

Acclimation of thermal physiology in natural populations of *Drosophila melanogaster*: a test of an optimality model

B. S. COOPER*¹, M. CZARNOLESKI*[†] & M. J. ANGILLETTA JR*²

*Department of Biology, Indiana State University, Terre Haute, IN, USA

[†]Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland

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Abstract

Many organisms modify their physiological functions by acclimating to changes in their environment. Recent studies of thermal physiology have been influenced by verbal models that fail to consider the selective advantage of acclimation and thus make no predictions about variation in acclimation capacity. We used a quantitative model of optimal plasticity to generate predictions about the capacity of *Drosophila melanogaster* to acclimate to developmental temperature. This model predicts that the ability to acclimate thermal sensitivity should evolve when temperature varies greatly among generations. Based on the model, we expected that flies from the highly seasonal environment of New Jersey would acclimate thermal sensitivity more than would flies from the less seasonal environment of Florida. When raised at constant and fluctuating temperatures, flies from these populations failed to adjust their thermal optima in the way predicted by the model, suggesting that current assumptions about functional and genetic constraints should be reconsidered.

Introduction

Many forms of phenotypic plasticity enable organisms to enhance their fitness in a changing environment (Bradshaw, 1965; Schlichting, 1986; Via *et al.*, 1995). When exposed to novel conditions, organisms can adjust molecular and cellular structures to maintain capacities for behavioural and physiological performance – a form of plasticity known as acclimation (Prosser, 1991). For example, acclimation to temperature can improve energy assimilation (Yamori *et al.*, 2006), locomotor performance (Wilson & Franklin, 2000), environmental tolerance (Dahlgaard *et al.*, 1998) and even mating success (Wilson *et al.*, 2007). These types of responses led to the

beneficial acclimation hypothesis, which predicts that acclimation should generally improve performance in novel environments (Leroi *et al.*, 1994; Huey & Berrigan, 1996). Yet, many species do not acclimate according to the beneficial acclimation hypothesis when exposed to novel thermal environments (reviewed by Angilletta, 2009). Even when acclimation fails to enhance performance, researchers often resort to post hoc adaptive explanations (cf. Huey & Berrigan, 1996), instead of considering that the cost of acclimation might outweigh the benefit (but see Hoffmann, 1995; Scott *et al.*, 1997; Huey *et al.*, 1999; Angilletta, 2009).

Huey & Berrigan (1996) proposed a strong inference approach to the study of acclimation, in which one distinguishes among several alternatives to the beneficial acclimation hypothesis. For example, the *optimal-developmental-temperature hypothesis* predicts that a certain body temperature during development will lead to adults that outperform adults that developed at other temperatures. The *colder-is-better hypothesis* predicts that individuals reared at low temperatures will outperform those reared at high temperatures; the *warmer-is-better hypothesis*

Correspondence: Brandon S. Cooper, Department of Biology, Indiana University, Bloomington, IN 47405, USA.
Tel.: 812 236 4532; fax: 812 855 6082;
e-mail: brascoop@indiana.edu

¹Present address: Department of Biology, Indiana University, Bloomington, IN 47405, USA.

²Present address: School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA.

predicts the opposite pattern. Huey *et al.* (1999) recommended an explicit statistical framework to determine whether the thermal sensitivity of performance supports one or more of these hypotheses. Although these ideas have greatly influenced the design and interpretation of recent experiments (Deere & Chown, 2006; Marais & Chown, 2008; Steigenga & Fischer, 2009; Clusella-Trullas *et al.*, 2010), these hypotheses were not derived from formal analyses of the selective advantage of acclimation.

By drawing on a theory of evolution in heterogeneous environments, we can infer the selective advantages of acclimation (Janzen, 1967; Levins, 1968; Gabriel & Lynch, 1992; Gabriel *et al.*, 2005). This theory focuses on the evolution of reaction norms – relationships between continuous environmental variables and traits that relate to fitness (e.g. survivorship and fecundity). Here, we interpret the reaction norm as a thermal performance curve (*sensu* Huey & Stevenson, 1979), a concept that physiologists commonly invoke when describing the relationship between body temperature and organismal performance (Fig. 1a). Performance curves are generally defined by two parameters, which have clear biological interpretations: (i) a thermal optimum, or the temperature that enables maximal perfor-

mance and (ii) a performance breadth, or the range of temperatures that enables performance that equals or exceeds a certain level (e.g. 80% of the maximum). In theory, these parameters either remain fixed throughout ontogeny (Lynch & Gabriel, 1987) or respond to the developmental environment (Gabriel & Lynch, 1992). The latter scenario corresponds to what physiologists refer to as developmental acclimation. Gabriel & Lynch (1992) showed that developmental acclimation of the thermal optimum increases fitness by enabling organisms in a heterogeneous environment to adjust their physiology to current conditions (see Fig. 1b). Their model tells us that the selective advantage of developmental acclimation depends on a specific component of environmental variation: the variation experienced among generations. Such variation results from thermal changes among seasons or organismal dispersal among environments. Thus, organisms that evolved in fluctuating environments should possess a greater capacity to acclimate than do organisms that evolved in relatively stable environments, assuming that the majority of environmental variation occurs among generations and sufficient genetic variation exists within populations.

We can test Gabriel and Lynch's model by comparing the capacity for acclimation between populations that experience different levels of thermal variation among generations. Although comparative studies of acclimation are less common than studies of individual populations, physiologists have compared the thermal acclimation of photosynthetic rate among populations of plants (Gunderson *et al.*, 2000; Cunningham & Read, 2003; Eggert *et al.*, 2006). In contrast to the model's prediction, tropical and temperate plants generally have similar capacities to modify their thermal optimum and performance breadth during development (reviewed by Angilletta, 2009). Analogous studies of acclimation do not exist for animals, but researchers have compared the acclimation of heat and cold tolerances among tropical and temperate populations of fruit flies (*Drosophila* spp.). As with plants, no qualitative differences in acclimation capacity were found between flies from tropical and temperate environments (Ayrinhac *et al.*, 2004; Hoffmann *et al.*, 2005). On one hand, the failure of the model to explain these patterns suggests that we should seriously question its assumptions about phenotypic trade-offs, genetic constraints and population dynamics. On the other hand, rates of photosynthesis and tolerances of thermal extremes are unlikely to map directly onto fitness in a way envisioned by the model. Gabriel and Lynch assumed a direct relationship between performance and fitness, such that one must interpret performance as a rate of survival or fecundity (reviewed by Angilletta, 2009). Therefore, we would benefit greatly from additional tests of the theory before investing the time to develop more complex models.

We tested Gabriel and Lynch's model by comparing the capacity for developmental acclimation between popula-

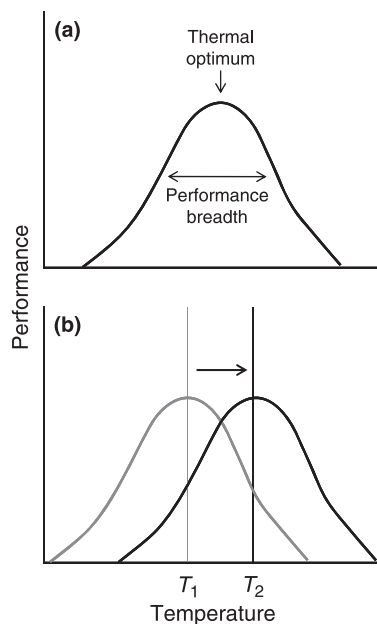


Fig. 1 A thermal performance curve describes the relationship between body temperature and organismal performance (e.g. fecundity), usually characterized by a thermal optimum and a performance breadth (a). When the body temperature of an organism changes from T_1 to T_2 , the new body temperature can cause a loss of performance (b). Acclimation can enhance performance at the new temperature; in this plot, acclimation results in a shift in the thermal optimum from T_1 to T_2 (i.e. a shift from the grey performance curve to the black performance curve).

tions of fruit flies (*Drosophila melanogaster*) that experience markedly different levels of thermal variation among generations. We sampled flies from a highly seasonal, temperate environment in New Jersey, USA, and a more stable, subtropical environment in Florida, USA. To make explicit predictions, we modelled air temperatures at each site and estimated thermal variation among generations. Based on these calculations, we predicted that flies from New Jersey would display a higher capacity for developmental acclimation than would flies from Florida. To evaluate this prediction, we measured the thermal sensitivity of fecundity in flies that had developed in ecologically relevant thermal treatments. Our calculations of thermal heterogeneity, our use of naturalistic treatments and our measurements of fecundity make this experiment a unique test of the theory of developmental acclimation.

Material and methods

Calculating thermal variation among generations

To make predictions about the relative capacities for acclimation, we estimated the thermal variation among generations of flies in Marlton, New Jersey, and Miami, Florida. For each site, we used daily minimal and maximal air temperatures for 2003–2008 from the nearest weather station (Stations 284229 and 83163 of the United States Historical Climatology Network, Williams *et al.*, 2006). Missing data, although rare, were filled by interpolating linearly between temperatures for previous and subsequent days. Following procedures outlined by Campbell & Norman (1998), we estimated hourly air temperatures by fitting a sinusoidal function to daily minima and maxima. Once hourly profiles were obtained, we modelled the development of flies in each environment to estimate the number of generations per year and the temperatures experienced among generations. Generation times were calculated from the number of degree-hours required to develop from hatching to adulthood; degree-hour thresholds were based on rates of development for females at constant temperatures of 18, 22, 25 and 29 °C, averaged among eight populations (Worthen, 1996). Based on these data, we defined a generation as the period required to accumulate 2470 degree-hours. Degree-hours (D) were calculated as

$$D = T_h - T_{\min},$$

where T_h equals the hourly temperature, and T_{\min} equals the minimal temperature required for development. Because *D. melanogaster* initiates diapause at 12 °C (Emerson *et al.*, 2009), we set T_{\min} equal to this temperature; our qualitative predictions about thermal variation were unaffected by small changes in T_{\min} .

Starting with data for January of 2003, we divided the years into generations by summing degree-hours until

we reached the number required for development (i.e. 2470 degree-hours per generation). When T_h was below T_{\min} , developmental rate was set to zero and no degree-hours accrued. For each generation, we calculated the mean of the hourly air temperatures. Then, we calculated the grand mean of air temperature for all generations; the standard deviation of this mean was considered an estimate of thermal variation among generations. By comparing these standard deviations between sites, we could infer the relative selective pressures on the capacity for developmental acclimation.

Our calculations account for the different activity periods of flies in Florida and New Jersey. For example, temperatures during certain months regularly fall below 12 °C (T_{\min}) in New Jersey. These temperatures below T_{\min} did not contribute to development when we computed generation times. Thus, some generations in New Jersey lasted many months, whereas multiple, uninterrupted generations passed in Florida. In this way, we indirectly modelled periods of diapause or dormancy.

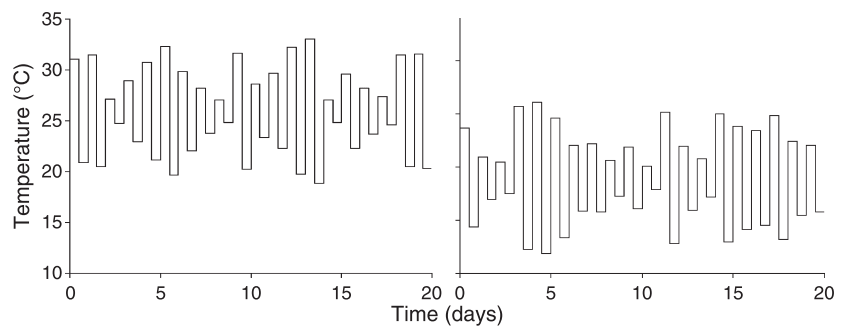
Establishing isofemale lines

In August of 2008, we used banana-bait traps to collect females of *D. melanogaster* from Marlton, NJ and Miami, FL. Multiple traps were scattered throughout a 10-km radius at each site to ensure adequate sampling. To form isofemale lines, each female was placed in a vial containing instant medium (Formula 424, Carolina Biological Supply, Burlington, NC, USA). Vials were shipped overnight to Indiana State University, where they were maintained at 21 °C with a 12 : 12 light cycle. Male offspring were examined for species designation. Thereafter, flies from each isofemale line were transferred every 3 weeks to fresh vials with a standard medium (recipe of the Bloomington Stock Center, Indiana University).

Thermal treatments

We raised flies from all isofemale lines in three thermal treatments (Fig. 2): (i) a relatively warm, constant environment (25 °C), which approximates the stable conditions of Miami; (ii) a relatively warm, stochastic environment (25 ± 7 °C), which approximates summer conditions in New Jersey; and (iii) a relatively cool, stochastic environment (18 ± 7 °C), which approximates spring/autumn conditions in New Jersey. Temperatures within each incubator were monitored with data loggers (iButton Thermochron, Dallas Semiconductors, Dallas, TX, USA). Flies in each treatment experienced a 12 : 12 light cycle. Thermal treatments were based on air temperatures recorded near the sites of collection (Stations 28229 and 83163, Williams *et al.*, 2006). This choice of thermal treatments ensured ecological relevance and provided realistic thermal cues for acclimation. However, Gabriel and Lynch's model predicts similar patterns of

Fig. 2 Minimal and maximal temperatures were changed daily to create stochastic environments with mean temperatures of 25 °C (left panel) and 18 °C (right panel). Flies were also allowed to develop at a constant temperature of 25 °C (not shown).



acclimation for flies developing in constant and stochastic environments, as long as the mean temperature remains the same.

We exposed flies from our isofemale lines to these thermal treatments after three generations at 21 °C. One virgin male and female from each line were paired in a fresh vial. After 5 days, the females were transferred individually to new vials and were provided a drop of active yeast to stimulate oviposition. Forty-eight hours later, we began a series of daily transfers to acquire a vial of eggs for each thermal treatment. Each day, females were transferred to a new vial containing active yeast and were given 24 h at 21 °C to oviposit. To transfer flies without anaesthesia, we simply inverted a fresh vial over each old vial and allowed the female to move into the fresh vial. At the end of the oviposition period, each vial was randomly assigned to one of the three treatments. Through randomization, a similar number of vials from each population were placed into each thermal treatment on all 3 days. In total, 17 and 14 isofemale lines were included from New Jersey and Florida, respectively.

Thermal sensitivity of fecundity

We compared the thermal sensitivities of fecundity among females from the three thermal treatments. To avoid confounding the effects of temperature and age on fecundity, we used siblings to estimate a reaction norm for each line (Scheiner, 2002). Newly emerged females were collected daily from every vial in each treatment. Once collected, these females were placed individually in vials with newly emerged males to stimulate oviposition. To control for paternal effects, the males in our experiment were from an isofemale line derived from a laboratory population (Oregon R strain, Carolina Biological Supply); this control line was established in August of 2008 and was cultured at 21 °C prior to use. After exposure to males, the females were placed individually in new vials and were provided hydrated active yeast to further stimulate oviposition. Forty-eight hours later, we measured the daily fecundity of each female at one of eight temperatures (14, 18, 21, 25, 28, 30, 33 or 36 °C). During this time, flies experienced a 12 : 12 light cycle. For most lines, we collected sufficient females to measure the fecundity of two individuals at each temperature.

Daily fecundity was measured by placing females in vials with a substrate of grape agar. This agar was poured into Petri dishes (35 mm × 10 mm) and after cooling, a drop of hydrated active yeast was placed in the centre of each dish. A vial containing a female was then inverted onto a dish to make a sealed chamber. Prior to this procedure, small holes were punched in the bottom of the vial to prevent hypoxia during the experiment. Given the number of flies, we initiated measures of fecundity in temporal blocks; each hour, equivalent numbers of females from each population were placed in laying chambers and were assigned to each of the eight temperatures. At the start of the hour, all flies within the block were placed at their respective temperature. Because of variation in eclosion time among lines, this process was performed over a 4-day period with the aim of controlling for age. Whenever possible, females were measured at 8 days of age; rarely, we substituted a female of a different yet similar age. Because of thermal effects on developmental time, we measured the fecundities of flies from the colder developmental environment about a week after measuring the fecundities of flies from the two warmer developmental environments.

To characterize the performance curve for each isofemale line, we calculated the breadth and area as defined by Gilchrist (1996). Breadths (B) were calculated as

$$B = \sqrt{\sum_{i=1}^N \left[\frac{P_i(T_i - T_{\text{opt}})}{P_{\text{max}}} \right]^2},$$

where N equals the number of temperatures, T_i equals temperature i , P_i equals performance at T_i in eggs day⁻¹, P_{max} equals the maximal rate of performance and T_{opt} equals the temperature at which the maximal rate was achieved. Area was estimated by summing the rates of performance at the eight temperatures.

Statistical analyses

We used a nested analysis of variance to compare thermal sensitivities among treatments and populations. Population, developmental environment and body temperature were fixed factors, and isofemale line was a random factor nested within populations. Following

this analysis, we conducted an ordered heterogeneity test (Rice & Gaines, 1994) to determine whether the developmental acclimation of thermal sensitivity matched the predictions of Gabriel and Lynch's model (Fig. 3). We also used ANOVA to compare breadths and areas of performance curves. When main effects were significant, Tukey's test was used to determine pairwise differences between means. All analyses were performed with Statistica for Windows 6.0 (StatSoft, 2003). Descriptive statistics are reported as mean \pm 95 confidence interval.

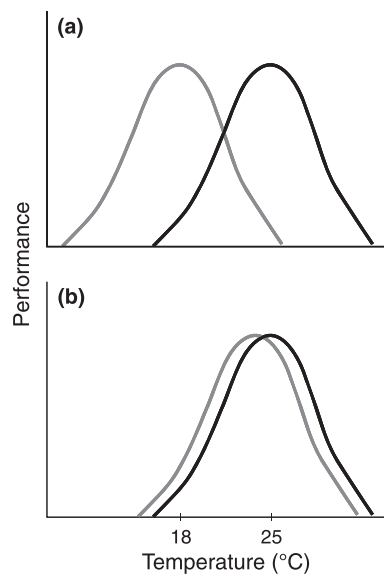


Fig. 3 Predictions of the beneficial acclimation hypothesis (Leroi *et al.*, 1994) and an optimality model (Gabriel & Lynch, 1992) for flies developing at mean temperatures of 18° (grey) and 25 °C (black). The beneficial acclimation hypothesis predicts that flies from any population will display a high capacity for acclimation (a). The optimality model predicts that flies from New Jersey will have a high capacity to acclimate (a), whereas flies from Florida will have a low capacity for acclimation (b). Note that the optimality model predicts similar patterns of acclimation for flies developing in constant and stochastic environments, as long as the mean temperature remains the same.

Results

Using our computer model, we estimated that flies from Marlton, New Jersey, experience nearly twice as much thermal variation among generations as do flies from Miami, Florida. Specifically, the standard deviations of air temperature among generations during 2003-2008 were 5.7 and 3.3 °C in New Jersey and Florida, respectively. Not surprisingly, flies in New Jersey experience more thermal variation because of the greater seasonality at higher latitudes (see Fig. 4). Based on this estimation, Gabriel and Lynch's model predicts that flies from New Jersey should have a greater capacity for developmental acclimation than do flies from Florida (Fig. 3). Alternatively, the beneficial acclimation hypothesis predicts that both populations should display an equal and high capacity for acclimation.

The fecundity of flies from both populations depended strongly on body temperature ($MS = 148\,462$, $F_{7,1315} = 355.32$, $P < 0.001$; see Table 1), but flies did not display a capacity to acclimate their thermal optima as predicted by Gabriel and Lynch's model or the beneficial acclimation hypothesis. Flies from Florida produced the most eggs at 28 °C, regardless of their temperature during development (76 ± 7 eggs day⁻¹). Similarly, flies from New Jersey produced the most eggs at 28 °C (95 ± 7 eggs day⁻¹) after developing at a constant temperature of 25 °C, but produced the most eggs at 30 °C (76 ± 8 and 90 ± 10 eggs day⁻¹ for treatments averaging 18 and 25 °C, respectively) after developing in stochastic environments. An ordered heterogeneity test confirmed that these empirical patterns did not match the predicted patterns ($r_s P_c = -0.20$; $P > 0.85$). In particular, flies from New Jersey did not display any capacity to shift their thermal optima for fecundity in response to their mean developmental temperature. In fact, both flies from New Jersey and flies from Florida displayed nearly identical patterns of variation among developmental treatments (Fig. 5). Flies that developed at a mean temperature of 25 °C had a greater area under their performance curve than did flies that developed at a mean temperature of 18 °C ($MS = 74\,052$, $F_{2,83} = 10.43$, $P < 0.0001$). However, performance breadths did not differ significantly among treatments ($MS = 10.06$, $F_{2,83} = 2.66$, $P = 0.07$).

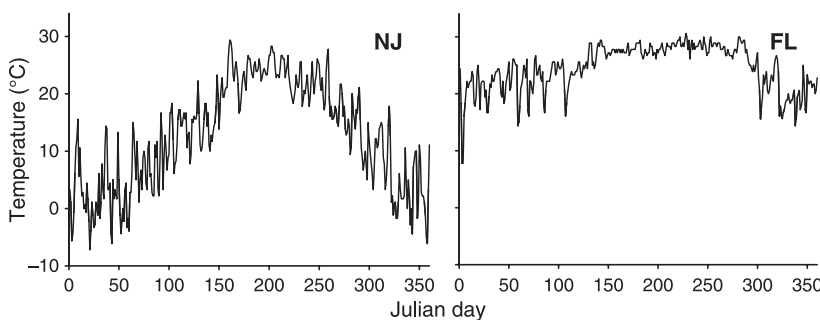


Fig. 4 Daily mean air temperatures recorded during 2008 near our sites in New Jersey (NJ) and Florida (FL). Data were obtained from the United States Historical Climatology Network (Stations 284229 and 83163; Williams *et al.*, 2006).

Table 1 Nested ANOVA describing the effects of population, isofemale line, developmental environment and body temperature on the daily fecundity of *Drosophila melanogaster*.

Factor(s)	d.f.	MS	F	P
Intercept	1, 28.2	2 570 105	690.33	< 0.001
Population	1, 28.2	26 632	7.16	0.01
Body temperature	7, 1315	148 462	355.32	< 0.001
Developmental environment	2, 1315	20 000	47.87	< 0.001
Line (population)	28, 1315	3824	9.15	< 0.001
Population × body temperature	7, 1315	1178	2.82	0.006
Population × developmental environment	2, 1315	383	0.92	0.400
Developmental environment × body temperature	14, 1315	1119	2.68	< 0.001
Population × developmental environment × body temperature	14, 1315	520	1.24	0.237

Performance curves differed between populations but in a way that countered our expectation based on theory. Flies from New Jersey were more fecund than were flies from Florida across the entire range of body temperatures, regardless of the developmental environment ($MS = 26\,632$, $F_{1,28.2} = 7.16$, $P = 0.01$; see Table 1 and Fig. 5). On average, flies from New Jersey produced nearly 20% more eggs than did flies from Florida (48 ± 2.5 vs. 40 ± 2.9 eggs), as reflected by the significantly larger breadth ($MS = 29.18$, $F_{1,83} = 7.72$, $P < 0.01$) and area ($MS = 95\,695$, $F_{1,83} = 13.48$, $P < 0.001$) of the performance curve. Fecundity also varied among isofemale lines within populations (Table 1), but either environmental or genetic effects could have caused this variation.

Discussion

Neither the beneficial acclimation hypothesis nor Gabriel and Lynch's model can account for the patterns observed in our experiment. First, neither flies from New Jersey

nor Florida adjusted their physiologies to perform maximally at the temperature of their developmental environment, as predicted by the beneficial acclimation hypothesis. Instead, the thermal optimum for fecundity was 28 or 30 °C, regardless of the developmental environment (see Fig. 5). Second, flies from New Jersey did not display a greater capacity for acclimation as predicted by Gabriel and Lynch's model. Finally, flies that developed at a mean temperature of 25 °C were more fecund over the range of 14–36 °C than were flies that developed at a mean temperature of 18 °C, regardless of their population of origin (see Fig. 5).

Because we examined only a pair of populations, we should consider factors that might have weakened our inference about the evolution of thermal acclimation. Garland & Adolph (1994) noted that many factors differ between populations, such that phenotypic divergence might be attributed to factors other than the one (or few) of interest to biologists. In our case, we tested hypotheses about a very complex trait: how the thermal sensitivity of fecundity responds to developmental temperature. Many factors such as resource availability, predation risk and competitive interactions could affect the evolution of fecundity (Roff, 1992); nevertheless, these factors should influence the *mean fecundity* at each temperature (i.e. the height of the performance curve) rather than the capacity to shift the *thermal optimum of fecundity* in response to developmental temperature. **Only thermal variation among generations exerts a clear selective pressure on the capacity to shift the thermal optimum of performance (Gabriel & Lynch, 1992).** Moreover, we observed no evidence of divergence in acclimation capacity between populations. Thus, we need not worry about falsely attributing a pattern of evolutionary divergence to the selective pressures described by Gabriel and Lynch. To explore potential reasons why the current theory cannot account for our observations, we focus the remainder of our discussion on the assumptions of Gabriel and Lynch's model, ranging from selective pressures to genetic constraints. **The assumptions of the beneficial acclimation hypothesis have already been criticized extensively by others (Huey & Berrigan, 1996; Wilson & Franklin, 2002; Angilletta, 2009).**

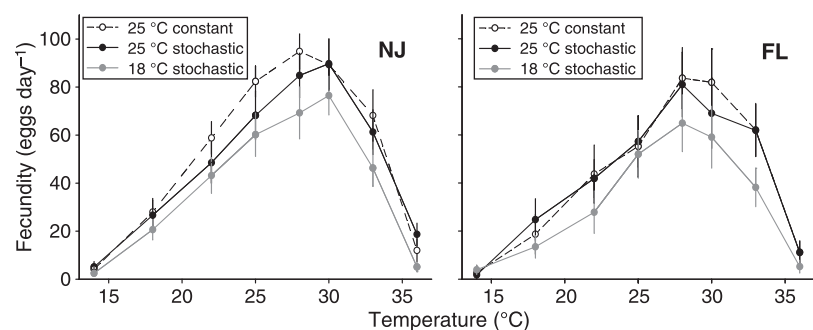


Fig. 5 Thermal sensitivities of fecundity for flies that developed in one of three thermal treatments. Data are means for 17 and 14 isofemale lines from New Jersey (NJ) and Florida (FL), respectively. Error bars are 95% confidence intervals.

Possibly, we failed to predict variation in acclimation capacity, because we could not define selective environments appropriately. The predictions of Gabriel and Lynch's model depend greatly on the variation in body temperature among generations. As we could not measure the temperatures of developing flies in natural environments, we used air temperatures from weather stations to predict selective pressures on acclimation (see Fig. 4). When calculating thermal variation among generations, we assumed that changes in air temperature reflected changes in body temperature. Considering that convection dominates the heat exchange of *D. melanogaster*, air temperature provides a reasonable estimate of body temperature (Huey & Pascual, 2009). However, larvae likely experience temperatures that exceed the air temperatures recorded by weather stations (Feder *et al.*, 1997). This caveat does not change our qualitative prediction about the relative capacities for acclimation, because larvae should still experience variation in body temperature that is proportional to variation in air temperature (Feder *et al.*, 1997). Moreover, our estimation of thermal variation among generations was conservative in the sense that we accounted for differences in activity time by only counting temperatures above 12 °C (see Materials and methods). Thus, we conclude that flies in temperate zones experience higher levels of thermal variation among generations than do flies in subtropical zones.

Current models of acclimation ignore some important constraints on development. For example, Gabriel and Lynch's model assumes that organisms have perfect cues to trigger acclimation. If organisms receive inaccurate signals of environmental change, they would fail to evolve acclimation capacities or fail to express such capacities in artificial environments. In our experiment, flies in some treatments experienced stochastic temperatures, but flies in all treatments experienced a constant photoperiod (12L : 12D). For organisms that regulate the timing of developmental events, photoperiod offers the most accurate cue, because photoperiodic cycles have remained constant throughout geological time (Bradshaw & Holzapfel, 2007). Furthermore, photoperiod and temperature interact to determine the initiation of diapause in *D. melanogaster* (Schmidt *et al.*, 2005), which can directly influence survival during winter. Because we wanted to isolate the effects of temperature from those of photoperiod, we chose not to manipulate photoperiod in our experiment. In the future, we could conduct similar experiments in which temperature and photoperiod covary in an ecologically relevant manner (e.g. Tsuji, 1988). By comparing the results of such an experiment to the results of our experiment, we can see how photoperiodic cues influence the capacity for thermal acclimation. Nevertheless, other researchers have documented thermal acclimation under conditions of constant photoperiod (e.g. Huey *et al.*, 1995; Nunney & Cheung, 1997; Geister & Fischer, 2007).

Gene flow between populations can limit adaptation to local environments (Lenormand, 2002) or produce novel selective pressures by exposing genotypes to multiple environments (Gabriel & Lynch, 1992). If acclimation cannot evolve, gene flow would impede local adaptation and favour thermal generalists (Lynch & Gabriel, 1987). A thermal generalist could persist in either New Jersey or Florida but would achieve less fitness in each environment than would thermal specialists. If acclimation can evolve, gene flow would increase the selective advantage of acclimation by amplifying thermal variation among generations (Gabriel & Lynch, 1992). Genotypes that acclimate to local conditions would share the fitness advantages enjoyed by specialists, as long as the cost of acclimation did not offset the benefits. In the case of our flies, extensive gene flow between populations in New Jersey and Florida could have favoured a high capacity for acclimation in both populations (Gabriel & Lynch, 1992). Still, the failure of flies from either population to acclimate their thermal optima does not accord with the strategy favoured by gene flow (Gabriel & Lynch, 1992). Therefore, gene flow alone cannot explain the outcome of our experiment.

Very likely, genetic constraints inhibit populations from reaching the global optimum portrayed by current models of thermal adaptation. To maintain performance across thermally varying environments, cells must evolve mechanisms to detect environmental change and regulate gene expression. The latter mechanisms would require suites of genes that code for temperature-specific isoforms of proteins (Somero, 1995; Mitten, 1997). Gene duplication, mutation and recombination can create genotypes with novel acclimation strategies. Similarly, relaxed selection of conditionally expressed genes or modules could lead to the accumulation of novel genetic material that can increase in frequency during periods of environmental stasis (Snell-Rood *et al.*, 2010). But some populations will undoubtedly fail to evolve the capacity to acclimate all types of performances. Indeed, a more likely outcome of evolution would be some mechanism for regulating the expression of existing genes, such that an organism alters its mean performance over a broad range of temperatures instead of shifting its thermal optimum (Angilletta *et al.*, 2003). This process would involve quantitative changes in the concentrations of proteins rather than qualitative changes in the forms of proteins (Somero, 1995). In our experiment, flies shifted their mean daily fecundity according to their developmental temperature. Similar patterns of acclimation have been observed in other species of plants and animals (reviewed by Angilletta, 2009), suggesting that the capacity to shift mean performance during development evolves more readily than the capacity to shift the thermal optimum. The widespread occurrence of such phenomena could represent evolution towards a local optimum resulting from limited genetic variation for the acclimation of thermal optima.

Overall, flies reared in our warm treatments were more fecund than were flies reared in our cool treatment. This pattern seems counter-intuitive when one considers the thermal plasticity of body size and its potential effect on fecundity. In populations of *D. melanogaster*, including those studied by us (Czarnoleski *et al.*, unpublished data), development at 25 °C generally results in smaller size at maturity than does development at 18 °C (French *et al.*, 1998). Smaller animals usually suffer lower fecundity (Roff, 2002), presumably because they can store less energy. In *D. melanogaster*, ovariole number increases with increasing thorax length, although the strength of this relationship depends on nutritional conditions (Bergland *et al.*, 2008). Therefore, we might have expected flies in our warmer treatments to emerge smaller and lay fewer eggs. Yet, warmer environments also cause the production of smaller eggs (Azevedo *et al.*, 1996), which might have offset an effect of body size on fecundity. Furthermore, greater fecundity can result from the allocation of resources to reproduction at the expense of competing functions, such as growth (Angilletta *et al.*, 2003). Because poor survival during adulthood favours the allocation of resources to reproduction (Cichoń & Kozłowski, 2000), poor survival in warm environments might have generated the pattern that we observed. By incorporating these allocation processes in models of thermal adaptation, we might better predict the evolution of acclimation in heterogeneous environments.

We have shown that current theory cannot account for the acclimation of thermal sensitivity in *D. melanogaster*. Our study of fecundity accords with previous studies that focused on nonreproductive performances, such as photosynthetic rate and thermal tolerances (reviewed by Angilletta, 2009). Therefore, rather than continuing to amass data for additional traits and taxa, we should work towards new quantitative models that posit more realistic constraints on the evolution of acclimation. To do so, we need to evaluate the genetic basis and molecular mechanisms of acclimation (e.g. Montooth *et al.*, 2006), because these factors inform our assumptions about genetic and developmental constraints. For example, if most of the genetic variation in performance curves reflects variation in mean performance, the pattern of acclimation that we observed in *D. melanogaster* might represent the best strategy among those present in natural populations. Despite decades of studying the acclimation of plants and animals, physiologists know virtually nothing about the genetic variance of acclimation within and among populations (Huey *et al.*, 1999; Angilletta, 2009). Moreover, classic debates about the genetic architecture of phenotypic plasticity (Snell-Rood *et al.*, 2010; Via *et al.*, 1995) have yet to be resolved with respect to physiological acclimation. Because understanding the link between the genotype and the phenotype remains a major challenge (Schwenk *et al.*, 2009), studies that characterize the selective advantage of

acclimation while investigating the underlying genetic architecture would simultaneously advance the current theory of acclimation and address long-standing questions about the evolution of plasticity.

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References

- Angilletta, M.J. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford.
- Angilletta, M.J., Wilson, R.S., Navas, C.A. & James, R.S. 2003. Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* **18**: 234–240.
- Ayrinhac, A., Debat, V., Gibert, P., Kister, A.G., Legout, H., Moreteau, B., Vergilino, R. & David, J.R. 2004. Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Funct. Ecol.* **18**: 700–706.
- Azevedo, R.B.R., French, V. & Partridge, L. 1996. Thermal evolution of egg size in *Drosophila melanogaster*. *Evolution* **50**: 2338–2345.
- Bergland, A.O., Genissel, A., Nuzhdin, S.V. & Tatar, M. 2008. Quantitative trait loci affecting phenotypic plasticity and the allometric relationship of ovariole number and thorax length in *Drosophila melanogaster*. *Genetics* **180**: 567–582.
- Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* **13**: 115–155.
- Bradshaw, W.E. & Holzapfel, C.M. 2007. Evolution of animal photoperiodism. *Annu. Rev. Ecol. Evol. Syst.* **38**: 1–25.
- Campbell, J.S. & Norman, J.M. 1998. *An Introduction to Environmental Biophysics*. Springer, New York.
- Cichoń, M. & Kozłowski, J. 2000. Ageing and typical survivorship curves result from optimal resource allocation. *Evol. Ecol. Res.* **2**: 857–870.
- Clusella-Trullas, S., Terblanche, J.S. & Chown, S.L. 2010. Phenotypic plasticity of locomotion performance in the seed harvester *Messor capensis* (Formicidae). *Physiol. Biochem. Zool.* **83**: 519–530.
- Cunningham, S.C. & Read, J. 2003. Do temperate rainforest trees have a greater ability to acclimate to changing temperatures than tropical rainforest trees? *New Phytol.* **157**: 55–64.
- Dahlgard, J., Loeschcke, V., Michalak, P. & Justesen, J. 1998. Induced thermotolerance and associated expression of the heat-shock protein Hsp70 in adult *Drosophila melanogaster*. *Funct. Ecol.* **12**: 786–793.

- Deere, J.A. & Chown, S.L. 2006. Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. *Am. Nat.* **168**: 630–644.
- Eggert, A., Visser, R.J.W., Van Hasselt, P.R. & Breeman, A.M. 2006. Differences in acclimation potential of photosynthesis in seven isolates of the tropical to warm temperate macrophyte *Valonia utricularis* (Chlorophyta). *Phycologia* **45**: 546–556.
- Emerson, K.J., Bradshaw, W.E. & Holzapfel, C.M. 2009. Complications of complexity: integrating environmental, genetic and hormonal control of insect diapause. *Trends Genet.* **25**: 217–225.
- Feder, M.E., Blair, N. & Figueras, H. 1997. Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. *Funct. Ecol.* **11**: 90–100.
- French, V., Feast, M. & Partridge, L. 1998. Body size and cell size in *Drosophila*: the developmental response to temperature. *J. Insect Physiol.* **44**: 1081–1089.
- Gabriel, W. & Lynch, M. 1992. The selective advantage of reaction norms for environmental tolerance. *J. Evol. Biol.* **5**: 41–59.
- Gabriel, W., Luttbegg, B., Sih, A. & Tollrian, R. 2005. Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. *Am. Nat.* **166**: 339–353.
- Garland, T. & Adolph, S.C. 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* **67**: 797–828.
- Geister, T.L. & Fischer, K. 2007. Testing the beneficial acclimation hypothesis: temperature effects on mating success in a butterfly. *Behav. Ecol.* **18**: 658–664.
- Gilchrist, G.W. 1996. A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* **50**: 1560–1572.
- Gunderson, C.A., Norby, R.J. & Wulschleger, S.D. 2000. Acclimation of photosynthesis and respiration to simulated climatic warming in northern and southern populations of *Acer saccharum*: laboratory and field evidence. *Tree Physiol.* **20**: 87–96.
- Hoffmann, A.A. 1995. Acclimation: increasing survival at a cost. *Trends Ecol. Evol.* **10**: 1–2.
- Hoffmann, A.A., Shirriffs, J. & Scott, M. 2005. Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Funct. Ecol.* **19**: 222–227.
- Huey, R.B. & Berrigan, D. 1996. Testing evolutionary hypotheses of acclimation. In: *Animals and Temperature: Phenotypic and Evolutionary Adaptation* (I.A. Johnston & A.F. Bennett, eds), pp. 205–237. Cambridge University Press, Cambridge.
- Huey, R.B. & Pascual, M. 2009. Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. *Ecology* **90**: 1715–1720.
- Huey, R.B. & Stevenson, R.D. 1979. Integrating thermal physiology and ecology of ectotherms: discussion of approaches. *Am. Zool.* **19**: 357–366.
- Huey, R.B., Wakefield, T., Crill, W.D. & Gilchrist, G.W. 1995. Within-generation and between-generation effects of temperature on early fecundity of *Drosophila-melanogaster*. *Heredity* **74**: 216–223.
- Huey, R.B., Berrigan, D., Gilchrist, G.W. & Herron, J.C. 1999. Testing the adaptive significance of acclimation: a strong inference approach. *Am. Zool.* **39**: 323–336.
- Janzen, D.H. 1967. Why mountain passes are higher in tropics. *Am. Nat.* **101**: 233.
- Lenormand, T. 2002. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **17**: 183–189.
- Leroi, A.M., Bennett, A.F. & Lenski, R.E. 1994. Temperature-acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proc. Natl. Acad. Sci. USA* **91**: 1917–1921.
- Levins, R. 1968. *Evolution in Changing Environments: Some Theoretical Explorations*. Princeton University Press, Princeton.
- Lynch, M. & Gabriel, W. 1987. Environmental tolerance. *Am. Nat.* **129**: 283–303.
- Marais, E. & Chown, S.L. 2008. Beneficial acclimation and the Bogert effect. *Ecol. Lett.* **11**: 1027–1036.
- Mitten, J.B. 1997. *Selection in Natural Populations*. Oxford University Press, Oxford.
- Montooth, K.L., Siebenthal, K.T. & Clark, A.G. 2006. Membrane lipid physiology and toxin catabolism underlie ethanol and acetic acid tolerance in *Drosophila melanogaster*. *J. Exp. Biol.* **209**: 3837–3850.
- Nunney, L. & Cheung, W. 1997. The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: evidence for adaptive plasticity. *Evolution* **51**: 1529–1535.
- Prosser, C.L. 1991. *Environmental and Metabolic Animal Physiology*. Wiley-Liss, New York.
- Rice, W.R. & Gaines, S.D. 1994. Extending nondirectional heterogeneity tests to evaluate simply ordered alternative hypotheses. *Proc. Natl. Acad. Sci. USA* **91**: 225–226.
- Roff, D.A. 1992. *The Evolution of Life Histories: Theory and Analysis*. Chapman and Hall, Inc., Routledge.
- Roff, D.A. 2002. *Life History Evolution*. Sinauer Associates, Inc., Sunderland.
- Scheiner, S.M. 2002. Selection experiments and the study of phenotypic plasticity. *J. Evol. Biol.* **15**: 889–898.
- Schlichting, C.D. 1986. The evolution of phenotypic plasticity in plants. *Annu. Rev. Ecol. Evol. Syst.* **17**: 667–693.
- Schmidt, P.S., Paaby, A.B. & Heschel, M.S. 2005. Genetic variance for diapause expression and associated life histories in *Drosophila melanogaster*. *Evolution* **59**: 2616–2625.
- Schwenk, K., Padilla, D.K., Bakken, G.S. & Full, R.J. 2009. Grand challenges in organismal biology. *Integr. Comp. Biol.* **49**: 7–14.
- Scott, M., Berrigan, D. & Hoffmann, A.A. 1997. Costs and benefits of acclimation to elevated temperature in *Trichogramma carverae*. *Entomol. Exp. Appl.* **85**: 211–219.
- Snell-Rood, E.C., Van Dyken, J.D., Cruickshank, T., Wade, M.J. & Moczek, A.P. 2010. Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity. *Bioessays* **32**: 71–81.
- Somero, G.N. 1995. Proteins and temperature. *Annu. Rev. Physiol.* **57**: 43–68.
- StatSoft, I. 2003. STATISTICA (data analysis software system), version 6. <http://www.statsoft.com>.
- Steigenga, M.J. & Fischer, K. 2009. Fitness consequences of variation in developmental temperature in a butterfly. *J. Therm. Biol.* **34**: 244–249.
- Tsuji, J.S. 1988. Thermal acclimation of metabolism in *Sceloporus* lizards from different latitudes. *Physiol. Zool.* **61**: 241–253.
- Via, S., Gomulkiewicz, R., de Jong, G., Scheiner, S.M., Schlichting, C.D. & van Tienderen, P.H. 1995. Adaptive phenotypic

- plasticity: consensus and controversy. *Trends Ecol. Evol.* **10**: 212–217.
- Williams, C.N. Jr, Menne, M.J., Vose, R.S. & Easterling, D.R. 2006. United States historical climatology network daily temperature, precipitation, and snow data. ORNL/CDIAC-118, NDP-070. Available on-line (<http://cdiac.ornl.gov/epubs/ndp/ushcn/usa.html>) from the Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.
- Wilson, R.S. & Franklin, C.E. 2000. Inability of adult *Limnodynastes peronii* (Amphibia : Anura) to thermally acclimate locomotor performance. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **127**: 21–28.
- Wilson, R.S. & Franklin, C.E. 2002. Testing the beneficial acclimation hypothesis. *Trends Ecol. Evol.* **17**: 66–70.
- Wilson, R.S., Hammill, E. & Johnston, I.A. 2007. Competition moderates the benefits of thermal acclimation to reproductive performance in male eastern mosquitofish. *Proc. Biol. Sci.* **274**: 1199–1204.
- Worthen, W.B. 1996. Latitudinal variation in developmental time and mass in *Drosophila melanogaster*. *Evolution* **50**: 2523–2529.
- Yamori, W., Suzuki, K., Noguchi, K., Nakai, M. & Terashima, I. 2006. Effects of Rubisco kinetics and Rubisco activation state on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. *Plant Cell Environ.* **29**: 1659–1670.

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