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TEMPORAL VARIATION FAVORS THE EVOLUTION OF GENERALISTS IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

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In variable environments, selection should favor generalists that maintain fitness across a range of conditions. However, costs of adaptation may generate fitness trade-offs and lead to some compromise between specialization and generalization that maximizes fitness. Here, we evaluate the evolution of specialization and generalization in 20 populations of *Drosophila melanogaster* experimentally evolved in constant and variable thermal environments for 3 years. We developed genotypes from each population at two temperatures after which we measured fecundity across eight temperatures. We predicted that constant environments would select for thermal specialists and that variable environments would select for thermal generalists. Contrary to our predictions, specialists and generalists did not evolve in constant and spatially variable environments, respectively. However, temporal variation produced a type of generalist that has rarely been considered by theoretical models of developmental plasticity. Specifically, genotypes from the temporally variable selective environment were more fecund across all temperatures than were genotypes from other environments. These patterns suggest certain allelic effects and should inspire new directions for modeling adaptation to fluctuating environments.

KEY WORDS: **Acclimation, experimental evolution, phenotypic plasticity, specialist–generalist trade-offs, thermal adaptation.**

In changing environments, selection should favor generalists that perform well over a wide range of conditions (Levins 1968; Gilchrist 1995). A generalist either expresses a fixed phenotype that functions under diverse conditions or plastically remodels its phenotype when the environment changes (Via and Lande 1985; Lynch and Gabriel 1987; Gabriel and Lynch 1992; Gomulkiewicz and Kirkpatrick 1992; Gavrilets and Scheiner 1993; Gabriel et al. 2005). In theory, genotypes that perform over a broader range of environments will perform worse in the optimal environment—that is, a jack-of-all environments is a master of none (Lynch and Gabriel 1987). This trade-off can stem from multiple processes. For example, an allele can increase fitness in one environment at a cost to fitness in another environment (Remold 2012). Furthermore, neutral mutations that accumulate during periods of environmental stasis can be deleterious when expressed in other environments (Kassen 2002). Thus, in variable environments, selection should favor plastic genotypes that outcompete fixed generalists by matching their phenotype to the

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current environment, as long as genetic variation for plasticity exists and costs of plasticity are low (Via and Lande 1985; Futuyma and Moreno 1988; Van Tienderen 1991; Scheiner 1993). The type of plasticity that evolves should depend on the scale at which the organism experiences environmental variation; environments that vary greatly across generations favor developmentally plastic genotypes (Gabriel and Lynch 1992), whereas those that vary greatly within generations favor reversibly plastic genotypes (Gabriel 1999).

Although theory predicts that variable environments should favor genotypes with greater plasticity, the capacity for thermal plasticity rarely matches the degree of thermal variation that a population experiences (Stillman 2003; Deere and Chown 2006; Angilletta 2009). For instance, variable temperatures should favor genotypes that more readily match their thermal phenotypes to the current temperature relative to genotypes from more stable thermal environments. Despite this prediction, genotypes from more stable thermal environments often exhibit the same degree of plasticity as those from variable ones (Hoffmann and Watson 1993; Bubliy et al. 2002; Klok and Chown 2003; Fangue et al. 2006; Cooper et al. 2010, 2012b). Moreover, genotypes from stable environments can even exhibit greater plasticity (Cunningham and Read 2002). These data suggest that selection acts differently than theory predicts or that some unknown factor constrains adaptation (Angilletta 2009).

Experimental evolution has been used to control and replicate environmental conditions that should select for generalists (Bull and Wang 2010). When compared under fluctuating conditions, genotypes evolved in fluctuating environments usually outcompeted genotypes evolved in constant environments (Kassen and Bell 1998; Weaver et al. 1999; Hughes et al. 2007b; Duncan et al. 2011). Although these experiments support the prediction that variable environments select for generalists, they could not distinguish between the evolution of fixed versus plastic generalists. This limitation stems from the use of viruses and unicellular organisms with short generations, which enables the measurement of fitness but makes the measurement of performance in one environment after development in another environment onerous. While measuring fitness may be more difficult, experimentally evolving organisms with longer generation times in constant and variable environments enables one to evaluate the evolution of plasticity as well as divergence in components of fitness among populations from different environments.

Here, we use experimental evolution to study the evolution of thermal plasticity in 20 populations of *Drosophila melanogaster* that evolved at either constant (16°C for 32 generations, and 25°C for 64 generations), or variable (temporally or spatially for an intermediate number of generations) temperatures (Yeaman et al. 2010). We tested two predictions derived from theoretical models. First, we tested whether temporal and spatial variations in temperature favored the evolution of plasticity (Gabriel and Lynch 1992); specifically, we expected that populations from variable environments would evolve the ability to alter their thermal physiology during development (i.e., shift their thermal optimum for performance toward the current temperature). Second, we tested whether constant environments favored specialists that perform best at the temperature of their selective environment (Lynch and Gabriel 1987; Gabriel and Lynch 1992; Gilchrist 1995). To test these predictions, we measured daily fecundity, a major component of fitness, across a range of thermal environments for genotypes from all 20 populations after development in two environments, 16°C and 25°C.

Materials and Methods

EXPERIMENTALLY EVOLVED POPULATIONS

The establishment of our experimentally evolved populations is described in detail in Yeaman et al. (2010), and the origin of the isofemale lines is described in Cooper et al. (2012a). Briefly, experimental populations were derived from flies captured in September 2005 in a certified organic orchard near Cawston (BC, Canada). From this sample of ∼2000 adults, 400 mated females were transferred individually into vials and were given time to oviposit. Virgin females that emerged from these vials were used to make isofemale lines. Of these lines, 149 were used to found a large breeding population from which, after nine generations, the selection lines were created. During this time, oviposition bottles were randomly transferred among cages to facilitate random mating. In February of 2006, 400 bottles were added to the cages of the breeding population, removed 2 days later, and then redistributed among 40 new cages.

To create the experimentally evolved populations, two cages were assigned to each of the five replicate populations within each of the following selective environments: (1) constant 16°C environment (C); (2) constant 25°C environment (H); (3) spatial heterogeneity with migration (S); and (4) temporal heterogeneity (T). In total, five populations were kept in each of the four selective environments with two replicate cages maintained for each population (eight bottles per cage with four bottles exchanged every 4 weeks between paired cages). For each population in the temporal selective environment, two paired cages were maintained and moved together between 25° and 16°C every 4 weeks. For each population in the spatially heterogeneous environment, paired cages remained separated with one cage maintained at 25°C and one at 16°C; migration consisted of four bottles being exchanged reciprocally every 4 weeks between the paired cages in the different environments. Cages at 16°C migrated every generation and cages at 25°C migrated every other generation. For cages maintained at 25°C (or 16°C), a new generation began every 2 (or 4) weeks. Populations consisted of 2000–4000 flies at

the end of each generation, but were sometimes as low as ∼800 (Yeaman et al. 2010). The photoperiod for all treatments was 12:12 h cycle, which precluded the use of photoperiodic cues to predict future environmental conditions during acclimation. Ultimately, five populations evolved for over 3 years in each of the four selective environments. This amounts to 32 generations at constant 16°C, 64 generations at 25°C, and an intermediate number of generations for populations experiencing temporal or spatial variability.

The creation of isofemale lines is a convenient method for examining reaction norms in *Drosophila* (David et al. 2004). In August 2009, isofemales lines were created from the five populations within each of the four selective environments (Cooper et al. 2012a). To establish isofemale lines, a virgin female was mated to one virgin male sibling within each line for two generations. Twenty isofemale lines were created from each of the five population in each of the four selective environments (400 total lines) and maintained on a standard cornmeal-yeast medium at 20.5°C (12:12 L:D). Lines have been transferred into fresh vials every 3 weeks since their inception. Creating isofemale lines enabled us to minimize evolution while maintaining genetic variation among lines within each population.

THERMAL SENSITIVITY OF FECUNDITY

Evolutionary models of plasticity assume a direct relationship between performance and fitness (Gabriel and Lynch 1992). To test this model, the performance curves of genotypes must be measured following development in different environments. We recorded fecundity of each isofemale line over 24 h because this measure includes both the total eggs laid and the female's survival in the (sometimes stressful) measurement temperature. Also, exposure of only 24 h also served to reduce any potential effect of acclimation to the measurement temperature.

We compared thermal sensitivities of fecundity of 7-day-old females after developing at 16°C and 25°C. Prior to the experimental generation, the densities of the isofemale lines were controlled by placing two males and two females into a vial and restricting the period of egg laying to 48 h. After two generations of density control, 9-day-old females from each line were transferred in pairs to new vials and allowed to lay. The eggs from these females from each line were allowed to develop from egg to adult in the two thermal treatments (16°C and 25°C). Upon emergence, virgin females were collected from each isofemale line and transferred to vials with newly emerged Canton S males (Bloomington Stock Center, Indiana University, IN), which we cultured at 20.5°C for several generations (Cooper et al. 2010). These males served to induce egg laying in experimental females while controlling for any paternal effects that might impact our measures of female fecundity. After 5 days, females were transferred individually to fresh vials with a drop of active yeast to stimulate egg laying.

To measure fecundity, 2 days later 7-day-old females were transferred individually and without anesthesia to oviposition chambers to lay eggs for 24 h (Cooper et al. 2010). These chambers consisted of a small dish of grape agar (Yang et al. 2008) and a drop of activated yeast to stimulate oviposition. A vial was inverted onto the dish to seal one female within each chamber. Prior to this procedure, small holes were drilled in the vials to prevent hypoxia during the experiment. Given the number of flies in the experiment, we initiated measures of fecundity in temporal blocks; each hour, an equivalent number of females from each population were placed individually in oviposition chambers and were randomly assigned to one of the eight measurement temperatures: 14°C, 16°C, 20.5°C, 25°C, 28°C, 30°C, 32°C, or 36°C (± 0.5°C). An individual fly was assayed at only one temperature and this procedure continued until all females of the appropriate age were processed for that day. After 24 h, the chambers were removed from the incubators and were frozen at −20°C. Because of variation in eclosion time among lines, this process was performed over a period of several days, controlling for the age of each female (7 days). Because of thermal effects on developmental time, we measured fecundities of flies that developed at 16°C after measuring fecundities of flies that developed at 25°C.

Later, oviposition chambers were thawed and the number of eggs in each chamber were counted under a dissecting microscope. The number of eggs laid in 24 h served as our measure of fecundity. For each isofemale line, only one female per measurement temperature was used which precluded the estimation of a random effect of line (David et al. 2004). In a few cases, fecundities of multiple females were recorded and these data were averaged within isofemale lines. The number of isofemale lines used for each of the four selective environments varied only slightly among treatments. For development at 16°C, we used 68, 60, 66, and 69 isofemale lines from the C, H, S, and T selective environments, respectively. For development at 25°C, we used 66, 63, 67, and 67 isofemale lines from the C, H, S, and T selective environments, respectively. A minimum of 10 isofemale lines per population was used in each of the developmental treatments.

ESTIMATING FEMALE SIZE

To control for the effect of body size on fecundity, we used wing size as a proxy for body size. Flies from each isofemale line were reared at 16°C and 25°C. Several days after emergence, each female was euthanized under CO_2 and her left wing was removed with a pair of fine forceps. Wings were mounted on glass slides with double-sided tape (Hoffman and Shirriffs 2002). We digitized each wing and used the software TpsDIG2 (Rohlf 2001) to identify 12 landmarks (Yeaman et al. 2010; Fig. 1). Based on these landmarks, we used MorphoJ (Klingenberg 2008)

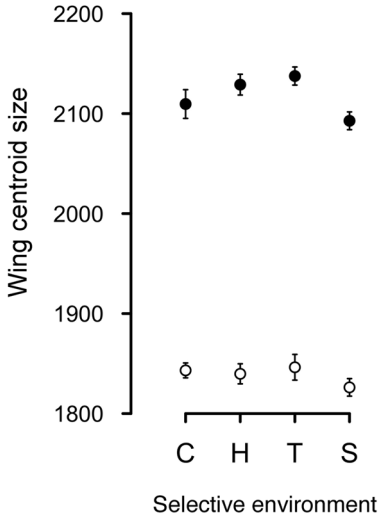

Figure 1. Wing sizes of flies evolved in constant 16°C (C), constant 25°C (H), temporally variable (T), and spatially variable (S) environments. Data are mean centroid size of populations after developing at 16°C (closed circles) and 25°C (open circles). Error bars denote standard errors.

to calculate the size of the wing's centroid: the square root of the sum of the squared coordinates of the landmarks (Hoffmann and Shirriffs 2002; Kellermann et al. 2006).

STATISTICAL ANALYSES

We used generalized linear mixed modeling to estimate the sources of variation in fecundity. Because fecundity was best approximated by an over-dispersed Poisson distribution, we fitted a model with a negative binomial error structure (Bolker et al. 2009). We also improved the model by specifying that the data were zero inflated (Zuur et al. 2009). This analysis was performed using the glmmADMB library (Skaug et al. 2006) of the R Statistical software (R Development Core Team 2013). Wing size was a covariate. Population nested within selective environment was a random factor. Selective environment, developmental temperature, and measurement temperature were fixed factors. We used model selection to obtain the most parsimonious model. We deleted terms based on their significance of the Wald's F-test (Bolker et al. 2009). Fecundities at different levels within factors were compared using Wald's statistic.

To examine the effect of selective and developmental environment on total fecundity, we added together the number of eggs laid at each of the eight measurement temperatures for each isofemale line after development in 16°C and 25°C (Gilchrist 1996). Isofemale lines that were missing values from one or more measurement temperatures were dropped from this part of the analysis. This left 169 isofemale lines with egg-laying data recorded at each measurement temperature (98 and 71 lines developing at 16°C and 25°C, respectively). Total fecundity data were square root transformed to improve normality and it was appropriate to use a linear mixed model for this section of our analyses. Population nested within selective environment was fitted as a random effect using restricted maximum likelihood and Akaike information criterion (AIC) was used to determine the structure (Zuur et al. 2009). Fixed effects were fit by maximum likelihood and their significance tested with log-likelihood ratio tests. We performed a planned contrast to examine the difference in total fecundity between the T population and the C, H, and S populations after development at 25°C. We used the same linear mixed model procedure to examine the effect of development temperature and selection environment on wing size and wing size on total fecundity. For the effect of wing size on total fecundity, isofemale lines in each developmental temperature were analyzed separately.

Results

THERMAL PLASTICITY OF WING SIZE

Wing size of female *D. melanogaster* was strongly affected by acclimation temperature during development. Flies developing at 16°C had an approximately 13% larger wing, on average, than did flies developing at 25°C (Fig. 1, $t = 36.9$, $P < 0.001$). Neither selective environment nor an interaction between selection and developmental temperature improved the model fit ($\chi^2_3 = 6.7$, $P = 0.08$, $\chi^2_3 = 2.6$, $P = 0.4$). Isofemale lines with larger wings were not more fecund; centroid size did not explain significant variation in the total fecundity of isofemale lines after developing at 16°C ($\chi^2_1 = 0.1$, $P = 0.6$) or 25°C ($\chi^2_1 = 0.8$, $P = 0.3$).

NO EVIDENCE FOR THERMAL SPECIALIZATION OF FECUNDITY

We did not find any evidence of thermal specialization in populations evolved in constant treatments. Despite selection at 16°C for 32 generations, the C populations did not lay more eggs than the H, T, and S populations when females were reared and tested at 16°C (Fig. 2, $z = 0.034$, $P = 0.9$). In fact, fecundity of the C populations was greatest near 25°C, an environment never experienced during the period of experimental evolution. This result contrasts the prediction that selection should shift the thermal optimum for performance to the mean of the selective environment (Gilchrist 1995, 2000). Similarly, the H lines did not exhibit higher fecundity than C, T, or S selection populations at 25°C after developing at this temperature (Fig. 2, $z = 0.09$, $P = 0.9$).

THERMAL PLASTICITY OF FECUNDITY

Mean fecundity of females greatly depended on measurement and developmental temperatures for all selective environments (Fig. 2 and Table 1). In general, the fecundity of all of the populations

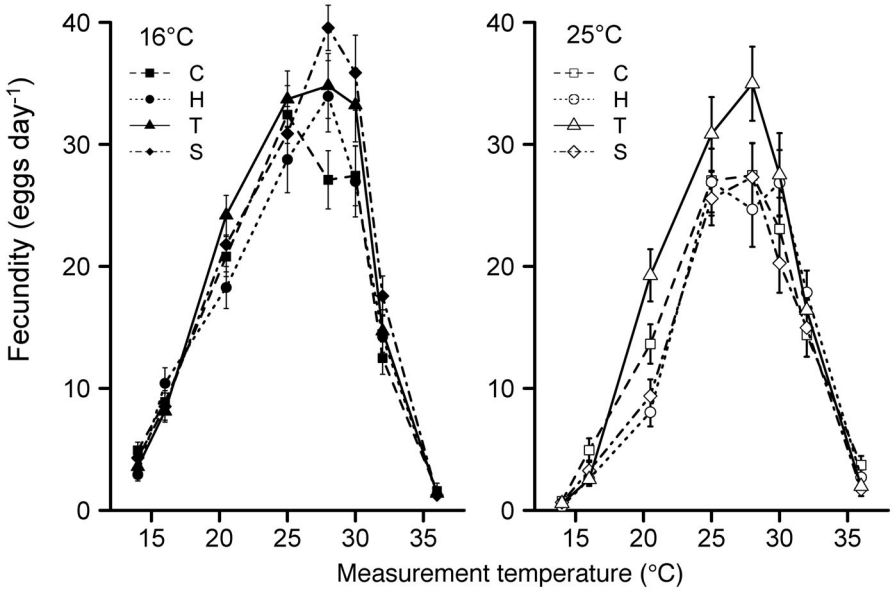

Figure 2. Thermal sensitivities of fecundity in flies after development at 16°C (left) and 25°C (right). Data are mean fecundity of isofemale lines from the four selective environments: constant 16°C (square), constant 25°C (circle), temporally variable (triangle), and spatially variable (diamond) environments. Error bars denote standard errors.

Table 1. Results of tests used to select terms from the mixed effect model of fecundity.

Fixed effect	df	Wald's F-value	P-value
Wing size	1	0.61	0.43
Measurement temperature	7	2778	<0.001
Development temperature	1	5.20	0.02
Selection environment	3	4.78	0.18
Development temperature × measurement temperature	7	538.73	<0.001
Selection Environment × development temperature	3	8.34	0.04
Selection environment × measurement temperature	21	61.23	<0.001
Selection environment × development temperature × measurement temperature	21	10.34	0.97

increased rapidly with measurement temperature as expected and peaked at or below 30°C regardless of developmental environment (Fig. 2 and Table 1). After developing at 16°C, isofemale lines had greater fecundity than flies developing at 25°C ($z = 11.56$, $P < 0.02$). However, flies developing at 16°C and those developing at 25°C laid the most eggs at 25–28°C, indicating populations have not diverged in the plasticity of the thermal optima for fecundity. When measured at 25°C, flies that developed at 16°C laid as many or more eggs than did flies that developed at 25°C. This effect of developmental environment on fecundity was greatest at low temperature (14°C: Wald test, all $P < 0.05$). Importantly, when comparing the T lines after developing at 16°C and 25°C, we see little or no difference in fecundity when measured at temperatures above 16°C (Wald test, $P = 0.27$–0.97), suggesting that the developmental plasticity of these lines diverged from that of other lines (see Fig. 2; Wald's $F = 8.34$, $P = 0.04$).

EVOLUTION OF THERMAL PLASTICITY

Populations evolved in temporally and spatially variable environments did not evolve increased plasticity of their thermal optima for fecundity as predicted by models of optimal plasticity. Among the temporally and spatially variable populations, we saw no increase in the plasticity of the thermal optima relative to flies evolved in constant environments: maximum egg laying occurred at ~28°C for flies regardless of developmental temperature (Fig. 2).

After developing at 25°C, temporal populations laid more eggs across all eight measurement temperatures than C, H, and S populations (Fig. 3; $z = 7.41$, $\chi^2_1 = 54.9$, $P < 0.001$). Among the T isofemale lines, the mean total number of eggs laid was similar among flies that developed at 16°C and 25°C (16°C: 142.9 ± 12.9 eggs, 25°C: 150.2 ± 26.9 eggs). In contrast, the total eggs laid in isofemale lines from the C, H, and S populations decreased approximately 20–40% from development at 16–25°C. After development at 16°C, the total eggs laid did not differ among selective populations (all $P > 0.2$). Together these data confirm that T populations lay more eggs when measured across a thermal

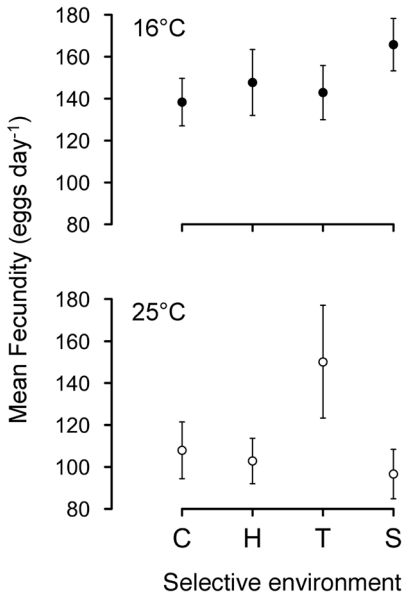

Figure 3. Mean fecundity of flies after development at 16°C (top) and 25°C (bottom). Data show the mean of the total fecundity across all measurement temperatures for populations evolved in 16°C (C), constant 25°C (H), temporally variable (T), and spatially variable environments (S). Error bars denote standard errors.

range (14–36°C) than the other selective populations, but only after development at 25°C.

Discussion

Selection in constant environments should favor specialists that are most fit under the narrow range of conditions experienced through evolutionary time, whereas selection in a variable environment should favor generalists with a larger breadth of fitness (Lynch and Gabriel 1987; Gabriel and Lynch 1992; Gilchrist 1995). Populations exposed to novel environments often evolve genotypes that outcompete their ancestors (Bennett et al. 1992; Hughes et al. 2007a; Legros and Koella 2010). However, the hypothetical benefits of specialization in constant environments have rarely been documented (Bennett et al. 1992; Hughes et al. 2007a, but see Hughes et al. 2007b). Instead, populations in fluctuating environments frequently evolved generalists that were as fit or more fit than specialists under all conditions tested (Reboud and Bell 1997; Weaver et al. 1999; Hughes et al. 2007b; Legros and Koella 2010; Duncan et al. 2011; Ketola et. al 2013; Long et. al 2013).

We found no evidence that specialists evolved in the 10 populations from constant environments after evolution at 16°C and 25°C for 32 and 64 generations, respectively. We also saw no evidence that generalists evolved in spatially variable environments. However, populations that had evolved in temporally variable environments were more fecund than were genotypes from other environments (see Fig. 2, right). This pattern corresponds to an increase in the area of the performance curve during development at 25°C (Fig. 3). This form of generalization has rarely been considered by theoretical models of phenotypic plasticity. Only Gilchrist (1995) examined the selective pressures on performance curves when generalists have a greater mean performance across temperatures (i.e., a greater area under the curve); not surprisingly, a positive correlation between the breadth and the area of a performance curve enhances selection for generalists in fluctuating environments (Gilchrist 1995). A similar condition during our experiment could explain the evolution of generalists in temporally variable environments.

Our findings prompt important questions about thermal adaptation. Why did our populations in temporally varying environments diverge from others whereas those in spatially varying environments did not? Moreover, why did populations in temporally varying environments evolve greater fecundity at 25°C and populations at a constant 25°C did not? And what are the genetic mechanisms that enabled mean fecundity to evolve in certain populations?

Variable environments should select for generalists, but spatial and temporal variations exert different degrees of selection pressure. When the environment varies temporally across generations, alleles periodically experience selection in each environmental state. When the environment varies spatially, some alleles escape negative selection until their carriers disperse to a region with novel environmental conditions. As a result, alleles that increase fitness in variable environments should spread more rapidly in temporally changing environments than in spatially variable ones (Kassen 2002). In our experiment, all genotypes in the T populations experienced a change in selection regime every four weeks, whereas genotypes in the S populations had only a 50% chance of migration. In the S populations, we found no evidence for adaptation to spatial selection. Migrants should have lower fitness than residents because they disperse from populations evolving in other conditions (Palmer and Feldman 2011). In contrast to this expectation, genotypes from the S populations did not have lower fecundity than those from the H or C populations. These data suggest that migration between 16°C and 25°C in the S populations did not introduce deleterious alleles in to each subpopulation (i.e., each of the paired cages that comprise a population).

When developing at 25°C, genotypes that evolved in the temporally variable environment were more fecund than those that evolved at a constant 25°C. Although this result seems counterintuitive and fails to conform with predictions of current models, the pattern directly corresponds to other observations. In a

previous study of these same populations, T populations expressed greater plasticity of cell membranes than did C and H populations; specifically, T populations developed membranes with greater concentrations of phosphatidylcholine at 25°C (Cooper et al. 2012a), a response that maintains membrane fluidity at warm temperatures (Hazel 1995; Hochachka and Somero 2002). This pattern of cellular divergence at 25°C matches that observed in our experiment, where T lines were more fecund than other lines only when developed at 25°C. These patterns suggest that divergence in membrane fluidity contributed to the differences in fecundity that we observed. However, further studies are needed to identify the proximate causes of these phenotypes and the mutations that underlie them. Still, this hypothesis seems likely given that core functions of cells can be perturbed by changes in membrane fluidity including the production of ATP (Hochachka and Somero 2002).

Fluctuating environments should favor alleles that confer high fecundity across a wide range of conditions (Via et al. 1995). Most alleles increase a genotype's fitness under specific conditions (environmentally specific alleles), but some alleles can increase fitness simply when the environment varies (environmentally sensitive alleles). By comparing plasticities of genotypes among our populations, we might infer something about the allelic effects that underlie the evolution of plasticity (Via 1993). If an environmentally specific allele was involved, the fecundity of T lines that developed and oviposited at 25°C would not have exceeded that of H lines in the same conditions. Instead, flies from T lines laid more eggs at 25°C than did flies from all other populations, which supports the selective advantage of an allele with greater environmental sensitivity with an associated cost during periods of environmental constancy (Cooper et al. 2012a; Via 1993; Van Tienderen 1991). This could explain why we see the evolution of greater fecundity in T lines in the home environment of the H lines.

Although we do not know the degree of thermal plasticity in the ancestral population, selection on standing variation likely explains the patterns that emerged during our experiment. On the one hand, environmentally sensitive alleles would experience strong positive selection in temporally varying environments (Gabriel and Lynch 1992). On the other hand, positive selection on these alleles would be relaxed in constant environments and such alleles would decrease in frequency if plasticity imposes a cost (Van Tienderen 1991; Van Dyken and Wade 2010). A combination of relaxed selection in constant environments and positive selection in the temporally variable environment likely led to differences in fecundity among our populations. Comparative studies of natural populations that experience different degrees of thermal variability (e.g., Berger et al. 2013; Klepsatel et al. 2013) will enable us to disentangle the contributions of relaxed and positive selection to the differences in our experiment, and to ultimately construct more sophisticated models of plasticity.

In our experiment, thermal specialization did not evolve in constant environments despite the strong selective pressure on fecundity (compare fecundities at 16°C and 25°C in Fig. 2). Although laboratory selection has been shown to generate rapid divergence in physiological traits (Burke and Rose 2009; Gibbs 1999; Hoffmann and Parsons 1993; Rose et al. 1992), adaptation proceeds slowly when standing genetic variance is low (McGuigan and Blows 2010) and mutations must accumulate sequentially (Hoffmann 2010; Lynch 2010). Given the duration of our experiment, divergence of thermal optima among populations would have required considerable standing genetic variation (Barrett and Schluter 2008). The lack of divergence in thermal optima for fecundity in C and H populations, at either developmental temperature, suggests that the ancestral population lacked sufficient genetic variance in the thermal optimum. Still, a previous study revealed that genotypes from the C and H populations produced more offspring that survived to adulthood in their respective selection environment, indicating that rates of embryonic or larval survival diverged between populations (Yeaman et al. 2010).

Although we have focused on trade-offs between specialists and generalists, evaluating other assumptions of the current theory will help us to predict adaptation to changing environments. For example, irreversible and reversible forms of plasticity have been modeled separately, precluding the possibility of genetic correlations between these traits (Gabriel and Lynch 1992; Gabriel et al. 2005). Similarly, the allocation of resources can cause trade-offs between fecundity and stress resistance in *D. melanogaster* (Service and Rose 1985; Chippindale et al. 1996). In our experiment, the greater fecundity in the T populations could have involved the re-allocation of resources (allocation trade-off; sensu Angilletta et al. 2003), such as when fecundity increases as egg size decreases. Optimality models, which omit these complications, fail to explain results such as ours (Angilletta 2009). Taking a completely different approach, for example, constructing and parameterizing a genotype-phenotype model that simulates the evolution of plasticity using the Wright–Fisher model may be a particularly fruitful approach for modeling the invasion of modifiers of thermal reaction norms (Draghi and Whitlock 2012). Ultimately, future studies that determine whether thermal traits covary across episodes of selection or that quantify trade-offs among traits, life stages, or environments could reveal the functional constraints on the evolution of thermal plasticity.

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DATA ARCHIVING

The doi for our data is doi:10.5061/dryad.7gs24.

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