

Community context modifies response of host-parasitoid interactions to phenological mismatch under warming

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Abstract

Climate change is altering the relative timing of species interactions by shifting when species appear in a community and by accelerating developmental rates. However, phenological shifts may be mediated through community contexts, such as intraspecific competition and alternative resource species, which can prolong the otherwise shortened windows of availability. Using a combination of laboratory experiments and dynamic simulations, we quantified how the effects of phenological shifts in *Drosophila*-parasitoid interactions differed with concurrent changes in temperature, intraspecific competition, and the presence of alternative host species. We found that community context, particularly the presence of alternative host species, supported interaction persistence across a wider range of phenological shifts than pairwise interactions. Parasitism rates declined under warming, which limited the ability of community contexts to manage mismatched interactions. These results demonstrate that ongoing declines in insect diversity may exacerbate the effects of phenological shifts in ecological communities under future global warming temperatures.

Introduction

The activities of interacting species are often coordinated in time and any shifts in the timing of ontogenetic or phenological events are hypothesized to cause fitness declines in interacting species, affecting population and community dynamics (Cushing 1990; Miller-Rushing *et al.* 2010; Yang & Rudolf 2010). Evidence is mounting that climate change is altering the timing of environmental cues (e.g., temperature and precipitation) that species use to initiate key life history events, such as seasonal emergence or activity (Parmesan & Yohe 2003; Forrest 2016). Furthermore, differential responses across species within the same community indicate that the temporal coordination of species interactions is changing, which raises concerns that interacting species are becoming temporally “mismatched” (Visser & Both 2005; Both *et al.* 2009; Kharouba *et al.* 2018). Such mismatches can lead to major changes in the outcome of species interactions and population dynamics, but our understanding of the mechanisms linking phenological asynchrony to their immediate and long-term consequences is unclear (Van der Putten *et al.* 2010).

The intricacies of how unequal shifts in emergence phenologies disrupt the timing of species interactions are critical in order to understand how climate change can decouple species interactions (Kerby *et al.* 2012; Johansson *et al.* 2015). For many species interactions the outcome depends, in part, on the developmental stages of interacting individuals. For instance, predators tend to attack specific developmental stages of their prey, and pollinators require plants to be in a certain flowering phase (Stacconi *et al.* 2015). Thus, the extent that interaction strength and resulting dynamics change with shifts in phenology depends on how much the outcome varies across developmental stages (e.g., the extent traits of individuals change

during development) and how long individuals remain available to interact (e.g., window of vulnerability) (Memmott *et al.* 2007; Miller & Rudolf 2011; Johansson *et al.* 2015). Besides contributing to shifts in phenologies, warmer temperatures also accelerate growth rates, which shortens the amount of time spent within each developmental stage (Kingsolver *et al.* 2011). This ultimately narrows and shifts the window of vulnerability for predator-prey interactions, or magnifies size differences in competitive interactions (Benrey & Denno 1997; Rudolf & Singh 2013), potentially exacerbating the effects of shifts in the emergence phenology of interacting species (but see (Tuda & Shimada 1995). For example, faster development rates of prey in warmer temperatures can result in natural enemies missing their window of opportunity to attack, even if the relative phenological relationship remains unchanged (Klapwijk *et al.* 2010; Ren *et al.* 2020). Thus, abiotic or biotic environmental conditions that change growth rates, or the temporal availability of resources are likely to modify how the strength of species interactions scales with phenological shifts, though few studies have quantified these effects.

Phenological shifts are taking place across a variety of community contexts, several of which are known to alter growth rates and the temporal availability of interacting species. For example, strength of intraspecific competition (e.g. resource limitation or host density) and presence of alternative resource species, can affect growth rates and temporal availability at a similar magnitude to changes attributed to global warming (Benrey & Denno 1997; Wolf *et al.* 2017). Development rates often slow down when levels of resource competition are high, prolonging the number of days which prey species remain vulnerable to attack (Barker & Podger 1970; Benrey & Denno 1997). Similarly, the presence of alternative resource species, with complementary development rates or phenologies, can help prolong the total period during which resources remain available (Wolf *et al.* 2017). However, most phenological studies have ignored community contexts beyond pairwise interactions, which does not reflect the reality that consumers typically utilize multiple resource taxa and that levels of resource competition can show high spatial and temporal variation (Nakazawa & Doi 2012; Revilla *et al.* 2014). Furthermore, ongoing global declines in insect diversity and abundance make these two aspects of community context particularly imperative to study (Forister *et al.* 2019; Salcido *et al.* 2020). Such community contexts may act as a buffer against severe changes in the strength of interactions and promote interaction persistence in the face of shifting phenologies (Yachi & Loreau 1999; Timberlake *et al.* 2019; Olliff-Yang *et al.* 2020), yet it's uncertain how these processes will act in future temperatures predicted by climate change. Therefore, there is a pressing need for studies that integrate relative interaction timing with concurrent shifts in temperature and community context.

Here, we use laboratory experiments (Fig. 1) and dynamic simulations to examine how warming and community context together modify the effects of phenological shifts on the strength and dynamics of host-parasitoid interactions. Parasitoids play an important role in determining host population dynamics and are commonly used as biological control agents, yet few studies have investigated the impacts of climate change on the relative timing of host-parasitoid interactions (Klapwijk *et al.* 2010; Dyer *et al.* 2013; Jeffs & Lewis 2013). Using a native *Drosophila* -parasitoid system from seasonal tropical forests of North Queensland, Australia (Jeffs *et al.* 2021), we experimentally delayed the emergence phenology of parasitoids relative to their hosts and assessed how resource competition and presence of an alternative host species modified the effect of delayed emergence in ambient (24°C) and predicted warming temperatures (28°C) (Shukla *et al.* 2019). It is important to note, that previous phenological studies have focused on how increased temperature acts as the environmental cue that drives shifts in emergence phenology among interacting species. However, elevated temperatures often persist throughout growing seasons. Thus, temperature is not only the cue; it is also the context in which mismatched ecological interactions must proceed. Therefore, we decoupled the effects of temperature and phenology to quantify the effects of different phenological shifts in current ambient and predicted warming temperatures. Additionally, we applied parameters derived from the single-generation experiment to simulate host-parasitoid population dynamics over 100 generations under our experimental conditions using an age-structured Nicholson-Bailey host-parasitoid model to examine their impacts on long-term persistence. Together, this allowed us to assess (1) how temperature alters the effects of phenological shifts on the outcome of host-parasitoid interactions, (2) to what extent resource limitation and (3) the presence of an alternative host species modifies effects of phenological shifts under warming, and (4) how

community contexts and temperature interact to affect long-term persistence of host-parasitoid interactions. Combining experimental and modeling approaches helped identify which conditions were favorable over a single generation, but detrimental for long-term persistence of host-parasitoid interactions. The results represent an important step toward understanding how warming and community context interact to modify the effects of phenological shifts on the strength and dynamics of species interactions, which is critical for predicting how ecological communities will respond to climate change.

Materials and Methods

Study system

Insects and their parasitoids are an excellent system for studying effects of climate change on species interactions (Hance *et al.* 2007; Jeffs & Lewis 2013). The strength of host-parasitoid interactions are predicted to be particularly sensitive to phenological shifts and global warming due to 1) age-related vulnerability of host to successful parasitism and 2) high thermal sensitivity given their obligate ties to host thermal performance (Tuda & Shimada 1995; Hance *et al.* 2007). Furthermore, the duration of host vulnerability and degree of phenological synchrony have been suggested to influence local stability within host-parasitoid systems. Stable host-parasitoid interactions require that not all hosts have been parasitized, so some degree of asynchrony may be adaptive (Singer & Parmesan 2010). Thus, precise timing is critical for these tightly co-evolved interactions and any shift in the optimal temporal overlap is hypothesized to have significant effects on long-term persistence (Tuda & Shimada 1995).

We used a native *Drosophila* -parasitoid system from the seasonal tropical forests of North Queensland, Australia. Two species of *Drosophila*, *D. sulfurigaster* and *D. birchii*, were collected from the field in March 2018 and maintained at the Czech Academy of Sciences (CAS) as a collection of isofemale lines (Shipped to the Czech Republic under permit no. PWS2016-AU-002018 from Australian Government, Department of the Environment). These species coexist across elevational gradients that span 900m at Kirrama (S18° 12.676' E145° 47.530') and Paluma (S19° 00.386' E146° 12.732') mountain ranges in Queensland, and are attacked by a shared suite of parasitoid wasp species (Jeffs *et al.* 2021). Individuals used in this experiment were collected as eggs from mass bred population cages, which originated from eight isofemale lines from each fly species respectively.

We used two hymenopteran parasitoid species, collected from the same tropical Australian locations: *Aso-bara* sp.1 (Braconidae: Alysiinae; strain KHB from site Kirrama, elevation 900m, reference voucher no. USNMENT01557097, reference sequence BOLD process ID: DROP043-21, and *Ganaspis* sp.1 (Figitidae: Eucolinae; strain 69B from site Kirrama, elevation 900, reference voucher no. USNMENT01557100, USNMENT01557297 reference sequence BOLD process ID: DROP164-21) (Lue *et al.* 2021). These species await description by taxonomists but are precisely identified above so that this study can be linked to species names once available. Each parasitoid species specializes on larval stages of *Drosophila* and are known to successfully attack *D. birchii* and *D. sulfurigaster*. All parasitoids were maintained on *D. melanogaster*, so no parasitoid used in this experiment had prior experience with *D. sulfurigaster* or *D. birchii*, and thus no acquired oviposition preference.

Experimental design

To examine how shifts in relative emergence time varied with simultaneous changes in temperature and community contexts, we experimentally delayed the emergence of parasitoids relative to the *Drosophila* larvae in current and expected warming temperatures, and in high and low levels of resource competition in a fully factorial design with 4 emergence times x 2 temperatures x 2 levels of competition. Additionally, the two host species were reared in isolation or with the other host species to examine effects of an alternative resource. Thus, we had a total of three *Drosophila* species combinations and two wasp species for a total of 96 unique treatments. Each treatment consisted of at least five replicates and initiated over the course of five days, which were represented as blocks in our statistical analyses.

We reared *Drosophila* larvae in current ambient ($22.9^{\circ}\text{C} \pm 0.47\text{SD}$ with $69\% \pm 4.34\text{SD}$ relative humidity) and

predicted warming ($27.4^{\circ}\text{C} \pm 1.15\text{SD}$ with $60\% \pm 10.1\text{SD}$ relative humidity) temperatures (climate change models predict a $1-6^{\circ}\text{C}$ increase in temperatures by 2070 in Australia (Hughes 2003)) in 12-hour light/12-hour dark photoperiod. Levels of intraspecific competition among *Drosophila* larvae were manipulated by providing 2ml or 20ml of fly medium. A total of 100 *Drosophila* eggs were added to all vials resulting in densities of 50 individuals/ml for the high competition, and 5 individuals/ml for the low competition treatments (Nouhaud *et al.* 2018). We decided to manipulate the volume of food instead of the abundance of larvae to avoid any frequency dependent parasitism effects. Thus, we established four temperature/competition treatments that varied host development rates: (1) slowest development (24°C with 2ml food); (2) slow development (24°C with 20ml food); (3) fast development (28°C with 2ml food); and (4) fastest development (28°C with 20ml of food). This process was repeated for both *Drosophila* species in isolation and when grown together for alternative host species treatments, where the 100 total eggs were composed of equal proportions of the two species (e.g., 50 eggs *D. sulfurigaster* and 50 eggs *D. birchii*).

We manipulated four levels of phenological relationships between host and parasitoids in 2-day intervals. The parasitoid either “emerged” at the same time (e.g., 0 day) as its host or 2-, 4-, or 6-days later. At each parasitoid emergence time, *Drosophila* larvae were exposed to three mated female and three male parasitoids of a single species of parasitoid. After 48-hours the parasitoids were removed from the vials and then maintained under the same temperature and light conditions described above. This allowed a more precise measurement of the vulnerability window and largely prevented the complete mortality of all *Drosophila* . To measure development times, emerges were recorded daily and stored in 95% ethanol until all hosts or parasitoids emerged (Fig. 1).

All experiments were set up in 2.8cm (diameter) x 9cm (height) glass vials, with a 4ml base layer of 1.5% agar gel in each vial to reduce excess desiccation of the fly medium and provide potential refuges from parasitoid attack. Control treatments consisted of ten unexposed vials for each host species combination ($n = 3$; *D. birchii*, *D. sulfurigaster*, *D. sulfurigaster* -*D. birchii*). These unexposed vials were used to calculate the average development time (egg-to-adult) and host survival in the absence of parasitoids across treatments and species.

Response variables

We counted the total number of adult *Drosophila* (d_i) and the number of adult parasitoids (p_i) that emerged from each vial. Proportion of host survival (HS) was estimated using $HS = d_i / T$, where T is the average abundance of flies that were reared in control treatments. In cases where $HS > 1$ (cases where more flies came out in the presence of parasitoids, compared to control treatments without parasitoid), we set $HS = 1$. Parasitism rate (PR) was estimated using $PR = p_i / T$. In cases where $PR > 1$ (cases where more parasitoids emerged than flies in control treatment), we set $PR = 1$. Both estimates were used to help account for differences in the number of *Drosophila* that a given temperature and competition treatment supported.

Statistical analysis

All statistical analyses were performed using R statistical software v. 3.6.0 (R Core Team 2014). We used Generalized Linear Mixed Models (GLMM) to analyze host development time, host survival (HS), and parasitism rate (PR) as response variables. Replicates were initiated over the course of five blocks of time; thus, we applied a mixed model approach, in which block was used as a random intercept. For host survival and parasitism rates, we included an observation level random effect (OLRE) to meet overdispersion and heteroscedasticity model assumptions. In all further analyses, the alternative host species treatment of *D. birchii* and *D. sulfurigaster* were combined to form its own unique species identification (*D. birchii*-*D. sulfurigaster*).

We modeled host development times using data from control vials (i.e., no parasitoids) only, with fixed factors of temperature, competition, and host species combination, and all potential interactions between the three factors. Log transformed development time data improved model fit and helped meet assumptions of normality. We modeled host survival and parasitism rates as a function of the cofactors using a Binomial GLMM with a logit link function. All statistical models included a three-way interaction between temperature, resource

competition, and phenological delay. We then compared models that incorporated interactions with host or parasitoid species and included all nested two-way interactions. We selected the model which minimized AICc using the *bblme* package in R (Bolker & Bolker 2019). We used the *DHARMA* (Hartig 2019) package to statistically test whether any assumptions of normality, non-constant error variance, and overdispersion were violated. *DHARMA* simulates scaled (quantile) residuals for mixed models and provides built-in tests to inspect model assumptions. Post-hoc multiple comparisons were performed using the *emmeans* package (Lenth 2019) and P-values were adjusted using the Tukey method when necessary. All figures were generated using the *ggplot2* R package (Wickham 2011).

Dynamic model and simulation

Host and parasitoid emergence counts were used to parameterize a stochastic age-structured Nicholson-Bailey model. We calculated the number of generations host and parasitoid populations persisted before going extinct and statistically compared which factors promoted the probability of host-parasitoid persistence. The methods used for developing the mathematical model, fitting with data, simulating host-parasitoid interactions across generations, and statistical analyses are described in **SI Materials and Methods**.

Results

Host development times and survival across temperatures and community contexts without parasitoids

In the absence of parasitoids, warming and competition levels altered mean host development times by up to four days (30%) (Fig. S1, Fig. S2). On average, shortening of developmental time due to warming was more pronounced (Mean = -2 ± 0.2 days) than its extension due to increased competition (Mean = $+1.2 \pm 0.2$ days); and the changes were consistent in direction for all species combinations (Table S1). Survival rates were always highest in 24°C and low competition treatments across all host species combinations, but species responses to warming and competition varied (Fig. S3, Table S2 & Table S3). For example, *D. birchii* survival (Post hoc odds ratio (OR) = 7.54, $P < 0.001$) declined significantly more in elevated temperatures than *D. sulfurigaster* (OR = 2.17, $P = 0.002$) (Fig. S3).

Effects of phenological shifts across temperatures

The effect of parasitoids on average host survival depended on the relative emergence time of parasitoids, and its interaction with warming (Fig. 2A, & Fig. S4B; Table S4; note that warming alone did not alter average host survival). Probability of host survival was at or near zero when host and parasitoids emerged simultaneously and increased significantly through the first four days of delay, followed by a plateau between four and six days ($P > 0.05$) of parasitoid delay (Fig. 2A). Increased development rates in elevated temperatures magnified differences in host survival across phenological delays for the first four days of phenological delays: the largest increase in host survival occurred between 2- and 4-days (OR = 22.36, $P < 0.001$) in ambient temperatures, compared to 0- and 2-days (OR = 31.11, $P < 0.001$) in warming (Fig. 2A). Interestingly, even with late phenological delays, parasitoids continued to inflict mortality on hosts, as rates of host survival rarely exceeded 80% of what was attained in trials without parasitoids.

Like host survival, parasitism rates differed significantly across phenological delays, with rates of parasitism declining as phenological delay increased ($P < 0.05$ for all phenological delays). Warming significantly reduced parasitism rates, but the decline in parasitism was not coupled with increased host survival. When emergence phenologies matched, host survival was at or near 0% in both temperature treatments, but rates of successful parasitism ultimately exceeded 50% in ambient temperature, compared to a maximum of 10% under warming. Consequently, even though hosts were developing faster in elevated temperatures, differences in parasitism rates across phenological delays were diminished (Fig. 2B & Fig. S4A; Table S5), since the chance of parasitism was already less than 5% with just a two-day delay. Together, these results suggest that warming reduced the effects of phenological shifts on parasitism rates due to significant declines in parasitoid performance and, to a lesser extent, because of increased development rates in hosts.

Modifying effects of competition across phenological shifts and temperature

The effects of temperature and relative emergence time on host survival presented above did not vary with levels of resource competition (Table S4). Alongside shifts in relative emergence time, resource competition had the strongest effect on host survival (Table S4), where hosts had a 22% (95% CI: 0.16, 0.30%) chance of survival at high levels of resource competition, compared to 42% (95% CI: 0.33, 0.51%) at low levels of competition (OR = 0.40, $P < 0.001$). Importantly, the relationship between phenological shift and survival varied significantly across competition treatments. For example, delaying parasitoid emergence by two days (relative to 0-day delay) increased survival by 9% in high competition compared to a 34% increase at low competition levels.

In contrast, the effects of temperature and phenological shifts on parasitism rates depended on levels of resource competition (Table S5). Parasitism rates became significantly greater in high competition treatments at the 2-day delay (OR = 2.17, $P = 0.032$) in elevated temperatures, compared to the 6-day delay in ambient temperatures (OR = 2.61, $P = 0.01$). Notably, competition was unable to increase parasitoid recruitment with delays longer than 2-days under warming. Thus, even though competition slowed development rates, including in higher temperatures, host species did not remain susceptible to parasitism across a broader range of phenological delays under warming. Together, we revealed that host survival was determined primarily by shifts in resource competition, while elevated temperatures were primarily responsible for difference in parasitism rates.

Effect of alternative host species across phenological shifts and temperature

On average, single-species treatments, with *D. sulfurigaster* (probability = 0.53 (95% CI: 0.43, 0.63)) and *D. birchii* (probability = 0.17 (95% CI: 0.12, 0.24)) had the highest and lowest probabilities of host survival respectively, while the treatment with both species present showed an intermediate probability of survival (Probability = 0.29 (95% CI: 0.22, 0.38)) (Fig. S5). Parasitism rates showed similar patterns across single (*D. sulfurigaster* probability = 0.03 (95% CI: 0.02, 0.05%) and *D. birchii* probability = 0.04 (95% CI: 0.03, 0.07)) and multiple host species combinations (*sulfurigaster-birchii* probability = 0.05 (95% CI: 0.03, 0.07)), with average parasitism rates being intermediate when multiple host species were present. While average host survival rates differed significantly among host species combinations ($P < 0.05$), average parasitism rates showed no differences. Thus, the average development time, host survival, and parasitism rates of the *sulfurigaster-birchii* combination were comparable to the pooled mean values of the two host species in isolation. However, the probability of host survival varied across host species combinations and this effect varied with temperature and phenological delay (Table S4; Fig. S5 & Fig S6). Interestingly, each species in isolation showed significant differences in survival across temperatures at each relative delay, but when an alternative host species was present, host survival rates did not significantly differ across temperatures at any relative delay ($P > 0.05$). This is likely due to opposing responses to phenological delay, temperatures, and resource competition between *D. birchii* and *D. sulfurigaster*. These results suggest that within a single generation the presence of alternative host species dampened differences in the probability of host survival and parasitism rates across both temperatures and levels of resource competition, but not phenological shifts.

Simulation results: changes in host-parasitoid persistence with phenological shifts, warming, and community context

The persistence of host-parasitoid interactions was less probable if parasitoids were perfectly synchronized with their hosts or if the shifts in relative emergence times became too delayed (e.g., delay 6-days) (Fig. 3). However, the effects of phenological delays on host-parasitoid persistence varied significantly with warming; that is, the probability of parasitoid persistence was significantly greater at the 4-day delay window in ambient temperatures (OR = 2093.8, $P < 0.001$), while a delay of 2-days resulted in the highest chance of persistence under warming (OR = 0.49, $P = 0.0013$). We also found that under simulated warming, the chances of parasitoid persistence were limited to a single delay window (2-day), while two windows of delay (2- and 4-day) indicated parasitoid persistence in 24°C, supporting predictions that accelerated development rates narrow the window of vulnerability in host-parasitoid interactions. Importantly, warming reduced the probability of host-parasitoid persistence, irrespective of phenological relationships (OR = 83.5, $P < 0.001$).

Our simulations indicated that high levels of resource competition among hosts did not increase the number of relative emergence times that supported parasitoid persistence in either ambient or elevated temperatures. Instead, the chances of parasitoids persisting for extended generations was significantly greater when competition levels were low, and this was consistent across all phenological delays, except 0-day (OR = 1.8, $P = 0.19$). Importantly, the presence of alternative host species (i.e., the *sulfurigaster-birchii* combination) significantly increased the probability of parasitoids persisting when averaged across all phenological shifts and temperatures (Fig. 3). This suggests, that while our single generation experiment identified intermediate levels of survival and parasitism rates when alternative host species were present, those differences in survival and parasitism rates had meaningful, positive effects on host-parasitoid coexistence across multiple generations.

Discussion

While we recognize that climate change is altering phenological relationships among interacting species, the consequences for species interactions in different community contexts are unclear (Forrest & Miller-Rushing 2010). Here we show that delays in the emergence of parasitoids, relative to their insect hosts, significantly alters the strength and persistence of host-parasitoid interactions; and the magnitude of these effects varied across temperatures and the community context in which the interaction took place. Importantly, modifications in the outcome of the host-parasitoid interaction were due to both changes in host development times and declines in host and parasitoid performance under warming, irrespective of shifts in development rates. Taken together, these findings demonstrate that modifying the effects of phenological shifts on host-parasitoid interactions, via community contexts, could help maintain availability of prey (e.g., adaptive capacity) when hosts and parasitoids become temporally mismatched, but these benefits may not be realized in predicted warmer environments due to significant declines in performances of hosts and parasitoids. Thus, accounting for interactive effects between climate and community context is necessary when trying to predict the immediate and long-term consequences of phenological shifts on biotic communities.

Effects of phenological shifts on outcomes of host-parasitoid interactions

Phenological shifts had the strongest effect on the strength and persistence of the host-parasitoid interactions and this effect persisted even after accounting for changes across temperatures, resource competition, and presence of alternative host species. This is consistent with other host-parasitoid studies which have highlighted the importance of windows of vulnerability and correct stage (age) in determining the immediate and long-term effects of interactions (Tuda & Shimada 1995; Benrey & Denno 1997; Chi & Su 2006; Stacconi *et al.* 2015). Thus, any phenological shift that makes this system more or less asynchronous could lead to the destabilization of interactions (Rasmussen *et al.* 2014; Ren *et al.* 2020), which suggest that some degree of temporal asynchrony may be required in order to maintain the stability of specialized, antagonistic interactions, such as host-parasitoid or plant-herbivore interactions (Boege & Marquis 2006; Visser *et al.* 2012; Ren *et al.* 2020).

Phenological shifts in a community context perspective

Community context proved to be a major modifying factor on the immediate and long-term consequences of phenological shifts in our study. Recent work has suggested that employing community contexts that extend the duration resources are available could be a useful management tool to help safeguard against climate-driven phenological mismatches in species interactions, such as plant-pollinator (Timberlake *et al.* 2019; Olliff-Yang *et al.* 2020). While increasing intraspecific competition extended the duration of *Drosophila*'s larval stage, our study indicates that it is not likely to promote host-parasitoid interaction persistence. Limited resources not only slowed host development, but increased host mortality, reducing the recruitment potential for the next generation of parasitoids. Other studies have found that, in addition to declines in individual survival, host populations experiencing strong competition may also be of lower value for parasitoids, reducing population growth to rates that are unable to support long term parasitoid persistence (Jones *et al.* 2009; Cuny *et al.* 2019). Furthermore, if parasitized hosts are more inclined to density-dependent mortality, as has been observed in some (Ives & Settle 1996), but not all host-parasitoid systems (Spataro

& Bernstein 2004; White *et al.* 2007), this effect is likely to be amplified. Overall, these results suggest that extending the temporal availability of hosts, via competition, is unlikely to dampen the effects of phenological shifts, and that shifts towards later emergence times of parasitoids may reduce host-parasitoid persistence if parasitoids struggle to establish on high-density (e.g., low quality) host populations.

Most phenological studies have examined consequences of phenological shifts between pairs of interacting species, rarely considering whether outcomes of phenological shifts differ when more than two species are involved. Recent theoretical (Nakazawa & Doi 2012; Revilla *et al.* 2014; Takimoto & Sato 2020) and experimental (Timberlake *et al.* 2019; Olliff-Yang *et al.* 2020) studies have pointed out this gap and suggest that the presence of alternative resources should weaken the effects of phenological mismatches, especially if those resources have complementary phenologies. By combining experimental and theoretical approaches, our results helped fill these gaps and showed that multispecies host communities were more likely to support the persistence of parasitoid populations across a broader range of phenological shifts, temperatures, and competition levels than pairwise interactions. Moreover, we found that the presence of an alternative host diminishes differences in average host survival and parasitism rates across different biotic and abiotic factors. Like in other studies, temporal complementarity between species (e.g., variation in growth rates), and a diversity of responses to environmental changes were major stabilizing mechanisms in our system (Zhang *et al.* 2013; Craven *et al.* 2018; Sasaki *et al.* 2019), though this is the first example using a host-parasitoid system. While most studies recognize that functional redundancy and trait diversity at any given time can positively affect community resilience to environmental changes, it is rarely considered that these community parameters continuously change during the development of individuals within the community. Future studies should explicitly consider that the degree of functional redundancy within a community will likely vary during the development of individuals within communities. Regardless, our results suggest that the presence of alternative host species serves as an important buffer to phenological shifts in ecological communities, as it can provide complementary phenologies, increases functional redundancy, and can dampen community responses to variation in biotic and abiotic environments (Yachi & Loreau 1999; Yang 2020).

Complex effects of elevated temperatures on consequences of phenological shifts

Our results show that the effects of phenological shifts varied with warming, but to what extent and why differed between hosts and parasitoids. While growth rates of both host species increased with warming, the declines in parasitism rates and increased host survival across phenological shifts did not scale with differences in growth rates. Thus, other factors, besides variation in growth rates among temperatures, community contexts, and species contribute to differences in effects of phenological shifts across treatments. Our dynamic model revealed that host-parasitoid interactions were less likely to persist in predicted global warming temperatures and identified that some host and parasitoid species were completely unable to support interactions under warming, even when parasitoid survival, host fecundity, and phenological relationships were ideal (Fig. 3; Fig S7, Fig. S9, Fig. S10). This highlights that physiological changes in elevated temperatures, beyond shifts in development rates, can considerably modify the effect of phenological shifts on species interactions. For example, regardless of the developmental relationship, warming may compromise immune systems or the ability to heal from injuries (i.e., oviposition punctures) (Catalan *et al.* 2012; Abram *et al.* 2019), though other studies have found that hosts are more able to successfully defend themselves against immature parasitoids in warmer temperatures (Thomas & Blanford 2003; Wojda 2017). In addition, parasitoids usually show lower thermal tolerances relative to their hosts (Kurban 1998; Fellowes *et al.* 1999; Zamani *et al.* 2007; Agosta *et al.* 2018; Thierry *et al.* 2019), both of which support our current findings. These discoveries have important implications for persistence of key trophic interactions and top-down control, as such interruptions to top down processes has been identified as key consequences of climate change in terrestrial and aquatic systems (Shurin *et al.* 2012; Velthuis *et al.* 2017; Derocles *et al.* 2018).

Conclusion

While long-term observational studies of phenological responses to climate warming are extremely valuable to understand how ongoing phenological shifts alter species interactions, experimental approaches combined with parameterized dynamic models can help disentangle the roles that biotic and abiotic environmental

variations play in altering the strength and persistence of species interactions in future climatic conditions. Our combined experimental and model simulation study showed that the strength and persistence of species interactions are sensitive to phenological shifts among interacting species, but to what extent varied with community context. We found that the presence of another host species helps promote long-term persistence of species interactions across a range of phenological relationships, temperatures, and levels of resource competition. Thus, the loss of biodiversity may exacerbate these effects and its preservation should be a priority, given its important role in maintaining species interactions in the face of ongoing phenological shifts, increased temperatures, and changes in other biotic environmental factors (Forister *et al.* 2019). Incorporating community contexts into future phenological research will be critical if we are to understand and accurately predict the consequences of phenological shifts on entire ecological communities.

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Figures

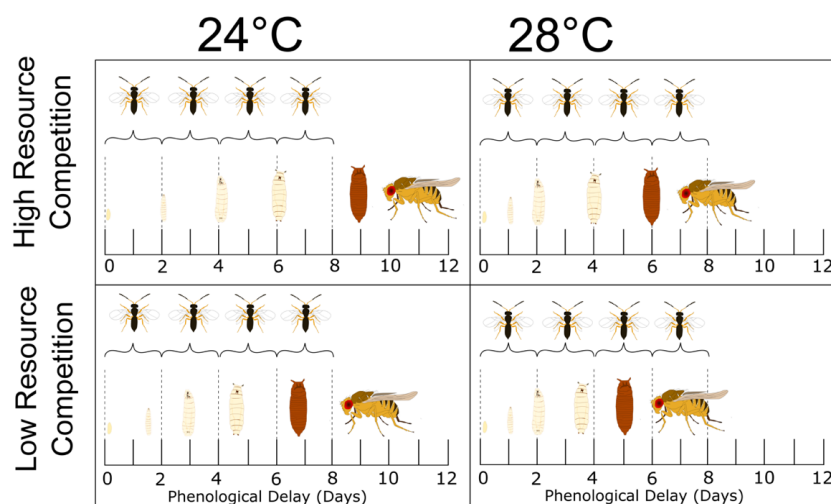


Figure 1: Schematic representation of the experimental design. To modify the development rates and temporal availability of *Drosophila*, 100 *Drosophila* larvae were reared in either current ambient (24°C) or predicted warming (28°C) temperatures, and in either high (50 indiv/ml food) or low (5 indiv/ml food) levels of resource competition. Differences in development rates and developmental stages in the control treatments (e.g., without parasitoids) are presented as *Drosophila* transitioning from larval (beige), to pupal (brown), to adult flies. This was replicated with *Drosophila sulfurigaster* and *D. birchii* alone and the two *Drosophila* species together for the alternative host species treatment. Female parasitoid wasps, from a single parasitoid species, were then introduced to vials in two-day intervals (e.g., 0, 2, 4, 6 days), as shown with the dotted lines, to mimic a phenological delay in emergence times between *Drosophila* and parasitoids. Parasitoids remained in vials with hosts for 48-hours and this was repeated with both parasitoid species (e.g., *Asobara sp.* and *Ganaspis sp.*). Each unique treatment was replicated 5-6 times.

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Figure 2: Association between phenological delay, temperature, and resource competition for average host survival and parasitism rates. For all representations, the predicted probabilities of (A) host survival (Table S4) and (B) parasitism rates (Table S5) are displayed as a function of phenological delay from independent mixed effects logistic regressions. The bar plots represent the estimated marginal means, averaged over all host and parasitoid species combinations with 95% confidence intervals. Points display the observed means of (A) host survival and (B) parasitism rates for each host-parasitoid combination separately. All effects were tested using Tukey's HSD test for multiple comparison and are provided within the text.

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Figure 3: Observed association between phenological delay, temperature, and resource competition for the persistence of *Drosophila*-parasitoid interactions in the dynamic simulation. Each point (n = 9599) overlaid on

the boxplot represents the proportion of replicated simulations ($n = 100$) in which host-parasitoid interactions persisted for at least 10 generations within each host species combination. Data for both parasitoid species are presented together within each host species facet. Many proportions are at or near zero, thus some points are not visible. We restricted the y-axis to show all values less than 0.3, but several observed proportions reach a value higher than what are presented in this figure. All levels of host net reproductive rate (1-10) and adult parasitoid survival rate (0.1-1) are included in this figure. Data is split across host species to help differentiate effects of phenological delay in pairwise and multispecies systems. Predicted probabilities (not shown) of host-parasitoid persistence across a wide range of parameters were less than 1%, but interspecific combinations of host species had significantly greater odds of persisting 10 generations then either species alone. Statistical analysis is provided in **SI Materials and Methods** . All effects were tested using Tukey's HSD test for multiple comparison.

