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Original Article

Overwintering drives rapid adaptation in *Drosophila* with potential costs to insecticide resistance

Eric G. Prileson [1], Bianca Campagnari [1,2], Catherine I. Clare [1], Amir R. Gabidulin [1], René S. Shahmohamadloo [1], Seth M. Rudman [1]

[1]School of Biological Sciences, Washington State University Vancouver, Vancouver, United States
[2]Department of Evolution, Ecology, and Organismal Biology, University of California Riverside, Riverside, United States

Corresponding author: Washington State University Vancouver, School of Biological Sciences, 14204 NE Salmon Creek Avenue, Vancouver, WA, 98686, United States. Email: eric.prileson@wsu.edu

Abstract

Winter is a formidable challenge for ectotherms that inhabit temperate climates. The extent to which winter conditions drive rapid adaptation, and separately, how selection from novel stressors affects adaptation to winter, remain poorly understood. Here, we use replicate populations of *Drosophila melanogaster* in a field experiment to test (i) whether winter conditions drive rapid adaptation and (ii) for trade-offs between insecticide resistance and overwintering survival. Following a longitudinal field experiment investigating the evolution of insecticide resistance, we tracked subsequent evolution during an overwintering period. In unexposed control populations, we detected parallel evolutionary shifts indicative of adaptation to winter conditions in multiple traits, including body size and fecundity. Additionally, populations that had evolved insecticide resistance during the growing season were more likely to go extinct than control populations. Further, both control and resistant populations showed patterns of lower resistance following the winter period, suggestive of a trade-off between overwintering success and insecticide resistance. Rapid evolutionary responses to winter conditions, and potential costs of resistance, provide important context for understanding overwintering performance in temperate insects with implications for pest management and ecosystem services.

Keywords: overwintering, adaptation, *Drosophila*, insecticide resistance, trade-offs

Introduction

Rapid adaptation is critical to organismal responses to environmental change and the maintenance of biodiversity (Exposito-Alonso et al., 2022; Urban et al., 2016). There has been considerable work to understand the ecological drivers of rapid adaptation, including biotic factors such as predation, competition, and mutualism (Li et al., 2021; Reznick & Endler, 1982; Schluter, 2000). Similarly, abiotic factors, including variation in temperature, have been studied as drivers of rapid adaptation with direct implications for responses to climate change (Bradshaw & Holzhapfl, 2010; Huey et al., 2021; Radchuk et al., 2019). In terrestrial ectotherms, there is extensive study on the ecology and physiological challenges associated with winter (Denlinger & Lee, 2010; Marchand, 1987; Sinclair et al., 2003), but the role of winter conditions in driving rapid adaptation has received comparatively little attention (but see Campbell-Staton et al., 2017; Marshall et al., 2020; Williams et al., 2015). Further, there are even fewer studies with sufficient population-level replication to detect parallel changes indicative of adaptation to winter conditions. Although there is clear evidence of ecological and demographic effects of winter conditions, including population declines and life-history trade-offs between winter stress tolerance and summer reproduction (Boulétreau-merle & Fouillet, 2002; Marshall & Sinclair, 2009), there is little known about how overwintering selection shapes evolutionary trajectories.

The fate of overwintering ectotherm populations is largely dependent on both physiology and demography. The physiological mechanisms enabling ectotherms to survive challenging winter conditions are well-documented (Sinclair et al., 2003; Teets et al., 2023; Toxopeus & Sinclair, 2018). In addition to putatively adaptive physiological responses, for multivoltine species, population size and number of generations per year are strongly influenced by seasonal fluctuations in temperature (Altermatt, 2010; Roff, 1980; Shpak et al., 2010; Tauber & Tauber, 1981). These demographic shifts are important for ecological interactions (Markow & O'Grady, 2008; Parmesan, 2006; Thackeray et al., 2016) but also for the capacity for rapid adaptation, because they alter genetic diversity of populations (Barrett & Schluter, 2008; Crozier & Dwyer, 2006). Across winter, populations can be subject to severe bottlenecks (Chen et al., 2006; Kinnison & Hairston, 2007; Lawton et al., 2022) as both demographic effects of genetic drift and the prevalence of adaptive physiological traits likely play a role in overwintering persistence.

There is evidence of physiological responses to co-occurrence of low temperature and natural stressors such as desiccation or pathogen exposure (Le Bourg et al., 2009;

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reviewed in Sinclair et al., 2013; Zhang et al., 2011). However, how the emergence of novel, strong, directional selective agents—such as those imposed anthropogenically—interacts with natural stressors to shape ecological and evolutionary outcomes (e.g., insecticide resistance and low temperature) is less clear (ffrench-Constant & Bass, 2017; Kliot & Ghanim, 2012). For example, there could be positive correlational selection and genetic covariance between insecticide resistance and overwintering survival (Lande & Arnold, 1983; Service & Rose, 1985; Sinervo & Svensson, 2002). Alternatively, there could be antagonism of insecticide resistance and overwintering rendering resistant populations less likely to survive the overwintering period (McKenzie, 1994; McKenzie et al., 1990), possibly due to pleiotropy and/or fitness trade-offs (Crow, 1957; Roush & McKenzie, 1987). Any correlations are important considerations for agriculture, as there is a need to understand the mechanisms that facilitate or constrain insecticide resistance evolution (Baker et al., 2007; Sparks et al., 2012).

The common fruit fly (*Drosophila melanogaster*, Meigen 1830) makes an excellent system to study natural selection across winter. Beyond the exceptional molecular and population genetic tools and amenability as a model organism, *D. melanogaster* is among the species most studied in overwintering contexts including responses to low temperature (Hoffmann et al., 2003a; MacMillan & Sinclair, 2011; Overgaard et al., 2014; Rako & Hoffman, 2006; Schmidt et al., 2005). Despite a tropical ancestral origin, local populations of *D. melanogaster* can overwinter even at high latitudes, though they may use human dwellings or compost piles as overwintering refugia (Ives, 1970). Mortality in winter can exceed 90%, though field survival data are scarce (Ives, 1970; Izquierdo, 1991; Mitrovski & Hoffman, 2001; Nunez et al., 2024). Spatial patterns of genomic variation suggest that high-latitude populations exhibit patterns of local adaptation and maintain relatively high effective population sizes overwinter (Cogni et al., 2015; Collett & Jarman, 2001; Ives, 1970; Machado et al., 2021). Thus, it is uncertain how evolution across winter allows *D. melanogaster* to maintain genetic variation and expand successfully in spring despite population bottlenecks.

Low temperature responses of flies show patterns of latitudinal variation in putative winter adaptations (Gibert et al., 2001; Hoffman et al., 2003b; Kellerman et al., 2012; Overgaard et al., 2014). These include traits for maintaining homeostasis under subzero conditions (Denlinger & Lee, 2010; Hoffman et al., 2003a; Sinclair et al., 2003; Teets et al., 2023), higher reproductive diapause incidence (Collett & Jarman, 2001; Schmidt et al., 2005), greater stress tolerance (Hoffman, 2010; MacMillan & Sinclair, 2011) due to life-history allocation trade-offs (Stearns, 1998), and larger body size based on laboratory and field studies (Angilletta, 2009; James et al., 1997; Partridge et al., 1994). Fall fly populations exhibit phenotypic (Behrman et al., 2015) and genomic signatures associated with summer selection (Bergland et al., 2014). Given that insecticide treatments are not commonly applied in winter, insecticide-resistant fall populations that overwinter as adults would face selective pressure from low temperatures while potentially incurring costs for carrying resistance (McKenzie, 1994; Roush & McKenzie, 1987). This could mean that resistant alleles decline in frequency across winter, an important factor for pest population control measures.

Assessment of rapid evolutionary adaptation and potential tradeoffs requires replicate populations that undergo selection in parallel; however, such studies are often carried out in controlled laboratory conditions (but see Hoffman et al., 2003a; Sgrò & Hoffman, 1998). Here, we use a field experiment to assess both the magnitude of overwintering adaptation and the potential for trade-offs or covariation with adaptation of insecticide resistance. Specifically we ask the following questions: (1) Does an overwintering period drive adaptation as measured by parallel genetic change across populations? (2) Is there a trade-off between selection during the overwintering period and prior selection for insecticide resistance? We hypothesized that strong overwinter mortality drives adaptation for greater overwintering performance (Izquierdo, 1991; Mitrovski & Hoffman, 2001). Therefore, we predicted that flies that successfully overwinter would evolve greater body size, reduced fecundity, and greater starvation tolerance. For our second question, we define a trade-off as the observed negative association between evolved resistance and phenotypic measures including survival: a broader definition of the term "trade-off" that describes the outcomes from rather than the mechanistic causes of a trade-off (Garland et al., 2022). We expected that overwintering survival would negatively covary with the prior evolution of resistance to an insecticide (McKenzie, 1994; Miyo et al., 2000). We predicted that overwintering selection would reduce insecticide resistance and, in turn, that resistant populations would have lower survival and reduced performance of cold tolerance traits.

Replicated field experiments can feature environmental conditions that closely resemble those found in nature. They also allow for larger population sizes than is typically feasible in laboratory evolution experiments, increasing evolutionary realism. In this vein, we established *D. melanogaster* populations into 40 replicated outdoor mesocosms in the summer and tested for parallel phenotypic evolution across independent populations. With a subset of these original populations, we then used repeated common garden rearing following an overwintering period to test whether overwintering drove adaptation and for any negative correlation between insecticide resistance and overwintering performance.

Materials and methods

Multigenerational experiment and fly populations

We tested fly populations as part of a multigenerational selection experiment following previously described protocols outlined in Rudman et al. (2022). *Drosophila melanogaster* populations were founded from 100 DGRP lines (*Drosophila* Genetic Reference Panel; Mackay et al., 2012) with 5 males and 10 females from each line placed into a population cage. This "hybrid swarm" population was allowed to mate, facilitating recombination, and grow at density-controlled conditions for nine generations prior to release outdoors. This breeding design decreases linkage disequilibrium through recombination of the founder haplotypes (Weller et al., 2021). Populations were reared for approximately nine generations in outdoor mesocosms located 45.729 N, −122.633 W (from here on "orchard") from July

17 until October 28, 2023. Throughout the experiment, all populations were fed a modified Bloomington recipe media.

Fly populations included 10 cages fed control media (from here on "control" populations with an approximate average population size of 43,000 flies across the season) and 10 populations fed exclusively media treated with a 0.0375 μg L^{-1} concentration of the organic insecticide spinosad (Entrust; Dow AgroSciences, Indianapolis, IN, USA) (average seasonal population of 7,000 flies). This concentration corresponds with the lethal dose at which 50% of flies saw mortality based on dose–response assays conducted on founder flies under standardized laboratory conditions (see supplementary information in Shahmohamadloo et al., 2025). Spinosad is a widely used insecticide for fruit crops and is applied prior to and during fruit ripening (Scott et al., 2024). Spinosad targets nicotinic acetylcholine neuronal membrane receptors (nAChR), leading to neuronal overexcitation and larval mortality (Martelli et al., 2022; Perry et al., 2007; Salgado, 1998). Five of 10 insecticide-exposed populations went extinct during the growing season. Five persisted—possibly via evolutionary rescue through the evolution of spinosad resistance from standing genetic variation (Perry et al., 2007, 2021; hereafter "resistant" populations). While resistance to spinosad is likely polygenic, it might include variation in target-site proteins, though the magnitude of evolved resistance in this study is modest compared to what can be observed in agricultural settings (Gress & Zalom, 2019).

Overwintering experimental design

At the end of the growing season, we collected eggs from eight outdoor control and five outdoor resistant populations, reared flies indoors under common garden conditions for two generations, and conducted fall phenotypic assays (Figure 1). From these common garden-reared populations, we collected approximately 2,000 adult flies per population for the overwintering experiment.

The orchard experiences a relatively mild temperate winter typical of the western Pacific Northwest, USA, but it still presents challenging conditions for *D. melanogaster* including occasional subfreezing air temperatures (Figure S1). To provide a favorable environment, we used 5-L clear plastic containers with a small, screened opening to allow aeration and added layers of hay pellets, ash wood chips, and cotton for insulation. We then transferred flies from each independent population and treatment type into an overwintering container and then placed the overwintering flies into an outdoor rearing cage. After 5 weeks in the outdoor cage, we transferred the flies to a greenhouse and then an indoor incubator to simulate a spring-like phenology cue in both temperature and photoperiod. Following the overwintering and spring acclimation period, we expanded post-overwintering flies for two generations and began our phenotyping trials (see Figure 1 and supplemental material for full details and timing of the experimental design).

Experimental constraints influenced aspects of the design, including use of containers, relatively brief overwintering period, and a lack of thermal refugia. While these choices do impact realism, they were largely made to balance severity of the overall winter environment populations experienced and the necessity of containing individuals in a way that facilitated collection following overwintering. There is evidence that *D. melanogaster* changes dietary preference in fall to polyunsaturated fats to enhance overwinter survival (Brankatschk et al., 2018), but our use of consistent diet precluded any change. Regardless of design decisions, fly populations still experienced strong selection from temperature and photoperiodic effects consistent with winter in a high-latitude environment.

Phenotypic assays

To assess temporal evolutionary responses during the growing season, eggs were collected from each replicate mesocosm in density-controlled 200-ml bottles at regular intervals (August, September, and October) and reared in common garden conditions as described earlier. The following phenotypes were assayed per replicate mesocosm: (1) Insecticide resistance measured as survivorship to adulthood on 0.0375 μg L^{-1} concentration spinosad: the proportion of eggs (30 eggs per vial) that survived to adulthood in three replicate vials; (2) fecundity: the total eggs laid by five females over 3 days, measured in each of three replicate bottles, and scored following Gabidulin & Rudman (2025); (3) starvation tolerance: the time to starvation for three replicate vials containing 10 males each on agar-only media; and (4) adult body size: measured as the average dry mass of three pools of five females, dried at 55 °C for 24 hr. We repeated these phenotypic assays for the post-overwintering period but also included a fifth measure: chill coma recovery time (CCRT), a static measure of cold tolerance widely used in assessing low temperature responses in *D. melanogaster* (Gibert & Huey, 2001). We immersed 15 female flies in three replicates per cage in an ice water bath to induce chill coma at 0° C for 2 hr following Macdonald et al. (2004) and Rako and Hoffman (2006). We manually scored recovery at 20 °C as the time to the minute when flies were able to right themselves.

Statistical analyses

To test adaptation across winter, we compared phenotypic measures from the populations collected in October (from here on "fall") and following the overwintering period (from here on "post-overwintering"). We modeled each phenotypic measure as the response variable, time point as our fixed-effect predictor variable, and considered cage (i.e., individual population) as a random effect. To assess a potential trade-off in resistance, we tested for differences in survival and evolutionary divergence in traits across time points between outdoor-reared resistant and control populations. Here, each phenotypic measure was the response variable, time point and population type were treated as fixed effects along with their interaction, and cage was treated as a random effect. Since CCRT was only measured in post-overwintering populations, we tested cold tolerance between overwintered control and resistant populations in a model with population type as the predictor variable and cage as a random effect.

To model each phenotypic measure for each question, we used generalized linear mixed-effects models constructed with the *glmmTMB* function from the *glmmTMB* package (Brooks et al., 2017; see supplemental material for details on each model). Model diagnostics were conducted using the *simulateResiduals* and *testDispersion* functions from the *DHARMa* package (Hartig & Lohse, 2022). Our model

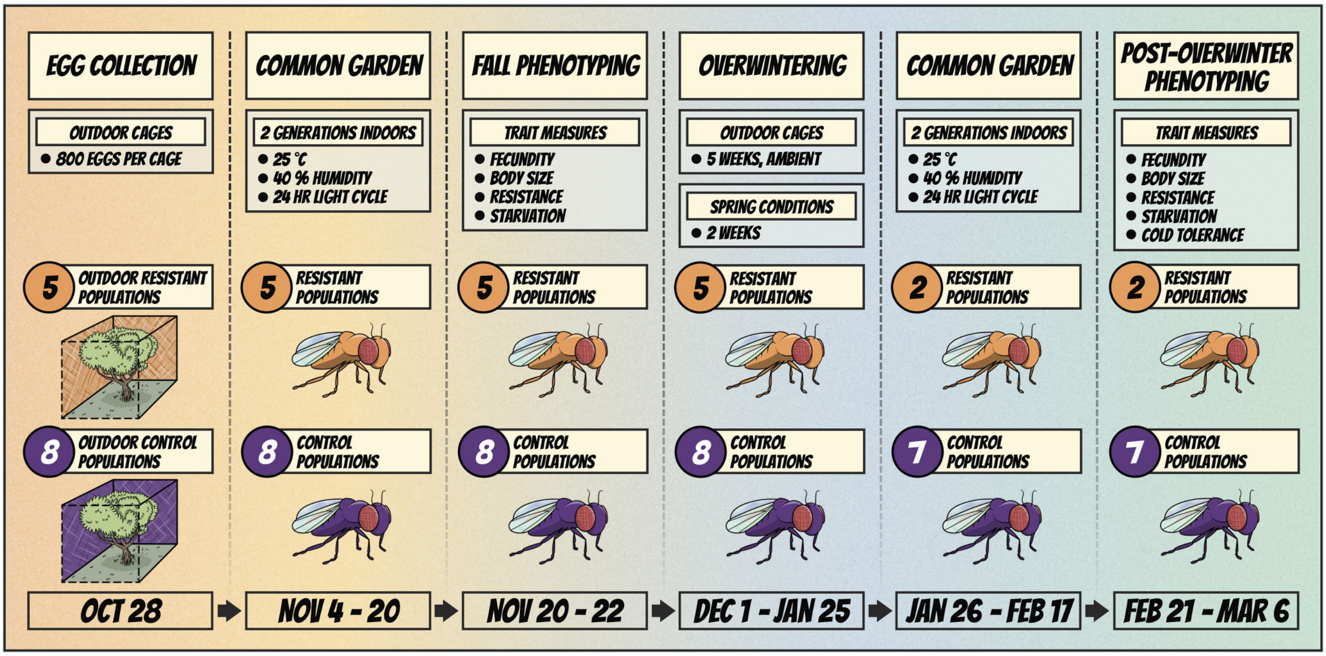

Figure 1. Timeline of the overwintering experimental design. At the end of the growing season, we collected approximately 800 eggs per cage from eight outdoor control and five outdoor resistant populations. We then reared flies indoors under common garden conditions for two generations and then conducted phenotyping for the fall time point. From this same generation of flies, we collected approximately 2,000 adults per population and placed them in overwintering containers with a non-spinosad-treated food resource held within a large 8-m^3 outdoor rearing cage. After 5 weeks in the outdoor overwintering trial, we transferred the flies to a greenhouse and then an indoor incubator to simulate spring-like phenology cues in both temperature and photoperiod and to encourage reproduction (see Table S1 for rearing conditions). During the overwintering and spring, four populations (three resistant and one control) were extirpated, leaving a final sample size of two resistant and seven control populations. We then collected 800 eggs from these overwintered flies and reared them under common garden conditions. We expanded these post-overwintering flies for two generations at a standard low density (1,500 eggs/300 ml of media), and 3–5 days after eclosion, we conducted phenotyping assays of fecundity, body size, cold tolerance, insecticide resistance, and starvation tolerance (see main text and Table S2 for details).

contrasting control and resistant populations was unable to meet assumptions of homoscedasticity, and efforts to correct for this did not improve model fits. We opted to retain original model designs but deemphasized the focus on significance relative to effect size. To do so, we prioritized effect size interpretation in the results but still report *p*-values from significance tests for completeness. We determined significance of the predictors using log-likelihood ratio tests in the *Anova* function with the type = "III" argument from the *car* package (Fox & Weisberg, 2019) and calculated effect sizes as *Hedges' g* using the *hedges_g* function from the *effectsize* package (Ben-Shachar et al., 2020). All analyses were conducted in R version 4.3.3 (R Core Team, 2024).

Results

Field survival of overwintering flies

All populations experienced severe mortality of >98% from a starting population of 2,000 adult flies (Figure 2), with 1.67% of control and 0.62% of resistant flies surviving the overwinter trial. Overall, 60% of resistant and 12.5% of control populations went extinct during the overwintering period and there was a non-significant trend of population type on survival ($\chi^2 = 5.35, p = 0.0689$).

Adaptation across winter

We found evidence for parallel temporal evolution in most phenotypes in control populations. Compared to fall populations, post-overwintering populations exhibited smaller dry mass (Figure 3A; estimate = −0.398, *SE* = ±0.0606; $\chi^2 = 43.2, p < 0.0001$), greater fecundity (Figure 3B; estimate = 0.515, *SE* = ±0.0543; $\chi^2 = 136.7, p < 0.0001$), and decreased spinosad resistance (Figure 3D; estimate = −24.9, *SE* = ±2.08; $\chi^2 = 174.7, p < 0.0001$). There was no difference in starvation tolerance between fall and post-overwintering populations (Figure 3C; estimate = 3.57; *SE* = ±2.85; $\chi^2 = 1.56, p = 0.212$).

Potential for trade-offs between winter conditions and insecticide resistance

To test for trade-offs between overwintering selection and insecticide resistance, we compared phenotypic performance in fitness-associated traits between fall and post-overwintering control and resistant populations. Extinction, particularly the high proportion observed in resistant populations, reduced our statistical power for this contrast. Given this unbalanced contrast and overlapping phenotypic distributions, we interpret the results with the effect size difference between population types (*Hedges' g*: control minus resistant; with 95% confidence intervals) at both time points. We report these values to show the magnitude of the differences in groups but note that biological inference is limited due to these constraints. For completeness, we also report the model outputs of predictors along with associated test statistics and *p*-values (Tables S3 and S4).

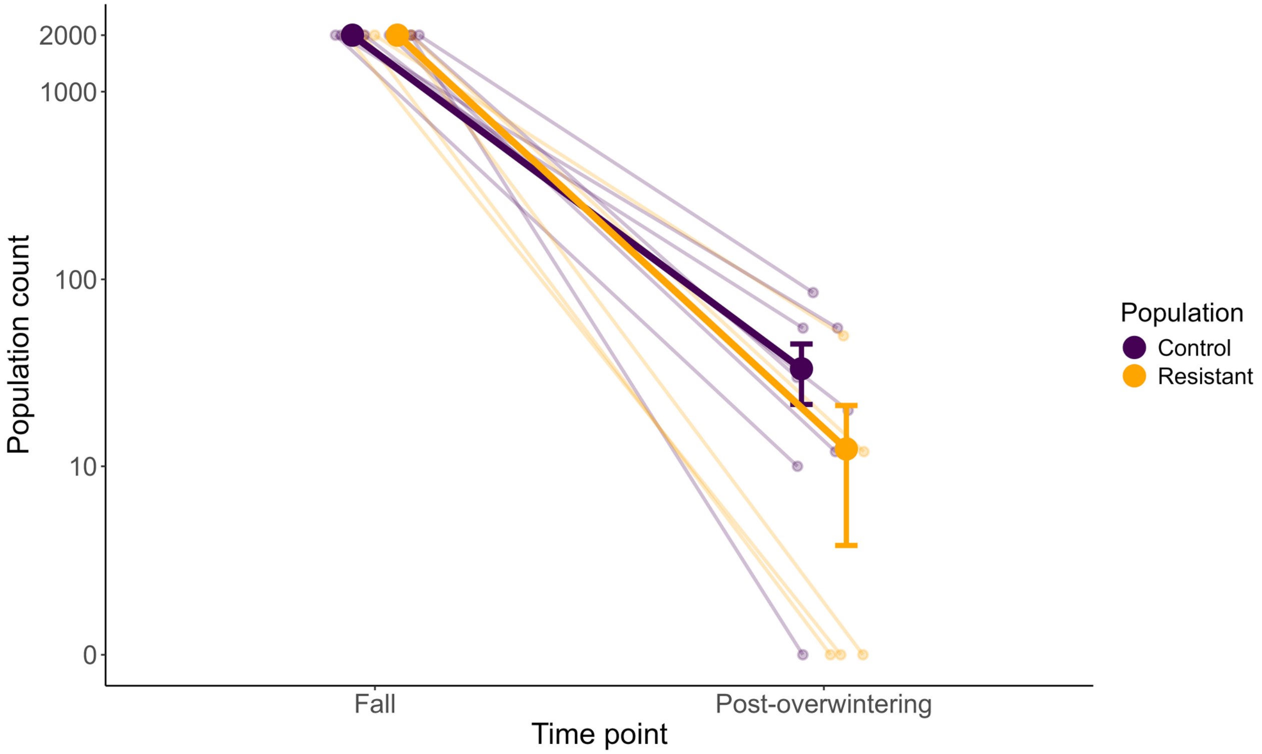

Figure 2. Population counts of flies before and after the overwintering period. The population count on the y axis is on a "pseudo" natural log scale that can accommodate the zero values that indicate population mortality. Large points show the mean number of surviving flies per population type ± 1 *SE* with colors corresponding to each population type; note: *SE* bars for the founder cages are within the size of the point). Smaller points jittered behind the means show the average number of flies per replicate cage, while lines connecting each point show the decline in population size across all replicates.

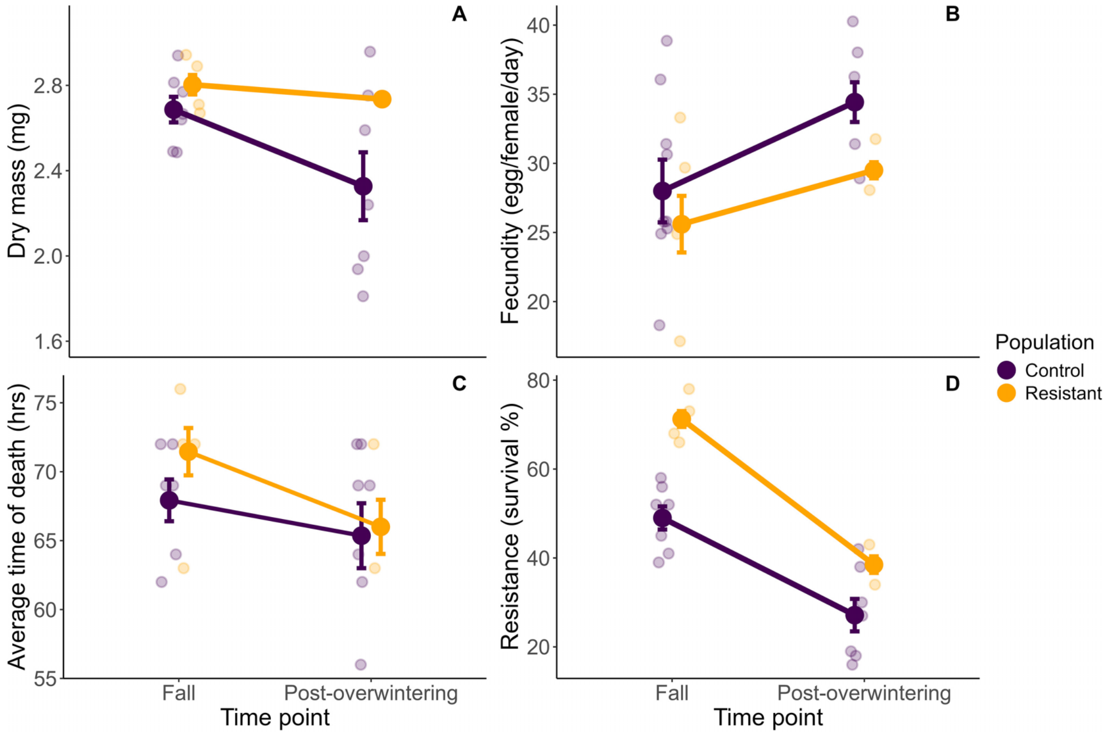

Figure 3. Phenotypic measures of outdoor control populations and outdoor resistant populations of *D. melanogaster* measured in the fall and following the overwintering experiment. Large points show the mean trait value of each population type ± 1 *SE*. Smaller points jittered behind the means show raw data values for each independent population's phenotypic measure. (A) Average dry mass in milligrams. (B) Average fecundity across a 3-day assay. (C) Starvation tolerance measured as average time to mortality. (D) Spinosad resistance measured as the percent egg-to-adult survival on insecticide media. Note: In panel A, raw data points fall within the size of the post-overwintering resistant mean point.

We found that for both body size and fecundity, the phenotypic difference between control and resistant populations increased after overwintering. Specifically, control populations showed a greater reduction in dry mass and a greater increase in fecundity relative to resistant populations (dry mass: Figure 3A; control minus resistant *Hedges' g* fall = −0.78 [−1.49, −0.05], post-overwintering = −1.05 [−1.97, −0.12]; fecundity: Figure 3B; control minus resistant *Hedges' g* fall = 0.41 [0.04, 0.078], post-overwintering = 1.42 [0.85, 1.98]). To directly test for a negative correlation between winter conditions and spinosad resistance, we compared egg-to-adult survival on spinosad for both population types between time points. We found that the magnitude of greater survival for resistant over control populations declined in post-overwintering populations (Figure 3D; control minus resistant *Hedges' g* fall = −3.52 [−4.10, −2.94], post-overwinter = −1.90 [−2.30, −1.49]). We did not find any differences in starvation tolerance (Figure 3C; Table S3; control minus resistant *Hedges' g* fall = −0.29 [−0.99, 0.41]; overwinter = −0.37 [−1.25, 0.52]) nor were there differences in CCRT between population types following the winter period (Figure S2 and Table S4; control minus resistant *Hedges' g* post-overwinter = −0.42 [−0.87, 0.04]).

Discussion

Winter can lead to dramatic declines in temperate insect populations, and the mechanisms that allow population persistence, including rapid adaptation, are poorly understood. Additionally, it is important to understand how insecticide-resistant populations respond to winter selection and if survival negatively covaries with resistance. From overwintered *D. melanogaster* in outdoor mesocosms, we detected evidence for adaptation in multiple phenotypic traits across the winter period, and a putative trade-off between the evolution of insecticide resistance and winter survival. These findings demonstrate that winter conditions can pose strong selective pressures and suggest that this selection may act against spinosad-resistant genotypes. Our findings have implications for insecticide use in pest management, impacts to non-target species, and overwintering evolution in a changing climate.

Adaptation across winter

In the outdoor-reared control populations, there was repeated evolution of multiple traits but not always in the predicted direction. From both insect thermal physiology and field data of *Drosophila* (Chown & Nicolson, 2004; James et al., 1997), we expected that post-overwintering populations would evolve larger body size and reduced fecundity when compared to fall populations, but instead the reverse was true. One explanation could be that many post-overwintering *Drosophila* females can carry male gametes within their spermatheca from fall matings (Boulétreau-merle & Fouillet, 2002; Collet & Jarman, 2001). Previous work suggests that fall populations are shaped by several generations of selection during the summer and early fall, while spring populations reflect selection across the winter (Behrman et al., 2015; Machado et al., 2021). Thus, females who successfully overwinter could produce maladapted offspring (Collet & Jarman, 2001).

Alternatively, body size and fecundity could respond in a counter-gradient fashion to the winter period. Larger body size can covary with lower temperature (i.e., Partridge & Coyne, 1997; Partridge et al., 1994) and fecundity can trade off with stress tolerance and diapause incidence, a pattern found in *D. melanogaster* across latitudinal gradients (Angilletta, 2009; Denlinger & Lee, 2010; Schmidt & Conde, 2006). However, reverse clines have been documented in nearly equal numbers in arthropods (Blanckenhorn & Demont, 2004; reviewed in Shelomi, 2012). In a counter-gradient scenario, overwintering selection in high-latitude *D. melanogaster* might favor traits such as greater fecundity or a rapid change into and out of reproductive diapause (Schmidt & Paaby, 2008) as this could allow populations to jumpstart and access resources more quickly when spring conditions become favorable (Bachmann et al., 2020; Conover et al., 2009).

Potential costs for overwintering-resistant populations

The evolution of resistance during the growing season was associated with reduced overwinter performance that is suggestive of a trade-off. First, a greater number of resistant fly populations went extinct across winter relative to control populations. Second, the difference in survival on spinosad between resistant and control populations decreased from fall to post-overwintering when exposed to spinosad, suggesting that resistant flies that survived the winter might have reduced resistance. In our study, we likely observed polygenic resistance adaptation, but target-site-mediated resistance could have unique pleiotropic effects on fitness including greater oxidative damage (Weber et al., 2012), decreased longevity, altered lipid environments, and vision loss (Martelli et al., 2022; Perry et al., 2015). These detrimental, sublethal effects of resistance could negatively interact with low temperature stress of overwintering, leading to the greater mortality we observed (ffrench-constant & Bass, 2017). While these patterns are consistent with costs of resistance, the small number of surviving resistant populations ($n = 2$) limits our statistical power and precludes strong inferences. We further note that the observed decline in resistance following overwintering could also reflect temporal variation in assay conditions rather than evolutionary change (Gray et al., 2025; Stone et al., 2020) and so we interpret these patterns cautiously.

Surprisingly, we did not find the expected differences in cold and starvation tolerance between resistant and control populations as drivers of lower overwintering survival. One possibility is that our measure of cold tolerance (CCRT) did not fully capture variation in low temperature performance (Andersen et al., 2015; Garcia et al., 2020). Although reduced survival in resistant populations might have been driven by variation in membrane fluidity (Brankatschk et al., 2018), since this is a factor in CCRT (Teets et al., 2023) that was consistent between population types, we think this is less likely. We interpret these phenotypic results carefully, however, as these findings might be due to survivorship bias and the reduced sample size for overwintered resistant populations limited the power to detect trait differences.

The genomic architecture of adaptation to seasonal variation has been well-studied in *D. melanogaster*, with several field studies and experiments demonstrating a largely polygenic basis of rapid adaptation (Bergland et al., 2014; Machado et al., 2021; Rudman et al., 2022). Future work determining the genomic basis of adaptation to overwintering, including the prominence of structural variants (many cosmopolitan inversions were present in the founder population used for this experiment; Table S5; Machado et al., 2021; Nunez et al., 2024), could elucidate any fluctuating selection associated with temporal variation and insecticide exposure.

Interactions and implications of overwintering, resistance, and pest management

Overall, there is little known about interactions between insecticide resistance and overwintering insect biology, and the evidence to date is mixed. In the Colorado potato beetle (*Leptinotarsa decemlineata*, Say 1824), a major agricultural pest, resistant populations have shown stimulated investment in fat body tissue that increase metabolic fuel and inherit epigenetic effects that elicit general stress responses that increase overwintering success (Brevik et al., 2018; Lehmann et al., 2014; Sinclair, Sinclair, 2015). In other studies, however, resistant beetles have exhibited lower overwintering survival due to maladaptive behavioral responses (Ferro et al., 1999; Piiroinen et al., 2013). Antagonistic responses between winter stress and insecticide resistance have been observed in insecticide-resistant green peach aphids (*Myzus persicae*, Sulzer 1776) and northern house mosquitos (*Culex pipiens*, Linnaeus 1758), which showed lower survival overwinter and differential success in finding overwintering refugia, respectively (Bourguet et al., 2004; reviewed in Kliot & Ghanim, 2012). Given the limited and mixed evidence among insect taxa, more research with population-level replication is needed to test whether negative correlations between winter survival and insecticide resistance impact population dynamics of pests.

Trade-offs between overwintering and insecticide resistance have important implications for both rapid adaptation and population dynamics under climate change. If resistance evolution is strongly temperature dependent, climate warming could substantially alter the dynamics of resistance evolution (Easterling et al., 2000; IPCC, 2023; Williams et al., 2015). One area of concern is that relaxed selection from winter could lead to greater population-level resistance and range expansions of overwintering pest populations. Indeed, with milder winter conditions, resistant pests have expanded into higher latitudes including the diamondback moth (Ma et al., 2021), multiple tick species (Molaei et al., 2022), and Colorado potato beetle (Piiroinen et al., 2013). Given that winter climate change is also associated with greater variability and population decline (Sinclair et al., 2013; Williams et al., 2015), our findings underscore the importance of monitoring and managing resistance in a rapidly changing world.

Conclusion

In this study, we add important evolutionary context for the well-studied ecology and physiology of overwintering ectotherms. Notably for *D. melanogaster*, adaptation leading to population persistence (and maintenance of genetic variation) overwinter seems to be critical for the "spring reset" where greater fecundity can allow rapid population growth and recolonization of resources at the start of the next growing season (Behrman et al., 2015; Machado et al., 2021). That winter led to greater mortality in resistant populations demonstrates potential costs to spinosad-resistant flies in their ability to make this same reset. More broadly, this work has implications for understanding seasonal demographics of important agricultural pollinators, management of pest species, and rapid adaptation of ectotherms in temperate climates.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Data are available via the Open Science Framework: DOI: 10.17605/OSF.IO/MW9R6, https://osf.io/mw9r6/

Author contributions

E.G.P.: Conceptualization, Investigation, Formal analysis, Visualization, Writing—Original draft, and Writing—Reviewing and editing; B.C.: Investigation, Writing—Reviewing and editing; C.I.C.: Investigation, Writing—Reviewing and editing; A.R.G.: Investigation, Writing—Reviewing and editing; R.S.S.: Investigation, Writing—Reviewing and editing; S.M.R.: Conceptualization, Investigation, Formal analysis, Project administration, and Writing—Reviewing and editing

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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