




LETTER

Simulated climate warming causes asymmetric responses in insect life-history timing potentially disrupting a classic ecological speciation system

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Abstract

Climate change may alter phenology within populations with cascading consequences for community interactions and on-going evolutionary processes. Here, we measured the response to climate warming in two sympatric, recently diverged (~170 years) populations of *Rhagoletis pomonella* flies specialized on different host fruits (hawthorn and apple) and their parasitoid wasp communities. We tested whether warmer temperatures affect dormancy regulation and its consequences for synchrony across trophic levels and temporal isolation between divergent populations. Under warmer temperatures, both fly populations developed earlier. However, warming significantly increased the proportion of maladaptive pre-winter development in apple, but not hawthorn, flies. Parasitoid phenology was less affected, potentially generating ecological asynchrony. Observed shifts in fly phenology under warming may decrease temporal isolation, potentially limiting on-going divergence. Our findings of complex sensitivity of life-history timing to changing temperatures predict that coming decades may see multifaceted ecological and evolutionary changes in temporal specialist communities.

KEYWORDS

climate change, diapause, parasitoids, phenology, *Rhagoletis*, Tephritidae

INTRODUCTION

A major challenge in predicting the ecological and evolutionary consequences of climate change is understanding how responses within populations affect community interactions. Within populations, common responses to climate change include shifts in geographic distributions and phenology (Bradshaw & Holzapfel, 2008; Cohen et al., 2018; Parmesan, 2006). Differential local adaptation, reduced gene flow, and associated genetic differentiation can result in population differences in life history and phenological responses (Forrest & Miller-Rushing, 2010). When interacting species respond differently to climate change, phenological mismatches are likely (Renner & Zohner, 2018; Rudolf, 2019). This may

be particularly true for specialist organisms that may have only brief temporal windows for interactions, with little margin for phenological error.

Life-history timing is a crucial axis of ecological adaptation for temperate organisms. Seasonal environments can pose stark fitness consequences for populations in the absence of phenological adaptation (Visser & Gienapp, 2019). Phenological mismatches between populations and abiotic conditions, such as the timing and duration of winter (Williams et al., 2015), can result in low survivorship (Miller-Rushing et al., 2010) or low reproductive success with offset breeding (Visser et al., 2004) or flowering (Wheeler et al., 2015) times. Moreover, phenological mismatches across biotic interactions may have fitness consequences across trophic

interactions (Both et al., 2009; Damien & Tougeron, 2019), pollination (Hegelend et al., 2009), seed dispersal (Gordon et al., 2019), or competition (Zettlemoyer et al., 2019). Such phenological mismatches among interacting species may be common, because different species rely on different sets of environmental cues (e.g., from photoperiod, temperature, aridity, and other organisms) to regulate life-history timing to withstand unfavourable conditions and synchronize life cycles with ephemeral resources (Tauber & Tauber, 1976; Wilsterman et al., 2021). Adaptive timing of life history, such as initiation of dormancy, involves the integration of a series of environmental cues (Denlinger, 2002). Important ecological interactions among distantly related organisms are often superimposed over adaptation to shared seasonal regimes via non-homologous physiological or developmental mechanisms. For instance, the phenology of a plant/insect interaction may be governed by vernalization and flowering time for a plant species (e.g., Vest et al. 2021) and a combination of diapause and development rate for a butterfly species (e.g., Posledovich et al., 2015). Thus, the ramifications of altered seasonality may be multifaceted, and phenological effects on populations may ripple through entire communities.

Studies of phenological change over recent decades of anthropogenic climate change have shown that altered seasonality of populations may already be driving ecological asynchrony (Root et al., 2003). However, robust experimental tests examining predicted future climatic conditions face tradeoffs between biological realism and experimental precision (Diamond, 1986). Warming experiments performed in situ, where temperature is manipulated in the field, have provided important insights (e.g., Diamond et al., 2016; Hoffman et al. 2010; Wadgymar et al., 2015). However, it can be difficult in practice to impose treatments on biologically relevant spatial and temporal scales that manipulate conditions adequately across ecological interactions. This is particularly true for animal species without spatially constraining them in ways that hamper biological realism. For instance, in situ warming of annual plants may reveal variation in plastic responses of flowering time, but the consequences for future fitness may be difficult to discern if their pollinators are developing under current local conditions. Thus, while warming experiments can demonstrate altered phenology affecting evolutionary and ecological processes within populations, it remains difficult to test the consequences of these altered phenotypes on interactions between species across trophic levels in communities. Moreover, these broader consequences of altered phenology may not only affect species interactions across trophic levels or between competitors but may influence mating systems and reproductive dynamics among populations as well. Divergent life-history timing can play an important role in speciation by promoting temporal (allochronic) isolation between populations adapted to different seasonal regimes

(Taylor & Friesen, 2017). The phenological effects of climate change may facilitate or hinder this important mechanism of ecological speciation and thus affect processes generating new biodiversity in communities and ecosystems.

Here, we used an ideal system for testing the potential for ecological and evolutionary effects of climate warming on temporal specialist insect populations that allowed us to impose realistic climate manipulations in the laboratory across two trophic levels. The Tephritid fruit fly *Rhagoletis pomonella* (Walsh) is a textbook example of ecological speciation-in-action (Dres & Mallet, 2002) and a model for rapid seasonal adaptation (Dowle et al., 2020). A recently derived, partially reproductively isolated host race of these flies evolved after shifting to introduced domesticated apples (*Malus pumila* Mill.) from ancestral downy hawthorn fruit (*Crataegus mollis* Scheele) ~170 years ago in eastern North America (Bush, 1969; Feder et al., 1988; Michel et al., 2010). Both populations are monophagous specialists, with a single generation per year and life cycles synchronized to the availability of ripe host fruit. At sympatric sites, the fruiting time of apple trees is earlier than hawthorns by ~3–4 weeks, and the phenology of each host race is adapted to these temporally distinct resources (Dambroski et al., 2007). The corresponding difference in eclosion timing of the short-lived adult flies drives temporal reproductive isolation between the host races (Feder et al., 1994). The combination of temporal isolation and prezygotic isolation due to divergent chemosensory adaptation (Dambroski et al., 2005) limits on-going hybridization between apple and hawthorn flies at sympatric sites to 4–6% (Feder et al., 1994). Genetic differentiation in this system is based on consistent frequency differences in shared alleles rather than fixed variants between host races (Meyers et al., 2020; Powell et al., 2013).

Both populations of flies are attacked by three species of specialist parasitoid wasps in the family Braconidae: *Diachasma alboeum*, *Diachasmimorpha mellea*, and *Utetes canaliculatis* (Forbes et al., 2010; Hood et al., 2015), abbreviated Da, Dm, and Uc, hereafter. Collectively, the flies and wasps represent one of the best supported cases of “sequential” ecological speciation, where the initiation of speciation at one trophic level triggers subsequent ecological speciation at higher trophic levels (Forbes et al., 2009). Moreover, each wasp species is also temporally specialized within fly hosts via divergent life-history timing, which generates reproductive isolation between wasps attacking different hosts (Hood et al., 2015). Seasonal timing of adult emergence in this system is largely governed by the regulation of diapause—an eco-physiological state of dormancy (Košťál, 2006). The environmentally sensitive periods that affect the initiation, maintenance, and termination of diapause development occur after the flies have formed their puparia (Powell et al., 2020; Ragland et al., 2009), and both flies and

parasitoids spend >85% of their life cycles in diapause inside sessile, behaviourally inert puparia. Thus, we can manipulate seasonal temperature regimes in biologically relevant treatments across this insect community during this key life stage dictating phenology.

As univoltine temporal specialists, the ability of *Rhagoletis* and their parasitoids to initiate and maintain diapause in the face of developmentally permissive temperatures before winter is critical for adaptive life cycle timing (Hahn & Denlinger, 2011). The initiation and initial pre-winter maintenance of diapause are physiologically facultative in these populations. Responses to environmental cues are variable such that some fly and wasp individuals express maladaptive phenotypes that either forgo diapause initiation completely or fail to maintain metabolic and developmental suppression in high temperature conditions (Figure 1; Dambroski & Feder, 2007). In nature, flies expressing either phenotype are ecologically doomed; flies forgoing diapause eclose as adults when host fruits are no longer available, and flies with short diapause lengths increase metabolism, exhaust nutrient stores, and die before overwintering.

In this study, we conducted a simulated climate warming experiment on natural apple and hawthorn fly and parasitoid communities at a long-studied sympatric field site at Urbana, Illinois, USA (Dowle et al., 2020; McPherson et al., 1988). Insects were exposed to daily

and seasonal temperature fluctuations reflecting either control (ten-year average of soil temperature) or elevated temperature (3°C warmer, see methods below). The tractability of this system allowed us to impose these experimental conditions across the entirety of the life stages affecting phenology in these species, including the initiation and termination of diapause. We tested how realistic simulated warming affected both flies and wasps in the community at three different levels: (i) fitness changes based on maladaptive pre-winter diapause initiation and maintenance and phenological shifts within populations, (ii) phenological synchrony of communities across trophic levels, and (iii) the strength of prezygotic reproductive (temporal) isolation between divergently adapted populations.

METHODS

Field collections, insect husbandry, and experimental conditions

In August and September of 2017, we collected apple and hawthorn populations of *R. pomonella*, respectively, from infested fruit from wild populations in Urbana, IL during peak infestation for both plants. The apple and hawthorn sites are located ~1300m apart, well within the

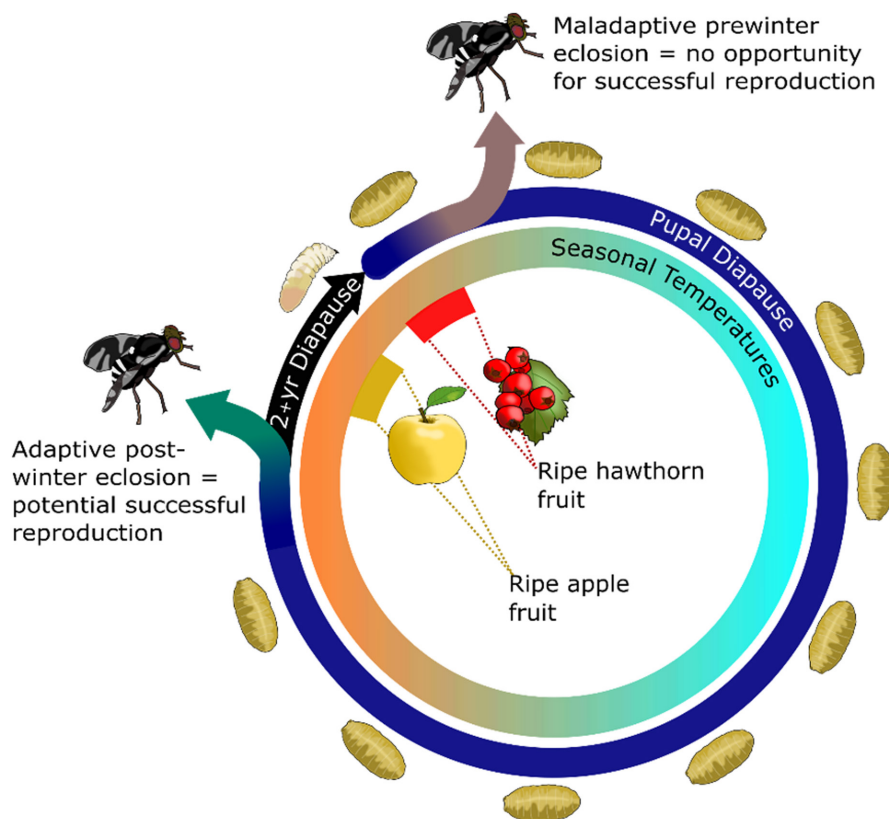


FIGURE 1 The lifecycle of *R. pomonella* flies, showing the potential for maladaptive pre-winter adult eclosion due to either a failure to enter pupal diapause or premature termination of diapause and the potential for multi-year (2+ year) diapause flies that remain in dormancy across the entire growing season.

flight range of these flies (Roitberg et al., 1984). Field collection procedures followed long-established methods for *R. pomonella* (e.g., Powell et al., 2020) and are described along with husbandry methods in the [supplementary methods](#). A total of 1885 and 1719 apple puparia and 1453 and 1422 hawthorn puparia were monitored daily for fly and wasp eclosion in the control and warming treatments, respectively. Seasonal and daily temperature fluctuations for the control treatment were based on 10-year averages from 2007 to 2016 of 10 cm soil temperature data from the NOAA weather station at Watseka, IL (40.79, -87.76) closest to the Urbana, IL site, with the warming treatment based on predictions for the region 50 to 100 years in the future (IPCC, 2013; Kunkel et al., 2013; Pryor et al., 2013) (see details in [supplementary methods](#); Figure 2).

Analysis

Statistical analyses were performed in RStudio version 1.1.463 (RStudio Team, Boston, MA, USA) using packages *survival* 3.5-5 (Grambsch & Therneau, 1994), *survminer* 0.4.9 (Kassambara et al., 2018), *ggplot2* 3.4.2 (Wickham, 2016), and *MASS* 7.3-60 (Venables & Ripley, 2002). To test for differences in phenology, we used accelerated failure time (AFT) analysis, a type of survival analysis that uses survival regression models to test for differences in the timing of the probability of events (Fox, 2001). Details of our AFT analysis can be found in the [supplementary methods](#).

To determine fitness changes between control and warming treatments, we tested for differences in the

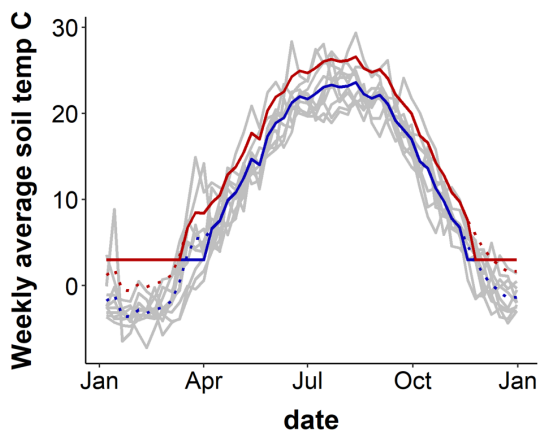


FIGURE 2 Experimental treatments based on weekly soil temperature averages in Celsius across the year. Control temperatures are shown in the blue line, and Warming temperatures are shown in red. Lines reflect the midpoint temperatures for the daily fluctuations synced with photoperiod (see [supplementary methods](#)). Dotted lines represent the soil temperature trends during the overwintering phase of the experiment (when temperatures dipped below the physiological threshold for *R. pomonella* of 6°C (Neilson, 1962) in the respective treatment, see [supplementary methods](#)). Grey lines show each year from 2007 to 2016.

proportions of outcomes within flies and wasps using chi-square tests. In flies, possible outcomes were: eclosed pre-winter, eclosed post-winter, remained alive as pupa (multi-year diapausing flies), died as pupa, and died as pharate adult prior to eclosion. In wasps, possible outcomes were: eclosed pre-winter, eclosed post-winter, died as larva, died as pupa, and died as pharate adult. Each of these outcomes results in a fitness of zero for the respective fly or wasp but potentially reflects different underlying phenotypes on which selection might act on, such as the propensity for non-diapause development for flies eclosing before winter versus the strength of metabolic suppression during dormancy for flies that die as pharate adults after successfully entering and termination diapause but have insufficient nutrient stores to complete development.

We also calculated the magnitude of temporal premating reproductive isolation (RI) between apple and hawthorn flies in the respective temperature treatments, accounting for the effects of sex on eclosion time using a model of “co-occurrence” premating isolation from Sobel & Chen (2014), representing the proportional decrease in potential reproduction between groups compared to panmixia (random mating between and within groups). Details of this approach are described in the supplementary material (Figure S1).

RESULTS

Phenological responses of flies

The apple and hawthorn fly populations differed in their pre-winter diapause initiation and maintenance responses to warming. No hawthorn fly eclosed before simulated winter in the control or warming temperature treatment (Figure 3). In contrast, 4.6% of apple flies eclosed pre-winter in the control treatment, while 24% eclosed in the warming treatment, which is over a 5-fold increase ($X^2=281$, $df=1$, $p<0.0001$, Figure 3). Importantly, the prewinter eclosion times of all apple

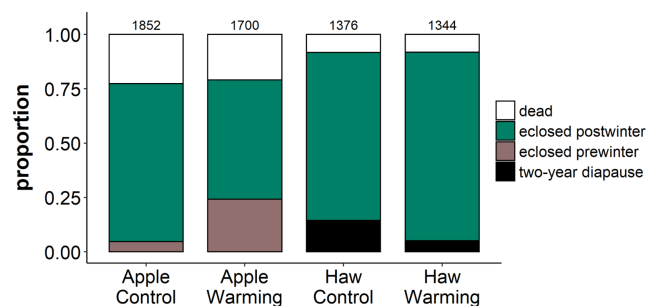


FIGURE 3 The fates of *R. pomonella* pupae from the apple and hawthorn (Haw) host races for both control and warming conditions with total sample sizes at the top of the bars. Proportions exclude parasitoid wasps, identified by successful adult eclosion or in post-mortem dissections.

flies were later than the peak mating and oviposition time of the sympatric hawthorn population at Urbana. The earliest prewinter apple fly eclosed on September 16, while peak mating and oviposition in hawthorn flies occurs in late August to early September. *Rhagoletis pomonella* adults take 10–14 days after eclosion to sexually mature (Prokopy & Bush, 1973), pushing reproduction of prewinter-eclosing apple flies into early October. Even if some of the pre-winter apple adults were receptive to using hawthorn as a host (Forbes et al., 2005), the temporal window of availability for hawthorns would have already closed.

While simulated warming did not induce non-diapause development in hawthorn flies, it did affect another aspect of diapause. Typically, a subset of *R. pomonella* flies eclose after two or more yearly cycles of chilling and heating rather than one (Phipps & Dirks, 1933). Multi-year diapause generates a seed bank of pupae in dormancy, likely representing a bet hedge against annual variability in host fruit set (Menu et al., 2000). Under warming, significantly fewer hawthorn flies remained in diapause at the end of the experiment (control: 14.4%, warming: 4.9%, $X^2=66.37$, $df=1$, $p<0.0001$, Figure 3). In the apple flies, no multi-year class pupae were observed in the control or warming treatment (Figure 3).

Taken together, the results suggest that the warming temperature treatment affected what could be considered the front end of the diapause phenotype in the apple race by inducing an increased portion of individuals out of their normal one-year life cycle into non-diapause development (Figure 3). Alternately, the warming temperature affected the back end for the hawthorn race, by causing an increased portion of individuals to shift from a multi- to one-year cycle (Figure 3). These shifts did not change overall survivorship in the experiment between control and warm treatments within apple or hawthorn flies (Figure 3). However, the reduced proportion

of multi-year diapausing hawthorn flies and increased numbers of non-diapausing apple flies in the warm temperature treatment are both maladaptive in nature and may adversely affect the long-term persistence of local populations in the face of climate change.

Simulated warming also affected the timing of post-winter adult eclosion (Figure 4). The best-fit accelerated failure time model retained a significant main effect of temperature ($z=10.44$; $p<0.0001$), with eclosion curves shifting earlier for both fly populations (Figure 4a). However, a significant population by temperature interaction term ($z=4.76$, $p<0.0001$) indicated that the shift was more pronounced for apple flies (Figure 4a). In addition to shifting median eclosion earlier, the warm treatment also caused 59% and 85% increases in the variance of eclosion times in apple and hawthorn flies, respectively (Fligner-Killeen test for homogeneity of variances in non-normal data, both host associations: $X^2>69$, $df=1$, $p<2.2\times 10^{-16}$; Figure 4b,c). The pronounced bimodality in the eclosion curves is likely reflective of the complex mix of categorical and continuous variation in gene-by-environment interaction-driven diapause phenotypes known to exist in this system (Calvert et al., 2022; Dambroski & Feder, 2007), and the same pattern occurred in the next year when the same treatments were used for a follow-up genomic response experiment (Lackey & Powell, 2019).

The asymmetric shifts in post-winter eclosion time of the apple and hawthorn flies have implications for on-going divergence in this incipient speciation system. The change in post-winter eclosion distributions under warming conditions led to greater overlap of sexually active adults (i.e., increased potential for gene flow) in the warming compared to control treatment (Figure 6a,b). We calculated temporal RI to be 0.44 (95% CI: 0.31–0.56) in control and 0.33 (95% CI: 0.23–0.43) in the warming treatment, a 25% reduction.

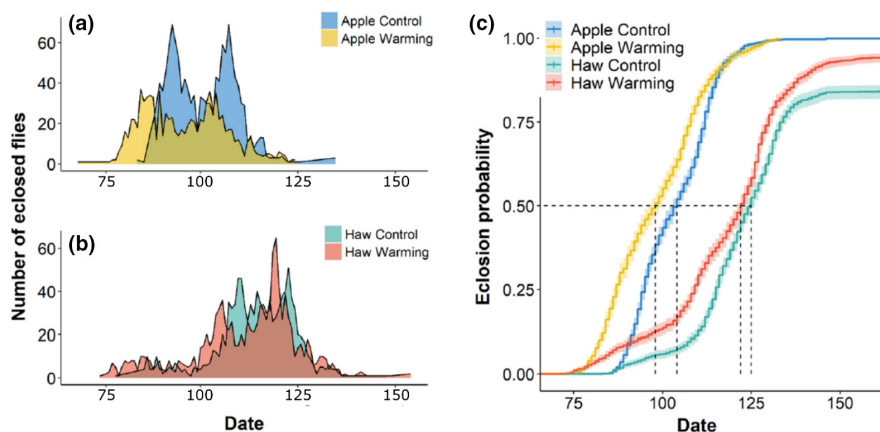


FIGURE 4 Post-winter adult eclosion distributions for (a) apple flies and (b) hawthorn flies. (c) Cumulative eclosion probability curves for apple and hawthorn flies reared under control and warming temperature regimes with days to eclosion since spring equinox, with confidence envelopes representing 95% CI from the best fit accelerated failure time model. Dashed lines show days to eclosion at median eclosion probability for each population and temperature regime.

Phenological responses of parasitoids

Eclosion times of wasps did not differ between the control and warming treatments (Figure 5), implying that parasitoids may not be as sensitive to increase temperature compared to flies. However, the sample size for apple fly parasitoids was small ($n=13$), limiting our ability to detect significant differences if any exist. For hawthorn wasps ($n=134$), accelerated failure time eclosion probability curves for control and warming treatments crossed each other and had overlapping 95% confidence intervals across the range of eclosion dates (Figure 5). Thus, the curves showed no indication of separation at any point across their distributions (Figure 5b). We also examined accelerated failure time eclosion probability curves for the three hawthorn wasps separately and they overlapped for each taxon between control and warming treatments (all model effect of temperature $\beta < |0.02|$, all $p > 0.2$, Figures S2a–c). Thus, the lack of eclosion time response was not specific to a particular wasp species but was general for all parasitoids. To examine our statistical power to detect a shift in eclosion due to warming in hawthorn wasps, we performed a permutation analysis in which eclosion times for hawthorn wasps were simulated to be shifted a day or more earlier in the warming treatment than the control (see [supplementary material](#)). Our observed experimental results showed a weaker response coefficient than 94.4% of coefficients simulated from a 1-day shift and 98.9% from a simulated 2-day shift

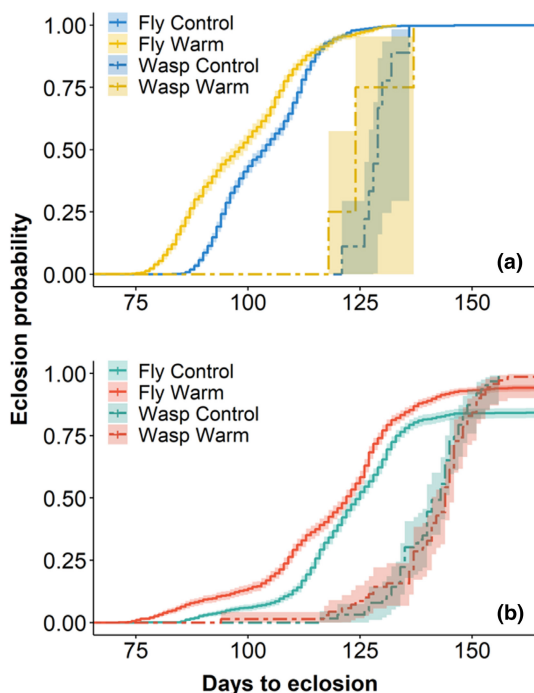


FIGURE 5 Cumulative eclosion probability curves for both flies and parasitoids reared from (a) apple and (b) hawthorn control and warming temperature regimes with days to eclosion since spring equinox, with confidence envelopes representing 95% CI from the best fit accelerated failure time model.

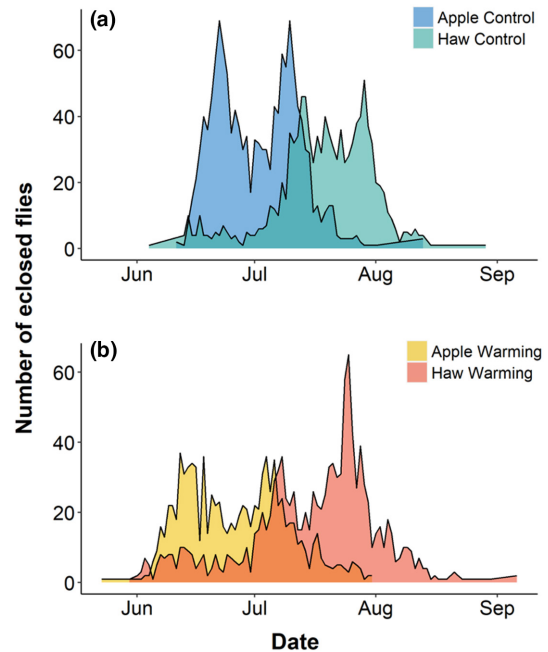


FIGURE 6 Post-winter eclosion distributions for apple and hawthorn flies in (a) control and (b) warming conditions, showing the overlap in phenology that determines the magnitude of temporal pre-zygotic reproductive isolation between the two host races.

(Figure S3). Thus, if the warming treatment induced a modest shift in eclosion time in the wasps, obscured by sampling error in our experiment, our observed results are still much weaker than would be expected by chance.

Combining eclosion data across temperature treatments showed that the three hawthorn wasp species differed in their eclosion phenologies ($F=18.75$, $p=7.7 \times 10^{-8}$, Figure S4), as expected from previous research demonstrating temporal niche partitioning within the community (Hood et al., 2021). The relative order of eclosion of the wasps from earliest to latest was Uc, Dm, and Da. Moreover, like their fly hosts, all three species of parasitoid wasps are physiologically capable of forgoing diapause and entering direct adult development under prolonged periods of pre-winter heating in the laboratory (Hood, 2016; McIntyre et al., 2023). However, no apple or hawthorn parasitoid in the control or warm treatment eclosed before winter.

DISCUSSION

Fitness consequences within fly populations are asymmetric

Despite diverging only in recent historical times (Bush, 1966) and experiencing the same abiotic factors in sympatry, the apple and hawthorn host races, as well as their parasitoids, showed asymmetric responses to experimental warming. Apple flies showed higher propensities for non-diapause development, resulting in maladaptive

pre-winter eclosion of adult flies in the autumn when host fruit is no longer available for oviposition. This decrease in population fitness may result in decreased population size in the short term but also alteration of the selection regime on the thermal sensitivity of diapause. Hawthorn flies showed decreases in the proportion of individuals manifesting a two-year diapause phenotype that may have more context-dependent outcomes for fitness. The initial pulse of increased one-year diapause flies may increase population size in a given generation but make the population more vulnerable to annual changes in fruit set by decreasing the capacity for ecological bet-hedging. These results highlight how difficult it may be to generalize responses to climate change across not only species but conspecific populations. The regulation of diapause is driven by complex gene-by-environment interactions, involving the integration of multiple environmental cues with multiple physiological systems and gene co-regulatory networks (Emerson et al., 2009). Thus, how climate change affects this critical feature of seasonal adaptation in insects may depend on population-specific thermal reaction norms underlying the regulation of different phases of diapause development. As specialist insects are perhaps the most diverse group of organisms on the planet (Forbes et al., 2018; Strong et al., 1984) and diapause is widespread among insects (Tauber et al., 1986), these results may have generalizable implications for some of the major ecological effects of climate change on a large proportion of terrestrial animal biodiversity. While the regulation of diapause development is the major determinant of adult eclosion phenology in *Rhagoletis* (Powell et al., 2020), assessing how other life stages, e.g., larval development within fruit, sexual maturation, and longevity may be affected by warming will give a more complete picture of the fitness and demographic consequences of altered seasonal regimes.

Interactions across trophic levels

The phenological shift in the flies coupled with the apparent lack of shift in the parasitoids suggests that climate warming may cause phenological asynchrony across trophic levels. All three parasitoid species have shorter adult lifespans than the flies (<10 days vs. ~30 days; Hood et al., 2015). Thus, an earlier shift in fly phenology of just a few days without a compensatory shift in the wasps could result in substantially decreased availability of hosts at the right life stages for successful parasitoid attack (Hood et al., 2015). Reduced top-down control could allow fly populations to increase, potentially intensifying competition within populations for host plant resources. However, shifted phenology in fly populations will also generate stronger selection on wasps to match a new phenological optimum. The strength and direction of such selection depends on whether host-parasitoid phenological relationships are currently

optimal from the perspective of the parasitoids (Singer & Parmesan, 2010). Gene-by-environment interactions affecting phenology in the parasitoids have been less well studied than those of the flies. However, eclosion time in the wasps has been shown to be genetically based and polygenic (Forbes et al., 2009; Hood et al., 2015), like the flies (Doellman et al., 2019), and, thus, these parasitoids may have the capacity for rapid adaptation, tracking phenological shifts in their host flies. The general implication of these results for insect communities may be that different thermal sensitivities of key life-history timing traits across interacting species are likely to perturb community synchrony away from present conditions. In diffuse, generalized host-parasitoid networks with multivoltine parasitoids, such as those in highly diverse oak-galling cynipid communities (e.g., Zhang et al., 2022), may have little effect on overall parasitoid attack rates on given host. However, in highly specialized parasitoid communities, like those surrounding some Lepidoptera (e.g., Kankare et al., 2005) or goldenrod gall flies (e.g., Heard et al., 2006), asymmetric responses are likely to have major effects on interaction outcomes and thus demographic success of either hosts or parasitoids.

Simulated warming had different effects on both the position and shape of adult eclosion phenology distributions between apple and hawthorn flies (Figure 4). How these differences manifest as fitness consequences and how natural selection may act on those fitness regimes under future conditions will also depend on how the fruiting phenology of its hosts are affected by the same conditions. While experimental warming of insects along with their host plants across some life stages is possible in certain study systems (e.g., Posledovich et al., 2018) such an experiment on the apple and hawthorn trees in Urbana, IL is logistically unfeasible. However, previous empirical and theoretical work on apple and other fruit trees in Rosacea speak to possible effects of predicted warming on host fruit phenology. Studies of apple phenology over past decades have shown a pattern of earlier flowering and fruiting through time (e.g., Fujisawa & Kobayashi, 2010; Wolfe et al., 2005). However, models of apple phenology predict that long-term trends heading into an even warmer climate may be more complicated, due in part to antagonistic effects of warming during different seasons (Darbyshire et al., 2017). While warmer springs accelerate apple flowering times, shorter, milder winters have an opposing effect on phenology; thus, models predict advancing, stationary, or even receding timing depending on localized climate projections (Darbyshire et al., 2014). Moreover, such models predict that the effects of future climatic conditions on apples will be highly variable across geography and apple cultivars (Legave et al., 2015). The drivers of phenological variation in downy hawthorn have not been studied directly. However, more distantly related Rosacea fruit trees, such as *Prunus* sp., appear to show similar patterns of high intraspecific variation in phenological responses

to seasonal temperature regimes, with contrasting effects of winter length and spring temperatures (Castède et al., 2014; Doi et al., 2010). In the midwestern United States, where our study site is located, latitudinal variation in fruiting phenology for apple and downy hawthorn trees runs in opposite directions, with apples fruiting earlier and hawthorns later moving North to South (Feder & Bush, 1989). Thus, as the prevailing seasonal regimes become warmer and more erratic in coming decades, the differences in post-winter phenology effects between apple and hawthorn flies observed in our experiment may be superimposed on highly variable effects on fruiting phenology, both between host plants and across geographic sites.

Phenological shifts reduce temporal isolation between fly populations

Shifts in phenology due to warmer rearing temperatures lead to predicted greater temporal overlap of apple and hawthorn flies, with a 25% reduction in the strength of temporal isolation. Because temporal isolation is an early-acting form for premating isolation, it can have disproportionate effects on total RI compared to later-acting barriers (Ramsey et al., 2003). In this system, temporal isolation acts alongside divergence in olfactory host plant preference (Dambroski et al., 2005) to drive total prezygotic reproductive isolation of ~93% (Lackey et al., 2023). Cases of incomplete ecological speciation may exist in a fragile equilibrium between migration and divergent selection (Nosil et al., 2009). Thus, increased migration stemming from weakened temporal isolation between apple and hawthorn flies could lead to increased genetic homogenization and even the possible collapse of differentiation between the host races. However, increased seasonal overlap between the flies is ultimately dependent on convergence in the fruiting phenologies of apple and hawthorn trees. Further study is required, but as discussed above, a balance between the effects of warmer growing seasons and winters may result in host fruiting times remaining similar or even diverging under future conditions. If true and given the extensive standing genetic variation in *R. pomonella* (Doellman et al., 2018), then divergent selection on eclosion timing in F1 offspring could counter increased migration rates, potentially maintaining or strengthening current levels of temporal RI. Our results highlight the precarious environmental sensitivity of temporal isolation. The increased frequency of extreme heat anomalies expected under climate change (Cai et al., 2014) may lead to more frequent and stronger oscillations in genetic divergence between apple and hawthorn flies and their associated parasitoids in the coming years. As temporal isolation may be a major mechanism of ecological speciation across a wide diversity of taxa (Taylor & Friesen, 2017) and increased variance in phenology may be a common

outcome of climate change (Zettlemyer & Demarche, 2022), increased gene flow between diverging populations may be likely in many systems. Similar patterns have also been observed in plants (Franks & Weis, 2009).

Certain populations of *R. pomonella* may be well poised for rapid adaptation to climate change. Standing variation in the form of latitudinal clines in diapause phenotypes (Dambroski & Feder, 2007) and chromosomal inversions associated with these traits exist in both host races (Doellman et al., 2019). These clines allow apple and hawthorn flies to track shifts in host phenology. Just as poleward range shifts of whole species may be an important response to a warming world (Pelini et al., 2009), shifting positions and slopes of genetic clines for dormancy traits may be another important consequence for systems with geographic variation in dormancy traits (Bradshaw & Holzapfel, 2006). As the genetic differences between the host races are superimposed over these clines (Feder et al., 1990), these shifts in the shapes of diapause responses to temperature may have further consequences for patterns of divergent selection and gene flow between apple and hawthorn flies in the future. Notably, the apple population at Urbana, IL is near the southern range limit of apple flies (Bush, 1969), potentially limiting the amount of standing variation available for adapting to even earlier and hotter seasonality. Moreover, antagonistic genetic correlations among pre- and post-winter diapause traits (Calvert et al., 2022) may pose serious constraints on adaptation to novel conditions in this system.

CONCLUSIONS

The asymmetric responses in diapause development and timing we observed in the *R. pomonella* system imply that temperate insect communities may be particularly vulnerable to fitness declines, altered species interactions, and the breakdown of divergent local adaptation. Even species or populations that may appear physiologically inert to increased temperatures, such as the parasitoid wasps here, may suffer fitness consequences as the result of asynchrony with interacting species. The proximate effects of climate change may ripple across ecological communities in complex ways, involving not just demographics and the temporal synchrony of species interactions but potentially altering the evolutionary landscape of divergent adaptation and speciation. Ecological theory predicts that specialist taxa may be at greater risk to environmental instability than generalists (Futuyma & Mereno, 1988; Kassen, 2002). Importantly, response to climate change also predicts a potential loss of biodiversity in both parasitoid and fly populations, though via different mechanisms. In the parasitoids, potential phenological mismatch of hosts and the very narrow adult life stage of parasitoids threatens biodiversity through fitness declines and perhaps even local

extinction in the parasitoids during hotter years. In the flies, the on-going evolutionary process of ecological speciation may be at risk of collapse due to population-specific shifts in phenological distributions that erode temporal isolation. The parameter space under which ecological speciation can proceed in sympatry is narrow (Nosil et al., 2009; Powell et al., 2014) and systems with environmentally sensitive isolating mechanisms may be pushed into unfavourable parameter space by climate change. It is possible that some phenological effects of future warming may increase opportunities of ecological speciation. However, as increased variance in phenology within populations is an expected outcome of plastic response to climate change (Nussey et al., 2007), we expect that increased overlap in seasonal mating between diverging populations to be common.

Taken together, our results highlight the difficulty of predicting the ecological and evolutionary ramifications of climate change. If responses to climate warming are highly varied and complex, even among populations that have been diverging for only ~170 years, still share all their genomic variation (Doellman et al., 2019; Powell et al., 2022), and occur in the same habitat and location, we have a long way to go in understanding which species and communities may be most vulnerable to altered seasonality. Our results further suggest that these outcomes may be governed by nuanced effects of differences in thermal reaction norms for life-history traits acting across organisms' life cycles, and identifying maladaptive tipping points in these systems should be a goal of future empirical work on insects and climate change. Coupled with empirical evidence for long-term declines in insect abundance (Wagner, 2020), the complex effects of simulated climate warming demonstrated here portend that the coming decades may see multifaceted changes to on-going ecological and evolutionary processes in the most diverse group of organisms on Earth.

AUTHOR CONTRIBUTIONS

THQP, JLF, GJR, and DAH conceived of the study, THQP and ACRL designed the experiment, THQP, ACRL, and GJR made the field collections, ACRL, PMD, and THQP executed the experiment, ACRL and PMD analysed the data, the manuscript was written by THQP and ACRL with input from all authors.

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DATA AVAILABILITY STATEMENT

Data from the study (all information collected about individual puparia across the experiment) and R code used in the analyses are accessioned in DRYAD. data: <https://doi.org/10.5061/dryad.hmgqnk9ng> analysis code: <https://doi.org/10.5281/zenodo.7884308>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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