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

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Running Head: Trophic interactions depend on context

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Abstract. Ecological communities are composed of a multitude of interacting species, forming complex networks of interactions. Current global changes are altering community composition and, as such, we need to understand if the mechanisms structuring species interactions are consistent across different species compositions. However, it is a challenge to identify 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 (i.e., host-parasitoid, exploitative competition, alternative host, and combined exploitative competition and alternative host modules) to measure the influence of structure and identity on the outcome of host-parasitoid interactions. In a laboratory experiment, we produced nine different species assemblage combinations per community module using a pool of three *Drosophila* host and three larval parasitoid species. We found that multiple parasitoid species enhanced host suppression due to sampling effect and weaker interspecific than intraspecific competition between parasitoids. 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 on parasitoid performance were species-specific and dependent on the identity of co-occurring species. Consequently, multiple parasitoid species generally strengthen top down-control, but performance of the parasitoids depends on the identity of either the co-occurring parasitoid species, the alternative host species, both, or none. This implies that effects of co-occurring species should be taken into account for understanding ecosystem functioning and services. Understanding the effects of the biotic context on species interactions is essential when developing biological control strategies, especially in open agricultural systems.

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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). Effects of the biotic context on species interactions is particularly relevant to predicting natural pest control. Moreover, in response to environmental changes, such as climate warming, species ranges and phenologies are shifting (Parmesan and Yohe 2003). Differences in the sensitivity and responsiveness to these changes among species are disrupting historical patterns of interactions and co-occurrences, creating communities with novel species compositions (Alexander et al. 2015). To accurately predict top-down control and forecast the ecological consequences of changes in the biotic environment induced by global changes, we need to determine which aspects of species interactions are primarily driven by community structure, and which are driven by species identity.

Trophic (i.e., direct predation) and non-trophic (e.g., competition, facilitation) interactions act in combinations to shape communities (Thierry et al. 2019), and their dynamics (Kawatsu et al. 2021). For example, 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). Top-down control might be driven by a single influential predator species independently of predator diversity (Letourneau et al. 2009), be enhanced with multiple predator species (i.e., risk enhancement for the prey) if predators show some degree of niche differentiation (Greenop et al. 2018), or be weakened when multiple predator species are present (i.e., risk reduction for the prey)

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due to exploitative competition, interference or intraguild predation (Sih et al. 1998). Most studies looking at complex networks are observational (e.g., Tylianakis et al. 2007, Jeffs et al. 2021), and are typically unable to disentangle the potential mechanisms driving species interactions described above. Experimental systems are thus needed to separate 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 include pairwise predator-prey (direct interactions), two prey species sharing a common natural enemy (indirect interactions; i.e., apparent competition or mutualism; hereafter alternative host module), or two predator species attacking the same prey species (either direct or indirect interactions; 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 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

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depending on the aphid species considered, but did not vary species composition in the multiple predator treatment. Woodcock and Heard (2011) showed that risk of predation for planthoppers was reduced in systems where predators shared the same habitat domain, but did not manipulate the herbivore community. We need studies manipulating species assemblages at both trophic levels to understanding how the identity of co-occurring species affects the outcome of consumer-resource interactions.

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, ultimately 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 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). Under laboratory conditions, we reproduced isolated subsets of this community to look at direct and indirect interactions within host-parasitoid communities. We aimed to uncover general effects of community modules in our *Drosophila*-parasitoid system across different species assemblages, 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, alternative host module, and combined exploitative competition and alternative host module). We also explored the mechanisms behind the effects of community structure and composition on host-parasitoid interactions and top-down control on the hosts. Specifically, we tested the following hypotheses: (i) host suppression will be

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higher with the presence of multiple parasitoid species (i.e., exploitative competition module), due to an increased chance that a more efficient parasitoid species will be present (i.e., sampling effect) (Pedersen and Mills 2004, Letourneau et al. 2009). However, (ii) parasitoid performance might decrease with the presence of multiple parasitoid species due to increased larval competition in multiparasitism events (Harvey et al. 2013). (iii) Interactions between a focal host-parasitoid pair will weaken with the presence of an alternative host because of trait- and density- mediated effects (Werner and Peacor 2003). (iv) Combined effects of exploitative competition among parasitoids and alternative host species in the four-species module will differ from three-species modules depending on the identity of the co-occurring species (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,

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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 two-days-old larvae of *Drosophila melanogaster*. This host species was not used in the experiment, thus avoiding potential bias due to maternal effects.

Experimental design

To investigate the effects of community structure and species composition on host-parasitoid interactions, we used four community modules, and nine different species assemblages each (six host and six parasitoid assemblages, from the pool of three host species and three parasitoid species) (Figure 1). Each unique treatment was replicated six times. 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). Host and parasitoid densities were kept constant across treatments (i.e., substitutive design; e.g., Snyder et al. 2008). We also included control treatments for each host species to acquire baseline levels of survival in the absence of parasitoids (replicated eight times each).

To initiate the experiment, twenty-five eggs of each host species were placed in a single 90 mm high and 28 mm diameter glass vial with 10mL of food media. To collect *Drosophila* eggs, an egg-

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wash protocol was developed based on Nouhaud et al. 2018). One 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 emergences until the last individual emerged (up to 41 days for the species with the longest developmental time). We stopped collecting host emerges after five 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).

To explore parasitism strength depending on host density, we estimated parasitoid functional responses for each of the three parasitoid species on each of the three host species (nine host-parasitoid combinations). An additional 14,760 host eggs were collected for this experiment across 432 experimental vials. The method used to set the experiment was the same as described above, and both experiments were run in parallel. The detailed method is presented in Appendix S2, and the parasitoid functional responses are presented in Figure S2. The empirical results for the host

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density at 25 eggs per 10mL of food media also served to measure degree of infestation and successful parasitism rate for each of the nine host-parasitoid pairs (Appendix S3 Figure S3).

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, data were modeled using a beta binomial error distribution and a logit link function using the *glmmTMB* function from the *TMB* package (Lüdecke et al. 2019). We compared the AIC of models with or without zero inflation. We chose the models with the smaller AIC, which were the

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zero-inflated models. 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 alternative host 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 and Box ID as a random factor as previously described, 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 competition and alternative host), 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

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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).

To further investigate emergent effects of parasitoid diversity on host suppression, we compared the observed and expected effect magnitude of multiple parasitoid species on host suppression for each host species (n = 3), each heterospecific parasitoid combination (n = 3), and each replicate (n = 6), following the framework proposed by Schmitz (2007). Observed values smaller than estimated translate to risk enhancement for the host with multiple parasitoid species, while observed values bigger than estimated reflect risk reduction. Detailed methods and results are presented in Appendix S4. 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 hosts being parasitized (DI) by 48% (95% 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 competition and alternative host module/pairwise interaction = 1.32, P = 0.376). Moreover, effects of multiple parasitoid species on host suppression were greater than expected in four host-parasitoid cases (Figure S4). However, DI did not significantly change with the presence of an alternative host species ($\chi2_{(1)} = 0.56$, P = 0.452; OR alternative host module/pairwise interaction = 0.96, P = 0.984), and the two-way interaction between alternative hosts and exploitative competition treatments had no significant effect (community module model: $\chi2_{(1)} = 0.22$, P = 0.638) (Figure 2a).

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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 S5 and Appendix S5).

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 competition and alternative host treatments had a significant effect on SP for one out of the nine host-parasitoid pairs (*Ganaspis sp*. on *D. simulans*). Successful parasitism rate of two other host-parasitoid pairs significantly decreased with exploitative competition between parasitoid species (*Ganaspis sp*. on *D. birchii* and on *D. pallidifrons*), while SP significantly increased with the presence of an alternative host species in *Asobara sp*. on *D. simulans*. Successful parasitism rate for the rest of the host-parasitoid pairs did not significantly change 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 terms 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.*

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simulans. Effects of species assemblages on SP for each host-parasitoid pair are summarized in Table 2 and Figure 3, and presented in Appendix S6.

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 due to a combination of sampling effect and resource partitioning between parasitoid species. 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. These species-specific effects on parasitoid performance have important implications for biological control programs that often overlook effects of co-occurring species on biological control agent efficiency to suppress pests.

Positive effects of consumer diversity on top-down control

The presence of multiple parasitoid species generally increased host suppression (community module model). We found a greater top-down control than expected with multiple parasitoid species for four out of the nine species combinations, suggesting weaker interference between parasitoids heterospecific than between conspecific parasitoids (Snyder et al. 2008, Snyder and Tylianakis 2012). Weak predator interference can be obtained if predators show some degree of resource partitioning (Pedersen and Mills 2004), which have been reported in several experimental studies (reviewed in Letourneau et al. 2009). Resource partitioning can be more important than diversity *per se* to explain an increase in top-down control with multiple predator species (Finke and Snyder 2008). The positive effects of parasitoid diversity on host suppression were also driven

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by the presence of the most efficient parasitoid species for the focal host (e.g., presence of *Ganaspis sp*. for *D. simulans*: Figure S3a and Figure S5c), which could explain why there were no significant differences between observed and expected effect magnitude of multiple parasitoid species in some cases. 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). Here, both the sampling effect and resource partitioning seem to be in play to explain the increase of host suppression with increase in parasitoid diversity. Our results demonstrating the enhancement of top-down control with multiple parasitoid species across several species assemblages, in addition to the results from previous studies (reviewed in Letourneau et al. 2009), highlight the importance of preserving predator diversity for ecosystem functioning.

No indirect interactions 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. This suggests that host switching behaviors and any indirect interactions between host species were absent 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 effects of an alternative host species (Jones et al. 2009). Furthermore, frequency-dependent attack rates, in which a predator switches between two

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prey species depending on their relative densities (Bonsall and Hassell 1999) 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., alternative host module and both exploitative competition and alternative host module) on host-parasitoid interactions were tested over a single generation and with constant host density. Experimental studies manipulating alternative prey density over several generations are needed to detect indirect interactions between host species. However, we could expect that an experiment performed on our study system over multiple generations would have shown effects of an alternative host species on host suppression due to changes in host relative abundances. Indeed, we found that the strength of parasitism for our nine host-parasitoid pairs increased logistically with host density (i.e., type II parasitoid functional responses; Appendix S2 and Figure S2). Beyond a host density threshold, parasitoids cannot attack more hosts in a given time period. This threshold changes depending on both host and parasitoid identity (Figure S2), meaning that the number of hosts attacked for each species will change depending on host relative abundances and the identity of co-occurring 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 that variation in directionality and magnitude of the effects of a particular community

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module on host-parasitoid interactions depend on the species assemblage considered. This is particularly relevant to predicting natural top-down control, and the consequences of climate change driven shifts in community composition for ecosystem functioning. Potential effects of co-occurring species (alternative prey species and/or other predators) should be taken into consideration when developing biological control strategies, especially when choosing the biological control agent in open agricultural system. For example, presence of non-target species might weaken the biological control agent-pest interaction (Wajnberg et al. 2001). Even with no direct effects of presence of a non-target species, presence of a natural enemy to the alternative prey species could decrease the biological control agent performance, and prevent its establishment in the community. However, the differences between the number of hosts suppressed and the outcome for the parasitoid populations depending on the biotic context might be a unique feature of host-parasitoid systems. It remains to be determined if the identity of co-occurring species is as important in prey-predator systems where the death of the prey generally results in energy flow to the predators.

Successful parasitism rate increased in modules with a parasitoid competitor compared to the pairwise interaction in six species combinations (out of the 54 species combinations representing host-parasitoid pairs in modules with exploitative competition between parasitoid species), 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 (Poelman et al. 2014). Our case seems to be different because successful parasitism rates did not increase for both parties, which implies competitive interactions between parasitoids. Parasitoids can compete both as adults for oviposition (i.e., extrinsic competition or interference) and as larvae

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within a host (i.e., intrinsic competition) (Harvey et al. 2013). Extrinsic competition has 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). In our study, modules with the pairwise interaction in isolation had two parasitoid conspecifics (i.e., substitutive design). Our results suggest that in these six cases, where parasitoid performance increased with the presence of a competitor, interspecific extrinsic competition between parasitoids was weaker than intraspecific extrinsic competition. On another hand, intrinsic competition is the result of a super- or multi-parasitism event when two parasitoids (conspecifics or heterospecific, respectively) parasitize the same host individual. It is usually detrimental for the host survival, and would explain an increase in host suppression with multiple parasitoid species despite a decrease in parasitoid performance in some cases (i.e., unsuccessful parasitism but with host suppression; e.g., Thierry et al. 2021b).

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). For example, *D. simulans* often encapsulates parasitoid eggs, while encapsulation rates of *D. birchii* are low in our system. *Asobara sp*. rarely lay more than one egg in a same host individual but develop fast to avoid encapsulation, while super-parasitism is common for *Ganaspis sp.* and could be a strategy of this parasitoid to overcome host immune response. 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).

Conclusion

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

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interactions 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), 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. 2021a), 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).

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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.

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Table 1. Odds ratios of a successful parasitism in each community module (exploitative competition, alternative host, and both exploitative competition and alternative host) 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. Odds Ratios are presented with the significance of the effect: (***) P < 0.001, (**) P < 0.01, (*) P < 0.05, (ns) P > 0.05.

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 of successful parasitism 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. χ^2 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	***
Parasitoid 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 residuals		60		66		60		60		60		66		59		59		60	

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Figure legends

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) alternative host module (two host and one parasitoid species) and, d) both exploitative competition and alternative host 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) indirect interactions between hosts, and d) potential for all the above). Direct interaction between host species were not allowed. See (Thierry et al. 2019)(Thierry et al. 2019)(Thierry et al. 2019)(Thierry et al. 2019)(Thierry et al. 2019)(Thierry et al. 2019) for a detailed description of each interaction type

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

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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

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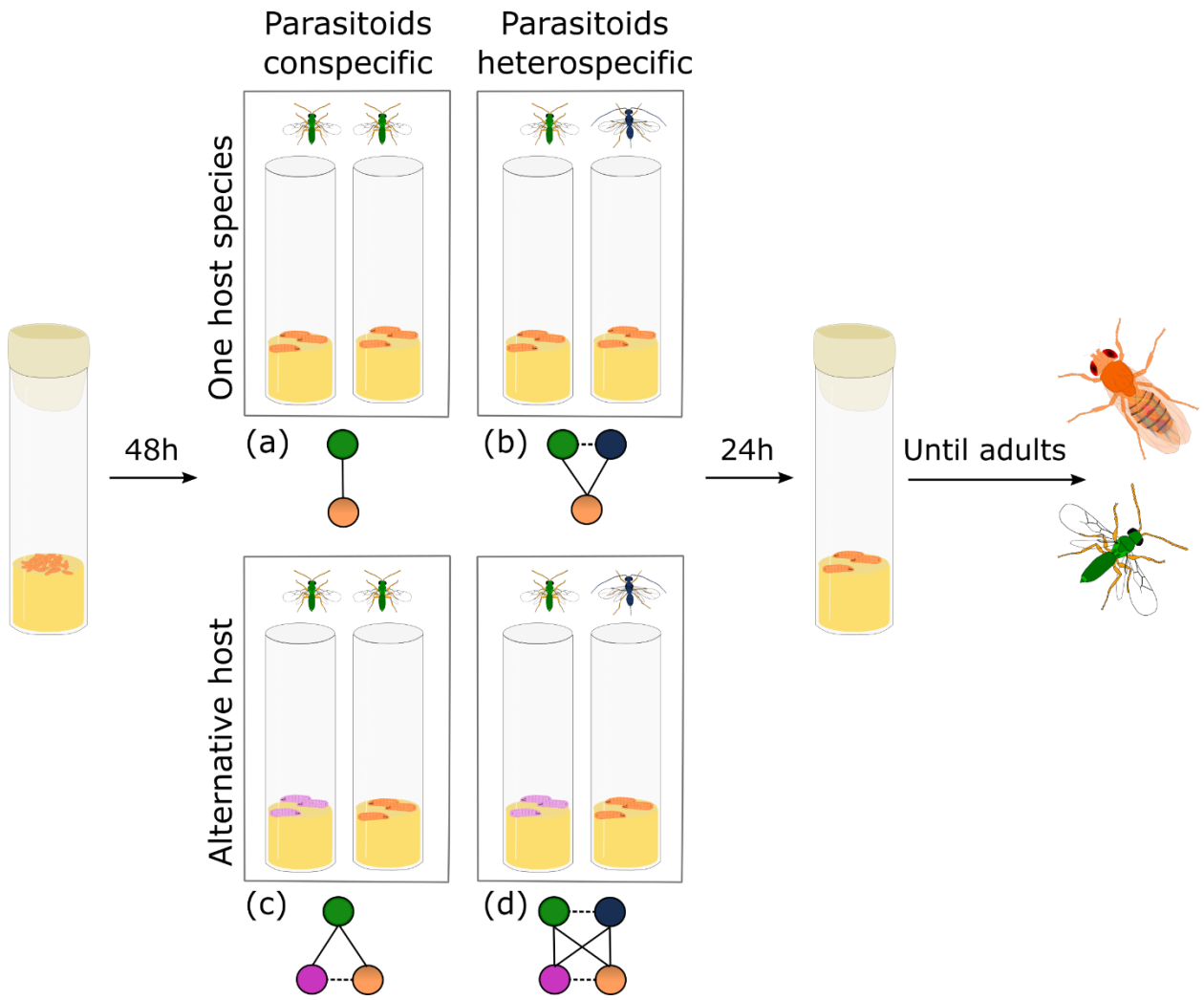

Figure 1.

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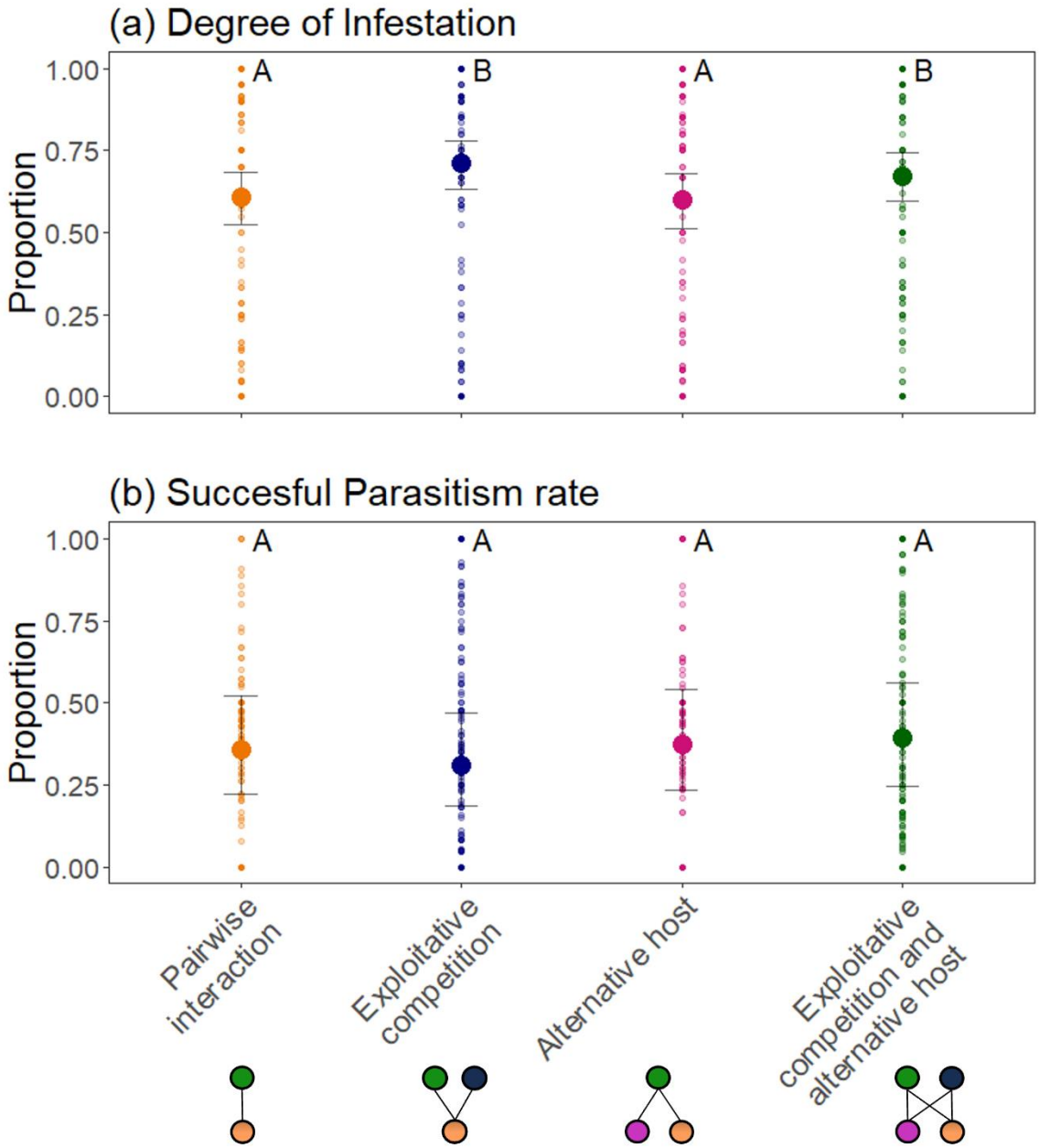

Figure 2.

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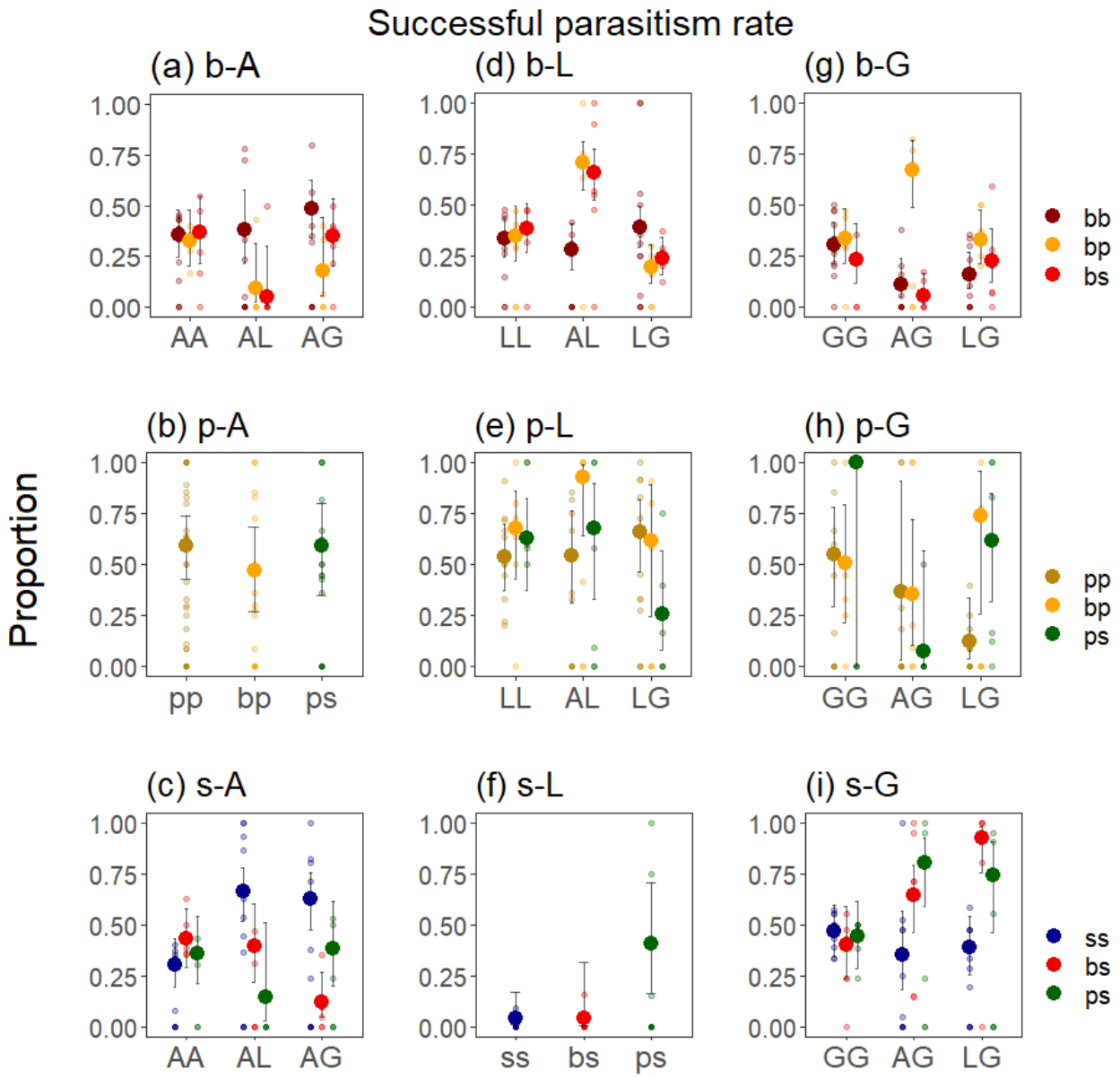

Figure 3.