–130. doi:10.1111/j.1600-0706.2008.16751.x
17. Paine RT. Food web complexity and species diversity. Am Nat. 1966;100: 65–75. doi:10.1086/282400
18. Gurevitch J, Morrison JA, Hedges L V. The interaction between competition and predation: a meta-analysis of field experiments. Am Nat. 2000;155: 435–453. doi:10.1086/303337
19. Chase JM, Abrams PA, P. GJ, Sebastian D, Peter C, D. HR, et al. The interaction between predation and competition: a review and synthesis. Ecol Lett. 2002;5: 302–315. doi:10.1046/j.1461-0248.2002.00315.x
20. Pilosof S, Porter MA, Pascual M, Kéfi S. The multilayer nature of ecological networks. Nat Ecol Evol. 2017;1: 101. doi:10.1038/s41559-017-0101
21. Veselý L, Boukal DS, Buřič M, Kuklina I, Fořt M, Yazicioglu B, et al. Temperature and prey density jointly influence trophic and non-trophic interactions in multiple predator communities. Freshw Biol. 2019; fwb.13387. doi:10.1111/fwb.13387
22. Sentis A, Hemptinne JL, Brodeur J. Effects of simulated heat waves on an experimental plant-herbivore-predator food chain. Glob Chang Biol. 2013;19: 833–842. doi:10.1111/gcb.12094
23. O'Connor MI, Gilbert B, Brown CJ. Theoretical predictions for how temperature affects the dynamics of interacting herbivores and plants. Am Nat. 2011;178: 626–638. doi:10.1086/662171
24. Gilbert B, Tunney TD, Mccann KS, Delong JP, Vasseur DA, Savage V, et al. A bioenergetic framework for the temperature dependence of trophic interactions. Wootton T, editor. Ecol Lett. 2014;17: 902–914. doi:10.1111/ele.12307
25. Gårdmark A, Huss M. Individual variation and interactions explain food web responses to global warming. Philos Trans R Soc B Biol Sci. 2020;375: 20190449. doi:10.1098/rstb.2019.0449
26. Tougeron K, Brodeur J, Le Lann C, Baaren J. How climate change affects the seasonal ecology of insect parasitoids. Ecol Entomol. 2019; een.12792. doi:10.1111/een.12792
27. Buse A, Dury SJ, Woodburn RJW, Perrins CM, Good JEG. Effects of elevated temperature on multi-species interactions: The case of Pedunculate Oak, Winter Moth and Tits. Funct Ecol. 1999;13: 74–82. doi:10.1046/j.1365-2435.1999.00010.x
28. Bideault A, Loreau M, Gravel D. Temperature modifies consumer-resource interaction strength through its effects on biological rates and body mass. Front Ecol Evol. 2019;7: 45. doi:10.3389/fevo.2019.00045
29. Gilman SE, Urban MC, Tewksbury J, Gilchrist GW, Holt RD. A framework for community interactions under climate change. Trends Ecol Evol. 2010;25: 325–331. doi:10.1016/j.tree.2010.03.002

30. Davis AJ, Jenkinson LS, Lawton JH, Shorrocks B, Wood S. Making mistakes when predicting shifts in species range in response to global warming. Nature. 1998;391: 783–786. doi:10.1038/35842

31. Tylianakis JM, Didham RK, Bascompte J, Wardle DA. Global change and species interactions in terrestrial ecosystems. Ecol Lett. 2008;11: 1351–1363. doi:10.1111/j.1461-0248.2008.01250.x

32. Berg MP, Toby Kiers E, Driessen G, van der Heijden M, Kooi BW, Kuenen F, et al. Adapt or disperse: Understanding species persistence in a changing world. Glob Chang Biol. 2010;16: 587–598. doi:10.1111/j.1365-2486.2009.02014.x

33. Sentis A, Gémard C, Jaugeon B, Boukal DS. Predator diversity and environmental change modify the strengths of trophic and nontrophic interactions. Glob Chang Biol. 2017;23: 2629–2640. doi:10.1111/gcb.13560

34. Dyer LA, Richards LA, Short SA, Dodson CD. Effects of CO2 and temperature on tritrophic interactions. PLoS One. 2013;8: e62528. doi:10.1371/journal.pone.0062528

35. Dancau T, Stemberger TLM, Clarke P, Gillespie DR. Can competition be superior to parasitism for biological control? The case of spotted wing *Drosophila* (*Drosophila suzukii*), *Drosophila melanogaster* and *Pachycrepoideus vindemmiae*. Biocontrol Sci Technol. 2017;27: 3–16. doi:10.1080/09583157.2016.1241982

36. Bartley TJ, McCann KS, Bieg C, Cazelles K, Granados M, Guzzo MM, et al. Food web rewiring in a changing world. Nat Ecol Evol. 2019; 1. doi:10.1038/s41559-018-0772-3

37. Seibold S, Cadotte MW, MacIvor JS, Thorn S, Müller J. The necessity of multitrophic approaches in community ecology. Trends Ecol Evol. 2018;33: 754–764. doi:10.1016/j.tree.2018.07.001

38. Godfray HCJ. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press; 1994.

39. Hance T, Baaren J van, Vernon P, Boivin G. Impact of extreme temperatures on parasitoids in a climate change perspective. Annu Rev Entomol. 2007;52: 107–126. doi:10.1146/annurev.ento.52.110405.091333

40. Le Lann C, Visser B, Mériaux M, Moiroux J, Baaren J van, Alphen JJM van, et al. Rising temperature reduces divergence in resource use strategies in coexisting parasitoid species. Oecologia. 2014;174: 967–977. doi:10.1007/s00442-013-2810-9

41. Fand BB, Tonnang HEZ, Bal SK, Dhawan AK. Shift in the manifestations of insect pests under predicted climatic change scenarios: key challenges and adaptation strategies. Advances in Crop Environment Interaction. Springer Singapore; 2018. pp. 389–404. doi:10.1007/978-981-13-1861-0_15

42. Thomson LJ, Macfadyen S, Hoffmann AA. Predicting the effects of climate change on natural enemies of agricultural pests. Biol Control. 2010;52: 296–306. doi:10.1016/j.biocontrol.2009.01.022

43. Stireman JO, Dyer LA, Janzen DH, Singer MS, Lill JT, Marquis RJ, et al. Climatic unpredictability and parasitism of caterpillars: implications of global warming. Proc Natl Acad Sci U S A. 2005;102: 17384–17387. doi:10.1073/pnas.0508839102

44. Jeffs CT, Lewis OT. Effects of climate warming on host–parasitoid interactions. Ecol Entomol. 2013;38: 209–218. doi:10.1111/een.12026

45. Fleury F, Ris N, Allemand R, Fouillet P, Carton Y, Boulétreau M. Ecological and genetic interactions in *Drosophila*-parasitoids communities: a case study with *D. melanogaster*, *D. simulans* and their common *Leptopilina* parasitoids in south-eastern France. In: Capy P, Gibert P, Boussy I, editors. Drosophila melanogaster, Drosophila simulans: So Similar, So Different. Springer Netherlands; 2004. pp. 181–194. Available: http://link.springer.com/chapter/10.1007/978-94-007-0965-2_15

46. Fox JW, Morin PJ. Effects of intra- and interspecific interactions on species responses to environmental change. J Anim Ecol. 2008;70: 80–90. doi:10.1111/j.1365-2656.2001.00478.x

47. Ockendon N, Baker DJ, Carr JA, White EC, Almond REA, Amano T, et al. Mechanisms underpinning climatic impacts on natural populations: Altered species interactions are more important than direct effects. Glob Chang Biol. 2014;20: 2221–2229. doi:10.1111/gcb.12559

48. Jeffs CT, Terry JCD, Higgie M, Jandová A, Konvičková H, Brown JJ, et al. Molecular analyses reveal consistent food web structure with elevation in rainforest *Drosophila* - parasitoid communities. Ecography (Cop). 2020; ecog.05390. doi:10.1111/ecog.05390

49. Kraaijeveld AR, Godfray HCJ. Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. Nature. 1997;389: 278–280. doi:10.1038/38483

50. Overgaard J, Kearney MR, Hoffmann AA. Sensitivity to thermal extremes in Australian *Drosophila* implies similar impacts of climate change on the distribution of widespread and tropical species. Glob Chang Biol. 2014;20: 1738–1750. doi:10.1111/gcb.12521

51. MacLean HJ, Sørensen JG, Kristensen TN, Loeschcke V, Beedholm K, Kellermann V, et al. Evolution and plasticity of thermal performance: An analysis of variation in thermal tolerance and fitness in 22 *Drosophila* species. Philos Trans R Soc B Biol Sci. 2019;374: 20180548. doi:10.1098/rstb.2018.0548

52. Grimaldi D, Jaenike J. Competition in natural populations of mycophagous *Drosophila*. Ecology. 1984;65: 1113–1120. doi:10.2307/1938319

53. Forbes RW, Sang JH. The ecological determinants of population growth in a *Drosophila* culture. I. Fecundity of adult flies. Proc R Soc London Ser B - Biol Sci. 1944;132: 258–277. doi:10.1098/rspb.1944.0017

54. Pachauri RK, Meyer L, Hallegatte France S, Bank W, Hegerl G, Brinkman S, et al. IPCC, 2014: Climate Change 2014: Synthesis Report.

Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change e [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland; 2014. Available: http://www.ipcc.ch.

55. Team RC. R: The R Project for statistical computing. 2017. Available: https://www.r-project.org/

56. Venables WN, Riplley BD. Modern Applied Statistics with S. Fourth Edi. Springer; 2002. doi:ISBN 0-387-95457-0

57. Fox J, Weisberg S. An {R} companion to applied regression. Third Edit. Sage; 2019. Available: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

58. Lüdecke D, Makowski D, Waggoner P. Performance: Assessment of regression models performance. 2019. Available: https://cran.r-project.org/package=performance

59. Hartig F. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. 2019. Available: http://florianhartig.github.io/DHARMa/

60. Lenth R V. Emmeans: Estimated marginal means, aka least-squares means. R Packag version. 2018.

61. Daufresne M, Boët P. Climate change impacts on structure and diversity of fish communities in rivers. Glob Chang Biol. 2007;13: 2467–2478. doi:10.1111/j.1365-2486.2007.01449.x

62. Kardol P, Campany CE, Souza L, Norby RJ, Weltzin JF, Classen AT. Climate change effects on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. Glob Chang Biol. 2010;16: 2676–2687. doi:10.1111/j.1365-2486.2010.02162.x

63. Villalpando SN, Williams RS, Norby RJ. Elevated air temperature alters an old-field insect community in a multifactor climate change experiment. Glob Chang Biol. 2009;15: 930–942. doi:10.1111/j.1365-2486.2008.01721.x

64. MacLean HJ, Overgaard J, Kristensen TN, Lyster C, Hessner L, Olsvig E, et al. Temperature preference across life stages and acclimation temperatures investigated in four species of *Drosophila*. J Therm Biol. 2019;86: 102428. doi:10.1016/j.jtherbio.2019.102428

65. Overgaard J, Kristensen TN, Mitchell KA, Hoffmann AA. Thermal tolerance in widespread and tropical *Drosophila* species: Does phenotypic plasticity increase with latitude? Am Nat. 2011;178. doi:10.1086/661780

66. Boulétreau M, Wajnberg E. Comparative responses of two sympatric parasitoid cynipids to the genetic and epigenetic variations of the larvae of their host, *Drosophila melanogaster*. Entomol Exp Appl. 1986;41: 107–114. doi:10.1111/j.1570-7458.1986.tb00516.x

67. Carton Y, Kitano H. Evolutionary relationships to parasitism by seven species of the *Drosophila melanogaster* subgroup. Biol J Linn Soc. 1981;16: 227–241. doi:10.1111/j.1095-8312.1981.tb01849.x

68. Linder JE, Owers KA, Promislow DEL. The effects of temperature on host–pathogen interactions in *D. melanogaster*: who benefits? J Insect Physiol. 2008;54: 297–308. doi:10.1016/j.jinsphys.2007.10.001
69. Flores-Mejia S, Fournier V, Cloutier C. Performance of a tri-trophic food web under different climate change scenarios. Food Webs. 2017;11: 1–12. doi:10.1016/j.fooweb.2017.05.001
70. Lavandero B, Tylianakis JM. Genotype matching in a parasitoid–host genotypic food web: an approach for measuring effects of environmental change. Mol Ecol. 2013;22: 229–238. doi:10.1111/mec.12100
71. Terry JCD, Chen J, Lewis OT. The effect of natural enemies on the coexistence of competing species - an empirical test using Bayesian modern coexistence theory. bioRxiv. 2020; 2020.08.27.270389. doi:10.1101/2020.08.27.270389
72. O´Brien EK, Higgie M, Jeffs CT, Hoffmann AA, Hrcek J, Lewis OT, et al. Interacting effects of the abiotic and biotic environment on fitness of rainforest Drosophila. bioRxiv. 2018; 395624. doi:10.1101/395624
73. Derocles SAP, Lunt DH, Berthe SCF, Nichols PC, Moss ED, Evans DM. Climate warming alters the structure of farmland tritrophic ecological networks and reduces crop yield. Mol Ecol. 2018;27: 4931–4946. doi:10.1111/mec.14903
74. Han P, Becker C, Sentis A, Rostás M, Desneux N, Lavoir AV. Global change-driven modulation of bottom–up forces and cascading effects on biocontrol services. Curr Opin Insect Sci. 2019;35: 27–33. doi:10.1016/j.cois.2019.05.005
75. Freeman BG, Lee-Yaw JA, Sunday JM, Hargreaves AL. Expanding, shifting and shrinking: The impact of global warming on species' elevational distributions. Glob Ecol Biogeogr. 2018;27: 1268–1276. doi:10.1111/geb.12774
76. Alexander JM, Diez JM, Levine JM. Novel competitors shape species' responses to climate change. Nature. 2015;525: 515–518. doi:10.1038/nature14952
77. Davis AJ, Lawton JH, Shorrocks B, Jenkinson LS. Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. J Anim Ecol. 1998;67: 600–612. doi:10.1046/j.1365-2656.1998.00223.x
78. IUCN. IUCN Red List of Threatened Species. 2012 [cited 21 Jan 2021] pp. 49-6872-49–6872. doi:10.5860/choice.49-6872

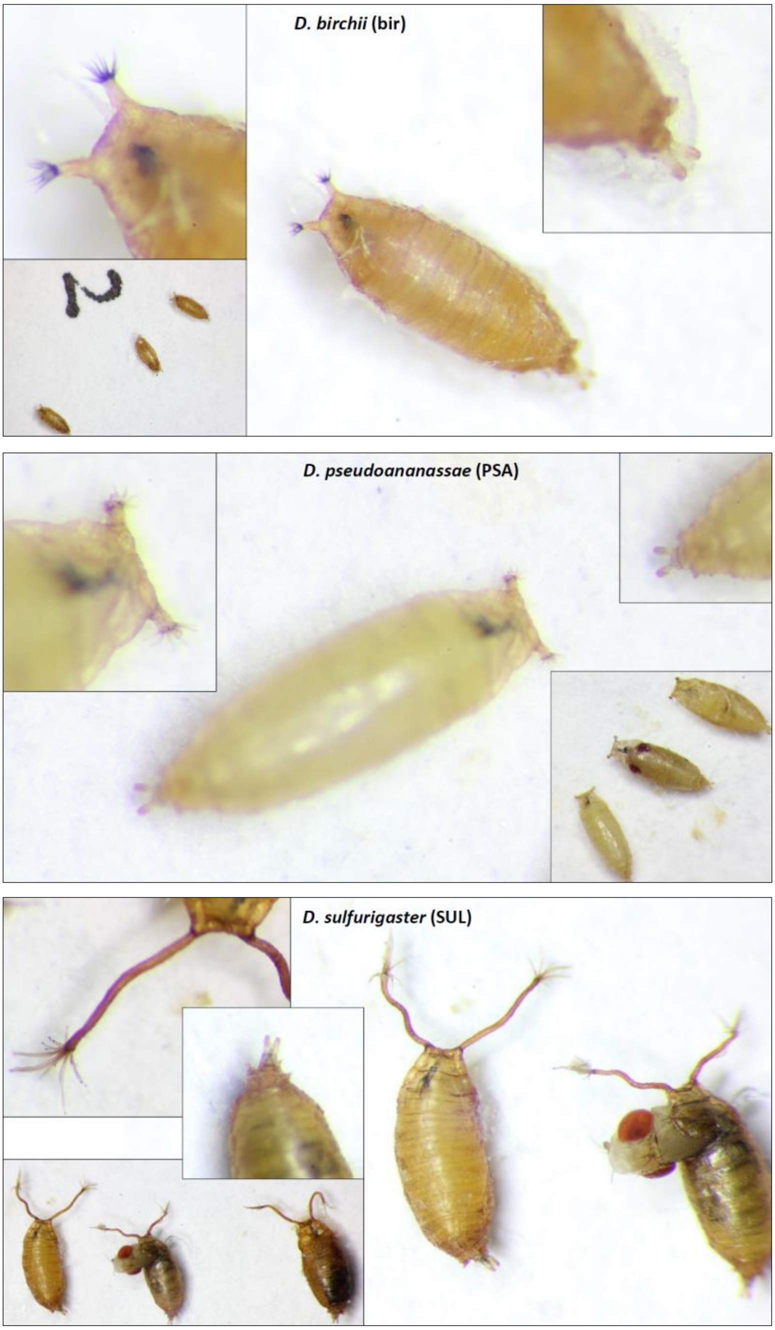
Supplementary Information

Supplementary Figure S1. Transparent plastic boxes (47cm x 30cm x 27.5cm) with three ventilation holes (15 cm in diameter) covered with insect-proof nylon mesh used as experimental unit allowing parasitoids to attack one of the three experimental vials containing 2-days-old host larvae for 72h

Supplementary Table S1. Mean number of offspring per species with 10, 30, 60, 90 or 180 adult hosts (1:1 sex ratio) in a 5 mL host-media glass vial. Choice of host number in the main experiment was based on these preliminary data to correspond to strong competition for all host species.

	Number of adult hosts	**Mean number of offspring ± SEM**
D. birchii	10	38 ± 28
	30	45 ± 9
	60	53 ± 19
	90	53 ± 10
	180	40 ± 17
D. pseudoananassae	10	46 ± 27
	30	90 ± 31
	60	126 ± 38
	90	94 ± 33
	180	107 ± 75
D. sulfurigaster	10	40 ± 35
	30	18 ± 11
	60	18 ± 13
	90	35 ± 27
	180	35 ± 19

Supplementary Figure S2. *Drosophila birchii*, *D. pseudoananassae*, and *D. sulfurigaster* pupae photography for morphological identification. Not in scale (photo credit to Jinlin Chen)



Supplementary Table S2. Number of observations per temperature, treatments (Intraspecific competition: no interaction between host species; Interspecific competition: direct competition between host species; Parasitism: intraspecific competition with parasitism; All interactions: interspecific competition with parasitism), and host species in the whole dataset, and with the reduced dataset used for analyses (excluding observations with fewer than 10 emerging insects or pupae).

	Whole dataset	**Reduced dataset**
23°C	48	41
27°C	48	42
Intraspecific competition	24	21
Interspecific competition	24	20
Parasitism	24	21
All interactions	24	21
D. birchii	32	32
D. pseudoananassae	32	32
D. sulfurigaster	32	19

Supplementary Table S3. Table showing the effect of temperature (23°C or 27°C), parasitism (presence or absence), competition between host species (intraspecific or interspecific), host species (n = 3), interactions between terms, and block (n = 4), on host abundances, and host frequencies for the whole dataset (without any deleted observations due to *D. sulfurigaster*). Degrees or freedom (Df) for each F-ratio are given for each factor and for the error. F values are presented with the significance of the effect: (***) P < 0.001, (**) P < 0.01, (*) P < 0.05, (ns) P > 0.05.

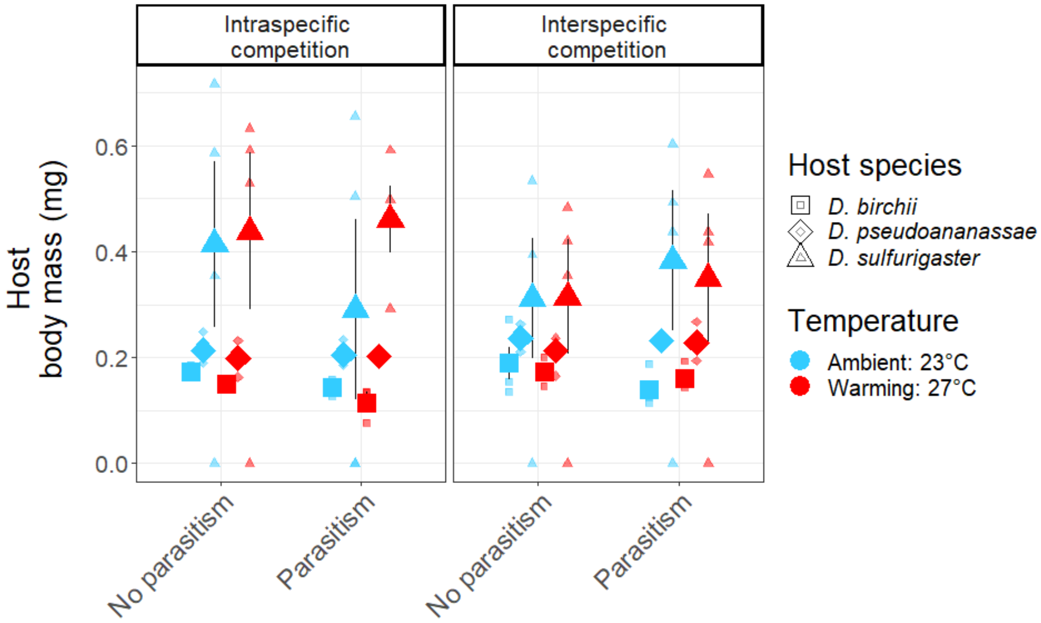
	Df	**Host abundance**		**Host frequency**		**Parasitism rate**	
Temperature	1	0.63	(ns)	0.21	(ns)	5.42	*
Parasitism	1	8.50	**	0.00	(ns)	-	-
Competition	1	0.11	(ns)	0.00	(ns)	1.25	(ns)
Host species	2	27.22	***	97.81	***	2.75	(ns)
Parasitoid species	1	-	-	-	-	2.68	(ns)
Temperature x Parasitism	1	0.53	(ns)	0.00	(ns)	-	-
Temperature x Competition	1	0.10	(ns)	0.00	(ns)	0.04	(ns)
Temperature x Host species	2	3.01	(ns)	20.05	***	-	-
Parasitism x competition	1	1.61	(ns)	0.00	(ns)	-	-
Host x parasitoid species	2	-	-	-	-	22.42	***
Block	3	1.02	(ns)	0.00	(ns)	1.66	(ns)
Df error		82		82		76	
R^2		0.74		0.05		0.10	

Supplementary Table S4. Summary table for mean (± SD) host abundances (Host ab.), individual host body mass (Host BM), total parasitism rate (PR), and parasitism rates of each parasitoid species (*Asobara* sp. and *Leptopilina* sp.) for each temperature (23 and 27°C), treatments (competition: intra or inter, parasitism: present or absent), and host species (*D. birchii*, *D. pseudoananassae*, *D. sulfurigaster*).

Temp	**Comp**	**Para**	**Host sp.**	**Host ab.**	**± SD**	**Host BM**	**± SD**	**PR**	**± SD**	**PR of Asb.**	**± SD**	**PR of Lept.**	**± SD**
23°C	**intra**	**no**	***D. birchii***	114	28	0.174	0.012	-	-	-	-	-	-
			D. pseud.	106	52	0.214	0.025	-	-	-	-	-	-
			D. sulfu.	32	4	0.651	0.092	-	-	-	-	-	-
		yes	***D. birchii***	57	70	0.143	0.022	0.30	0.30	0.29	0.29	0.01	0.01
			D. pseud.	70	69	0.205	0.021	0.30	0.18	0.05	0.09	0.26	0.18
			D. sulfu.	20	9	0.579	0.106	0	0	0	0	0	0
	inter	**no**	***D. birchii***	109	49	0.190	0.061	-	-	-	-	-	-
			D. pseud.	119	68	0.237	0.023	-	-	-	-	-	-
			D. sulfu.	46	16	0.416	0.108	-	-	-	-	-	-
		yes	***D. birchii***	45	14	0.139	0.034	0.31	0.14	0.17	0.12	0.14	0.12
			D. pseud.	65	64	0.232	0.010	0.26	0.19	0.04	0.04	0.22	0.17
			D. sulfu.	11	6	0.465	0.039	0.28	0.24	0.28	0.24	0.00	0.00
27°C	**intra**	**no**	***D. birchii***	69	20	0.151	0.004	-	-	-	-	-	-
			D. pseud.	184	36	0.199	0.030	-	-	-	-	-	-
			D. sulfu.	37	28	0.612	0.029	-	-	-	-	-	-
		yes	***D. birchii***	26	25	0.115	0.034	0.08	0.08	0.06	0.09	0.02	0.04
			D. pseud.	170	13	0.202	0.014	0.11	0.09	0.00	0.01	0.10	0.09

		D. sulfu.	31	31	0.517	0.067	0.28	0.36	0.28	0.36	0.00	0.00
inter	**no**	***D. birchii***	74	25	0.173	0.023	-	-	-	-	-	-
		D. pseud.	261	48	0.213	0.032	-	-	-	-	-	-
		D. sulfu.	37	2	0.388	0.046	-	-	-	-	-	-
	yes	***D. birchii***	27	11	0.161	0.022	0.25	0.14	0.16	0.10	0.08	0.06
		D. pseud.	130	33	0.229	0.031	0.16	0.10	0.02	0.02	0.14	0.10
		D. sulfu.	21	11	0.482	0.091	0.16	0.16	0.16	0.16	0.00	0.00

Supplementary Figure S3. Interactive effect of competition with host species, and with presence of parasitoids on mean host body mass. See Figure 1 for detailed description of the treatments. The small points represent the values from each block and each host-parasitoid pair, the large points represent the grand mean, and the bars represent standard errors of the means. Blue: ambient temperature (23°C), red: warming treatment (27°C)



Chapter IV

Warming decreases host survival with multiple parasitoids, but parasitoid performance also decreases

(manuscript)

Warming decreases host survival with multiple parasitoids, but parasitoid performance also decreases

Mélanie Thierry[*1,2], Nicholas A. Pardikes[2], Benjamin Rosenbaum[3,4], Miguel G. Ximénez-Embún[2], & Jan Hrček[1, 2]

[1]Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, [2]Department of Ecology, Institute of Entomology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic, [3]Theory in Biodiversity Science, German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Puschstr. 4, 04103 Leipzig, Germany, [4]Institute of Biodiversity, Friedrich Schiller University Jena, Dornburger Str. 159, 07743 Jena, Germany

[*] m.thierry@entu.cas.cz

Abstract. Community context and abiotic environments can impact the strength of species interactions, which in turn affects community dynamics. Therefore, we need to recognize the combined effects of these two factors on interaction strength in the face of current global environmental changes. Specifically, predator-prey interaction strength often depends on the presence of other natural enemies: it weakens with competition and interference or strengthens with facilitation. But effects of multiple predators on prey are likely to be altered by changes in the abiotic environment, leading to modified community dynamics and ecosystem functioning. Here, we investigate how warming alters the effects of multiple predators on prey suppression using a dynamic model, coupled with empirical laboratory experiments on a host-parasitoid community. We found that the multiple parasitoid effect on hosts was the average of the individual parasitoid effects at ambient temperature, but host suppression was higher than expected under warming. Our results were observed across different parasitoid assemblages, suggesting a general pattern for the temperature-dependence of species interaction strength. Our study highlights the importance of temperature as a modifying factor for the effect of multiple predators on prey suppression. Accounting for interactive effects between abiotic and biotic factors is imperative to better predict community dynamics in a rapidly changing world, and better preserve ecosystem functioning.

Keywords. Biodiversity-ecosystem functioning, global change, temperature, functional response, host-parasitoid networks, multiple predator effects

Introduction

The outcome of pairwise trophic interactions are influenced by other predator and prey species in the community (Wootton 1997, Kéfi et al. 2015). Yet, we still lack a clear understanding about how trophic interactions are influenced by co-occurring species within the community. It is crucial to address the effects of co-occurring species on interaction strength under climate changes since they disrupt species composition of communities (Parmesan 2006, Thierry et al. 2021). Moreover, effects of climate changes on ecological communities are mediated through effects on biotic interactions (Post 2013), yet little is known about the direct effects of warming on intra- and interspecific competitive interactions, and their cascading effects on other trophic levels.

The abiotic context is key for the outcome of species interactions (Davis et al. 1998, Song et al. 2020). Global warming can weaken strength of trophic interactions due to changes in metabolic rates (Rall et al. 2010), shifts in distributions and in phenology (Parmesan 2006), lethal effects on predators, or altered attack rates (Hance et al. 2007, Uszko et al. 2017, Thierry et al. 2019). But warming could also alter non-trophic interaction strength among predators. For instance, warming can induce changes in predator habitat use through phenotypic plasticity to adjust to the changing environment (Barton and Schmitz 2009, Schmitz and Barton 2014), which could lead to habitat overlap among predator species that were not previously interacting. Warming could also change predator foraging behavior, changing predator efficiency due to changes in encounter and attack rates (Uszko et al. 2017). Altered non-trophic interactions among predators would change the effects of multiple predators for top-down control (Sentis et al. 2017, Cuthbert et al. 2021).

The effects of multiple predators on prey suppression are often assumed to be additive, which would be true if predators have independent effects on prey (Sih et al. 1998, Schmitz 2007). However, direct and indirect interactions among predator species may cause effects to deviate from additivity. For example, the effects of multiple predators on prey can be synergistic due to niche complementarity or facilitation (i.e., risk enhancement), or antagonistic due to intraguild predation, competition, or interference (i.e., risk reduction). All such potential effects are referred to as multiple predator effects (MPEs; Soluk 1993). A meta-analysis from Griffin et al. (2013) revealed an overall positive effect of predator diversity on top-down control, suggesting a general pattern of niche complementarity (Northfield et al. 2010). Niche complementarity can be achieved when predators have complementary phenologies or habitat domains (Schmitz 2009). However, efficiency of a single species versus diverse predator assemblages on prey suppression remains unclear (May and Hassell 1988) because it depends on potential for niche differentiation between predators, and the overall efficiency of each predator for the focal prey species (Pedersen and Mills 2004). Emergent MPEs are particularly important in biological control where introduction of one or several predator species might result in contrasting effects from the main aim, i.e. risk reduction instead of risk enhancement (Tylianakis and Romo 2010).

Here, we use mathematical models following the Mccoy et al. (2012) framework in combination with a series of three laboratory experiments on *Drosophila simulans* and three of its co-occurring larval parasitoids to investigate the effects of temperature and predator community composition on top-down control. Host-parasitoid interactions are a particular type of predator-prey interaction in which parasitoid larvae develop inside or on an arthropod host from which it feeds on, while adults are free living (Godfray 2013). When parasitized, three outcomes are possible: the parasitoid successfully develops, the host successfully eliminates its parasitoid through immune response (i.e., encapsulation and melanization) and survival (Carton et al. 2008), or both parties die. When multiple parasitoids are present, they can compete extrinsically as adults for space and oviposition (i.e., interference), and intrinsically within a host (reviewed in Harvey et al. 2013). Intrinsic competition is the result of a super- and/or multiparasitism event when two parasitoids - conspecific or heterospecific respectively - parasitize the same host individual. In solitary parasitoids, such as the species used in the present study, only one individual completes its development in each host, suppressing the other(s) physically or physiologically. Parasitoids represent an excellent system to study how warming directly affects non-trophic intra- and interspecific interaction strength among predators because we can more easily look further at the mechanisms behind MPEs (i.e., host immune response, type of interactions among predators). In this study, we empirically measured trophic interaction strength across temperatures and parasitoid assemblages. We deducted emergent effects of multiple parasitoids by comparing empirical data with estimates in which multiple parasitoids would not interact (i.e., have additive effect on host suppression) using a mathematical model for multiple co-occurring parasitoids with a functional response approach (Mccoy et al. 2012, Sentis and Boukal 2018). With this framework, we addressed three specific questions: (1) Do multiple parasitoids have additive, synergetic, or antagonistic effects on host suppression? (2) To what extent does temperature modify the outcomes of MPEs? (3) Are changes in host immune response or shifts in parasitism rates causing emergent MPEs? Our results demonstrate the prevalent role of temperature for non-trophic interactions among parasitoids, with cascading effects on host suppression.

Materials and Methods

Biological system

Cultures of *Drosophila simulans* and their associated parasitoids collected from two tropical rainforest locations in North Queensland Australia: Paluma (S18° 59.031' E146° 14.096') and Kirrama Range (S18° 12.134' E145° 53.102'; both <100 m above sea level; Jeffs et al., 2021) were used for the experiments. *D. simulans* and parasitoid cultures were established between 2017 and 2018, identified using both morphology and DNA barcoding, and shipped to the Czech Republic under permit no. PWS2016-AU-002018 from Australian Government, Department of the Environment. All cultures were maintained at 23°C on a 12:12 hour light and dark cycle at Biology Centre, Czech Academy of Sciences. The three larval parasitoid species *Asobara sp*. (Braconidae: Alysiinae; strain KHB, reference voucher no. USNMENT01557097, reference sequence BOLD process ID: DROP043-21), *Leptopilina sp*. (Figitidae: Eucolinae; strain 111F, reference voucher no. USNMENT01557117, reference sequence BOLD process ID: DROP053-21), and *Ganaspis sp*. (Figitidae: Eucolinae; strain 84BC, reference voucher no. USNMENT01557102 and USNMENT01557297, reference sequence BOLD process ID: DROP164-21) were used (for more details on the parasitoid strains see Lue et al. 2021). *Drosophila simulans* isofemale lines were kept on standard *Drosophila* medium (corn flour, yeast, sugar, agar and methyl-4-hydroxybenzoate) for approximately 45 to 70 non-overlapping generations before the experiments. To revive genetic variation, five host lines were combined to establish two population cages of mass-bred lines prior the start of the experiments. Single parasitoid isofemale lines were used and maintained for approximately 25 to 40 non-overlapping generations prior to the start of the experiment by providing them every week with two-days-old larvae of a different *Drosophila* species – *Drosophila melanogaster*.

Experiments

To investigate the effects of warming on the strengths of trophic and non-trophic interactions, we used a functional response approach following the framework of Mccoy et al. (2012). We first obtained the parameters of each parasitoid functional response at ambient and warmed temperatures with single-parasitoid treatments (Experiment 1). Then, we used these functional response parameter estimates to predict trophic interaction strength for each temperature and parasitoid combination with the null hypothesis that parasitoids were not interacting, and thus had additive effects on host suppression. In Experiment 2 we empirically measured the effects of temperature and parasitoid combinations on trophic interaction strength and compared the predicted and observed values to deduct emergent effects of multiple parasitoids and their dependence on the temperature regime. The first blocks of Experiment 1 and entire Experiment 2 were performed in parallel, and controls and single-parasitoid treatments were common to both experiments. In Experiment 3, we investigated the mechanisms of multiple parasitoid effects (e.g., extrinsic competition, intrinsic competition, facilitation, or complementarity) by dissecting hosts rather than rearing them. This allowed us to measure rates of super- or multiparasitism, and melanization depending on the temperature regime and parasitoid combinations.

A total of 22,920 *D. simulans* eggs were collected: 13,120 for experiment 1, 4,800 for experiment 2 [of which 12,990 (73%) successfully emerged as adults (8,409 hosts and 4,581 parasitoids)], and 5,000 for experiment 3 from which 1,000 larvae were dissected.

Experiment 1: Single-parasitoid experiment

Eggs of *D. simulans* were placed in a single glass vial with 10mL of *Drosophila* media at six different densities (5, 10, 15, 25, 50 or 100 eggs per 10mL of food media in vial; Figure 1a). To collect *D. simulans* eggs, an egg-washing protocol was adapted from Nouhaud et al. (2018). The day before the egg-washing protocol was conducted, two egg-laying mediums (petri dishes with agar gel topped with yeast paste) were introduced in each population cage for flies to laying eggs overnight. Eggs were transferred in the experimental vials. Half of the vials were placed at ambient temperature (22.7°C ± 0.4), and the other half at warmed temperature (27.4°C ± 0.5).

After 48 hours, one single naïve mated three to five-days-old female parasitoid was placed in each vial with *D. simulans* larvae. Twenty-four hours later, parasitoids were removed. This was repeated for all three parasitoid species, temperatures, and host densities. Controls without parasitoids were run at the same time to obtain the baseline for host survival without parasitism. Vials were checked daily for emerges until the last emergence (up to 41 days for the species with the longest developmental time). We waited five consecutive days without any emerges to stop collecting, thus avoiding collecting the second generation. All emerges were collected, identified, sexed, and stored in 95% ethanol. Each treatment was replicated eight times across eight experimental blocks.

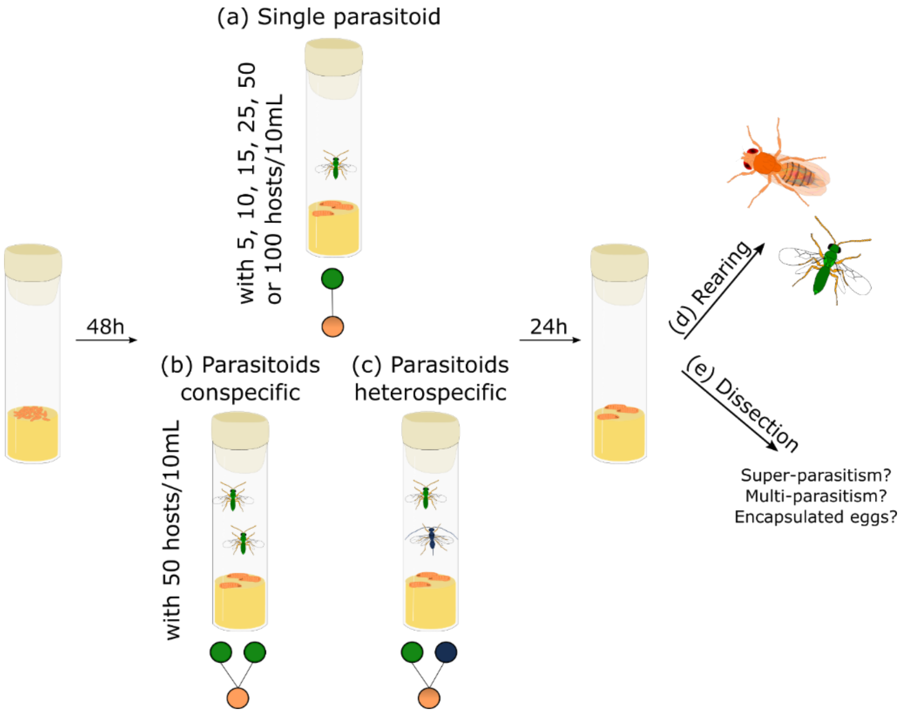


Figure 1. Schematic representation of the experimental design. (a) One single parasitoid female with either 5, 10, 25, 50 or 100 *D. simulans* per 10 mL of media, (b) two parasitoids conspecific or (c) two parasitoids heterospecific with 50 *D. simulans* per 10 mL of media. (d) Rearing until adults emerge for Experiments 1 and 2 (up to 41 days), or (e) dissection of 10 3rd instar larvae or pupae per vial 2, 3 or 4 days after infection for Experiment 3.

Experiment 2: Multiple parasitoids experiment

To investigate the effect of warming on MPEs, we manipulated parasitoid assemblages and temperature in a fully factorial design (Figure 1b and c). We followed the same protocol described above for Experiment 1, using 50 *D. simulans* eggs per vial with two female parasitoids either from the same (Figure 1b) or different species (Figure 1c). Each treatment was replicated eight times across two blocks.

Experiment 3: MPEs mechanisms

In a follow up experiment, we conducted a subset of the treatments described for Experiments 1 and 2 with *Asobara sp*. and *Ganaspis sp*. We used 50 *D. simulans* eggs per 10 mL of food media in vial (one parasitoid, two parasitoids conspecific and the two parasitoids heterospecific, resulting in five different parasitoid assemblages) under ambient and warming temperatures. Instead of rearing the insects to adults, we dissected 10 3rd instar larvae or pupae per vial (Figure 1e). Each host larva was individually transferred into a glass petri dish containing PBS and dissected under stereomicroscope. We recorded the number of parasitoid larvae and eggs of each species to assess super- and multiparasitism events, and, when possible, the number and identity of melanized eggs. Pictures of the eggs, larvae, and melanized eggs for each species observed during the experiment are presented in Supplemental Material S1. Each treatment was replicated ten times across two blocks. At 27°C, six replicates were dissected two days after infection and four three days after infection, and at 23°C, four replicates were dissected three days after infection and six four days after infection. Different times for dissection were chosen for each temperature to standardize parasitoid developmental stage and allow one parasitoid to win against its competitors in case of super- or multiparasitism events, while still being able to identify all the parasitoids that have parasitized the host. At the time of the dissection, multiple parasitoid larvae within a same host individual were sometimes still alive.

Data analysis and modelling

Experiment 1: Single-parasitoid experiment

We combined numerical simulations of host density dynamics accounting for host depletion (Rosenbaum & Rall, 2018):

$$\frac{dH}{dt} = -F(H)P,$$

with Bayesian parameter estimation using the *rstan* package (e.g. Sohlström et al. 2021). $P = 1$ is the parasitoid density, and $F(H)$ denotes the host density-dependent functional response. In the model fitting, MCMC was used to sample from the functional response's model parameters' posterior probability distribution $p(\theta|H_{\text{att}})$ given the observations H_{att}, based on the likelihood function $p(H_{att}|\theta)$ and prior distributions $p(\theta)$. H_{att} is the number of *D. simulans* attacked (the difference between adult hosts emerging from the controls without parasitoids and from the experiment). In each iteration, numerical solutions of the equation were computed with the built-in *Runge-Kutta* ODE solver, to predict densities $\widehat{H}_1$ after 1 day for each given initial host density, H_0. The likelihood was evaluated assuming a binomial distribution for observed numbers of attacked hosts H_{att} with $n = H_0$ trials and $p = \frac{H_0 - \widehat{H}_1}{H_0}$ success probability. Vague priors were used for all model parameters.

We fitted three different functional response models (Type II, Type III and generalized Type III), and retained the Type II functional response (Holling 1959) after model comparison (see Supplement Material S2). The equation for the instantaneous attack rate of a parasitoid is as follows:

$$F(H) = \frac{aH}{1 + ahH}$$

where a is the attack rate, and h is the handling time. Type II functional responses are thought to characterize the attack rate of many types of predators and parasitoids (Fernández-Arhex and Corley 2003). Parameter estimates and the functional responses for each species at each temperature are presented in Supplement Material S2 (Table S1 and Figure S2).

Experiment 2: Multiple parasitoids experiment

Host-parasitoid interaction strength was defined with the combination of Degree of Infestation (DI; i.e., host suppression) and Successful Parasitism rate (SP; i.e., parasitoid performance). Observed degree of infestation (DI_{obs}) and Successful parasitism rate (SP) were measured as:

$$DI_{obs} = 1 - \frac{H}{H_C} \; ; \; SP = \frac{P}{H_C - H}$$

where H is the number of adult hosts emerging from the experiment vial, H_C the mean number of adult hosts emerging from the controls without parasitoids, and P the number of parasitoid adults emerging from the experimental vial (Carton and Kitano 1981, Boulétreau and Wajnberg 1986). DI_{obs} was set to zero if the number of hosts emerging from the treatment was greater than the controls. If no parasitoid emerged or if the number of hosts attacked was estimated to be zero, SP was set to zero. If the number of parasitoids that emerged was greater than to the estimated number of hosts attacked, *SP* was set to one. For treatments with single parasitoid species, we assumed that each of the two parasitoid individuals were attacking the hosts equally, therefore the number of parasitoid adults emerging was divided by two to calculate individual successful parasitism rate.

Data were analyzed with generalized linear models (GLMs). Model assumptions were verified with the *DHARMa* package (Hartig 2019). To correct for overdispersion of the residuals and zero inflation, data were modeled using zero-inflation models with a beta binomial error distribution and a logit function using the *glmmTMB* function from the *TMB* package (Lüdecke et al. 2019). Two categories of predictor variables were used in separate models with temperature treatment (two levels: ambient and warming): (i) parasitoid treatment (three levels; single parasitoid, two parasitoid conspecific, and two parasitoids heterospecific), and (ii) parasitoid species assemblage (nine levels). For DI, two-way interactions between temperature and either parasitoid treatment or parasitoid assemblage were always kept in our models for better comparison with predicted DI values (see section below). For SP, these two-way interactions were tested and kept in our models if judged to be significant based on backward model selection using Likelihood-ratio tests. Significance of the effects was tested using Wald type III analysis of deviance with Likelihood-ratio tests. Factor levels were compared using Tukey's HSD *post hoc* comparisons of all means, and the *emmeans* package (Lenth 2018). Results for developmental rate are presented in Supplement Material S4 (Figure S4).

Estimation of multiple parasitoid effects

To predict the degree of infestation if parasitoids have independent effects on host suppression, we used the method develop by Mccoy et al. (2012) which takes into account host depletion. This method uses the functional responses obtained from Experiment 1 in a population-dynamic model to predict how host density changes in time as a function of initial density and parasitoid combination for each temperature. We thus calculated the estimated Degree of Infestation (DI_0) by integrating the aggregate attack rates over the duration of the experiment as host density declines. We first solved the equation

$$\frac{dH}{dt} = -\sum_{i=1}^{n} \frac{a_i H_t P_i}{1 + a_i h_i H_t}$$

similar to the equation described for Experiment 1, but adapted to n parasitoids. Then we calculated the estimated Degree of Infestation as

$$DI_0 = 1 - \frac{H_T}{H_0}$$

where H_0 is the initial host density, and H_T is the estimated host population at the end of the experiment (time $T = 1$ day). This methods allows a good estimate of DI_0 for the null hypothesis that predators do not interact (Sentis and Boukal 2018). The lower and upper confidence intervals around the predicted values were estimated with a global sensitivity analysis based on the functional response parameters estimates to generate a number of random parameter sets using a Latin hypercube sampling algorithm (Soetaert and Petzoldt 2010). The expected degree of infestation was calculated for each parameter set using the *sensRange* function in the R package *FME*. The 2.5% and the 97.5% quantiles of the values obtained from these simulations were used as 95% CIs around the predictions.

Predictions from the population dynamic model were then compared with the observed values (DI_{obs}). Estimated DI values greater than observed DI translate to risk reduction while estimates that were lower than observed DI reflects risk enhancement for the host with multiple parasitoids. We calculated the difference between DI_{obs} and DI_0, and investigated the effects of temperature (ambient versus warming), and parasitoid diversity (one or two species), and their interaction if significant, using an analysis of variance (ANOVA) with the *aov* function. We statistically compared the observed and estimated DI for each temperature regime using a quasibinomial GLM with DI_0 as an offset. A positive or negative significant intercept indicates that DI_0 values underestimate or overestimate DI_{obs}, respectively.

Experiment 3: MPEs mechanisms

The frequencies of super- and multiparasitism event, and melanization were calculated out of the 10 larvae dissected per vial (total of 1,000 larvae across 100 vials). Effects of temperature and parasitoid assemblages on these frequencies were analyzed with generalized linear mixed models (GLMMs). To correct for overdispersion of the residuals, data were modeled using a beta binomial error distribution and a logit function with the *glmmTMB* function. Temperature treatment (two levels: ambient and warming), and parasitoid species assemblage (nine levels) were used as predictor variables, and replicate (n = 10) was used as a random factor.

The two-way interaction between temperature and parasitoid assemblage was tested and kept in our models if judged to be significant based on backward model selection using Likelihood-ratio tests. Significance of the effects was tested using Wald type III analysis of deviance with Likelihood-ratio tests. Factor levels were compared using Tukey's HSD *post hoc* comparisons of all means. All analyses were performed using R 4.0.2 (Team 2017).

Results

Effects of multiple parasitoids on host suppression under warming

The degree of infestation observed in the experiment varied from the model estimations (Figure 2). Temperature significantly affected these differences ($F_{1,93}$ = 9.89, P = 0.002), but parasitoid diversity did not ($F_{1,93}$ = 0.08, P = 0.772), implying that number of parasitoids rather than their species identity is important for host suppression. The comparison of the estimated and observed DI revealed that, in most cases, the predicted DI overestimated the observed DI at ambient temperature (implying risk reduction with multiple parasitoids; but not significantly when looking at the intercept of the quasibinomial GLM with DI_0 as an offset: value ± SE: 0.18 ± 0.27, t value = 0.692, df = 942, P = 0.493), and significantly underestimated them under warming (implying risk enhancement for the host; value ± SE: 0.44 ± 0.20, t value = 2.139, df = 798, P = 0.038; Figure 2).

Effects of warming and parasitoid assemblages on the observed degree of infestation

Contrary to the emergent effects of multiple parasitoids on host suppression, the observed degree of infestation DI_{obs} was not significantly affected by temperature ($\chi2_{(1)}$ = 1.17, P = 0.279), or parasitoid treatment (single, two conspecific or two heterospecific parasitoid assemblages: $\chi2_{(2)}$ = 4.34, P = 0.114) due to species-specific effects. DI only varied with parasitoid species assemblages ($\chi2_{(8)}$ = 258.92, P < 0.0001). Infestation rates were the highest in assemblages with *Ganaspis sp.*, either alone, with a conspecific, or another parasitoid species (Figure S3). The interaction between temperature and parasitoid assemblages had no significant effect on DI_{obs} ($\chi2_{(1)} = 3.42$, $P = 0.166$), despite some observed variation (Figure S3).

Effect of warming and parasitoid assemblages on parasitoid performance

Despite having no effect on DI, parasitoid treatment (single, two conspecific or two heterospecific parasitoid assemblages) significantly affected successful parasitism rate, and the effect varied between parasitoid species (two-way interaction: $\chi2_{(4)} = 16.88$, $P = 0.002$; Table 1).

SP of *Ganaspis sp.* decreased by 95.7% (CI 93.6 - 97.8%) with the presence of a parasitoid conspecific (*Post hoc* Odds Ratio (OR) = 0.043, P < 0.0001), and by 83.4% (CI 75.4 - 91.3%) with the presence of a parasitoid heterospecific compared to when alone (OR = 0.166, P = 0.0007). However, it increased by 287.6% (CI 178.8 - 396.4%) when the parasitoid competitor was from another species compared to a conspecific (OR = 3.876, P < 0.0001). SP of *Asobara sp*. decreased by 55.2% (CI 41.5 - 69.7%) when a parasitoid conspecific was present compared to when alone (OR = 0.448, P = 0.036), but was not significantly affected by the presence of a parasitoid heterospecific (OR = 0.712, P = 0.484). There were no significant effects of parasitoid treatments for SP of *Leptopilina sp*. Effects of parasitoid assemblages on SP also varied between parasitoid species and are presented in Supplementary Material S5 (Table S2 and Figure S4).

Effects of temperature on SP also depended on the species (two-way interaction: $\chi2_{(2)} = 7.31$, $P = 0.026$). Only *Ganaspis sp.* was significantly affected by temperature, and its SP decreased by 58.8% (CI 69.8 - 47.8%) with warming (OR = 0.412, $\chi2_{(1)} = 10.17$, $P = 0.001$). However, all species developed faster under warming (Figure S4).

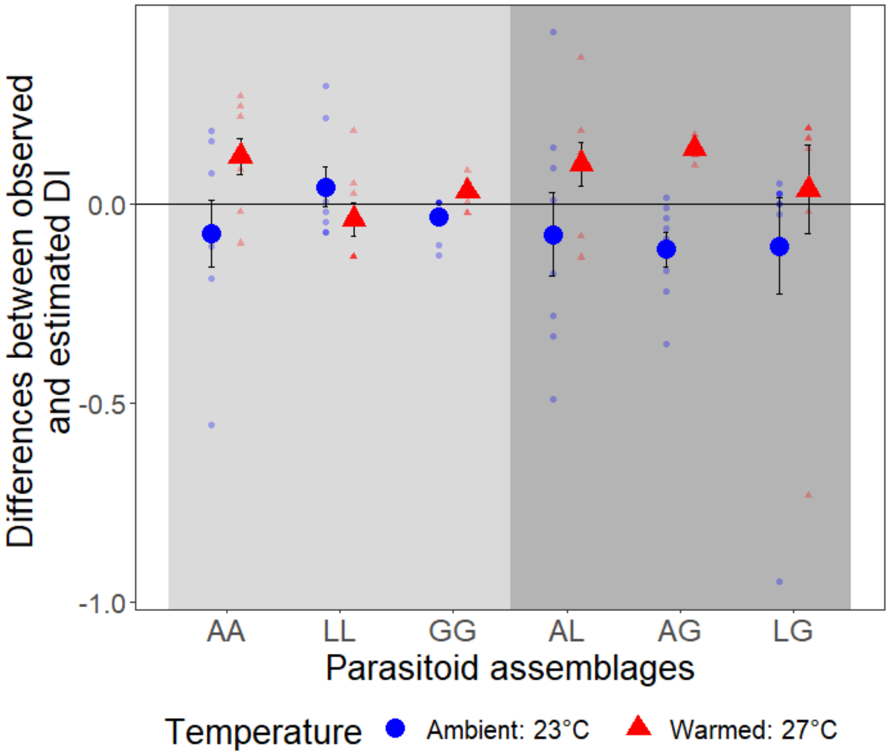


Figure 2. Differences between observed and estimated degree of infestation (DI) for each parasitoid assemblage and temperature. Negative values translate to risk reduction while positive values reflect risk enhancement for the host with multiple parasitoids. Light grey panel: two conspecific parasitoids, darker grey panel; two heterospecific parasitoids. Parasitoid abbreviations: A: *Asobara sp*., L: *Leptopilina sp*., and G: *Ganaspis sp*. Big dots represent the means (± SE), and small dots represent raw data.

Table 1. Odds ratios of a successful parasitism event between parasitoid treatments (1 parasitoid alone, 2 parasitoids conspecifics, and 2 parasitoids heterospecifics) for each parasitoid species. Results are averaged over both temperatures because there was no significant interaction between temperature and parasitoid treatments. Values less than or greater to one denote a decrease or an increase in the odds of successful parasitism, respectively. Significant differences are highlighted in bold.

Parasitoid species	Contrast	Odds Ratio	P-value
Ganaspis sp.	2 conspecifics/alone	**0.043**	**<0.0001**
	2 heterospecifics/alone	**0.166**	**0.0007**
	heterospecifics/conspecifics	**3.876**	**<0.0001**
Asobara sp.	2 conspecifics/alone	**0.448**	**0.036**
	2 heterospecifics/alone	0.711	0.484
	heterospecifics/conspecifics	1.589	0.251
Leptopilina sp.	2 conspecifics/alone	0.182	0.494
	2 heterospecifics/alone	0.871	0.994
	heterospecifics/conspecifics	4.764	0.295

Mechanisms of MPEs

Of the 1,000 larvae dissected, 868 were parasitized (presence of either one or both parasitoid species and/or trace of melanization).

The frequency of either super- or multiparasitism events, reflecting strength of intrinsic competition among parasitoids, was significantly affected by parasitoid assemblages ($\chi2_{(4)}$ = 103.67, P < 0.0001), but not by temperature ($\chi2_{(1)}$ = 3.24, P = 0.072) (Figure 3a). It increased by 619% (CI 469-769%) when *Ganaspis sp*. was with a conspecific (OR = 7.19, P < 0.0001), and by 199% (CI 139-258%) when it was associated with *Asobara sp*. (OR = 2.99, P < 0.0001). However, there was no significant difference in the frequency of superparasitism events when *Asobara sp*. was with a conspecific compared to when alone (OR = 1.59, P = 0.117), suggesting that this species avoid previously parasitized hosts to avoid intrinsic competition. Indeed, 90.6% of parasitized larvae were parasitized by *Asobara sp.*, but of these only 0.25% were super-parasitized.

52.4% of the parasitized larvae had evidence of melanization (traces, melanized egg, and/or melanized larvae), translating host immune response. The frequency of melanization was significantly affected by parasitoid assemblages ($\chi2_{(4)}$ = 88.20, P < 0.0001), and the interaction between temperature and parasitoid assemblages ($\chi2_{(4)}$ = 17.20, P = 0.002), but not by temperature alone (Figure 3b). At ambient temperature, the frequency of melanization significantly increased by 214% (CI 140-288%) when *Ganaspis sp*. was with *Asobara sp*. (OR = 3.14, P < 0.0001= 0.0002). Under warming, the frequency of melanization increased by 396% (CI 266-526%) when *Ganaspis sp*. was with *Asobara sp*. (OR = 4.96, P < 0.0001), and by 337% (CI 223-451%) when it was with another conspecific (OR = 4.37, P < 0.0001) compared to alone, but there was no significant difference between assemblages of conspecific and heterospecific parasitoids. Frequency of melanization was the lowest when *Ganaspis sp.* was alone, and significantly decreased by 60% (CI 49-71%) with elevate temperature (OR = 0.40, P = 0.0007).

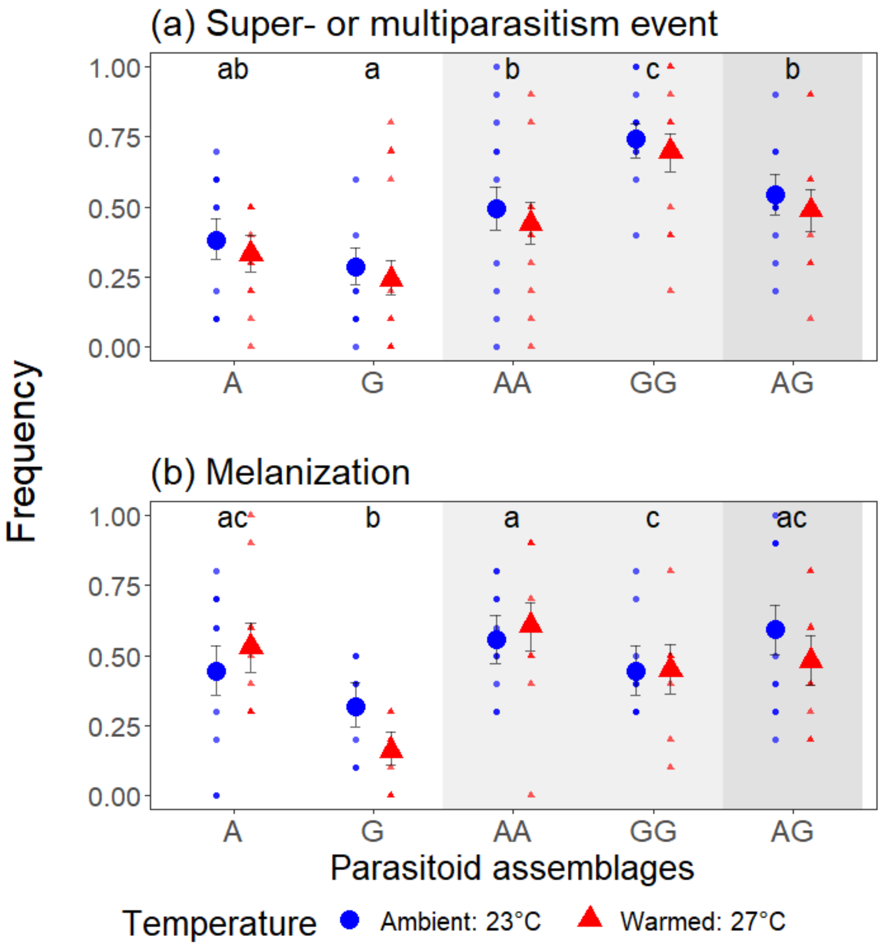


Figure 3. Frequency of (a) super- or multiparasitism event and of (b) melanization out of the 10 hosts dissected per vial for each parasitoid assemblage and temperature. Within each plot, different small letters denote significant differences between parasitoid assemblages. White panel: single parasitoid, light grey panel: two parasitoids conspecific, darker grey panel; two parasitoids heterospecific. Parasitoid abbreviations: A: *Asobara sp.*, and G: *Ganaspis sp*. Big dots represent the estimated means (± 95% CIs) and small dots represent raw data.

Discussion

Temperature alters the effects of multiple predators on risk of predation

The key result from our study is that temperature alters non-trophic interactions among predators, leading to predation risk enhancement for the prey under warming. Indeed, our mathematical model underestimated trophic interaction strength measured in multiple-predators' treatments at elevated temperature. Despite that host suppression was higher than expected with the presence of multiple parasitoids under warming, observed degree of infestation did not significantly vary with temperature. Studying the effects of warming on top-down control without investigating emergent MPEs would thus fail to uncover any effects.

Our results are in concordance with the Drieu and Rusch study (2017) in which predator diversity enhanced top-down control of insect pests in vineyard under warming due to functional complementarity among predator species, while effects were substitutive at ambient temperature. A recent study also found an effect of temperature on intraspecific multiple predator effects on an invasive Gammaridae species, but effects contrasted ours (risk enhancement at low temperature and risk reduction with warming) (Cuthbert et al. 2021). Another study on an aquatic food web found a general trend for predation risk reduction for the prey with multiple predators, but without any effect of temperature on those emergent MPEs (Sentis et al. 2017). All those studies, despite some discrepancy, indicate the importance of considering non-trophic interactions to predict the effect of predator density and diversity on trophic interaction strengths across systems. Our study contributes to these by showing the important effect of warming for both intra- and interspecific multiple predator effects, and across multiple predator assemblages. In addition to an increase in prey suppression with multiple predators under warming in terrestrial ecosystems, a diverse predator community also increases the chances of complementarity in face of environmental variations and disturbances (Macfadyen et al. 2011). Indeed, presence of multiple predator species could mitigate negative effects of warming on top-down control due to resource partitioning and/or functional redundancy (Drieu and Rusch 2017, Cebolla et al. 2018, Pepi and McMunn

2021). Preserving predator biodiversity should therefore be generally beneficial for top-down control under climate change.

Here, emergent MPEs were explored with constant initial prey density. But the unimodal relationship between prey density and trophic interaction strength (i.e., type II functional response) suggests that varying prey densities might lead to different outcomes for prey suppression with multiple predators (Sentis et al. 2017, Cuthbert et al. 2021). However, with prey densities too low or too high, one would fail to detect non-trophic interactions among predators, while intermediate prey density would result in high resource limitation, and thus antagonistic interactions among predators. The synergetic effects of multiple predators found in our study under warming suggested that our experimental design with a single prey density was adequate to detect emergent MPEs.

Mechanisms behind emergent multiple predator effects on the prey

Predation risk reduction translates to antagonistic interactions between predators, whereas risk enhancement reveals synergetic effects among predators, usually reflecting either niche partitioning or facilitation (Sih et al. 1998, Straub and Snyder 2008, Northfield et al. 2010). In predator-prey systems, it has been hypothesized that predators have a higher search rate at warmer temperature, with less time for interference (Lang et al. 2012). In our host-parasitoid system, we did not observe significant differences in intra- and interspecific competitive interaction strength (i.e., frequency of super- and multiparasitism event) between temperature regimes. There were also no significant differences in host immune response (i.e., frequency of melanization) between temperature regimes for treatments with multiple parasitoids. However, effects of multiple predators on host suppression were not additive under warming, suggesting change in the strength of non-trophic interactions among parasitoids not directly measured in this study. We thus tentatively hypothesize that the risk enhancement observed with warming could be due to weaker interference between adult parasitoids, similarly to predator-prey systems.

Here, the experiments were conducted in simplified laboratory conditions where parasitoids were forced to share the same habitat (i.e., a vial) and overlap in time, which does not allow for resource partitioning (Ives et al. 2005). This could have enhanced antagonistic interactions among predators

(Schmitz 2007), which seems to not be the case in our study. In nature however, warming could also change predator habitat use (Barton and Schmitz 2009, Schmitz and Barton 2014), and phenology (Renner and Zohner 2018, Abarca and Spahn 2021), leading to changes in MPEs. However, the impact of temperature on MPEs was consistent across parasitoid assemblages, suggesting a general pattern for synergistic effects with multiple natural enemies under warming in our system.

Parasitoid performance was not affected by temperature, but by parasitoid assemblage

Despite that multiple parasitoids enhanced host suppression under warming, successful parasitism rate was generally lower at both temperatures when another parasitoid individual was present. Wang et al. (2019) found that parasitoid species with the fastest development rate could eliminate its interspecific competitors. In our study, the slowest of the three, *Ganaspis sp*., performed best, but still had a lower success rate with the presence of another parasitoid. We found higher resistance of the hosts (i.e., frequency of melanization) when *Ganaspis sp.* was with another parasitoid compared to when alone, which might be the reason for the decrease in its performance. Another study on *Drosophila*-parasitoid interaction found contrasting results: a significant impact of thermal regime on parasitoid success despite no changes in degree of infestation (Delava et al. 2016). Long-term effects of warming on parasitoid populations are thus uncertain, and hosts from the next generation might benefit from lower parasitoid abundances.

No differences between inter- and intraspecific interactions

Similar to other studies, we did not find significant differences between treatments with multiple conspecifics or heterospecific predators for prey suppression (Finke and Snyder 2008, Lampropoulos et al. 2013, Griffin et al. 2015). Weaker effects of inter- than intraspecific competition have been observed on aphid suppression (Straub and Snyder 2008), and theory predicts that for species to coexist, interspecific interactions should be weaker than intraspecific interactions (Barabás et al. 2016). It is therefore important to look at both predator density and diversity on prey suppression, rather than only using a substitutive approach (i.e., keeping predator density constant), which might bias the results. When niche differentiation is allowed, for example with habitat heterogeneity or longer timeframe that include potential differences in phenology, increase in predator diversity should intensify prey suppression because of differences in prey use among species rather than because of diversity *per se* (Finke and Snyder 2008, Krey et al. 2021). Here, predator density intensified prey suppression at warmer temperature despite the small scale of the experiment. Allowing for differentiation in habitat domain between predator species might have yielded even higher prey suppression.

No effects of treatments on observed degree of infestation

Prey suppression was generally higher when predator assemblages included the best-performing species, *Ganaspis sp*., no matter the predator diversity or density, nor the temperature. A meta-analysis on the effects of predator diversity on prey suppression found a similar trend across the 46 studies taken into account (Griffin et al. 2013), but also found a general positive effect of multiple predators on top-down control. Contrastingly, a meta-analysis of 108 biological control projects against insect pests found no relationship between the number of agents released and biological control success for insect pests (Denoth et al. 2002). However, increasing predator diversity should be generally beneficial for top-down control by increasing the chances to have a performant natural enemy species in the mix, as it was the case in our study (i.e., sampling effect model; Myers et al. 1989). Moreover, presence of multiple species in the community could buffer any mismatch between predator and prey species induced by warming (Pardikes et al. 2021).

Ganaspis sp. was the most performant species to suppression *D. simulans* across treatments, but its performance decreased with warming, suggesting that parasitism rate and therefore host suppression could also decreased in the longer-term due to a decrease in parasitoid population.

Conclusion

Overall, pairwise interaction strength generally failed to accurately estimate the species interaction strength observed, indicating that non-trophic interactions must be considered to predict the effects of multiple predator on prey suppression, and in food web studies in general (Kéfi et al. 2012). Previous studies show altered MPEs with warming due to changes in resource partitioning (Barton and Schmitz 2009, Cuthbert et al. 2021), but our study is the first, to our knowledge, to show sign of direct effects of warming on both intra- and interspecific predator interactions and across predator assemblages, resulting in a higher top-down control at elevated temperature. Current global changes are eroding the biodiversity worldwide (Wagner et al. 2021), and predators are generally more at risk then their prey. A loss of predatory diversity could thus result in a decrease of top-down control, and further biodiversity erosion through cascading effects on lower trophic levels (Kehoe et al. 2020).

Acknowledgements

We thank Anna Mácová, Andrea Weberova, Grégoire Proudhom, and Joel Brown for their help during the setup of the experiments, and for Tereza Holicová and Vincent Montbel for their help dissecting the larvae. The drawings used for Figure 1 was made by Tereza Holicová. We acknowledge funding support from the Czech Science Foundation grant no. 20-30690S. BR acknowledges the support of iDiv funded by the German Research Foundation (DFG–FZT 118, 202548816).

Author contributions

MT conceived the project; NP and JH contributed to the experimental design; MT, NP, and MG collected the data; BR provided the methodology and R scripts to estimate functional responses. MT analyzed the data. All authors contributed critically to the drafts and gave final approval for publication.

Competing interests

The author(s) declare no competing interests.

Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

References

Abarca, M., and R. Spahn. 2021. Direct and indirect effects of altered temperature regimes and phenological mismatches on insect populations. Current Opinion in Insect Science.

Barabás, G., M. J. Michalska-Smith, and S. Allesina. 2016. The effect of intra- and interspecific Competition on coexistence in multispecies communities. The American Naturalist 188:E1–E12.

Barton, B. T., and O. J. Schmitz. 2009. Experimental warming transforms multiple predator effects in a grassland food web. Ecology Letters 12:1317–1325.

Boulétreau, M., and E. Wajnberg. 1986. Comparative responses of two sympatric parasitoid cynipids to the genetic and epigenetic variations of the larvae of their host, *Drosophila melanogaster*. Entomologia Experimentalis et Applicata 41:107–114.

Carton, Y., and H. Kitano. 1981. Evolutionary relationships to parasitism by seven species of the *Drosophila melanogaster* subgroup. Biological Journal of the Linnean Society 16:227–241.

Carton, Y., M. Poirié, and A. J. Nappi. 2008. Insect immune resistance to parasitoids. Insect Science 15:67–87.

Cebolla, R., A. Urbaneja, J. van Baaren, and A. Tena. 2018. Negative effect of global warming on biological control is mitigated by direct competition between sympatric parasitoids. Biological Control 122:60–66.

Cuthbert, R. N., R. J. Wasserman, T. Dalu, and E. Briski. 2021. Warming mediates intraspecific multiple predator effects from an invasive crustacean. Marine Biology 168:35.

Davis, A. J., J. H. Lawton, B. Shorrocks, and L. S. Jenkinson. 1998. Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. Journal of Animal Ecology 67:600–612.

Delava, E., F. Fleury, and P. Gibert. 2016. Effects of daily fluctuating temperatures on the *Drosophila–Leptopilina boulardi* parasitoid association. Journal of Thermal Biology 60:95–102.

Denoth, M., L. Frid, and J. H. Myers. 2002. Multiple agents in biological control: Improving the odds? Biological Control 24:20–30.

Drieu, R., and A. Rusch. 2017. Conserving species-rich predator assemblages strengthens natural pest control in a climate warming context. Agricultural and Forest Entomology 19:52–59.

Fernández-Arhex, V., and J. C. Corley. 2003. The functional response of parasitoids and its implications for biological control. Biocontrol Science and Technology 13:403–413.

Finke, D. L., and W. E. Snyder. 2008. Niche partitioning increases resource exploitation by diverse communities. Science 321:1488–1490.

Godfray, H. C. J. 2013. Parasitoids. Pages 674–682 Encyclopedia of Biodiversity: Second Edition.

Griffin, J. N., J. E. K. Byrnes, and B. J. Cardinale. 2013. Effects of predator richness on prey suppression: A meta-analysis. Ecology 94:2180–2187.

Griffin, J. N., B. J. Toscano, B. D. Griffen, and B. R. Silliman. 2015. Does relative abundance modify multiple predator effects? Basic and Applied Ecology 16:641–651.

Hance, T., J. van Baaren, P. Vernon, and G. Boivin. 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. Annual Review of Entomology 52:107–126.

Hartig, F. 2019. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.

Harvey, J. A., E. H. Poelman, and T. Tanaka. 2013. Intrinsic inter- and intraspecific competition in parasitoid wasps. Annual Review of Entomology 58:333–351.

Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. Canadian entomologist 91:385–398.

Ives, A. R., B. J. Cardinale, and W. E. Snyder. 2005. A synthesis of subdisciplines: Predator-prey interactions, and biodiversity and ecosystem functioning. Ecology Letters 8:102–116.

Jeffs, C. T., J. C. D. Terry, M. Higgie, A. Jandová, H. Konvičková, J. J. Brown, C. H. Lue, M. Schiffer, E. K. O'Brien, J. Bridle, J. Hrček, and O. T. Lewis. 2021. Molecular analyses reveal consistent food web structure with elevation in rainforest *Drosophila* - parasitoid communities. Ecography 44:403–413.

Kéfi, S., E. L. Berlow, E. A. Wieters, L. N. Joppa, S. A. Wood, U. Brose, and S. A. Navarrete. 2015. Network structure beyond food webs: Mapping non-trophic and trophic interactions on Chilean rocky shores. Ecology 96:291–303.

Kéfi, S., E. L. Berlow, E. A. Wieters, S. A. Navarrete, O. L. Petchey, S. A. Wood, A. Boit, L. N. Joppa, K. D. Lafferty, R. J. Williams, N. D. Martinez, B. A. Menge, C. A. Blanchette, A. C. Iles, and U. Brose. 2012. More than a meal… integrating non-feeding interactions into food webs. Ecology Letters 15:291–300.

Kehoe, R., E. Frago, and D. Sanders. 2020. Cascading extinctions as a hidden driver of insect decline. Ecological Entomology:een.12985.

Krey, K. L., O. M. Smith, E. G. Chapman, M. S. Crossley, D. W. Crowder, Z. Fu, J. D. Harwood, A. S. Jensen, C. A. Lynch, G. B. Snyder, and W. E. Snyder. 2021. Prey and predator biodiversity mediate aphid consumption by generalists. Biological Control 160:104650.

Lampropoulos, P. D., D. C. Perdikis, and A. A. Fantinou. 2013. Are multiple predator effects directed by prey availability? Basic and Applied Ecology 14:605–613.

Lang, B., B. C. Rall, and U. Brose. 2012. Warming effects on consumption and intraspecific interference competition depend on predator metabolism. Journal of Animal Ecology 81:516–523.

Lenth, R. V. 2018. Emmeans: Estimated marginal means, aka least-squares means. R package version.

Lüdecke, D., D. Makowski, and P. Waggoner. 2019. Performance: Assessment of regression models performance.

Lue, C.-H., M. L. Buffington, S. Scheffer, M. Lewis, T. A. Elliott, A. R. I. Lindsey, A. Driskell, A. Jandova, M. T. Kimura, Y. Carton, R. R. Kula, T. A. Schlenke, M. Mateos, S. Govind, J. Varaldi, E. Guerrier, M. Giorgini, X. Wang, K. Hoelmer, K. M. Daane, P. K. Abram, N. A. Pardikes, J. J. Brown, M. Thierry, M. Poirie, P. Goldstein, S. E. Miller, D. Tracey, J. S. Davis, F. M. Jiggins, B. Wertheim, O. Lewis, J. Leips, P. P. A. Staniczenko, and J. Hrcek. 2021. DROP: Molecular voucher database for identification of *Drosophila* parasitoids. Molecular Ecology Resources.

Macfadyen, S., P. G. Craze, A. Polaszek, K. van Achterberg, and J. Memmott. 2011. Parasitoid diversity reduces the variability in pest control services across time on farms. Proceedings of the Royal Society B: Biological Sciences 278:3387–3394.

May, R. M., and M. P. Hassell. 1988. Population dynamics and biological control. Philosophical Transactions of the Royal Society of London. B, Biological Sciences 318:129–169.

Mccoy, M. W., A. C. Stier, and C. W. Osenberg. 2012. Emergent effects of multiple predators on prey survival: The importance of depletion and the functional response. Ecology Letters 15:1449–1456.

Myers, J. H., C. Higgins, and E. Kovacs. 1989. How many insect species are necessary for the biological control of insects? Environmental Entomology 18:541–547.

Northfield, T. D., G. B. Snyder, A. R. Ives, and W. E. Snyder. 2010. Niche saturation reveals resource partitioning among consumers. Ecology Letters 13:338–348.

Nouhaud, P., F. Mallard, R. Poupardin, N. Barghi, and C. Schlötterer. 2018. High-throughput fecundity measurements in *Drosophila*. Scientific Reports 8:4469.

Pardikes, N., T. Revilla, C.-H. Lue, Thierry Mélanie, D. Soutos-Villaros, and J. Hrcek. 2021. Community context modifies response of host-parasitoid interactions to phenological mismatch under warming. Authorea.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.

Pedersen, B. S., and N. J. Mills. 2004. Single vs. multiple introduction in biological control: The roles of parasitoid efficiency, antagonism and niche overlap. Journal of Applied Ecology 41:973–984.

Pepi, A., and M. McMunn. 2021. Predator diversity and thermal niche complementarity attenuate indirect effects of warming on prey survival. American Naturalist.

Post, E. 2013. Ecology of Climate Change: The Importance of Biotic Interactions. Princeton University Press, Princeton, NJ.

Rall, B. Ö. C., O. Vucic-Pestic, R. B. Ehnes, M. Emmerson, and U. Brose. 2010. Temperature, predator-prey interaction strength and population stability. Global Change Biology 16:2145–2157.

Renner, S. S., and C. M. Zohner. 2018. Climate change and phenological mismatch in trophic interactions among plants, insects, and vertebrates. Annual Review of Ecology, Evolution, and Systematics 49:165–182.

Rosenbaum, B., and B. C. Rall. 2018. Fitting functional responses: Direct parameter estimation by simulating differential equations. Methods in Ecology and Evolution 9:2076–2090.

Schmitz, O. J. 2007. Predator diversity and trophic interactions. Ecology 88:2415–2426.

Schmitz, O. J. 2009. Effects of predator functional diversity on grassland ecosystem function. Ecology 90:2339–2345.

Schmitz, O. J., and B. T. Barton. 2014. Climate change effects on behavioral and physiological ecology of predator-prey interactions: Implications for conservation biological control. Biological Control 75:87–96.

Sentis, A., and D. S. Boukal. 2018. On the use of functional responses to quantify emergent multiple predator effects. Scientific Reports 8:1–12.

Sentis, A., C. Gémard, B. Jaugeon, and D. S. Boukal. 2017. Predator diversity and environmental change modify the strengths of trophic and nontrophic interactions. Global Change Biology 23:2629–2640.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends in Ecology and Evolution 13:350–355.

Soetaert, K., and T. Petzoldt. 2010. Inverse modelling, sensitivity and monte carlo analysis in R using package FME. Journal of Statistical Software 33:1–28.

Sohlström, E. H., L. C. Archer, B. Gallo, M. Jochum, R. L. Kordas, B. C. Rall, B. Rosenbaum, and E. J. O’Gorman. 2021. Thermal acclimation increases the stability of a predator-prey interaction in warmer environments. Global Change Biology:gcb.15715.

Soluk, D. A. 1993. Multiple predator effects: predicting combined functional response of stream fish and invertebrate predators. Ecology 74:219–225.

Song, C., S. Von Ahn, R. P. Rohr, and S. Saavedra. 2020. Towards a probabilistic understanding about the context-dependency of species interactions. Trends in Ecology and Evolution 35:384–396.

Straub, C. S., and W. E. Snyder. 2008. Increasing enemy biodiversity strengthens herbivore suppression on two plant species. Ecology 89:1605–1615.

Team, R. C. 2017, January 29. R: The R Project for statistical computing.

Thierry, M., J. Hrček, and O. T. Lewis. 2019. Mechanisms structuring host–parasitoid networks in a global warming context: a review. Ecological Entomology:een.12750.

Thierry, M., N. A. Pardikes, C. H. Lue, O. T. Lewis, and J. Hrček. 2021. Experimental warming influences species abundances in a Drosophila host community through direct effects on species performance rather than altered competition and parasitism. PLoS One 16:e0245029.

Tylianakis, J. M., and C. M. Romo. 2010. Natural enemy diversity and biological control: making sense of the context-dependency. Basic and Applied Ecology 11:657–668.

Uszko, W., S. Diehl, G. Englund, and P. Amarasekare. 2017, April 1. Effects of warming on predator–prey interactions – a resource-based approach and a theoretical synthesis. Blackwell Publishing Ltd.

Wagner, D. L., E. M. Grames, M. L. Forister, M. R. Berenbaum C, and D. Stopak. 2021. Insect decline in the Anthropocene: Death by a thousand cuts. Proceedings of the National Academy of Sciences 118.

Wang, X., B. N. Hogg, E. Hougardy, A. H. Nance, and K. M. Daane. 2019. Potential competitive outcomes among three solitary larval endoparasitoids as candidate agents for classical biological control of Drosophila suzukii. Biological Control 130:18–26.

Wootton, J. T. 1997. Estimates and tests of per capita interaction strength: Diet, abundance, and impact of intertidally foraging birds. Ecological Monographs 67:45–64.

Supplement Materials

Supplement Material S1: Identification of parasitoid eggs and larvae inside D. simulans 2, 3 or 4 days after infection.

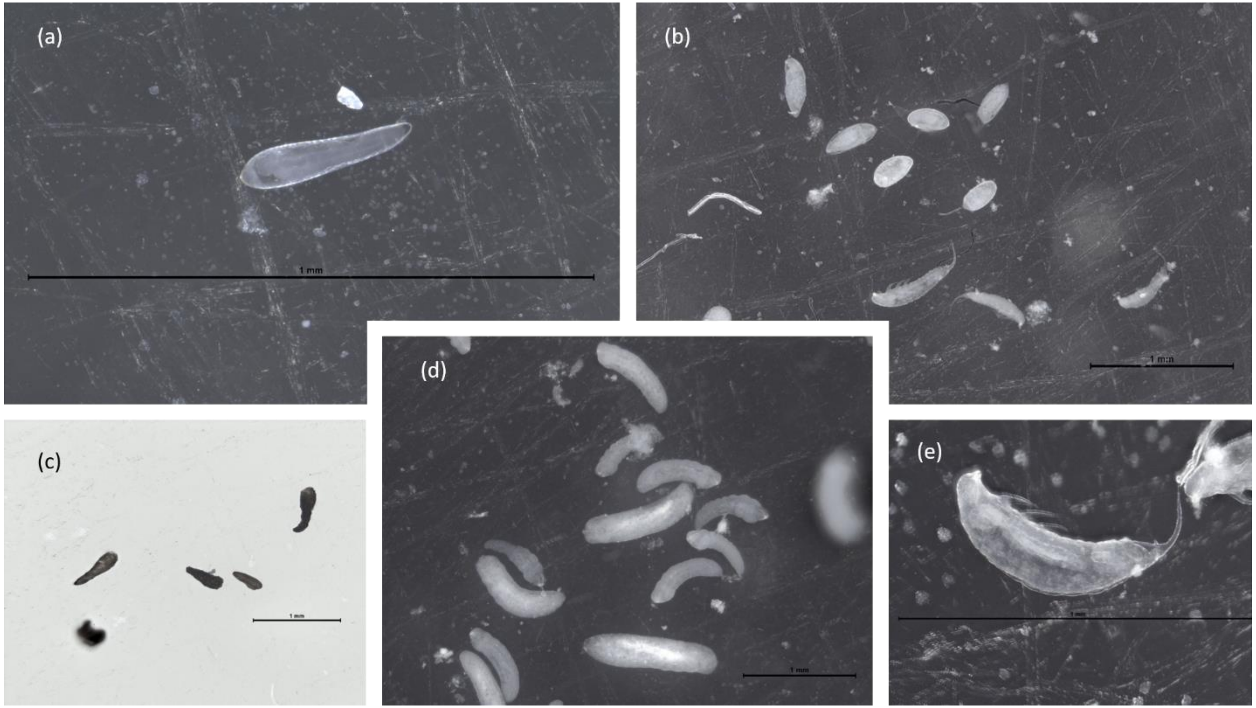


Figure S1. (a) *Asobara sp*. egg, (b) *Ganaspis sp*. eggs and larvae, (c) melanized eggs, (d) *Asobara sp*. larvae, (e) *Ganaspis sp*. larva. For all pictures, the bar scale represents 1 mm

Supplement Material S2: Parasitoid functional responses

We fitted three functional response models to the single-parasitoid experiments at all temperatures and for all parasitoid species. All three functional response models can be expressed by

$$F(H) = \frac{aH^{1+q}}{1 + ahH^{1+q}}$$

where (1) q = 0 defines a type II response, (2) q = 1 defines a type III response, and (3) a free parameter q defines a generalized type III response, that allows a continuous shift between type II and type III (Rosenbaum & Rall, 2018). We used the leave-one-out information criterion (LOOIC) for model comparison, which was computed from the log-likelihood values of posterior samples (*loo* package). Although type III and generalized type III responses had lower LOOIC scores than the type II response (differences ΔLOOIC = 0.7, SE = 30.6, and ΔLOOIC = 19.2, SE = 26.2, respectively), the differences were in the range of estimated uncertainty. Therefore, we chose the type II response as the most parsimonious model.

Table S1. Estimated parameters *a* search rate (day host^{-1}) and *h* handling time (day host^{-1}) of the type II functional response for each parasitoid species at each temperature ± standard error.

Species	**Temperature**	**a ± s.d.**	**h ± s.d.**
Asobara sp.	23°C	1.85 ± 0.16	0.029 ± 0.002
Asobara sp.	27°C	0.56 ± 0.05	0.008 ± 0.003
Ganaspis sp.	23°C	3.13 ± 0.21	0.002 ± 0.001
Ganaspis sp.	27°C	1.26 ± 0.05	0.001 ± 0.0004
Leptopilina sp.	23°C	1.67 ± 0.58	0.541 ± 0.064
Leptopilina sp.	27°C	0.08 ± 0.01	0.042 ± 0.026

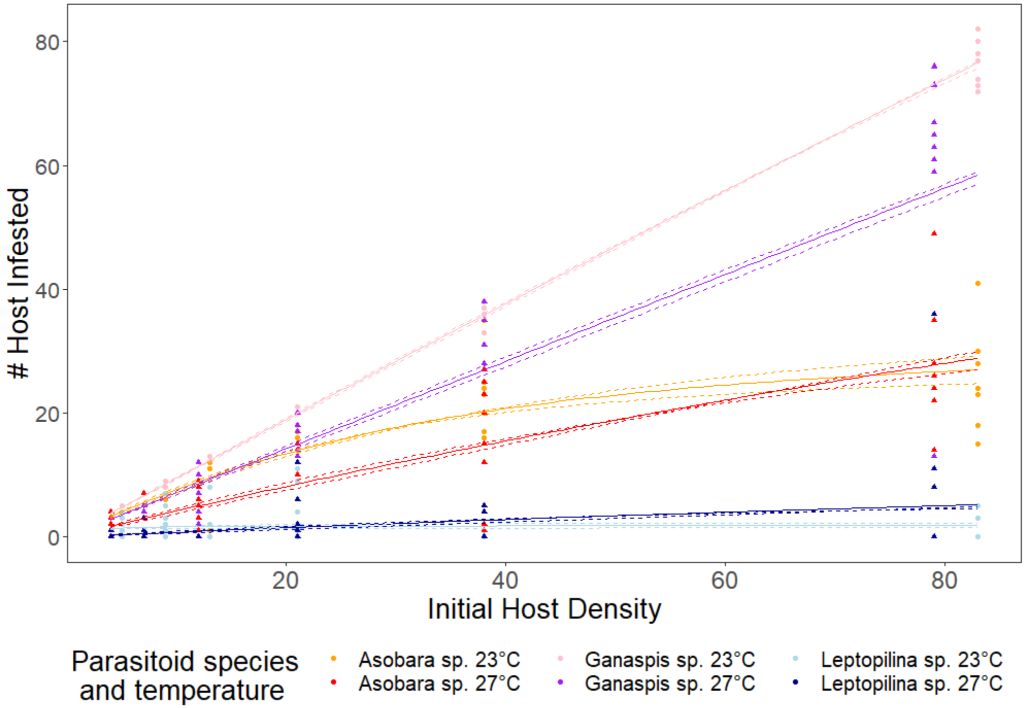


Figure S2. Type II functional responses of the three parasitoids at ambient (23 °C) and warmed (27 °C) temperature estimated from Experiment 1. Points represent observed values, solid lines correspond to the fitted functional responses, and dashed lines the 95% confidence intervals

Supplement Material S3: Effects of warming and parasitoid assemblage on parasitoid developmental rate

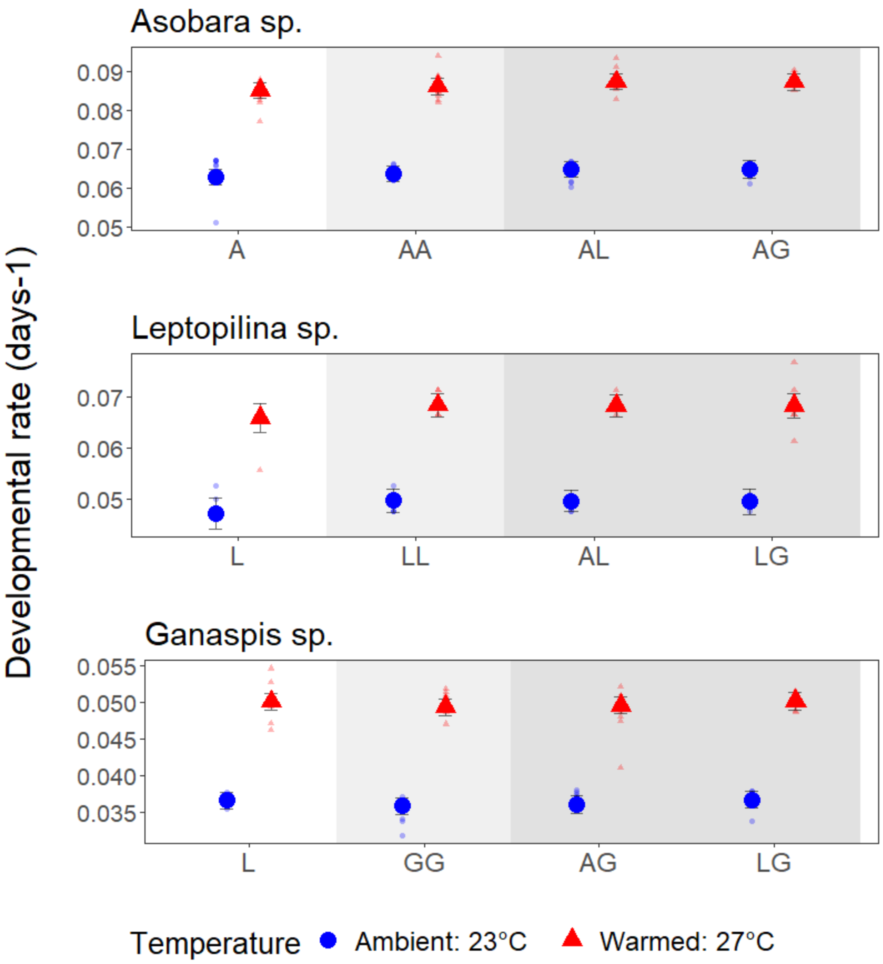


Figure S3. Development rate per day of each parasitoid species significantly increased with warming, but was not affected by parasitoid assemblage. White panel: single parasitoid, light grey panel: two parasitoids conspecific, darker grey panel; two parasitoids heterospecific. Parasitoid abbreviations: A: *Asobara sp*., L: *Leptopilina sp*., and G: *Ganaspis sp*. Big dots represent the estimated means (± 95% CIs) and small dots represent raw data. *Notes that y-axis scale varies between parasitoid species*

Supplement Material S4: Effect of warming and parasitoid assemblages on host degree of infestation

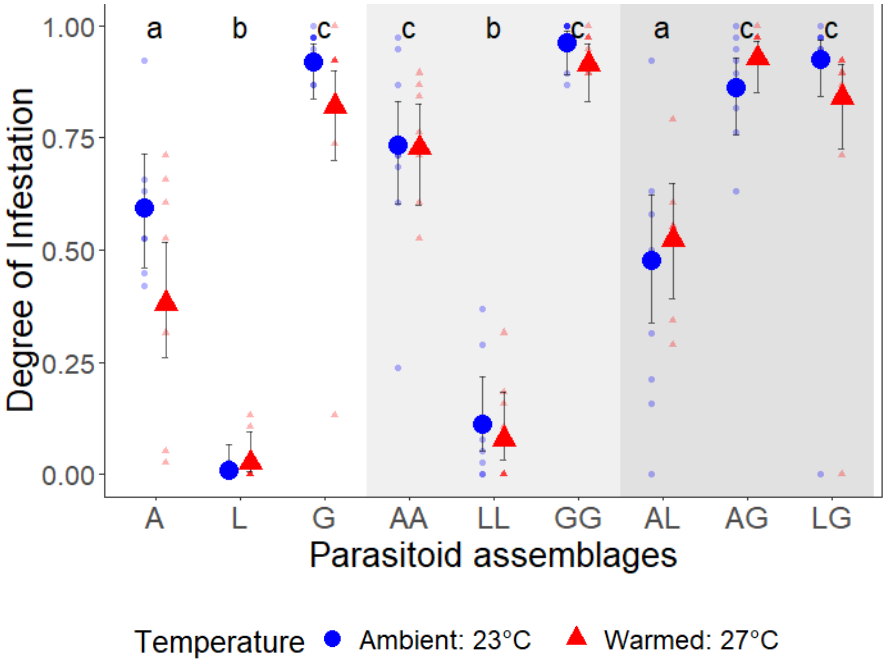


Figure S4. Degree of infestation for each parasitoid assemblage and temperature. Different small letters denote significant differences between parasitoid assemblages. White panel: single parasitoid, light grey panel: two parasitoids conspecific, darker grey panel; two parasitoids heterospecific. Parasitoid abbreviations: A: *Asobara sp*., L: *Leptopilina sp*., and G: *Ganaspis sp*. Big dots represent the estimated means (± 95% CIs) and small dots represent raw data.

Supplement Material S5: Effects of warming and parasitoid assemblage on successful parasitism rate

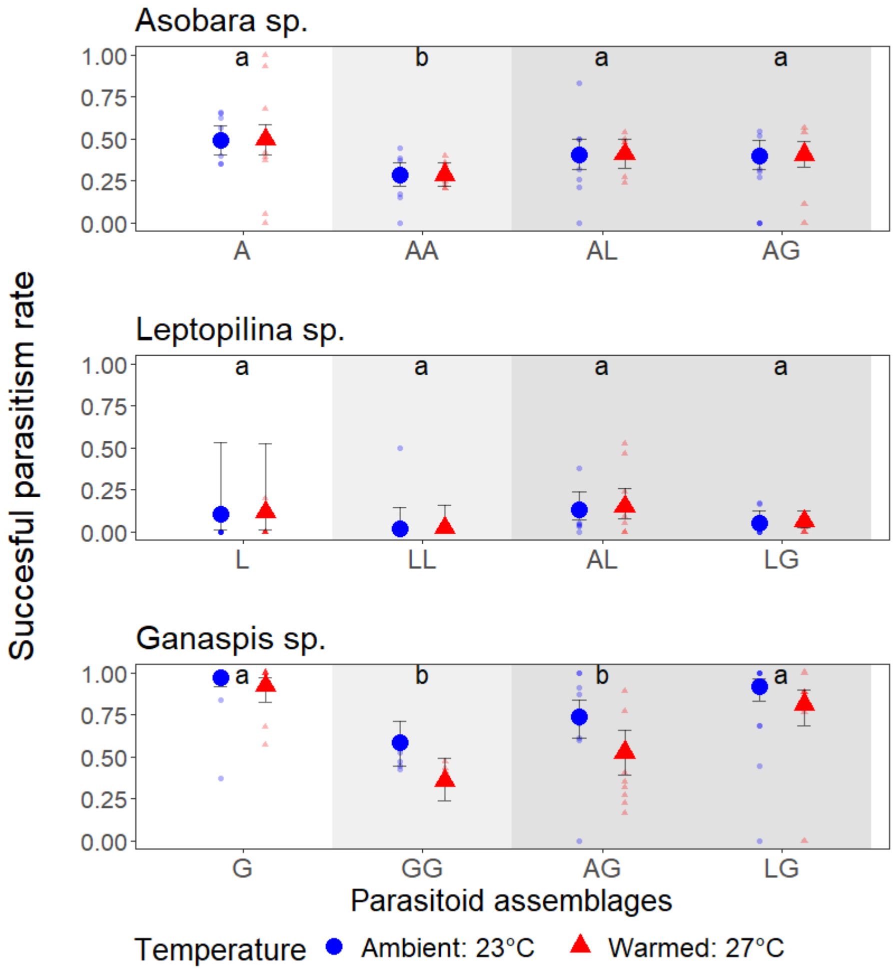


Figure S5. Probability of successful parasitism rate varied across parasitoid assemblage and temperature depending on the species identity. Within each parasitoid species, different small letters denote significant differences between parasitoid assemblages. White panel: single parasitoid, light grey panel: two parasitoids conspecific, darker grey panel; two parasitoids heterospecific. Parasitoid abbreviations: A: *Asobara sp*., L: *Leptopilina sp*., and G: *Ganaspis sp*.. Big dots represent the estimated means (± 95% CIs) and small dots represent raw data. Contrasts between parasitoid assemblages are presented in Table S2.

Table S2. Effects of parasitoid assemblages on successful parasitism rate for each parasitoid species. Abbreviations: A: *Asobara sp*., L: *Leptopilina sp*., and G: *Ganaspis sp*. Results are averaged over both temperatures because there was no significant interaction between temperature treatments and parasitoid assemblages. Significant differences are highlighted in bold.

Parasitoid species	**Contrast**	**Odds Ratio**	**P-value**
Asobara sp.	**AA/A**	**0.41**	**0.001**
	AL/A	0.71	0.434
	AL/AA	**1.73**	**0.080**
	AG/A	0.70	0.082
	AG/AA	1.70	0.082
	AG/AL	0.99	1.000
Ganaspis sp.	**GG/G**	**0.05**	**< 0.0001**
	AG/G	**0.10**	**0.0002**
	AG/GG	2.02	0.183
	LG/G	0.37	0.301
	LG/GG	**7.93**	**< 0.0001**
	LG/AG	**3.94**	**0.010**
Leptopilina sp.	LL/L	0.18	0.656
	AL/L	1.35	0.993
	AL/LL	7.51	0.231
	LG/L	0.51	0.931
	LG/LL	2.81	0.768
	LG/AL	0.38	0.124

Summary of results

In this thesis I probed the joint effects of abiotic and biotic environmental changes due to global warming on species interactions and communities. I focused on the effects of warming and changes in community structure and composition on host-parasitoid interactions and host communities. To disentangle the different drivers affecting interaction strengths and shaping communities, I used a new host-parasitoid model from Australian tropical rainforest that we established in the laboratory in 2017 (Jeffs et al. 2021). I used species that co-occur in the field, and a set of laboratory experiments that allowed me to control both the abiotic and biotic contexts in which species interacted. I was therefore able to test if those abiotic and biotic drivers had independent effects, worked in combination, or had antagonistic effects on species interactions and community structure.

In **Chapter 1** I first reviewed the wide array of trophic and non-trophic interactions that structure host-parasitoid communities. I discussed how these mechanisms are likely to be impacted by global warming. I tabulated and discussed published evidence for altered rates of parasitism with increasing temperature. I emphasized the little-known role of facultative endosymbionts in structuring host-parasitoid networks, and how these effects will interact with global warming. Finally, I provided suggestions for future research avenues aiming to understand the mechanisms structuring host-parasitoid networks in a global warming context.

Results from **Chapter 2** revealed a general positive effect of parasitoid diversity on top-down control. However, effects of community structure on parasitoid performance depended on the identity of co-occurring species. This chapter highlights the importance of the community species composition for the outcome of interactions. Current global changes alter both the structure and the composition of communities, and it is therefore important to consider both aspects to better predict the dynamics of ecological communities in a changing world.

In the experiment presented in **Chapter 3** I found that experimental warming significantly decreased parasitism for all host-parasitoid pairs considered, consistent with the general trend of a decrease in parasitism rate with warming observed in other studies and reviewed in Chapter 1. However, the effects of parasitism and competition on host communities did not vary with temperature. Instead, effects of experimental warming on host community structure were species-specific, with one host species dominating the community at warmer temperature, independently of parasitism and competition treatments. This chapter shows that temperature shaped our *Drosophila* host community directly through differences in species thermal performance, rather than altered biotic interactions.

With **Chapter 4** I showed that warming alters the effects of multiple parasitoids on host suppression. Previous studies on terrestrial and aquatic systems also found important effects of temperature for both trophic and non-trophic interaction strengths (Barton and Schmitz 2009, Drieu and Rusch 2017, Cuthbert et al. 2021), unveiling a potential general trend across ecosystems. It has been hypotheses that in food webs, the positive effect of multiple predators under warming would be due to an increase in predator search rate with elevated temperatures accompanied by a decrease in interference between predators (Lang et al. 2012). As I did not find significant differences between temperature regimes in the strength of intrinsic competition, but generally higher attack rates at ambient temperatures, our system might follow the same mechanisms. This chapter emphasizes that pairwise predator-prey interaction strength is context-dependent. Effects of environmental factors on non-trophic interactions are important to accurately predict effects of global change on ecosystem functioning.

Discussion & conclusions

DISCUSSION

Revealing the links between environmental factors and species interactions is crucial to understand the ecological consequences of global environmental changes for community structure and dynamics. However, the combined action of abiotic and biotic drivers in shaping communities is poorly understood. This thesis investigated how warming and changes in community composition affect how species interact, and how communities are structured. From the chapters presented here, several important points have emerged regarding community response to environmental changes.

The context-dependence of species interactions

The most prominent conclusion from my dissertation is that species interactions are strongly context-dependent.

Chapter 4 revealed a general trend for the temperature-dependence of trophic and non-trophic interactions by demonstrating these effects across multiple species assemblages. Our study adds to the body of evidence about the interactive effects between biotic and abiotic factors on species interaction strength (Barton and Schmitz 2009, Sentis et al. 2017, Cuthbert et al. 2021). This is particularly relevant in the context of current global changes that alter both the abiotic and biotic environment in which species interact. A diverse community could buffer potential negative effects of warming via functional redundancy (Cebolla et al. 2018) and complementarity (Pardikes et al. 2021). These results, and that of a field study (Drieu and Rusch 2017), suggest that the erosion of biodiversity worldwide due to global changes could lead to a loss of top-down control, with cascading extinctions at all trophic levels (Sanders et al. 2015).

Chapter 2 highlights the combined effect of community structure and composition on the outcome of species interactions. Indeed, effects of community structure on parasitoid performance depended on the identity of both the focal pair of interacting species, and of the co-occurring species in the community. Current global changes are reshaping communities worldwide, and disrupt historical patterns of interactions, disturbing ecosystem functioning (Burkle et al. 2013). These results suggest that with new co-occurring species, the outcome of interactions between focal species is likely to change, which will further alter population and community dynamics.

The context-dependence of species interactions is not a new concept (Chamberlain et al. 2014), but understanding its extent, and the interplay between the different environmental drivers, are of wide importance with ongoing global changes and declines in species diversity. This thesis tackles the complexity of the context-dependence of species interactions. Overall, I showed that the structure and composition of communities influence how species interact and respond to warming, and in turn that warming alters the effects of community-context on species interactions.

Multiple predators enhance prey suppression

Despite species-specific responses to changes in the environment, I found a general pattern for the effects of multiple parasitoids on host suppression. In **Chapters 2 and 4** I showed that an increase in parasitoid density and diversity enhanced top-down control. This is consistent with previous work on the effects of natural enemy diversity on arthropod suppression (reviewed in Letourneau et al. 2009), but we are the first to have demonstrated this general pattern with varying species composition at both tropic levels within the same study and biological model. When keeping parasitoid density constant (**Chapter 2**), this result was mainly explained by an increasing probability that a superior natural enemy species would be present in the community, also known as the sampling effect model (Myers et al. 1989). But when varying both parasitoid density and diversity (**Chapter 4**), multiple parasitoids, either from the same or a different species, consistently increased host suppression. These results, coupled with previous studies, hint at the prevalence of synergistic effects of multiple parasitoids on top-down control. Moreover, a diverse natural enemy community would increase ecosystem robustness in the face of perturbations such as land-use and climate change. Preserving biodiversity would therefore be beneficial for biological control. However, as the outcomes for parasitoids were species-specific, a future challenge will be to study such mechanisms in the longer-term to determine the ecological consequences of predator loss and mismatch on community dynamics and ecosystem functioning.

Effects of multiple predators on predation risk is altered by temperature

Multiple parasitoid effects on host suppression were consistently altered by warming in the experiment presented in **Chapter 4**. Indeed, despite the general trend of decreased parasitism rates with warming reviewed in **Chapter 1** and observed in the experiment from **Chapter 3**, and in another laboratory experiment on our system not presented in this thesis (Pardikes et al. 2021), multiple parasitoids caused higher host mortality than expected at elevated temperature in **Chapter 4**. Thus, despite that warming weakened trophic interactions and decrease parasitoid perfomance (**Chapter 3 and 4**), non-trophic interaction strength among natural enemies also seemed to decrease (**Chapter 4**). Another experimental study on an aquatic food web found an increase in trophic interaction strengths with warming, while intra- and interspecific interference among predators also weakened (Sentis et al. 2017). Despite opposite trends for trophic interaction strength across these systems, those findings and those of a few other studies (Barton and Schmitz 2009, Drieu and Rusch 2017, Cuthbert et al. 2021) highlight the need to consider effects of environmental factors on non-trophic interactions to accurately predict effects of global change on community dynamics and stability. This study is the first, to me knowledge, to demonstrate the temperature-dependence of multiple predator effects for prey suppression across several species assemblages. These results have particularly relevant implication for the fate of top-down control in natural communities where a multitude of natural enemies interact (Frost et al. 2016). With global changes, this important ecosystem function is likely to be altered directly by a decrease in trophic interaction strength with elevated temperature, and indirectly through changes in non-trophic interaction strengths among predators.

Direct and indirect effects of warming on communities

With **Chapters 2 and 4**, I have shown the interplay between abiotic and biotic drivers to influence the strength of species interactions. However, **Chapter 3** did not uncover an interactive effect between abiotic and biotic factors for the structure of our host community. Indeed, the response of the host community to warming was primarily driven by species sensitivity to temperature, rather than indirect effects through changes in biotic interactions. Parasitism rate consistently decreased with warming, and abundances of all host species were thus affected equally. Differences in their relative abundances depended solely on their thermal performance: with an increase in mean temperature, the species showing the highest performance at high temperature became the most abundant ones. Which species is dominating the community is therefore determined by species performance at a given temperature (Davis et al. 1998).

Chapter 3 showed that temperature can have a more direct effect on ecological communities than previously thought through its direct effect on individuals. However, my experimental design did not allow for variations in parasitoid density and diversity, and these results are therefore valid if we partition out the effects of co-occurring species on how species interactions respond to warming. However, as shown throughout the thesis, temperature strongly impacts parasitoid performance and the strength of non-trophic interactions among them. It is therefore expected that warming will also have cascading effects on host communities through parasitoid response to elevated temperatures. Moreover, changes in host relative abundances can lead to parasitoid species going extinct in the long term (Sanders et al. 2013), with further cascading extinctions at all trophic levels. Furthermore, in natural settings, natural enemies play an essential role in determining which prey will dominate the community through apparent competition (Fleury et al. 2004). A decrease in trophic interaction strength with warming (**Chapters 1 and 3**) would thus likely imply changes in the structure of host community in the longer-term. We could speculate that after several generations, the most abundant host species would be attacked the most via frequency-dependent effects, aggregative behavior, and parasitoid learning (Bonsall and Hassell 1999, van Veen et al. 2005, Ishii and Shimada 2012). I demonstrated the context-dependence of species interactions, and it would be surprising to not see such effects reflected in community structure.

FUTURE DIRECTIONS

It has become increasingly clear that we cannot accurately predict species' responses to global change without considering the joint effects on co-occurring species and their interactions, and in turn how effects of the biotic environment buffer or amplify how species and interactions respond to environmental changes. From my dissertation, we have learned that the general effects of multiple parasitoids on host suppression are altered by temperature. We have also seen how important the identity of co-occurring species is to accurately predict the effects of environmental changes on species interactions. Overall, this work adds to the growing body of evidence on the context-dependence of species interactions, and on the interplay between the abiotic and biotic contexts. Future studies on other systems should tell us which aspects of community response to global changes are general, and which aspects are species and system-specific.

Accurately forecasting how a whole ecosystem will respond to environmental changes is challenging as species responses are often asynchronous. The ecological context is important for the outcome of species interactions, and lies at the heart of reliably predicting community response to environmental changes. The development of molecular tools such as DNA barcoding has allowed us to easily sample and quantify trophic links (Hrček et al. 2011, Wirta et al. 2014), and could help us obtain a better picture of food web structure through time and space. However, in host-parasitoid networks the observed links from molecular methods do not necessarily inform us on the outcome of the interactions, which is crucial if we are to study the dynamics of these communities. Rearing is necessary to get an accurate knowledge of the realized trophic links in host-parasitoid networks. This is thus a unique system to understand what mechanisms constraint species' niches, and hence structure communities, by comparing results from these different methods. In addition to observational field studies on large networks, laboratory experiments on a smaller set of interacting species, such as the work presented in this thesis, are necessary to explicitly depict which aspects of environmental changes affect species interactions, and community structure and dynamics.

To predict the ecological consequences of global environmental changes, ecologists, myself included, tend to focus on the processes impacting communities of interacting species, while evolutionary biologists focus on the processes generating differences among populations and genotypes. Crucially, these studies taken in combination should enable us to predict and prepare for the disruption of ecological communities and ecosystem functioning due to anthropogenic perturbations more accurately.

References

Barton, B. T., and O. J. Schmitz. 2009. Experimental warming transforms multiple predator effects in a grassland food web. Ecology Letters 12:1317–1325.

Bonsall, M. B., and M. P. Hassell. 1999. Parasitoid-mediated effects: apparent competition and the persistence of host–parasitoid assemblages. Researches on Population Ecology 41:59–68.

Burkle, L. A., J. C. Marlin, and T. M. Knight. 2013. Plant-pollinator interactions over 120 years: Loss of species, co-occurrence, and function. Science 340:1611–1615.

Cebolla, R., A. Urbaneja, J. van Baaren, and A. Tena. 2018. Negative effect of global warming on biological control is mitigated by direct competition between sympatric parasitoids. Biological Control 122:60–66.

Chamberlain, S. A., J. L. Bronstein, and J. A. Rudgers. 2014. How context dependent are species interactions? Ecology Letters 17:881–890.

Cuthbert, R. N., R. J. Wasserman, T. Dalu, and E. Briski. 2021. Warming mediates intraspecific multiple predator effects from an invasive crustacean. Marine Biology 168:35.

Davis, A. J., J. H. Lawton, B. Shorrocks, and L. S. Jenkinson. 1998. Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. Journal of Animal Ecology 67:600–612.

Drieu, R., and A. Rusch. 2017. Conserving species-rich predator assemblages strengthens natural pest control in a climate warming context. Agricultural and Forest Entomology 19:52–59.

Fleury, F., N. Ris, R. Allemand, P. Fouillet, Y. Carton, and M. Boulétreau. 2004. Ecological and genetic interactions in Drosophila-parasitoids communities: a case study with *D. melanogaster*, *D. simulans* and their common *Leptopilina* parasitoids in south-eastern France. Pages 181–194 *in* P. Capy, P. Gibert, and I. Boussy, editors. Drosophila melanogaster, Drosophila simulans: So Similar, So Different. Springer Netherlands.

Frost, C. M., G. Peralta, T. A. Rand, R. K. Didham, A. Varsani, and J. M.

Tylianakis. 2016. Apparent competition drives community-wide parasitism rates and changes in host abundance across ecosystem boundaries. Nature Communications 7:12644.

Hrček, J., S. E. Miller, D. L. J. Quicke, and M. A. Smith. 2011. Molecular detection of trophic links in a complex insect host–parasitoid food web. Molecular Ecology Resources 11:786–794.

Ishii, Y., and M. Shimada. 2012. Learning predator promotes coexistence of prey species in host–parasitoid systems. Proceedings of the National Academy of Sciences 109:5116–5120.

Jeffs, C. T., J. C. D. Terry, M. Higgie, A. Jandová, H. Konvičková, J. J. Brown, C. H. Lue, M. Schiffer, E. K. O'Brien, J. Bridle, J. Hrček, and O. T. Lewis. 2021. Molecular analyses reveal consistent food web structure with elevation in rainforest *Drosophila* - parasitoid communities. Ecography 44:403–413.

Lang, B., B. C. Rall, and U. Brose. 2012. Warming effects on consumption and intraspecific interference competition depend on predator metabolism. Journal of Animal Ecology 81:516–523.

Letourneau, D. K., J. A. Jedlicka, S. G. Bothwell, and C. R. Moreno. 2009. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 40:573–592.

Myers, J. H., C. Higgins, and E. Kovacs. 1989. How many insect species are necessary for the biological control of insects? Environmental Entomology 18:541–547.

Pardikes, N., T. Revilla, C.-H. Lue, Thierry Mélanie, D. Soutos-Villaros, and J. Hrcek. 2021. Community context modifies response of host-parasitoid interactions to phenological mismatch under warming. Authorea.

Sanders, D., R. Kehoe, and F. J. F. van Veen. 2015. Experimental evidence for the population-dynamic mechanisms underlying extinction cascades of carnivores. Current Biology 25:3106–3109.

Sanders, D., L. Sutter, and F. J. F. van Veen. 2013. The loss of indirect interactions leads to cascading extinctions of carnivores. Ecology Letters 16:664–669.

Sentis, A., C. Gémard, B. Jaugeon, and D. S. Boukal. 2017. Predator diversity and environmental change modify the strengths of trophic and nontrophic interactions. Global Change Biology 23:2629–2640.

van Veen, F. J. F., P. D. van Holland, and H. C. J. Godfray. 2005. Stable coexistence in insect communities due to density- and trait-mediated indirect effects. Ecology 86:3182–3189.

Wirta, H. K., P. D. N. Hebert, R. Kaartinen, S. W. Prosser, G. Várkonyi, and T. Roslin. 2014. Complementary molecular information changes our perception of food web structure. Proceedings of the National Academy of Sciences 111:1885–1890.

Curriculum Vitae

Mélanie THIERRY

Email: melanie.thierry34@gmail.com
Date and place of birth: 6th November 1991 in Montpellier, France
Nationality: French

CURRENT POSITION

2017 - current PhD candidate at the University of South Bohemia and Biology Centre of the Czech Academy of Sciences, Czech Republic: *Mechanisms structuring host-parasitoid communities in a global warming perspective*. (Supervisors: Dr Jan Hrček, Biology Centre CAS and Pr. Owen Lewis, University of Oxford).

2020 – current Data manager for the LifeWebs project (host-parasitoid interaction data).

PROFESSIONAL EXPERIENCES

2020 Erasmus traineeship (2 months) at the German Centre for Integrative Biodiversity Research, Germany: *Stability-complexity domain of host-parasitoid networks.* (Collaborators: Prof. Ulrich Brose, Dr Benoit Gauzens, and Dr Benjamin Rosenbaum)
2017 - 2018 Field work (7 months): Collection of live insects in Australia to establish our *Drosophila* -parasitoid system. (Collaborator: Dr Megan Higgie, James Cook University, Australia)

2016 - 2017 Civic volunteer service (6 months) at Estuaire, France: *Dragonflies as indicators of wetlands quality* and collection of data for "L'Observatoire des vers luisants" and "L'Observatoire des Bourdons".

2016 Volunteer project (4 months) at Cloudbridge Natural Reserve, Costa Rica: *Effect of reforestation on bird communities in cloud forests.*

2015 MSc thesis at the French National Institute for Agricultural Research (INRA), France: *Effect of local and landscape factors on butterfly communities.* (DIVA 3 Levana project) (Supervisors: Dr Marie-Lise Benot, Bordeaux University and Dr Inge Van Halder, INRA)

2014 Volunteer mission (2 months) at Archelon, Greece: Monitoring of sea turtle population and public awareness.

2014 MSc project at Nature Midi-Pyrénées, France: *Creation of an identification key for the ladybugs.* (Supervisor: Pierre-Olivier Cochard)

2013 Volunteer internship (1 month) at the French National Hunting and Wildlife Agency, France: *Monitoring of the Caroux Espinouse's Bighorn sheep population.*

2012 Volunteer internship (2 months) at the French National Center for Scientific Research of Moulis (CNRS), France: *Monitoring of viviparous lizard populations and their dispersal.* (Supervisor: Dr Virginie Stevens)

EDUCATION

2015 MSc in Ecology and Biodiversity Management, Aix-Marseille University, France

2013 BSc in Ecology and Biology of Organisms, Montpellier 2 University, France

2013 CREPUQ exchange, Bishop's University, Canada

RESEARCH GRANTS & FELLOWSHIPS

2021 IBERA from the Czech Academy of Sciences (41,105 CZK)

2020 Erasmus traineeship fellowship (1,724 EUR)

2019 Principal investigator on GAJU grant n°04-134/2019/P: *Is the stabilizing effect of parasitoids on host-parasitoid networks reduced under global warming scenarios?* (Budget awarded: 129,000 CZK, 1-year-project)

RESEARCH OUTPUT

As of July 2021: IF = 3 and citations = 39

Peer-reviewed publications

Thierry M., Pardikes N., Lue C-H., Lewis O. & Hrček J. (2021) Experimental warming influences species abundances in a Drosophila host community through direct effects on species performance rather than altered competition and parasitism. *Plos One* DOI: **10.1371/journal.pone.0245029**.

Thierry M., Hrček J. & Lewis O. (2019) Mechanisms structuring host-parasitoid networks in a global warming context: a review. *Ecological Entomology*. DOI: **10.1111/een.12750**

Lue C-H., Buffington M., ..., **Thierry M.**, ... & Hrček J. (2021) DROP: Molecular voucher database for identification of Drosophila parasitoids. *Molecular Ecology Resources*. DOI: **10.1111/1755-0998.13435**

Van Halder I., **Thierry M.**, ... & Benot M. L. (2017) Trait-driven responses of grassland butterflies to habitat quality and matrix composition in mosaic agricultural landscapes. *Insect Conservation and Diversity*. DOI: **10.1111/icad.12200**

Publications submitted and in preparation

Thierry M., Pardikes N., Ximénez-Embùn M.G., Proudhom G., & Hrček J. (submitted) Multiple parasitoid species enhance top-down control, but parasitoid performance is context-dependent. Submitted in *Ecology*.

Thierry M., Pardikes N., Rosenbaum B., Ximénez-Embùn M.G., & Hrček J. (in prep.) Warming decreases host survival with multiple parasitoids, but parasitoid performance also decreases.

Thierry M., Gauzens B., Rosenbaum B., & Brose U. (in prep.) Complexity-stability domain of host-parasitoid networks.

Pardikes N.A., Revilla T., Lue C-H., **Thierry M.**, Souto D. & Hrček J. (submitted) Community context modifies response of host-parasitoid interactions to phenological mismatch under warming. Submitted in *Ecology Letters*. Preprint DOI: **10.22541/au.162454818.82806593**

Mugnai M., Trindade D.P.F., **Thierry M.**, Kaushik K., Hrček J. & Götzenberger L. (submitted) Environment and geographic distance drive community assembly of Atlantic European grasslands: insights from integrating taxonomic, functional and phylogenetic diversity. Submitted in *Journal of Biogeography*.

Lue C-H., Pardikes N., **Thierry M.**, Staniczenko P. & Hrcek J. (in prep.) Warming decreases the niche breadth of *Drosophila* parasitoids – Results from experiments on a host-parasitoid community.

Other publications

Mercier O., … **Thierry M.**, … Leriche, A. & Million A. (2017) Étude pilote pour la mise en place d'un protocole de suivi du Phyllodactyle d'Europe (Euleptes europaea) sur l'île de Port-Cros (Var, France). *Trav. Scient. P. N. Port-Cros*, 31: 189-211.

Thierry M. (2017) Les coccinelles de Midi-Pyrénées: approche des Chilocorinae, Coccinellinae et Epilachninae.

Participation at international conferences

2021 Entomological Society of America International Branch Virtual Symposium: *Beyond pairwise interactions: isolating the direct and indirect interactions that structure a host-parasitoid community.* (ePoster)

2019 4th Symposium on Ecological Networks (Paris, France): *Parasitism decreases with increased temperature and structures host-parasitoid networks through host preference and competition between parasitoids.* (talk)

SERVICES

Peer reviewer

- Scientific Reports (1)
- Biotropica (2)
- Population Ecology (1)

LANGUAGES

French (native)

English (TOEFL 2019, C1)

Spanish (B2), **Czech** (A1), and **German** (A1)

© for non-published parts Mélanie Thierry

e-mail: melanie.thierry34@gmail.com

Mechanisms structuring host-parasitoid communities in a global warming perspective
Ph.D. Thesis Series 2021, No. 14.

All rights reserved
For non-commercial use only

Printed in the Czech Republic by Typodesign
Edition of 15 copies

University of South Bohemia in České Budějovice
Faculty of Science
Branišovská 1760
CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 776 201
www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz