PROCEEDINGS B

royalsocietypublishing.org/rspb

CC BY

Research

Cite this article: Prileson EG, Campagnari B, Ruotsalainen B, Shahmohamadloo RS, Zetina C, Rudman SM. 2026 Insecticide resistance evolution and assisted gene flow interact to shape the evolution of plasticity. *Proc. R. Soc. B* **293**: 20262085.
https://doi.org/10.1098/rspb.2026.2085

Received: 18 April 2026
Accepted: 14 September 2026

Subject Category:
Evolution

Subject Areas:
evolution, genetics, ecology

Keywords:
phenotypic plasticity, evolution of plasticity, evolutionary rescue, rapid adaptation, insecticide resistance, assisted gene flow

Author for correspondence:
Eric G. Prileson
e-mail: eric.prileson@wsu.edu

Supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.8737484.

THE ROYAL SOCIETY PUBLISHING

Insecticide resistance evolution and assisted gene flow interact to shape the evolution of plasticity

Eric G. Prileson[1], Bianca Campagnari[1,2], Brynne Ruotsalainen[1], René S. Shahmohamadloo[1], Cristian Zetina[1] and Seth M. Rudman[1]

[1]School of Biological Sciences, Washington State University - Vancouver Campus, Vancouver, WA, USA
[2]University of California Riverside, Riverside, CA, USA

EGP, 0000-0003-1103-3900; RSS, 0000-0002-5373-9808

Adaptive phenotypic plasticity can bolster fitness in changing environments, but the extent to which plasticity evolves rapidly, and which forces shape its evolutionary trajectory, are largely unknown. To empirically study the evolution of plasticity, we first conducted a replicated field experiment in which *Drosophila melanogaster* populations adapted to insecticide exposure and a subset of these populations received high diversity assisted gene flow. We then reared individuals from each population across temperature and insecticide treatments in common garden conditions to test the following questions: (i) has prior selection and rapid adaptation of insecticide resistance led to evolved shifts in plasticity relative to naive populations? (ii) Does gene flow from genetically diverse populations contribute to adaptive plasticity evolution relative to gene flow-restricted low diversity populations? Both gene flow and prior evolution of resistance influenced the evolution of plasticity for multiple traits and were often maladaptive for resistant and gene flow-restricted populations, suggesting a trade-off between trait and plasticity evolution. Assisted gene flow minimized maladaptive plasticity potentially through relaxation of underlying epistatic or pleiotropic constraints. Together, these results demonstrate the dynamic interactions between trait evolution, the evolution of plasticity and forces that shape genetic diversity, with implications for conservation of threatened populations.

1. Introduction

When environmental conditions shift over short time-scales, they can exacerbate mismatches between organismal phenotypes and the environment. In the absence of compensatory mechanisms that reduce this mismatch, populations are likely to decline, potentially to extinction. To persist, populations can respond across generations via evolution but also more immediately within a generation through phenotypic plasticity—the capacity of a genotype to express different phenotypes in response to environmental variation (figure 1*A*) [1,2]. Following environmental shifts, these environmentally dependent traits could be adaptive if the plastic response moves the phenotype closer to new fitness optima [3–5]. On the other hand, plasticity might be maladaptive if the direction of plastic response opposes novel fitness optima or fails to minimize the phenotype–environment mismatch [6,7]. Complicating this picture is the fact that across generations, plasticity itself can evolve rapidly, with potentially strong effects on fitness (figure 1*A*) [1,8,9]. Determining the pace, direction and key drivers of the evolution of plasticity relative to trait evolution

© 2026 The Authors. Published by the Royal Society under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/, which permits unrestricted use, provided the original author and source are credited.

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

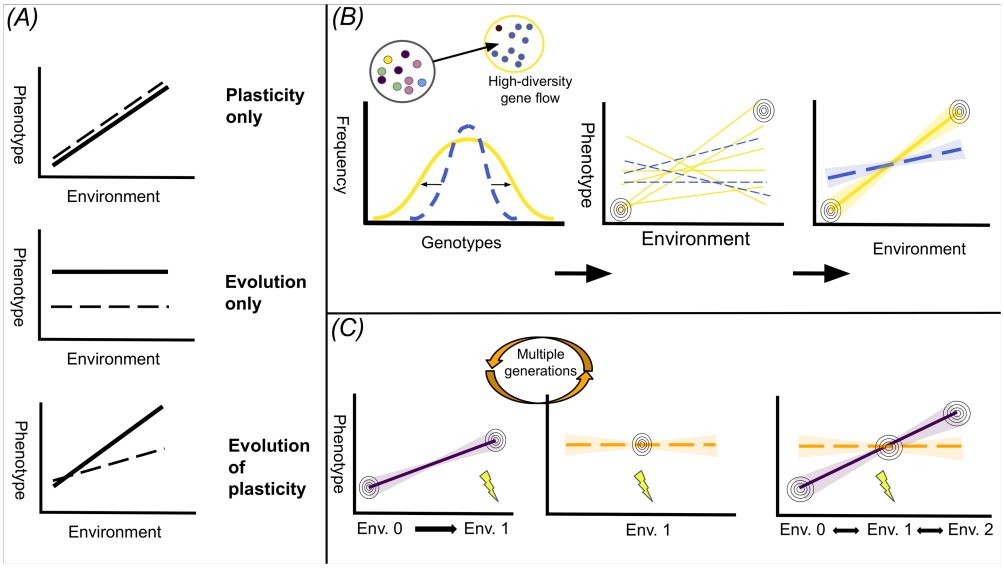


Figure 1. A simplified schematic of how linear reaction norms might differ between populations and processes underlying their evolution. *(A)* Three possible outcomes for population-level reaction norms across environments. Different populations (solid and dashed lines) can both express strong plastic traits (top), evolved differences with different intercepts but with no change in slopes (middle), or different intercepts and slopes (bottom). *(B)* The effect of genetic variation on the evolution of plasticity. An increase in the genetic diversity of a population, such as through assisted gene flow (left), can provide greater variation in reaction norms in diverse (solid lines) relative to less diverse (dashed lines) populations. For genetically diverse populations (centre; solid lines), there is a higher probability of reaction norms within the population matching optima across environments (concentric circles) compared with less diverse populations (dashed lines) and selection can move the average reaction norm closer to these optima (right). *(C)* Depiction of genetic assimilation. A population can express an adaptive plastic response between an ancestral environment (Left; Env. 0) and a novel one with a strong stressor (Env. 1; lightning bolt). If the novel stressor remains and the maintenance of plasticity is costly, strong selection (centre) could canalize the expression of the phenotype such that the plastic response is lost over generations (orange dashed line). If the canalized trait does not match optima under returns to ancestral (right; Env. 0) or new environments (Env. 2), this could lead to a trade-off compared with a plastic population (purple solid line).

is therefore critical to understanding its role in population responses to environmental change, including potential rescue from extinction.

Plasticity can evolve if there is standing genetic variation in sensitivities to the environment (i.e. reaction norms), a process called genetic accommodation (figure 1*B*; [1,10,11]). Fundamental evolutionary forces that alter genetic diversity then are likely to be key for the evolution of plasticity [12,13]. For example, within sub-populations, selection can decrease, whereas gene flow can increase genetic diversity on which selection can act relative to the broader metapopulation [14,15]. Assisted gene flow, or the directed introduction of new alleles, is one such process that can increase the diversity and probability of persistence for populations at risk of extinction [16,17]. There is conflicting evidence, however, regarding whether increased genetic variation on its own will increase the magnitude of plasticity [18–21] or if evolved plasticity will be adaptive because gene flow could swamp locally adapted populations with maladaptive alleles [22]. In addition to potential effects of demography, the evolution of plasticity could also be constrained by costs of plasticity and maladaptive reaction norms [20,23,24]. Further, plasticity could also be lost altogether if selection canalizes previously plastic traits through genetic assimilation (figure 1*C*; [25,26]; reviewed in [27]). Thus, while genetic variation can lead to the evolution of plasticity, complexities of the fitness landscape will dictate the change in direction or magnitude of population-level reaction norms and their potential to rescue populations.

Natural populations face complex environments including anthropogenic stressors, but it is unclear how adaptation for one stressor bolsters or constrains the evolution of plasticity in multiple environments [28,29]. Spatial or temporal decoupling of a stressor in different environments presents an opportunity to test for evolutionary constraints or plastic phenotypes that are otherwise cryptic [30,31]. For example, temperate insects in an agricultural environment contend with both insecticides and seasonal variation, but because insecticides are primarily applied during warm seasons, low temperatures and insecticide exposure might not co-occur. Energetic constraints then may lead to selection favouring alternative alleles at different time points owing to negative trait correlations (via pleiotropy or linkage) that oppose the direction of selection [32,33]. Indeed, prior work documenting greater extinction of resistant *Drosophila melanogaster* populations over winter [34] could be explained by a trade-off between resistance evolution and adaptive thermal plasticity. In addition, simultaneous exposure to both insecticide and low temperature could dramatically affect reaction norms and reveal interactions that are antagonistic or synergistic, with the combined effects cancelled out or amplified, respectively [35,36]. Given these different outcomes, there is a clear need to investigate how resistance evolution impacts the evolution of plasticity in multiple environments, but to do so requires substantial population-level replication and a system that allows for experimental manipulation.

The fruit fly *D. melanogaster* (Meigen 1830) is an excellent system in which to test the evolution of plasticity and to identify the factors underlying it. As a model system, *D. melanogaster* is amenable to large-scale evolution experiments that provide environmental realism without reducing replication [37,38] and it has been used to track rapid adaptation over seasonal time-scales

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

(i.e. adaptive tracking [39]). Further, the genetic resources of *D. melanogaster* allow for manipulation of genetic variation from a known genetic history. There is also abundant empirical evidence of developmental plastic responses in thermal physiology (reviewed in [10,40]), as well as genetic variation of plastic responses to low temperature [41–43]. When reared at low temperatures, *D. melanogaster* exhibits plastic traits that are putatively adaptive, including reduced fecundity [44,45], improved starvation tolerance [46], greater cold tolerance [47,48] and larger body sizes [49,50]. In addition, *D. melanogaster* can evolve resistance to insecticides [51–53], but it is unknown how resistance evolution impacts the evolution of plasticity. Sudden environmental stress such as insecticide exposure can lead to genetic assimilation in *D. melanogaster* ([25]; reviewed in [54]), but whether resistance is related to initial plasticity to insecticide exposure that becomes canalized is uncertain.

Here, we address how prior selection and assisted gene flow affect the evolution of plasticity in *D. melanogaster* by asking the following questions: (i) Does rapid evolution of insecticide resistance impact the evolution of plasticity relative to susceptible control populations? (ii) Within insecticide-resistant populations, does gene flow from a high-diversity population facilitate the evolution of adaptive plasticity relative to populations without gene flow? To test these questions, we used populations of *D. melanogaster* that differed in prior-evolved insecticide resistance and gene flow histories. Following evolution during an outdoor growing season and common garden rearing, we raised populations across temperature, insecticide exposure, and their combination to test the evolution of plasticity in stress response and fitness-associated phenotypes. Broadly, we expected directional selection and gene flow to shift the magnitude and direction of reaction norm slopes based on changes to genetic diversity [10,26], but we had divergent predictions for reaction norm evolution depending on the population and developmental environment. We predicted that across temperature, constraints from selection (for resistant populations) and reduced genetic diversity (from lack of gene flow) would lead to reaction norms that were putatively maladaptive. To test whether prior selection from insecticide leads to canalization, we predicted that across an insecticide treatment, resistant populations would have dampened plasticity but greater evolved trait values on the insecticide than susceptible populations. Together, these questions and our approach addressed how key processes affect the evolution of plasticity in complex environments with broader implications for responses to rapid environmental change.

2. Methods

(a) Multigenerational experiment and founding fly populations

To investigate the evolution of plasticity, we measured plasticity in fly populations following a multigenerational outdoor selection experiment in 2024 in Vancouver, WA, USA. *D. melanogaster* populations were founded from DGRP lines (*Drosophila* Genetic Reference Panel; [55]). Mesocosms were maintained at the Washington State University Vancouver campus orchard (45.729 N, −122.633 W; henceforth 'orchard') from 18 June to 24 October 2024, encompassing nine overlapping generations. Throughout the experiment, all populations were fed a modified Bloomington recipe following Rudman *et al.* [39].

We experimentally manipulated the genetic composition of the starting *D. melanogaster* populations. We first founded a high-diversity population from 100 DGRP laboratory fly lines (henceforth 'HD control') and low-diversity populations from 15 DGRP lines (henceforth 'LD control'). Each of these population types was recombined in the laboratory and reared for four generations on control medium prior to outdoor rearing. Additionally, a subset of the LD control populations was reared on medium treated with a 0.0375 µg l^{-1} concentration of the organic insecticide spinosad (Entrust; Dow AgroSciences, Indianapolis, IN, USA) as part of an artificial selection experiment for five generations (henceforth 'pre-adapted' flies). This concentration corresponds to the lethal dose at which 50% of flies experienced mortality based on dose–response assays conducted on founder flies under standardized laboratory conditions (see supplementary material in [53]).

(b) Selection event and gene flow manipulation

We allowed populations to expand for one generation outdoors in 8 m^3 mesocosms beginning on 18 June 2024. Then, on 5 July 2024, we introduced insecticide treatments to food media of 16 of the 36 LD control populations as a strong agent of directional selection (supplementary material, figure S1). For both the selection event and subsequent food treatments, insecticide medium was treated with a 0.0375 µg l^{-1} concentration of spinosad. Following two generations on the insecticide treatment, during which we observed strong mortality, we initiated gene flow treatments to test the influence of gene flow on rescue (see supplementary material, figure S1 and table S1, for details on timeline). In one of the insecticide-treated groups, we replaced 33% of the reproductive output with a matched number of eggs from the HD control population. This high diversity gene flow 'HDGF' treatment was conducted with six additions across 11 days to account for one *D. melanogaster* generation. This treatment simulated genetic rescue such that the influx of novel alleles from HD control populations increased total genetic diversity in the receiving cages. In a second insecticide-treated group, we again replaced one-third of the reproductive output but introduced 'pre-adapted' gene flow from the laboratory-adapted resistant population, such that gene flow probably increased the frequency of resistance alleles but not genetic diversity. We termed the population receiving pre-adapted flies as 'PAGF'. For both HDGF and PAGF populations, we removed an equal proportion of eggs laid by the original population to keep the total population size consistent before and after gene flow treatment. This ensured that any change was attributable to gene flow (HDGF) or changes in frequency of pre-adapted alleles (PAGF) and not direct demographic or competitive effects.

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

A key contrast for the HDGF population was a treatment founded with the same low-diversity populations that did not receive gene flow (we termed these 'NGF' for no gene flow). NGF flies that survived exhibited resistance to spinosad only via adaptation from standing genetic variation—an example of evolutionary rescue [56,57]. Collectively, based on survival when reared on insecticide media, we refer to the NGF, PAGF and HDGF populations as 'resistant' to spinosad. Because we did not manipulate genetic diversity through gene flow with PAGF populations, we pooled NGF and PAGF populations together as a 'resistant' group that we could compare with unexposed 'LD control' populations that had the same pool of genetic diversity. For the remainder of the growing season (approx. seven generations), resistant flies continued to be exposed to spinosad medium at the same dosage as the initial exposure.

(c) Collection and initial common garden rearing

After nine overlapping generations near the end of the growing season (6 and 24 October 2024; supplementary material, figure S1 and table S1), we collected approximately 800 eggs from each field population in density-controlled 200 ml plastic bottles from 10 LD control, 10 HD control, 8 NGF, 8 PAGF and 8 HDGF cages. We then reared flies indoors under common garden conditions at 25°C under 12 : 12 L : D lighting and 40% humidity for three generations to control for density and environmental effects among outdoor populations. We were able to successfully rear flies from eight LD control, four NGF, seven PAGF and four HDGF populations. Four of the original eight NGF and HDGF populations had insufficient population size for accurate sampling and were excluded. Upon eclosion of the third generation reared under common conditions, we collected 1500 eggs/300 ml, a standard non-stressful density for the developmental plasticity experiment.

(d) Laboratory rearing and full factorial design

We transferred freshly laid eggs to media vials at a standard low density (30 eggs/vial) in one of four conditions: (low temperature (18°C), control medium; low temperature, spinosad medium; warm temperature (25°C), control medium; warm temperature, spinosad medium); supplementary material, figure S1). At warm temperatures, flies developed in approximately two weeks and were monitored for eclosion and per cent survival. Flies developing at low temperature took approximately two weeks longer and thus phenotyping occurred in two non-overlapping time periods (supplementary material, table S1). Although 18°C is a non-stressful temperature for *D. melanogaster*, it can induce differential gene expression and phenotypes [58,59]. Three to five days following eclosion, we conducted our phenotypic assays for all combinations.

(e) Phenotypic assays

The following phenotypes were assayed per replicate population: (i) insecticide resistance measured as survivorship to adulthood: the proportion of eggs (30 eggs per vial) that survived to adulthood in three replicate vials; (ii) fecundity: the total eggs laid by a group of five females over 3 days, measured daily in each of three replicate bottles; (iii) starvation tolerance: the time to starvation for three replicate vials containing 10 males each on agar-only medium; (iv) adult body size, measured as the average dry mass of three pools of five females, dried at 55°C for 24 hours; (v) we also included chill coma recovery time (CCRT), a static measure of cold tolerance widely used in assessing low temperature responses in *D. melanogaster* [47]. As flies reared at 18°C largely did not experience chill coma at 4°C (supplementary material, table S2), we excluded this group from the cold tolerance assay and so CCRT was only compared among populations reared at 25°C across media treatments.

Determining when phenotypic reaction norms are adaptive or not is challenging because phenotypic responses may or may not affect fitness [60,61]. As we did not measure direct effects to fitness, we use the language 'consistent with adaptation' or 'putatively (mal)adaptive' and suggest that inferences of whether or not reaction norm evolution was truly adaptive be interpreted with caution. Nevertheless, trait performance in ectotherms including *D. melanogaster* can be strongly temperature dependent [62] and the degree of deviation from trait levels and expectations from thermal performance curves can be a proxy for fitness [4,63]. We therefore considered the plasticity of one population to be adaptive relative to another when reaction norms matched the magnitude and direction predicted by *D. melanogaster* and insect thermal physiology (supplementary material, table S3), although we note that these proxy measures serve as guidelines rather than hard and fast rules.

(f) Statistical analyses

Plasticity can be modelled as the linear change in means across environmental treatments (i.e. the reaction norm slope [64–66]) and we follow similar empirical approaches that approximate the reaction norm at the population level [67,68]. To model each phenotypic measure for each question, we used generalized linear mixed-effects models constructed with the *glmmTMB* function from the *glmmTMB* package [69]. Since the populations were common garden reared for three generations prior to developmental treatments, we consider the between-population variation in plastic responses to be owing to genetic differences, not within-generation environmental exposures, and that they represent evolved differences in plasticity ([65,70]; but see [71]). For each phenotypic response, a significant effect of population type, treatment condition (temperature and/or medium treatment), or their interaction would indicate evolution between populations, plasticity, or the evolution of plasticity, respectively.

For our first question of resistance evolution and plasticity, we sought to test the interaction between population type, medium treatment and temperature on phenotypic response. At low temperatures and insecticide treatment, however, mortality was much

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

higher for control populations (supplementary material, table S4). This reduced sample size prevented the comparison across both medium treatment and temperature for all control population phenotypes except resistance. We therefore constructed models with phenotype as the response variable, and either temperature, population and their interaction as the predictor variables, or separately, with medium treatment, population and their interaction as predictor variables. To test our second question of gene flow history and plasticity, we included medium treatment, population type and temperature in our models (with the exception of CCRT). Therefore, within each model, each phenotype was the response variable and temperature, medium treatment and population type were the fixed effect predictor variables, along with their pairwise interactions. For both questions, all models included individual population (cage) as a random intercept (see supplementary material for details on each model).

The total variance in reaction norms rather than the population level mean could be a key indicator of genetic variation of plasticity [66,72] and model results indicated potential differences in reaction norm variances between populations. We therefore ran Levene's test for heteroscedasticity using the *leveneTest()* function from the *car* package, but found no differences in variance (supplementary material, table S5).

We conducted model diagnostics using the *simulateResiduals* and *testDispersion* functions from the *DHARMa* package [73] and determined significance of the predictors using log-likelihood ratio tests in the *Anova* function with the type = 'III' argument from the *car* package [74]. When interaction terms were significant, *post-hoc* tests were conducted to identify differences between treatment levels and populations and were corrected for multiple comparisons using the *adjust = 'fdr'* argument in the *emmeans* function from the *emmeans* package [75]. All analyses were conducted in R v. 4.4.0 [76].

3. Results

As insecticide but not temperature served as both the agent of field selection and a treatment during development, we clarify the results into three distinct patterns of the evolution of plasticity: (i) thermal plasticity—a population-level difference in reaction norm across temperature; (ii) insecticide plasticity—a population-level difference in reaction norm across medium treatment. For most phenotypes, this reflects a novel developmental response but not the resistance phenotype that instead reflects the same selective history that defines the resistant populations; and (iii) environment-dependent plasticity—population-level differences in reaction norms during development in both treatments where reaction norm differences were dependent on both environments (i.e. a three-way interaction).

(a) Insecticide resistance and plasticity

The vast majority of flies developed successfully in three of the four treatments, but when exposed to the combination of low temperature and the insecticide, only 1.4% of flies in control populations survived to adulthood (supplementary material, table S4). With this reduced sample, we could only test the evolution of plasticity in the combined low temperature and insecticide treatment for the resistance phenotype (i.e. survival when exposed). For the remaining phenotypes, we considered evolutionary divergence of plasticity across temperature and media treatments separately.

We found that plasticity of resistance evolved between resistant and control populations, with a significant three-way interaction between population type, temperature and insecticide treatment (figure 2*A*; estimate = 14.4 ± 5.39 s.e., $\chi^2 = 7.15$, $p = 0.00751$). This result indicates that both thermal plasticity and insecticide plasticity diverged between populations, but also that they were environment-dependent. This pattern was driven by considerable mortality for all populations when reared at low temperatures, but only when also developing on insecticide medium (supplementary material, table S6). Although survival on spinosad declined at the cooler temperature, resistant populations still maintained higher survival than control populations (supplementary material, table S7).

(i) Responses across temperature

Across temperature on control medium, thermal plasticity evolved for starvation tolerance and body size with significant interactions between population type and temperature (figure 2*B*,*C*; supplementary material, tables S6 and S7). Control populations exhibited stronger plasticity, with improved starvation tolerance at low temperature (control—resistant at 18°C = 6.26 ± 3.06 s.e., z ratio = 2.04, $p = 0.0409$), whereas in resistant populations, low temperature did not induce a response (18 − 25°C = 0.559, ±2.93 s.e., z ratio = 0.191, $p = 0.851$). By contrast, body size declined at a greater rate in response to warmer temperatures for resistant populations, such that they exhibited a smaller size at 25°C relative to control (control − resistant 25°C: estimate = 0.142 ± 0.0540 s.e., z ratio = 2.63, $p = 0.00860$). We did not find evidence for the evolution of thermal plasticity for fecundity, but as expected, we did see a strong plastic effect of temperature on fecundity (supplementary material, figure S2; estimate = 18.2 ± 1.90 s.e., $\chi^2 = 92.2$, $p < 0.0001$).

(ii) Responses across insecticide

Across insecticide exposure at 25°C, there was evolutionary divergence in insecticide plasticity between populations for CCRT, starvation tolerance and body size, with a significant interaction between population type and insecticide treatment

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

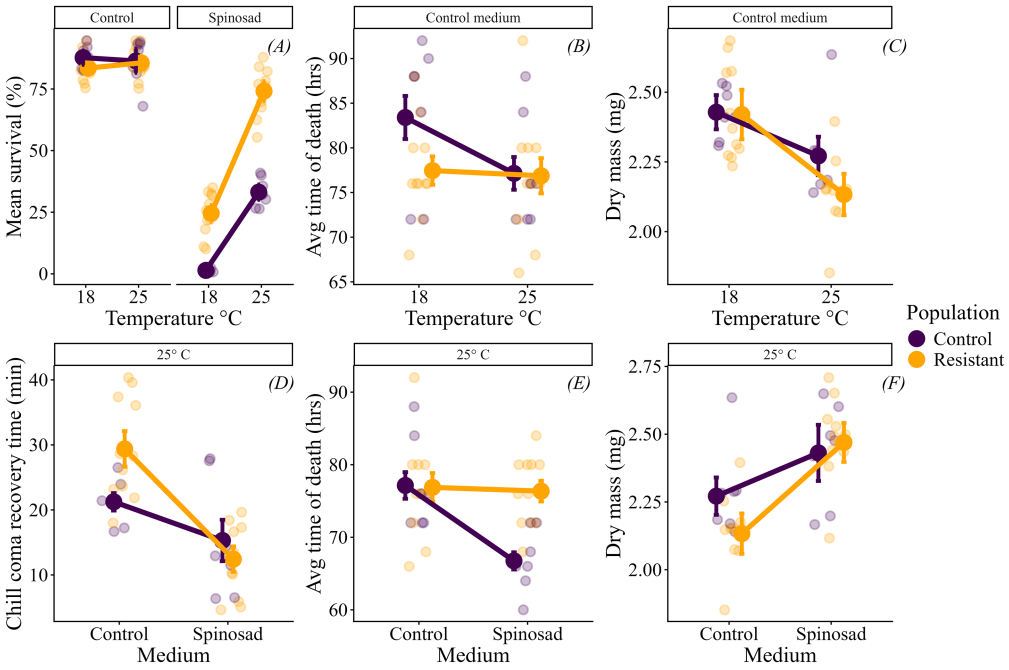


Figure 2. Phenotypic responses of control and resistant populations of *D. melanogaster* to temperature (top row) and media treatments (bottom row). Large points show the mean trait value of each population type ± 1 s.e. Smaller points jittered behind the means show raw data values for each independent population's phenotypic measure. *(A)* Spinosad resistance measured as the per cent egg-to-adult survival on insecticide media (note: inset panels denote media treatments). *(B)* Starvation tolerance measured as average time to mortality. *(C)* Average body size measured as dry mass in milligrams. *(D)* Cold tolerance measured as chill coma recovery time in minutes. *(E)* Starvation tolerance measured as average time to mortality across media treatment at 25°C. *(F)* Average body size measured as dry mass in milligrams.

(figure 2*D–F*; supplementary material, tables S6 and S7). For CCRT, both populations showed faster recovery time on spinosad medium (estimate −6.95 ± 1.15 s.e., $\chi^2 = 8.06$, $p = 0.00452$), but resistant populations exhibited stronger negative plasticity with a longer recovery time on control medium (figure 2*D*; control − resistant CCRT, control medium: −7.06 ± 3.44 s.e., *z*.ratio = −2.05, $p = 0.0403$). Control populations exhibited strong declines in starvation tolerance when exposed to spinosad, whereas resistant populations showed little plasticity (figure 2*E*; control − insecticide, control population = 10.3, ±2.92 s.e., *z* ratio = 3.52, $p = 0.000400$; resistant = 0.182, ±2.33 s.e., *z* ratio = 0.0780, $p = 0.938$) and maintained greater starvation tolerance (supplementary material, table S6). Control populations also expressed a non-significant pattern of greater decline in fecundity when exposed to insecticide (supplementary material, figure S3 and table S7) but resistant populations exhibited a greater increase in body size from a smaller starting point on control medium (figure 2*F*; resistant – control = −0.142 ± 0.0501 s.e., *z* ratio = 2.83, $p = 0.00460$).

(b) Effect of gene flow on plasticity

Gene flow from the high diversity population influenced the evolution of thermal and insecticide plasticity for fecundity, starvation tolerance and CCRT, with significant interactions of population type, temperature and/or medium treatment (figure 3*A–C*; supplementary material, table S8). Both populations exhibited higher fecundity as temperature increased, but the induced response was weaker for NGF populations on insecticide medium (figure 3*A*; supplementary material, table S9; HDGF − NGF 25°C = 5.47 ± 2.08 s.e., *z* ratio = −2.64, $p = 0.00840$). Starvation tolerance thermal plasticity evolved on insecticide but not control medium (figure 3*B*; supplementary material, tables S8 and S9), with greater starvation tolerance at 18°C and stronger thermal plasticity for HDGF populations across temperature compared with NGF populations (HDGF – NGF = −10.0 ± 3.66 s.e., *z* ratio = −2.73, $p = 0.00630$). Recovery from chill coma was faster on insecticide medium for both populations (figure 3*C*; CCRT estimate = −17.3 ± 0.864 s.e.; $\chi^2 = 398.7$, $p < 0.0001$) but on control medium, HDGF populations had a faster recovery time (supplementary material, table S9; HDGF – NGF = −10.0 ± 2.44 s.e., *z* ratio = 4.11, $p < 0.0001$).

For body size and resistance, there were plastic responses to temperature and insecticide, but reaction norms did not differ between population types within each treatment (figure 3*D*; supplementary material, figure S4 and table S9). As temperature increased, body size declined for both HDGF and NGF populations on control medium (18°C – 25°C NGF: 0.495 ± 0.0472, *z* ratio = 10.9, $p < 0.0001$; 18°C – 25°C HDGF: 0.257 ± 0.0485, *z* ratio = 5.52, $p < 0.0001$), but on insecticide medium, the direction of the plastic response was reversed (18°C – 25°C no gene flow: −0.200 ± 0.0519, *z* ratio = −4.001, $p < 0.0001$; 18°C – 25°C HDGF: −0.296 ± 0.0485, *z* ratio = −6.35, $p < 0.0001$). For resistance, both populations had high survival on control medium regardless of temperature, but on insecticide, resistance declined sharply at 18°C (supplementary material, figure S4 and table S9).

Last, we evaluated the extent to which gene flow from HD control populations reflected reaction norm variation in HDGF populations rather than post-gene flow divergence. We found that HD control populations exhibited similar reaction norms to HDGF populations (i.e. non-significant interaction terms) for most phenotypes (body size, fecundity, starvation tolerance) but

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

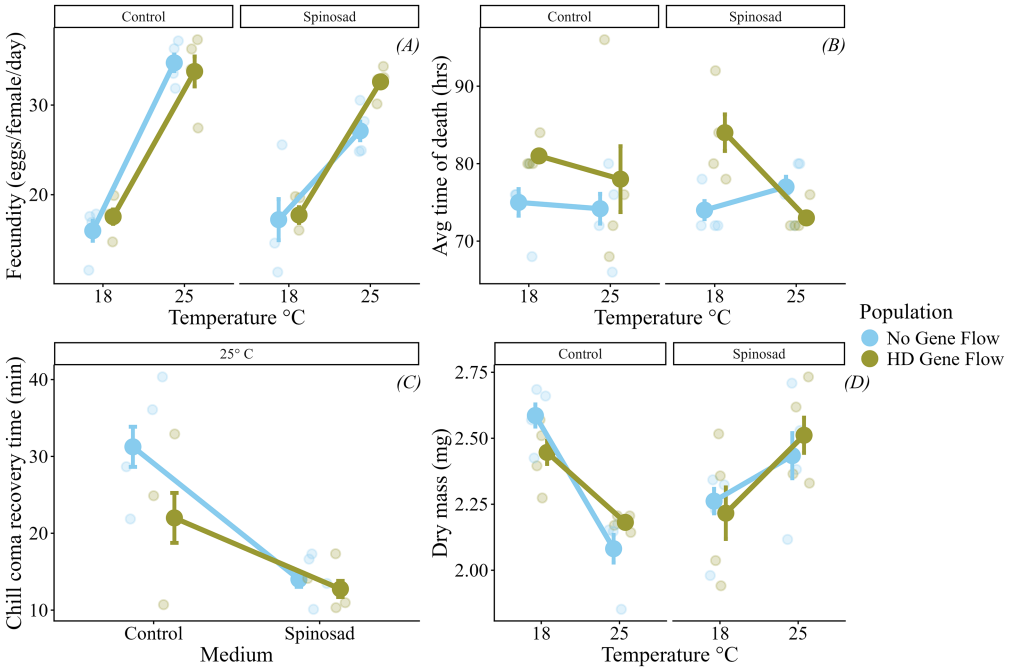


Figure 3. Phenotypic responses of *D. melanogaster* to temperature and media treatments (*x*-axis and inset panels) of resistant populations with divergent histories of gene flow: blue = NGF, gold = HDGF. Large points show the mean trait value of each population type ± 1 s.e. Inset panels denote media treatments. Smaller points jittered behind the means show raw data values for each independent population's phenotypic measure. *(A)* Average fecundity across a 3-day assay. *(B)* Starvation tolerance measured as average time to mortality. *(C)* Cold tolerance measured as chill coma recovery time in minutes. *(D)* Average body size measured as dry mass in milligrams.

they did differ in CCRT and resistance (supplementary material, tables S10 and S11). Although resistance was greater for HDGF populations as expected, surprisingly, HD control populations exhibited longer CCRT.

4. Discussion

Adaptive plasticity can be a critical component of how species respond and persist in the face of global change. Despite the potential importance of adaptive plasticity for the preservation of biodiversity, little is known about how plasticity evolves in the complex environments typical for many natural populations. We find that plasticity does evolve rapidly in fewer than 10 generations. Moreover, both gene flow and selection can affect the magnitude and direction of plasticity evolution, and for some phenotypes, the prior adaptation of insecticide resistance or lower diversity led to shifts that were putatively maladaptive. Together, these findings offer insight into how plasticity might evolve rapidly in fluctuating natural conditions, with implications for both agriculture and conservation.

In the absence of gene flow, evolutionary rescue can proceed from standing genetic variation, but demographic costs of selection might inhibit rescue or can leave populations less capable of longer-term persistence (reviewed in [77,78]). In our study, we tested how prior selection and adaptation for one stressor, an insecticide, affected the evolution of plasticity to a second unrelated environmental stressor, temperature. Although plasticity is expected to evolve more readily when stress responses are positively correlated, we found that both prior evolution of insecticide resistance and high-diversity gene flow influenced the evolution of thermal and insecticide plasticity, with shifts in reaction norm slope magnitude and/or direction. Although the evolved shifts were not always in the predicted magnitude or direction, this confirmed our expectation that broad-level evolutionary processes can drive the evolution of plasticity. Reaction norms for resistant and NGF populations in some traits were dampened and putatively maladaptive relative to control and HDGF populations, respectively. The influx of alleles provided by the high-diversity gene flow treatment, however, generally alleviated maladaptive plasticity, providing empirical evidence that assisted gene flow can contribute to the evolution of adaptive plasticity and play an integral part in evolutionary rescue. We delve into the specific evolutionary shifts in reaction norms for each question below and discuss the broader implications for these results.

(a) Resistance evolution and the evolution of plasticity

We had predicted that prior selection from insecticides would influence the evolution of plasticity and this was supported as there were shifts in reaction norm slope magnitude and direction between control and resistant populations. Specifically, there were shifts in reaction norms for starvation tolerance and body size across temperature, as well as in fecundity and cold tolerance across insecticide treatment (figure 2*B–D*; supplementary material, figure S3). Since resistant populations expressed worse cold tolerance on control medium and exhibited weaker starvation tolerance at the low temperature, this matched our prediction of a trade-off

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

between resistance evolution and thermal plasticity. In addition, across insecticide treatment, resistant populations expressed dampened plasticity for fecundity and starvation tolerance, consistent with our hypothesis of canalization. The evolved shifts in reaction norms, however, did not always match our predictions. Plastic responses for body size, for instance, were stronger in resistant relative to control populations and not consistent with maladaptation, suggesting that the adaptive nature of these shifts, and if plasticity is lost, is phenotype dependent.

Resistance to insecticides can evolve through many pathways and understanding this requires consideration of how a multifaceted genetic architecture might interact with the environment [79,80]. Consistent with this idea, spinosad resistance in *D. melanogaster* is probably polygenic [80,81] and reflects broad spectra of shifts in allele frequencies, including (either directly or indirectly) alleles related to thermal reaction norms. For example, allelic shifts underlying resistance potentially include pleiotropy or linkage that could explain the negative correlation between resistance and cold and starvation tolerance reaction norms [30,82]. Further, the intense exposure to spinosad during the growing season potentially selected for non-plastic traits in resistant populations, either through canalization or because costs of maintaining plasticity were high relative to non-labile traits [23,27]. Exposure to spinosad can lead to sub-lethal effects in naive *D. melanogaster* [53,83,84] and we found evidence of these effects with lower evolved fecundity and starvation tolerance for control populations. Surprisingly, both control and resistant populations exhibited a beneficial plastic cold tolerance response when exposed to spinosad at 25°C, suggesting that development with spinosad at warm temperatures might elicit a general stress response needed for future recovery from chill coma [31,85]. Considering that development at low temperature and spinosad was detrimental to survival, any cross-protective effect of insecticide exposure seems to be limited when encountering seasonal cooling.

(b) Gene flow and the evolution of plasticity

As expected, gene flow played a strong part in shaping the magnitude and direction of plasticity evolution. This was reflected in the largely parallel reaction norms between HD control and HDGF populations. This suggests that alleles from the donor population that increased genetic diversity provided a substantial basis for divergence, but that some of the variance probably occurred as a result of prior selection from spinosad exposure. In addition to divergence based on gene flow, for several traits, evolved shifts were putatively adaptive. Specifically, HDGF populations had greater fecundity at non-stressful temperatures and improved starvation tolerance at low temperatures (figure 3*A*,*B*), which are both consistent with adaptive life history responses [44,46]. As in the comparison of resistant and control populations, both gene flow populations showed improved cold tolerance when exposed to spinosad and NGF populations exhibited slower, putatively maladaptive recovery time on control medium (figure 3*C*). Intriguingly, the shifts in plasticity across temperature for fecundity and starvation tolerance occurred only when populations were exposed to spinosad during development. The induced response to temperature for NGF populations was weaker (in the case of fecundity) or absent (in the case of starvation tolerance). Although any magnified effect of both treatments was alleviated in the HDGF population for these phenotypes, this was not the case for body size or resistance (figure 3*D*; supplementary material, figure S4). Here, both populations exhibited similar plastic responses, with reduced body size and survival on spinosad at low temperature, evocative of the negative synergism found in the comparison of control and resistant populations.

Why did lower diversity result in the evolution of putatively maladaptive plasticity? Given reduced standing genetic variation relative to HDGF populations, the chances of matching phenotypic optima across temperatures and medium treatment were lower. This reinforces that rapid evolution of plasticity may largely be governed by similar processes to rapid evolution in other traits [12], although investigations of rates of plasticity evolution—relative to rates of mean shifts in traits—could reveal more complex patterns worthy of additional study. Broadly, expanding an understanding of the relationship between genetic diversity, the rate of plasticity evolution and the interaction between rapid trait evolution and plasticity evolution are critical to projecting organismal and population responses to environmental change.

(c) Implications for the evolution of plasticity in multiple environments

Insecticides can be strongly deleterious to key insect pollinators [86] and can interact with thermal physiology [87,88]. In our study, when both insecticide and low temperature were experienced during development, all populations showed a strong decline in survival. This is important because effects in only one environment (e.g. insecticide exposure in warm temperature) might hide potentially negative outcomes that are only elicited when experiencing a different environment (exposure in low temperatures). Such 'cryptic phenotypic plasticity' has been suggested as a potential source of variation for selection to act on and indeed it could impact fecundity and vital rates [89,90]. However, as we found, maladaptive effects to plasticity could also be exacerbated under differing thermal regimes and could in part explain why resistance might trade-off with overwintering success [34,91]. Although this is potentially good news for managing agricultural pests, it is equally concerning for beneficial insects.

(d) Limitations and future directions

There are important limitations to our study that should be considered when interpreting the results. First, the parallel evolution of reaction norms that we found is strong evidence for the action of natural selection driving the evolution of plasticity. From our study design, however, we cannot distinguish if plasticity in the traits we measured was the true target of selection or whether parallel shifts were due to correlated responses (i.e. pleiotropy or linkage). Second, our design structure had only two end points

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

of temperature and spinosad treatment [72,92]. This prevented us from detecting nonlinear shapes of reaction norms that might differ at greater or lower environmental values [20,93]. Third, our single sampling time point means that we had limited temporal resolution of the evolution of plasticity across the season. Lastly, we did not directly test for shifts at the genomic or transcriptomic level and so the mechanisms that underlie plasticity evolution remain untested. Future work can address each of these gaps by incorporating more than two environmental levels and sampling at multiple junctures to determine whether the speed, direction or magnitude of plasticity evolution varies over the growing season. Finally, a transcriptomics approach using RNAseq and candidate loci for resistance and low temperatures could reveal genomic mechanisms of plasticity evolution.

5. Conclusion

Strong selection from sudden environmental shifts can reduce genetic diversity and impact population recovery with the loss of potentially beneficial alleles, including those that are plastic [56,94]. As we show here, assisted gene flow can provide an influx of novel alleles, including those that contribute to adaptive plasticity, that temper the impact of prior selection [5,95]. Indeed, this type of genetic rescue has been at the heart of conservation efforts for threatened species such as tropical corals through assisted gene flow [16,96]. Thus, the evolution of plasticity through selection or gene flow could be a critical, but underappreciated, feature of evolutionary rescue (reviewed in [9,97]. The complex outcomes in multiple environments, however, underscore the need for further investigation into how plasticity evolves in a changing world.

Ethics. Study specimens are exempt from ethics committee approval.

Data accessibility. Data and code scripts available on the Open Science Framework [98].

Supplementary material is available online [99].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. E.G.P.: conceptualization, formal analysis, investigation, visualization, writing—original draft, writing—review and editing; B.C.: investigation, writing—review and editing; B.R.: investigation, writing—review and editing; R.S.S.: investigation, writing—review and editing; C.Z.: investigation, writing—review and editing; S.M.R.: conceptualization, funding acquisition, investigation, project administration, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. This work was supported by startup funding from Washington State University and a National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS) award number R35 GM147264 to S.M.R.

Acknowledgements. The authors are grateful to members of the laboratory for assistance with field and laboratory work and feedback on the project design. The authors also thank Simone Des Roches, Wes Dowd, Jonah Piova-Scott, Jeremiah Busch, Sarah Diamond and Ryan Martin for helpful comments with the manuscript draft and composition.

References

1. West-Eberhard MJ. 2003 *Developmental plasticity and evolution* [Internet]. New York, NY: Oxford University Press, Inc. http://ebookcentral.proquest.com/lib/wsu/detail.action?docID=422956.
2. DW Pfenning (ed). 2021 *Phenotypic plasticity & evolution: causes, consequences, controversies*. Boca Raton, FL: CRC Press. (doi:10.1201/9780429343001)
3. Merilä J, Hendry AP. 2014 Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl.* **7**, 1–14. (doi:10.1111/eva.12137)
4. Seebacher F, White CR, Franklin CE. 2015 Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim Change* **5**, 61–66. (doi:10.1038/nclimate2457)
5. Diamond SE, Martin RA. 2021 Buying time: plasticity and population persistence. In *Phenotypic plasticity & evolution* (ed. DW Pfennig), pp. 185–209. Boca Raton, FL: CRC Press. (doi:10.1201/9780429343001-11)
6. Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007 Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* **21**, 394–407. (doi:10.1111/j.1365-2435.2007.01283.x)
7. Brady SP *et al.* 2019 Causes of maladaptation. *Evol. Appl.* **12**, 1229–1242. (doi:10.1111/eva.12844)
8. Schlichting C, Pigliucci M. 1998 *Phenotypic evolution: a reaction norm perspective*. Sunderland, MA: Sinauer. (ISBN: 0-87893-799-4)
9. Chevin LM, Gallet R, Gomulkiewicz R, Holt RD, Fellous S. 2013 Phenotypic plasticity in evolutionary rescue experiments. *Phil. Trans. R. Soc. B* **368**, 20 120 089. (doi:10.1098/rstb.2012.0089)
10. Scheiner SM. 1993 Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Syst.* **24**, 35–68. (doi:10.1146/annurev.es.24.110193.000343)
11. Gomulkiewicz R, Kirkpatrick M. 1992 Quantitative genetics and the evolution of reaction norms. *Evolution* **46**, 390–411. (doi:10.1111/j.1558-5646.1992.tb02047.x)
12. Barrett R, Schluter D. 2008 Adaptation from standing genetic variation. *Trends Ecol. Evol.* **23**, 38–44. (doi:10.1016/j.tree.2007.09.008)
13. Gomulkiewicz R, Houle D. 2009 Demographic and genetic constraints on evolution. *Am. Nat.* **174**, E218–E229. (doi:10.1086/645086)
14. Wright S. 1931 Evolution in Mendelian populations. *Genetics* **16**, 97–159. (doi:10.1093/genetics/16.2.97)
15. Gillespie JH. 2004 *Population genetics: a concise guide*, 2nd edn. Baltimore, MD: Johns Hopkins University Press. (ISBN: 0801880092)
16. Aitken SN, Whitlock MC. 2013 Assisted gene flow to facilitate local adaptation to climate change. *Annu. Rev. Ecol. Evol. Syst.* **44**, 367–388. (doi:10.1146/annurev-ecolsys-110512-135747)
17. Aguilar-Gómez D, Yuan L, Zhang Y, Ochoa A, Culver M, Fitak RR, Onorato D, Lohmueller KE, Nielsen R. 2025 Genetic rescue of Florida panthers reduced homozygosity but did not swamp ancestral genotypes. *Proc. Natl Acad. Sci. USA* **122**, e2410945122. (doi:10.1073/pnas.2410945122)

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

18. Hairston NG, Jr, Holtmeier CL, Lampert W, Weider LJ, Post DM, Fischer JM, Cáceres CE, Fox JA, Gaedke U. 2001 Natural selection for grazer resistance to toxic cyanobacteria: evolution of phenotypic plasticity? *Evolution* **55**, 2203–2214. (doi:10.1111/j.0014-3820.2001.tb00736.x)
19. Sultan SE, Spencer HG, Schmitt AEJM. 2002 Metapopulation structure favors plasticity over local adaptation. *Am. Nat.* **160**, 271–283. (doi:10.1086/341015)
20. Murren CJ *et al.* 2014 Evolutionary change in continuous reaction norms. *Am. Nat.* **183**, 453–467. (doi:10.1086/675302)
21. David JR, Denis B, Francelle P, Lemaire A, Das A, Mohanty S, Gibert P, Yassin A. 2026 Phenotypic plasticity as a function of genetic polymorphism: thermal dominance reversal in *Drosophila* species with contrasting melanism. *Evolution* **80**, 127–142. (doi:10.1093/evolut/qpaf225)
22. Lenormand T. 2002 Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **17**, 183–189. (doi:10.1016/S0169-5347(02)02497-7)
23. Snell-Rood EC, Van Dyken JD, Cruickshank T, Wade MJ, Moczek AP. 2010 Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity. *Bioessays* **32**, 71–81. (doi:10.1002/bies.200900132)
24. Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. 2015 Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* **525**, 372–375. (doi:10.1038/nature15256)
25. Waddington CH. 1953 Genetic assimilation of an acquired character. *Evolution* **7**, 118–126. (doi:10.2307/2405747)
26. Lande R. 2009 Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J. Evol. Biol.* **22**, 1435–1446. (doi:10.1111/j.1420-9101.2009.01754.x)
27. Scheiner SM, Levis NA. 2021 The loss of phenotypic plasticity via natural selection: genetic assimilation. In *Phenotypic plasticity & evolution* (ed. DW Pfennig), pp. 161–182. Boca Raton, FL: CRC Press. (doi:10.1201/9780429343001-9)
28. Spielman D, Brook BW, Briscoe DA, Frankham R. 2004 Does inbreeding and loss of genetic diversity decrease disease resistance? *Conserv. Genet.* **5**, 439–448. (doi:10.1023/B:COGE.0000041030.76598.cd)
29. Bueno EM, McIlhenny CL, Chen YH. 2023 Cross-protection interactions in insect pests: implications for pest management in a changing climate. *Pest Mgmt Sci.* **79**, 9–20. (doi:10.1002/ps.7191)
30. Nyamukondiwa C, Terblanche JS, Marshall KE, Sinclair BJ. 2011 Basal cold but not heat tolerance constrains plasticity among *Drosophila* species (Diptera: Drosophilidae). *J. Evol. Biol.* **24**, 1927–1938. (doi:10.1111/j.1420-9101.2011.02324.x)
31. Sinclair BJ, Ferguson LV, Salehipour-shirazi G, MacMillan HA. 2013 Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integr. Comp. Biol.* **53**, 545–556. (doi:10.1093/icb/ict004)
32. Etterson JR, Shaw RG. 2001 Constraint to adaptive evolution in response to global warming. *Science* **294**, 151–154. (doi:10.1126/science.1063656)
33. Garland T, Downs CJ, Ives AR. 2022 Trade-offs (and Constraints) in organismal biology. *Physiol. Biochem. Zool.* **95**, 82–112. (doi:10.1086/717897)
34. Prileson EG, Campagnari B, Clare CI, Gabidulin AR, Shahmohamadloo RS, Rudman SM. 2026 Overwintering drives rapid adaptation in *Drosophila* with potential costs to insecticide resistance. *Evolution* **80**, 56–66. (doi:10.1093/evolut/qpaf205)
35. Piggott JJ, Townsend CR, Matthaei CD. 2015 Reconceptualizing synergism and antagonism among multiple stressors. *Ecol. Evol.* **5**, 1538–1547. (doi:10.1002/ece3.1465)
36. Bazzicalupo AL, Kahn PC, Ao E, Campbell J, Otto SP. 2025 Evolution of cross-tolerance to metals in yeast. *Proc. Natl Acad. Sci. USA* **122**, e2505337122. (doi:10.1073/pnas.2505337122)
37. Rudman SM *et al.* 2019 Microbiome composition shapes rapid genomic adaptation of *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **116**, 20 025–20 032. (doi:10.1073/pnas.1907787116)
38. Grainger TN, Rudman SM, Schmidt P, Levine JM. 2021 Competitive history shapes rapid evolution in a seasonal climate. *Proc. Natl Acad. Sci. USA* **118**, e2015772118. (doi:10.1073/pnas.2015772118)
39. Rudman SM, Greenblum SI, Rajpurohit S, Betancourt NJ, Hanna J, Tilk S, Yokoyama T, Petrov DA, Schmidt P. 2022 Direct observation of adaptive tracking on ecological time scales in *Drosophila*. *Science* **375**, eabj7484. (doi:10.1126/science.abj7484)
40. MacLean HJ, Sørensen JG, Kristensen TN, Loeschcke V, Beedholm K, Kellermann V, Overgaard J. 2019 Evolution and plasticity of thermal performance: an analysis of variation in thermal tolerance and fitness in 22 *Drosophila* species. *Phil. Trans. R. Soc. B* **374**, 20180548. (doi:10.1098/rstb.2018.0548)
41. Gerken AR, Eller OC, Hahn DA, Morgan TJ. 2015 Constraints, independence, and evolution of thermal plasticity: probing genetic architecture of long- and short-term thermal acclimation. *Proc. Natl Acad. Sci. USA* **112**, 4399–4404. (doi:10.1073/pnas.1503456112)
42. Teets NM, Hahn DA. 2018 Genetic variation in the shape of cold-survival curves in a single fly population suggests potential for selection from climate variability. *J. Evol. Biol.* **31**, 543–555. (doi:10.1111/jeb.13244)
43. Mathur V, Schmidt PS. 2017 Adaptive patterns of phenotypic plasticity in laboratory and field environments in *Drosophila melanogaster*. *Evolution* **71**, 465–474. (doi:10.1111/evo.13144)
44. Schmidt PS, Paaby AB, Heschel MS. 2005 Genetic variance for diapause expression and associated life histories in *Drosophila melanogaster*. *Evolution* **59**, 2616–2625. (doi:10.1111/j.0014-3820.2005.tb00974.x)
45. Marshall KE, Sinclair BJ. 2009 Repeated stress exposure results in a survival–reproduction trade-off in *Drosophila melanogaster*. *Proc. R. Soc. B* **277**, 963–969. (doi:10.1098/rspb.2009.1807)
46. Rion S, Kawecki TJ. 2007 Evolutionary biology of starvation resistance: what we have learned from *Drosophila*. *J. Evol. Biol.* **20**, 1655–1664. (doi:10.1111/j.1420-9101.2007.01405.x)
47. Gibert P, Huey R. 2001 Chill-coma temperature in *Drosophila*: effects of developmental temperature, latitude, and phylogeny. *Physiol. Biochem. Zool.* **74**, 429–434. (doi:10.1086/320429)
48. Ayrinhac A, Debat V, Gibert P, Kister AG, Legout H, Moreteau B, Vergilino R, David JR. 2004 Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Funct. Ecol.* **18**, 700–706. (doi:10.1111/j.0269-8463.2004.00904.x)
49. Partridge L, Barrie B, Fowler K, French V. 1994 Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* **48**, 1269–1276. (doi:10.1111/j.1558-5646.1994.tb05311.x)
50. James AC, Azevedo RBR, Partridge L. 1997 Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* **146**, 881–890. (doi:10.1093/genetics/146.3.881)
51. Perry T, McKenzie JA, Batterham P. 2007 A *Dα6* knockout strain of *Drosophila melanogaster* confers a high level of resistance to spinosad. *Insect Biochem. Mol. Biol.* **37**, 184–188. (doi:10.1016/j.ibmb.2006.11.009)

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

52. Karageorgi M *et al.* 2025 Beneficial reversal of dominance maintains a large-effect resistance polymorphism under fluctuating insecticide selection. *Nat. Ecol. Evol.* **9**, 2145–2160. (doi:10.1038/s41559-025-02853-x)
53. Shahmohamadloo RS, Gabidulin AR, Andrews ER, Rudman SM. 2025 Microbiome evolution plays a secondary role in host rapid adaptation. *bioRxiv* 2025.06.27.661976. (doi:10.1101/2025.06.27.661976)
54. Scharloo W. 1991 Canalization: genetic and developmental aspects. *Annu. Rev. Ecol. Syst.* **22**, 65–93. (doi:10.1146/annurev.es.22.110191.000433)
55. Mackay TFC *et al.* 2012 The *Drosophila melanogaster* genetic reference panel. *Nature* **482**, 173–178. (doi:10.1038/nature10811)
56. Gomulkiewicz R, Holt RD. 1995 When does evolution by natural selection prevent extinction? *Evolution* **49**, 201–207. (doi:10.1111/j.1558-5646.1995.tb05971.x)
57. Hufbauer RA, Szűcs M, Kasyon E, Youngberg C, Koontz MJ, Richards C, Tuff T, Melbourne BA. 2015 Three types of rescue can avert extinction in a changing environment. *Proc. Natl Acad. Sci. USA* **112**, 10 557–10 562. (doi:10.1073/pnas.1504732112)
58. Hoffmann AA, Watson M. 1993 Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. *Am. Nat.* **142**, S93–S113. (doi:10.1086/285525)
59. Chen J, Nolte V, Schlötterer C. 2015 Temperature stress mediates decanalization and dominance of gene expression in *Drosophila melanogaster*. *PLos Genet.* **11**, e1004883. (doi:10.1371/journal.pgen.1004883)
60. Sultan SE. 1995 Phenotypic plasticity and plant adaptation. *Acta Bot. Neerland.* **44**, 363–383. (doi:10.1111/j.1438-8677.1995.tb00793.x)
61. Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M. 2006 Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* **9**, 981–993. (doi:10.1111/j.1461-0248.2006.00950.x)
62. Huey RB, Stevenson RD. 1979 Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* **19**, 357–366. (doi:10.1093/icb/19.1.357)
63. Sinclair BJ, Williams CM, Terblanche JS. 2012 Variation in thermal performance among insect populations. *Physiol. Biochem. Zool.* **85**, 594–606. (doi:10.1086/665388)
64. Via S, Lande R. 1985 Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**, 505–522. (doi:10.1111/j.1558-5646.1985.tb00391.x)
65. Berrigan D, Scheiner SM. 2004 Modeling the evolution of plasticity. In *Phenotypic plasticity—functional and conceptual approaches* (eds TJ DeWitt, S Scheiner), pp. 82–97. Oxford, UK: Oxford University Press. (doi:10.1093/oso/9780195138962.003.0006)
66. Morrissey MB, Liefting M. 2016 Variation in reaction norms: statistical considerations and biological interpretation. *Evolution* **70**, 1944–1959. (doi:10.1111/evo.13003)
67. Nussey DH, Postma E, Gienapp P, Visser ME. 2005 Selection on heritable phenotypic plasticity in a wild bird population. *Science* **310**, 304–306. (doi:10.1126/science.1117004)
68. Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC. 2008 Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* **320**, 800–803. (doi:10.1126/science.1157174)
69. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM. 2017 GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **9**, 378–400. (doi:10.32614/RJ-2017-066)
70. Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH. 1995 Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.* **10**, 212–217. (doi:10.1016/S0169-5347(00)89061-8)
71. Harney E, González J. 2026 Transgenerational effects of heat shock on gene regulation and fitness-related traits in natural *Drosophila* populations. *Mol. Biol. Evol.* **43**, msag069. (doi:10.1093/molbev/msag069)
72. Windig JJ, de Kovel CGF, Jong G. 2004 Genetics and mechanics of plasticity. In *Phenotypic plasticity—functional and conceptual approaches* (eds TJ DeWitt, S Scheiner), pp. 31–49. Oxford, UK: Oxford University Press. (doi:10.1093/oso/9780195138962.003.0003)
73. Hartig F, Lohse L. 2022 *DHARMa: residual diagnostics for hierarchical (multi-level /mixed. Regression models*. https://CRAN.R-project.org/package=DHARMa.
74. Fox J, Weisberg S. 2019 *An R companion to applied regression* [Internet]. 3rd edn. Thousand Oaks, CA: Sage. https://www.john-fox.ca/Companion.
75. Lenth RV *et al.* 2022 *emmeans: estimated marginal means, aka least-squares means (1.8.3)*. [Internet]. https://CRAN.R-project.org/package=emmeans.
76. R Core Team. 2025 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.
77. Carlson SM, Cunningham CJ, Westley PAH. 2014 Evolutionary rescue in a changing world. *Trends Ecol. Evol.* **29**, 521–530. (doi:10.1016/j.tree.2014.06.005)
78. Nordstrom SW, Hufbauer RA, Olazcuaga L, Durkee LF, Melbourne BA. 2023 How density dependence, genetic erosion and the extinction vortex impact evolutionary rescue. *Proc. R Soc. B* **290**, 20 231 228. (doi:10.1098/rspb.2023.1228)
79. ffrench-Constant RH. 2013 The molecular genetics of insecticide resistance. *Genetics* **194**, 807–815. (doi:10.1534/genetics.112.141895)
80. Fournier-Level A *et al.* 2019 The spread of resistance to imidacloprid is restricted by thermotolerance in natural populations of *Drosophila melanogaster*. *Nat. Ecol. Evol.* **3**, 647–656. (doi:10.1038/s41559-019-0837-y)
81. Sabio MC, Alzogaray RA, Ortiz MSM, Fanara JJ. 2025 Genetic bases and robustness of the toxicological response to spinosad and lambda-cyhalothrin in *Drosophila melanogaster*. *Insect Sci.* (doi:10.1111/1744-7917.70107)
82. Kristensen TN, Kjeldal H, Schou MF, Nielsen JL. 2016 Proteomic data reveal a physiological basis for costs and benefits associated with thermal acclimation. *J. Exp. Biol.* **219**, 969–976. (doi:10.1242/jeb.132696)
83. Martelli F *et al.* 2022 Low doses of the organic insecticide spinosad trigger lysosomal defects, elevated ROS, lipid dysregulation, and neurodegeneration in flies. *eLife* **11**, e73812. (doi:10.7554/eLife.73812)
84. Weber AL, Khan GF, Magwire MM, Tabor CL, Mackay TFC, Anholt RRH. 2012 Genome-wide association analysis of oxidative stress resistance in *Drosophila melanogaster*. *PLoS ONE* **7**, e34745. (doi:10.1371/journal.pone.0034745)
85. MacMillan HA, Sinclair BJ. 2011 Mechanisms underlying insect chill-coma. *J. Insect Physiol.* **57**, 12–20. (doi:10.1016/j.jinsphys.2010.10.004)
86. Goulson D. 2015 Neonicotinoids impact bumblebee colony fitness in the field; a reanalysis of the UK's Food & Environment Research Agency 2012 experiment. *PeerJ* **3**, e854. (doi:10.7717/peerj.854)
87. Yang BJ, Liu ML, Zhang YX, Liu ZW. 2018 Effects of temperature on fitness costs in chlorpyrifos-resistant brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). *Insect Sci.* **25**, 409–417. (doi:10.1111/1744-7917.12432)
88. Engell Dahl J, Marti SL, Colinet H, Wiegand C, Holmstrup M, Renault D. 2021 Thermal plasticity and sensitivity to insecticides in populations of an invasive beetle: cyfluthrin increases vulnerability to extreme temperature. *Chemosphere* **274**, 129 905. (doi:10.1016/j.chemosphere.2021.129905)
89. Schlichting CD. 2008 Hidden reaction norms, cryptic genetic variation, and evolvability. *Ann. N.Y. Acad. Sci.* **1133**, 187–203. (doi:10.1196/annals.1438.010)

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026

90. Gibert P, Debat V, Ghalambor CK. 2019 Phenotypic plasticity, global change, and the speed of adaptive evolution. *Curr. Opin. Insect Sci.* **35**, 34–40. (doi:10.1016/j.cois.2019.06.007)
91. Marshall KE, Gotthard K, Williams CM. 2020 Evolutionary impacts of winter climate change on insects. *Curr. Opin. Insect Sci.* **41**, 54–62. (doi:10.1016/j.cois.2020.06.003)
92. Chevin L-M, Lande R. 2011 Adaptation to marginal habitats by evolution of increased phenotypic plasticity. *J. Evol. Biol.* **24**, 1462–1476. (doi:10.1111/j.1420-9101.2011.02279.x)
93. de Villemereuil P, Chevin LM. 2025 Partitioning the phenotypic and genetic variances of reaction norms. *Peer Comm. J.* **5**, e15. (doi:10.24072/pcjournal.515)
94. Bell G, Gonzalez A. 2009 Evolutionary rescue can prevent extinction following environmental change. *Ecol. Lett.* **12**, 942–948. (doi:10.1111/j.1461-0248.2009.01350.x)
95. Scheiner SM, Mindell DP. 2020 *The theory of evolution: principles, concepts, and assumptions* [Internet]. Chicago, IL: University of Chicago Press. http://ebookcentral.proquest.com/lib/wsu/detail.action?docID=6001963.
96. Baker AC *et al.* 2025 Proactive assisted gene flow for Caribbean corals in an era of rapid coral reef decline. *Science* **389**, 344–347. (doi:10.1126/science.adx5842)
97. Tengstedt ANB, Høye TT, Kristensen TN, Hansen MM. 2026 Evaluating inbreeding and assessing the risk of outbreeding depression in genetic rescue using whole-genome sequence data. *Proc. Natl Acad. Sci. USA* **123**, e2526216122. (doi:10.1073/pnas.2526216122)
98. Prileson EG *et al.* 2026 Insecticide resistance evolution and gene flow interact to shape the evolution of plasticity. (doi:10.17605/OSF.IO/5YCZK)
99. Prileson EG, Campagnari B, Ruotsalainen B, Shahmohamadloo RS, Zetina C, Rudman SM. 2026 Supplementary material from: Insecticide resistance evolution and assisted gene flow interact to shape the evolution of plasticity. Figshare. (doi:10.6084/m9.figshare.c.8737484)

Downloaded from royalsocietypublishing.org/rspb/article-pdf/doi/10.1098/rspb.2026.2085/6176363/rspb.2026.2085.pdf
by guest
on 11 November 2026