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Thermal acclimation of flies from three populations of *Drosophila melanogaster* fails to support the seasonality hypothesis

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ABSTRACT

In seasonal environments, natural selection should favor genotypes that acclimate to slow and predictable changes in temperature. Selective pressure for acclimation should be especially strong for animals that complete many generations per year, because seasonal warming or cooling causes offspring to experience different temperatures than their parents did. Here, we studied variation in acclimation capacity among three populations of *Drosophila melanogaster*. We used a reverse acclimation design to see whether developmental acclimation persisted throughout adulthood. Flies developed from fertilization to adulthood at either 16° or 26 °C. Then, flies either remained at the same temperature or moved to the other temperature for 7 days. We measured fecundity at seven temperatures ranging from 14° to 36°C. Genotypes from North Carolina and Vermont laid more eggs at 16 °C after spending the larval and adult stages at 16 °C, instead of 26 °C. In both populations, the benefit of acclimation to 16 °C during development was erased by acclimation to 26 °C during adulthood. In contrast to our prediction, genotypes from Indiana laid fewer eggs at 16 °C or 26 °C after developing at this temperature. Overall, these data provide only weak support for the models of optimal acclimation in seasonal environments.

1. Introduction

In changing environments, natural selection should favor genotypes that perform well over a wide range of conditions (Levins, 1968; Gilchrist 1995). These genotypes either expresse a generalized phenotype that functions under diverse conditions or a plastic phenotype that changes with the environment (Via and Lande 1985; Lynch and Gabriel 1987; Gabriel and Lynch 1992; Gomulkiewicz and Kirkpatrick 1992; Gabriel et al., 2005). To be a generalist, an organism must invest in cellular structures that extend the range of function to extreme conditions (Angilletta, 2009; Hochachka and Somero, 2002). However, plasticity enables a genotype to specialize its phenotype for current conditions. In exemplary cases, animals exposed to either a high or low temperature for a few weeks adjusted their thermal physiology to match their environmental temperature (Glanville and Seebacher, 2006; Widdows and Bayne, 1971). Such changes in physiology depend on isozymes, chaperones, and membranes (Hazel, 1995; Hoffmann et al., 2003; Thomashow, 1999), which require energy to produce. However, the energy needed to fuel acclimation may be less than the energy needed to maintain a generalized phenotype when the environment changes infrequently.

In theory, acclimation confers the greatest fitness when the environment changes slowly and predictably, enabling the organism to accurately match its phenotype to the current environment (Gabriel, 1999, 2005; Hendry, 2016). Therefore, seasonal changes in abiotic conditions select for genotypes that acclimate, as long as the energetic benefit of specialization outweighs the energetic cost of acclimation. This seasonality hypothesis (Kellermann et al., 2018), which emerged from mathematical modeling initiated by Levins (1968), has roots in Janzen's conceptual model of adaptation to temperature along latitudinal and altitudinal clines (Ghalambor et al., 2006; Janzen, 1967; Sheldon et al.). Studies of thermal acclimation have provided mixed evidence for the seasonality hypothesis, with stronger support coming from experimental populations than from natural ones. In the laboratory, Yeaman et al. (2010) exposed replicated populations of flies (*Drosophila melanogaster*) to either a constant environment or an environment whose temperature fluctuated between generations. After three years, genotypes from a population in the fluctuating environment could acclimate to developmental temperature more effectively than could genotypes from populations in constant environments (Cooper et al., 2012; Le Vinh Thuy et al., 2016). Although these experiments confirmed the selective pressures described by theoretical models, comparative studies of natural populations indicate that current models cannot explain patterns of acclimation (Gunderson and Stillman, 2015; Seebacher et al., 2015). Many researchers have compared the capacity for acclimation among populations from different

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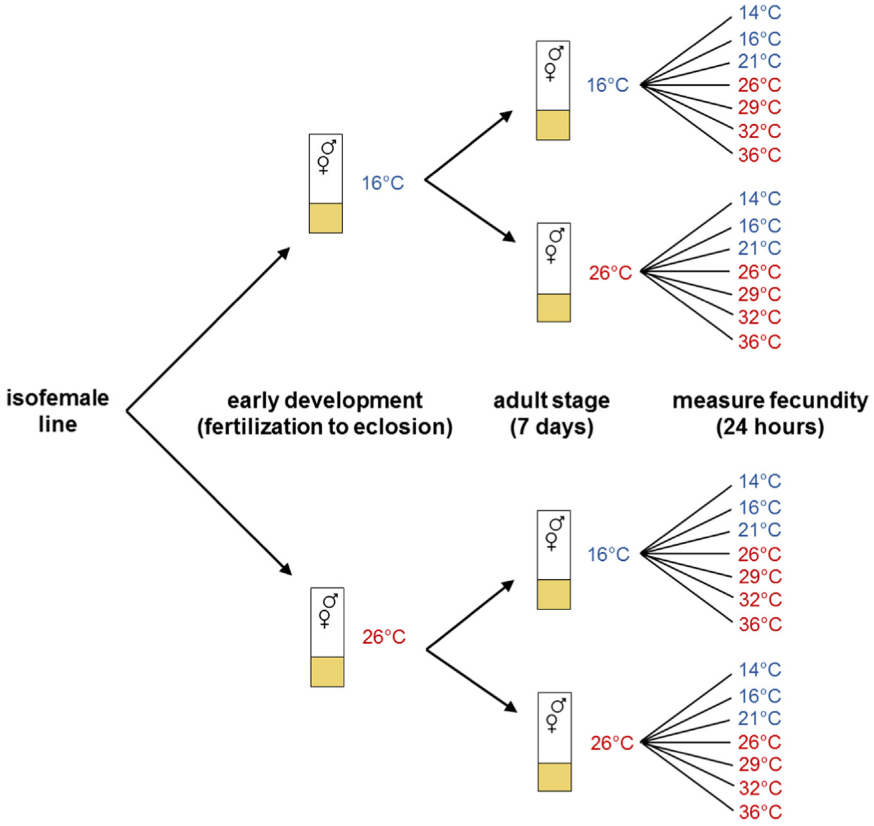

Fig. 1. A schematic of our experimental design showing the transfer of flies between treatments at each life stage.

latitudes, expecting genotypes from a higher latitude to acclimate more effectively (reviewed by Angilletta, 2009). Instead, genotypes from a low latitude often exhibit a similar capacity for acclimation as those from a high latitude (Bauerfeind et al., 2018; Bubliy et al., 2002; Cooper et al., 2010; Fangue et al., 2006; Hoffmann and Watson, 1993; Klok and Chown, 2003). In one case, genotypes from a tropical environment exhibit greater plasticity than did genotypes from a temperate environment (Cunningham and Read, 2002). Possibly, longer periods are needed in natural environments, where factors other than seasonality reduce the strength of selection for acclimation. However, a recent meta-analysis of hundreds of species also revealed mixed support for the seasonality hypothesis; a latitudinal effect on acclimation capacity was found among small organisms but not among large ones (Rohr et al., 2018). Thus, patterns of acclimation in natural populations paint a more complicated picture than the one predicted by models of optimal acclimation (Angilletta, 2009).

To test the seasonality hypothesis, we studied the capacity for acclimation by genotypes from three populations of *Drosophila melanogaster* along a latitudinal cline. This species has been a major focus of research on adaptation to environmental variation (Adrion et al., 2015), including the evolution of phenotypic plasticity (Condon et al., 2014; Overgaard et al., In press; Scheiner, 2002). Because *D. melanogaster* completes multiple generations each year (Cooper et al., 2014), each generation in a seasonal environment experiences different temperatures than the generation before does. Therefore, the environment at the highest latitude should have selected for genotypes that acclimate to temperature during development. Additionally, a fly moves from one microhabitat to another when passing between life stages. The larvae experience the thermal fluctuations in rotting fruits (Feder et al., 1997a), whereas adults avoid extreme temperatures by limiting activity to certain times of day (Huey and Pascual, 2009). To make matters worse, female flies will lay eggs in fruits likely to experience extreme temperatures, as evidence by the presence of dead larvae (Feder et al., 1997b). The ontogenetic switch in thermal conditions, punctuated by a period of metamorphosis, likely amplifies the opportunity for and the benefit of acclimation.

With these factors in mind, we designed a double-acclimation experiment, in which genotypes from each population experienced one temperature as larvae and another temperature as adults. For comparison, the same genotypes were raised at constant temperatures through both life stages. Then, we compared the thermal sensitivities of fecundity among flies from these acclimation treatments. Based on the seasonality hypothesis, we expected the thermal optimum for fecundity to diverge during acclimation, particularly for genotypes from the highest latitude exposed to the same temperature as larvae and adults.

2. Methods

2.1. Source of genotypes

The isofemale lines used in our experiment were obtained from researchers at Indiana University (Cooper et al., 2014). These lines were established by sampling flies from populations in three locations: Burlington, Vermont (44° 25' N); Bloomington, Indiana (39° 59' N); and Raleigh, North Carolina (35° 30' N). Among these locations, flies to the north experience a lower mean and greater variance of temperature (Cooper et al., 2014). Using a degree-day model, Cooper et al. (2014) estimated that flies complete 55, 88, and 123 generations in Vermont, Indiana, and North Carolina, respectively.

Flies were collected with banana-bait traps in the summer of 2010. Multiple traps were scattered throughout each site to ensure adequate sampling. Each female was placed in a separate vial containing instant medium (Formula 424, Carolina Biological Supply). Male offspring were examined to confirm the flies belonged to *D. melanogaster*. Flies of each isofemale line were shipped to Arizona State University in 2012. Thereafter, all lines were maintained at 21 °C with a 12:12 light cycle.

Table 1
All likely models included an effect of developmental temperature (dev temp) on wing size. For each model, we provide the Akaike information criterion (AIC) and the Akaike weight, which equals the probability that the model describes the data better than other models. All models contained an intercept and an error term associated with isofemale line.

Model	*Parameters*	*Log likelihood*	*AIC*	*ΔAIC*	*Akaike weight*
1) dev temp	4	− 638.3	1285.0	0.00	0.71
2) dev temp + population + dev temp · population)	8	− 635.4	1288.2	3.13	0.15
3) dev temp + population	6	− 637.8	1288.3	3.28	0.14

Flies from each vial were transferred every three weeks to fresh vials with a standard medium (recipe of the Bloomington Stock Center). In early 2013, we conducted our experiment with 20, 20, and 19 isofemale lines from Indiana, North Carolina, and Vermont, respectively.

2.2. Acclimation experiment

The seasonality hypothesis predicts that genotypes from temperate environments will acclimate their thermal optimum according to recent body temperatures. To test this hypothesis, we compared the thermal performance curves of genotypes exposed to different developmental temperatures. We used a reverse-acclimation design to determine whether acclimation during adulthood could reverse acclimation during early development. First, each fly developed at 16 ° or 26 °C through larval and pupal stages. After eclosion, flies either remained at the same temperature or switched to the other temperature for the first 7 d of the adult stage. This factorial design resulted in four treatments: 16 °C for larvae but 26 °C for adults; 26 °C for larvae but 16 °C for adults; 16 ° for both stages; and 26 °C for both stages.

We measured thermal sensitivity of daily fecundity after acclimation to each treatment (Fig. 1). Initially, we controlled the density of flies in each isofemale line 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 eggs. The offspring of these females were randomly assigned to an incubator set at either 16 ° or 26 °C for the duration of larval and pupal development (larval temperature). Vials were checked daily for newly eclosed flies. Upon eclosion, virgin females were collected and transferred to new vials assigned to either 16 ° or 26 °C (adult temperature). Females were paired with newly eclosed males of a known genotype (Canton S, Bloomington Stock Center, Indiana University, USA). These males served to induce reproduction while controlling for any paternal effects that might affect fecundity. Five days after eclosion, females were transferred individually to fresh vials with a drop of active yeast to stimulate oviposition. Two days later, we used these flies to compare the thermal sensitivity of performance among treatments and among populations.

We measured the fecundity of each female at a single temperature. Females from each isofemale line were transferred without anesthesia to oviposition chambers. Each chamber consisted of a small dish of grape agar (Yang et al., 2008) and a drop of activated yeast. A vial was inverted onto the dish to seal the female within the chamber. Prior to the experiment, small holes were drilled in the vials to prevent hypoxia. Oviposition chambers were randomly assigned to one of seven temperatures: 14 °, 16 °, 21 °, 26 °, 29 °, 32 ° or 36 °C (± 0.5 °C). After 24 h, the chambers were removed from the incubators and were frozen at −20 °C.

Due to variation in eclosion time among lines, we measured fecundity in temporal blocks. Each block included an equivalent number of females from each population and each acclimation treatment. Because of thermal effects on developmental time, we measured fecundities of flies that developed at 16 °C about a week after we measured fecundities of flies that developed at 26 °C.

2.3. Estimating body 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 raised at either 16° or 26 °C from the egg stage into the adult stage. 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. We digitized each wing and used the software TpsDIG2 to identify 12 landmarks described by Yeaman et al. (2010). Based on these landmarks, we used MorphoJ (Klingenberg, 2008) 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, 2007).

2.4. Statistical analyses

We modeled the effects of population, larval temperature, adult temperature, and measurement temperature on fecundity. In all analyses, isofemale line was included as a random factor. The need to include this factor was confirmed by comparing the AIC of a mixed model to that of a model without a random effect. In all cases, the random factor substantially improved the fit of a model to the data (ΔAIC > 2). Mixed models were fit with the R Statistical Software (R Core Team, 2017)

For analyses of wing size, we fit general linear mixed models using the package *lme4* (Bates et al., 2015). Following Burnham and Anderson (2002), we used multimodel averaging to estimate the most likely values of means. First, we used the *MuMIn* library (Bartoń, 2013) to fit all possible models to the data. Then, we calculated the Akaike information criterion and Akaike weight of each model (Table 1), the latter variable being the probability that the model best describes the data. Finally, we calculated the weighted average of each parameter including estimates from all models. The resulting values of parameters were used to calculate the most likely mean for each combination of factors.

For analyses of fecundity, we fit generalized linear mixed models with a Poisson distribution (Zuur et al., 2009). Because many flies failed to lay eggs at extreme body temperatures, we chose models with zero-inflation from the package *glmmADMBA* (Fournier et al., 2012; Skaug et al., 2016). When modeling fecundity, we controlled for body size by including the mean wing size of females from each isofemale line at each developmental temperature. We were unable to use multi-model averaging, because some models could not be fit to the data. Therefore, we used a model selection approach in which higher-order terms were dropped from the model until we arrived at a model with the lowest AIC (Table 2). We used the parameters of this model to calculate the expected fecundity for each combination of factors.

Table 2
The most likely model of fecundity included the main and interactive effects of population, larval temperature, adult temperature, and measurement temperature, but excluded the effect of wing size. Based on the data, a more complex or less complex model has essentially zero chance of being a better model. For each model, we provide the Akaike information criterion (AIC) and the Akaike weight, which equals the probability that the model describes the data better than other models. All models contained an intercept and an error term associated with isofemale line.

Model	*Parameters*	*Log likelihood*	*AIC*	*ΔAIC*	*Akaike weight*
1) model without wing size	85	− 9085	18340	0	1.00
2) model without wing size and the 4-way interaction	74	− 9158	18464	124	0.00
3) full model	86	− 9294	18760	420	0.00

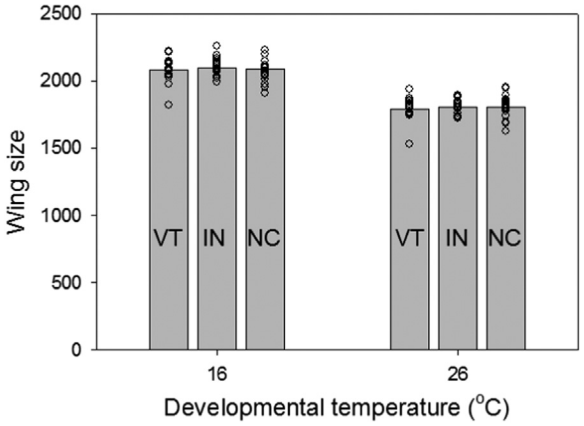

Fig. 2. In all populations, flies developed larger wings when raised at a lower temperature. Bars are mean centroid sizes estimated by multi-model averaging. Open circles are centroid sizes for the isofemale lines in each population. See Table 1 for statistical results.

3. Results

As expected from previous experiments (Azevedo et al., 2002; Partridge et al., 1994), the wing size of a female was strongly affected by her body temperature during development. The most likely model of wing size excluded an effect of population (Table 1), suggesting that developmental temperature was the predominant factor contributing to variation in wing size. On average, the wings of flies that developed at 16 °C were 16% larger than the wings of flies that developed at 26 °C (Fig. 2).

Daily fecundity strongly depended on temperature, peaking at temperatures between 26 ° and 33 °C, depending on the population and acclimation treatment (Fig. 3). We draw this conclusion from the most likely statistical model (Table 2), which includes the interactive effects of population, larval temperature, adult temperature, and measurement temperature (Table 3). This model fit the data far better than the full model, which also included wing size (ΔAIC = 420). Further simplification of the model, by dropping the 4-way interaction, severely reduced the quality of the fit (ΔAIC = 124).

At best, the patterns of acclimation weakly support the seasonality hypothesis. Genotypes from Vermont and North Carolina had greater fecundity at 16 °C after spending the larval and adult stages at 16 °C, instead of 26 °C (Fig. 3, top and bottom rows). For genotypes from North Carolina, this benefit was achieved through acclimation to low temperature only as an adult. In both populations, acclimation to 26 °C, as a larva or an adult, led to lower fecundity at 16 °C. Gains in performance at low temperatures were accompanied by a loss of performance at high temperatures. For genotypes from North Carolina, this tradeoff was evident at body temperatures between 26° and 29 °C. For genotypes from Vermont, the tradeoff occurred at the highest range of temperatures, 32–36 °C.

Acclimation of genotypes from Indiana was more difficult to explain. These genotypes exhibited greater plasticity in mean fecundity than in the thermal optimum or thermal limits of fecundity. This result can be seen from the very different heights of performance curves after acclimation to temperature as an adult (compare red and blue curves in middle row of Fig. 3). Counterintuitively, flies were most fecund after switching temperatures between larval and adult stages (either from 16 ° to 26 °C or from 26 ° to 16 °C). If anything, we expected the opposite pattern based on other researchers' ideas about beneficial acclimation (Huey and Berrigan, 1996).

4. Discussion

As the distribution of temperatures changes among seasons, genotypes that adjust their thermal optimum and thermal limits of performance should outcompete those that do not (Angilletta, 2009). This seasonality hypothesis stems from optimality models of reversible plasticity (Gabriel, 1999, 2005, 2006), which assume an energetic tradeoff limits the breadth of performance. Based on these models, we expected the thermal physiology of flies to correspond to the temperatures they experienced during development. When tested at 16 °C, flies that spent their life at 16 °C should have outperformed flies that spent their life at 26 °C. The opposite should have occurred when flies were tested at 26 °C. Importantly, we tested flies over the full range of temperatures that permit reproduction, to see whether acclimation affected the thermal limits and the thermal optimum of performance. We also attempted to reverse the acclimation of thermal physiology during the adult stage, by switching flies between temperatures at eclosion. Reverse acclimation should have reduced the difference in performance between flies that spent the larval stage at 16 °C and those that spent the larval stage at 26 °C. Our data for two of the three populations of flies partially support the seasonality hypothesis. Flies from North Carolina and Vermont had greater mean fecundities at 16 °C after spending the larval and adult stages at 16 °C (see Fig. 3). These same genotypes also had a lower fecundity at some high temperatures. This pattern was supported by the most likely model of fecundity, which contained a 4-way interaction that vastly improved the fit to the data relative to a simpler model (see Table 2). However, these patterns were not observed among flies from Vermont that experienced 26 °C as larvae and 16 °C as adults.

By predicting reversible acclimation between the larval stage and the adult stage, we assumed that flies regularly experience a change in temperature between stages. As with many species of insects, *D. melanogaster* shifts from one habitat to another between larval and adult stages (Kingsolver et al., 2011). Larvae reside within rotting organic material such as fruit (Feder et al., 1997a; McKenzie and McKechnie, 1979). Because of their small size, larvae cannot escape the boundary layer created by their habitat (Stevenson, 1985). Additionally, fruits generally weigh far less than the 10 kg needed to create thermal inertia (Stevenson, 1985). These environmental factors combine with slow movement of larvae to limit the potential for thermoregulation. By contrast, adults fly quickly between microclimates (Jones et al., 1987). Therefore, the mean and variance of larval temperature likely exceeds those of adult temperatures. Reversible acclimation would enable larvae to match the surrounding microclimate and adults to match opportunities for thermoregulation.

Reversible acclimation could only have occurred in flies from North Carolina, whose thermal sensitivity of fecundity depended on adult temperature regardless of the larval temperature. The absence of reversible acclimation in two populations accords with the findings of Cooper et al. (2014), who studied the plasticity of genotypes from the same populations. In their study, thermal acclimation during adulthood failed to erase variation in membrane composition generated by acclimation during larval development. Similarly, flies from Denmark acclimated to constant developmental temperatures far better than they acclimated to brief thermal stress during specific life stages (MacLean et al., 2017). Even when thermal acclimation occurs during adulthood, the response depends on the temperature during development (Bahrndorff et al., 2016). Arguably, developmental acclimation requires processes that limit reversible acclimation later in life (Beaman et al., 2016).

The seasonality hypothesis cannot account for the pattern of acclimation in flies from Indiana. Models of optimal acclimation assume that the area of a performance curve remains fixed during acclimation. In other words, as acclimation enhances performance at low temperatures, it should reduce performance at high temperatures. This assumption follows from the idea that organisms invest resources in cellular structures that enhance performance within a range of temperatures (Hochachka and Somero, 2002; Sørensen et al., 2003). Thus, a finite supply of resources imposes a tradeoff between investing in

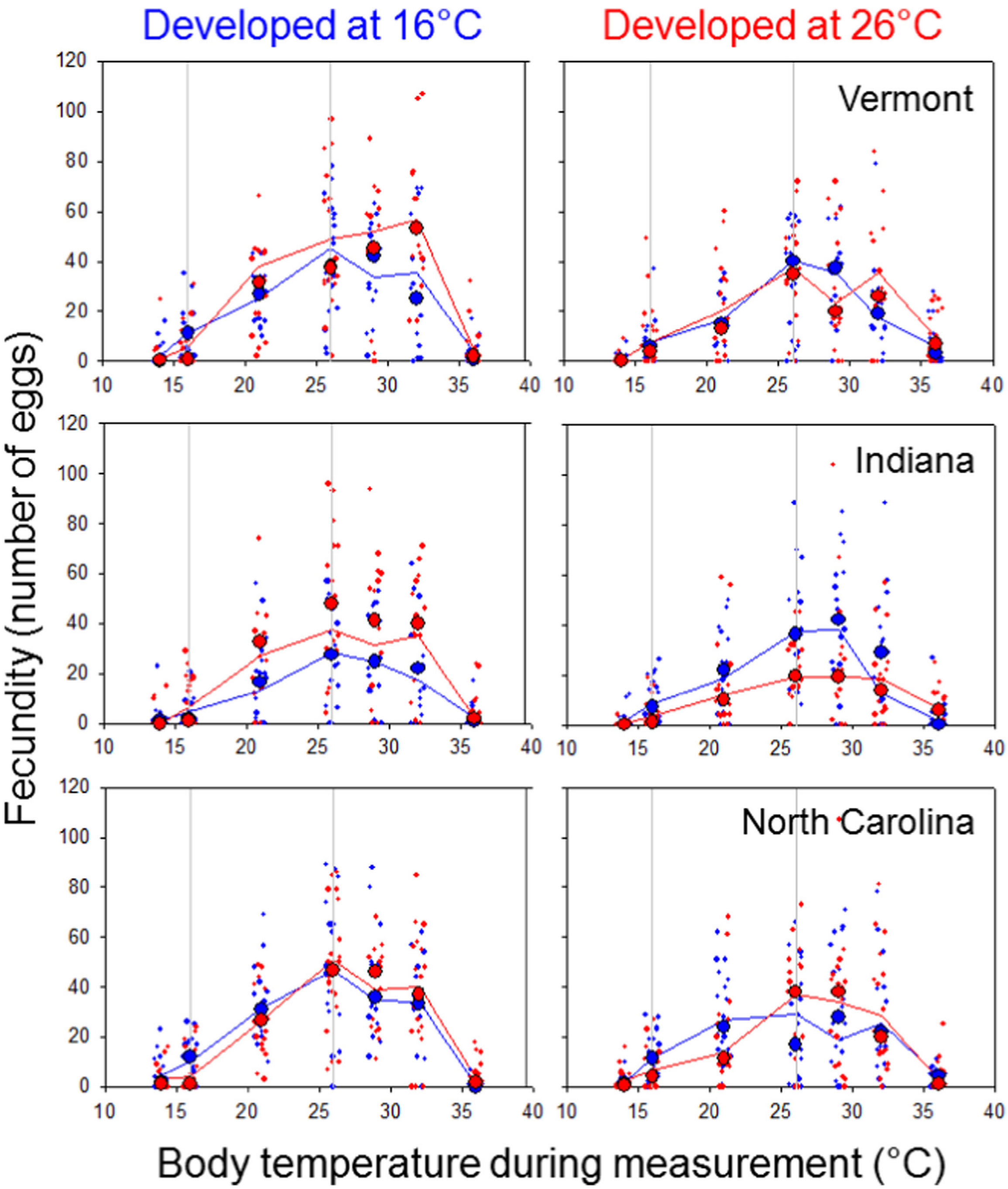

Fig. 3. Thermal sensitivities of fecundity in flies from three populations after spending the larval and adult stages at either 16 °C or 26 °C. Plots in the left-hand and right-hand columns contain data for flies that spent their larval stage at 16° and 26 °C, respectively. Plots in top, middle, and bottom rows show data for flies from Vermont (44° 25' N), North Carolina (35° 30' N), and Indiana (39° 59' N), respectively. In each plot, blue symbols and red symbols denote fecundities of flies that spent their adult stage at 16 ° and 26 °C, respectively. An enlarged symbol highlights the median fecundity at each temperature. Solid lines depict the expected fecundities calculated from the most likely statistical model. Gray vertical bars serve as references at 16 ° and 26 °C to compare data among plots.

performance at low temperatures versus performance at high temperatures (Huey and Hertz, 1984). Despite this assumption, acclimation often alters the area of a performance curve (Angilletta, 2009). In our study, genotypes from Indiana laid more eggs at 21–32 °C after acclimation to certain conditions. Specifically, flies that switched from one temperature to another between stages outperformed flies that remained at one temperature throughout development. This result supports neither the seasonality hypothesis nor the beneficial acclimation hypothesis, which predicts that flies acclimate to the same temperature during larval and adult stages would perform best (Huey and Berrigan, 1996; Leroi et al., 1994; Wilson and Franklin, 2002). Moreover, the magnitude of this acclimation response far exceeded that of acclimation responses in flies from North Carolina or Vermont. Other researchers have reported counter-intuitive patterns of acclimation in *D. melanogaster*. In an experiment performed by Crill et al. (1996), flies that developed at 18 °C had a higher thermal optimum than did flies that developed at 25 °C. In some species of *Drosophila*, a higher developmental temperature produced adults that preferred lower body temperatures, (Yamamoto and Ohba, 1984). Future research should focus on unknown developmental constraints or novel selective pressures that might explain these seemingly maladaptive patterns.

In general, acclimation responses fail to support a key prediction of

Table 3

Parameters of the most likely model of fecundity. This generalized linear model predicts the natural logarithm of expected fecundity. The intercept of the model (bold font) equals the logarithm of fecundity for a certain combination of conditions: a fly from Indiana (IN) at a measurement temperature of 14 °C, after spending larval and adult stages at 16 °C. Other values in the table quantify the effects of deviating from the conditions defined by the intercept. These deviations include the following: 1) the main effects of population, larval temperature, adult temperature and measurement temperature (black font); 2) two-way interactions (blue font), 3) three-way interactions (green font), and four-way interactions (red font). In each cell, the standard error around the effect is given in parentheses. To obtain the logarithm of the expected fecundity, one must add the appropriate main effects and interactions to the intercept, based on the levels of population, larval temperature, adult temperature, and measurement temperature. For details, consult Quinn and Keough (2002) and Zuur et al. (2009).

Effect			Measurement temperature						
Pop	Larval temp	Adult temp	14 °C	16 °C	21 °C	26 °C	29 °C	32 °C	36 °C
IN	16 °C	16 °C	**0.0239 (0.2643)**	1.5664 (0.2610)	2.5788 (0.2505)	3.3254 (0.2472)	3.1784 (0.2474)	2.8498 (0.2565)	0.7629 (0.2861)
		26 °C	−1.9925 (0.7516)	2.3237 (0.7622)	2.7046 (0.7557)	2.2754 (0.7542)	2.2369 (0.7546)	2.6740 (0.7573)	2.0331 (0.7847)
	26 °C		0.5346 (0.3002)	0.0157 (0.3258)	−0.2255 (0.3127)	−0.2659 (0.3068)	−0.0954 (0.3075)	− 0.9017 (0.3216)	−0.9829 (0.3957)
	26 °C	26 °C	0.9109 (0.8344)	−2.2510 (0.8577)	−2.0812 (0.8434)	−1.8660 (0.8394)	−1.8526 (0.8424)	− 1.1685 (0.8460)	0.5296 (0.8949)
NC			1.4952 (0.3032)	−0.7520 (0.2915)	−0.6465 (0.2760)	−0.9953 (0.2720)	−1.1493 (0.2731)	− 0.8423 (0.2816)	−2.6675 (0.4294)
NC		26 °C	1.5986 (0.7706)	−2.7633 (0.7921)	−2.4869 (0.7772)	−1.7926 (0.7748)	−1.7280 (0.7757)	− 2.1209 (0.7784)	0.0756 (0.8665)
NC	26 °C		−1.6121 (0.3662)	1.1873 (0.3992)	1.1263 (0.3816)	0.8674 (0.3764)	0.5604 (0.3790)	1.6896 0.3901	3.8991 (0.5529)
NC	26 °C	26 °C	−0.0538 (0.8812)	1.6986 (0.9190)	0.7552 (0.8958)	1.1560 (0.8892)	1.4742 (0.8926)	0.2574 (0.8957)	−3.5066 (1.0114)
VT			0.5614 (0.3524)	0.2707 (0.3414)	0.0589 (0.3292)	−0.0980 (0.3232)	−0.2448 (0.3275)	0.1418 (0.3343)	−0.1687 (0.3793)
VT		26 °C	1.2673 (0.8216)	−2.3005 (0.8402)	−1.5628 (0.8282)	−1.4751 (0.8247)	−1.0799 (0.8269)	− 1.4822 0.8290	−1.1762 (0.8721)
VT	26 °C		−1.1676 (0.4314)	0.1801 (0.4638)	0.4456 (0.4472)	0.7824 (0.4378)	0.7734 (0.4415)	0.8003 (0.4540)	2.2036 (0.5294)
VT	26 °C	26 °C	−0.1861 (0.9548)	2.2663 (0.9905)	1.1250 (0.9689)	0.9936 (0.9615)	0.2783 (0.9663)	0.6957 (0.9700)	−0.9311 (1.0323)

the seasonality hypothesis: the thermal optimum of genotypes from seasonal environments should acclimate. Acclimation often affects an organism's thermal breadth (Hoffmann et al., 2003; Rohr et al., 2018; Schou et al., 2017) but rarely affects its thermal optimum (Angilletta, 2009; Deere and Chown, 2006). Moreover, acclimation of thermal breadth does not seem restricted to genotypes from seasonal environments. In comparative studies, genotypes from tropical environments often acclimate just as well as (or better than) genotypes from temperate environments (Cooper et al., 2010; Cunningham and Read, 2003; Nilsson-Ortman and Johansson, 2017; Overgaard et al., In press; but see Shah et al., 2017). This surprising pattern might stem from selective pressures generated by dispersal in a metapopulation. When individuals disperse along latitudinal clines, natural selection would favor alleles that contribute to developmental acclimation because some individuals will end up in seasonal environments (Adrion et al., 2015). The same type of selective pressure should occur if individuals migrate along altitudinal clines within tropical environment (Janzen, 1967; Shah et al., 2017). In *Drosophila*, the potential for migration along latitudinal clines seems high given the history of expansion on multiple continents (Hoffmann and Weeks, 2007; Huey et al., 2000). However, we doubt that migration alone can explain the discrepancy between predicted and observed patterns of acclimation; only a fraction of a population will disperse to seasonal environments and the remainder will experience selection against acclimation or dispersal (Hua, 2016).

More likely, genetic constraints limit the evolution of acclimation capacity. Optimality models assume the presence of additive genetic variation for reaction norms (Gilchrist, 2000), but genetic variation often limits adaptation in ways that optimality models fail to consider (Gilchrist, 1996; Kingsolver et al., 2001, 2004). Either natural selection or genetic drift can eliminate the genetic variation needed to evolve a generalized thermal niche (Hoffmann, 2010). For *D. melanogaster*, genetic drift seems unlikely given its large populations. However, genetic constraints likely prevent the evolution of phenotypic plasticity in some populations. Acclimation, like any form of phenotypic plasticity, requires mutations that enable an organism to sense and respond to environmental change (DeWitt et al., 1998). These mutations may occur infrequently or contribute little to the mean fitness of a genotype (Chevin and Hoffmann, 2017). Experimental evolution of *D. melanogaster* in fluctuating environments has failed to enhance the plasticity of some traits but not others (Condon et al., 2014; Cooper et al., 2012; Le Vinh Thuy et al., 2016; Manenti et al., 2015). The selective pressure for plasticity likely varies among traits, but the genetic variation for plasticity must vary as well. The absences of genetic variation for phenotypic plasticity could explain why the frequencies of alleles fluctuate seasonally in *D. melanogaster* (Bergland et al., 2014). Given our inability to explain patterns of thermal acclimation in natural or experimental populations, the genetics and evolution of phenotypic plasticity remain important areas of research in thermal adaptation.

Finally, we should note a common feature of acclimation experiments that complicates the interpretation of results, including those presented here. When exposing genotypes to different temperatures, researchers usually control for factors such as photoperiod. This experimental design makes sense only if temperature serves as the only cue for acclimation. In some species, temperature drives acclimation more than photoperiod does (Condon et al., 2010). Still, some species probably rely on photoperiod because temperature fluctuates within seasons (Dillon et al., 2009). Acclimation responses to photoperiod are common among animals and evolve during climate change (Bradshaw and Holzapfel, 2006, 2008). Therefore, future experiments should couple photoperiodic cues with thermal cues to minimize the chance of missing ecologically relevant patterns of acclimation.

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