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Developmental plasticity of thermal tolerances in temperate and subtropical populations of *Drosophila melanogaster*

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ABSTRACT

Variation in temperature imposes selection pressures on organisms. In variable environments, organisms must adopt fixed or plastic strategies that enable persistence over a broad range of temperatures. In coarse-grained environments, where the thermal variation among generations exceeds that within generations, selection should favor developmental plasticity. Here, we compare the degree of developmental plasticity of thermal tolerances between populations of *Drosophila melanogaster* from environments with relatively high (Marlton, NJ, USA) and relatively low (Miami, FL, USA) variance in temperature among generations. We predicted that flies from Marlton would exhibit a greater plasticity of thermal tolerances than would flies from Miami. Flies from both populations were reared in three ecologically relevant treatments, after which we assessed knockdown and chill-coma recovery times. Flies from both populations responded plastically to temperature, but flies from New Jersey did not exhibit greater plasticity. Our results complement previous comparative studies and indicate that selection favors plasticity of thermal tolerances equally in these populations.

1. Introduction

The temperatures of all environments fluctuate to some degree, but temporal variation in temperature generally increases with latitude (Janzen, 1967; Piper and Stewart, 1996; Ghalambor et al., 2006). Thermal fluctuations, ranging from sudden changes within days to gradual changes among seasons, impose selection pressures on organisms (Angilletta, 2009). In variable environments, selection favors genotypes that perform well over a wide range of temperatures (Gilchrist, 1995; Levins, 1968), resulting in tradeoffs between physiological performance and other aspects of the life history (Angilletta et al., 2003). However, when genetic variation for plasticity exists, populations in variable environments can evolve a capacity to adjust their thermal physiology according to the current environment (Gabriel and Lynch, 1992; Gabriel, 2005). Such plasticity enables an individual to maintain fitness in a changing environment, particularly when different conditions favor different phenotypes (Via and Lande, 1985; Gomulkiewicz and Kirkpatrick, 1992; Via 1993).

The type of plasticity that will evolve depends upon how the organism experiences environmental variation. According to models of optimal plasticity, natural selection favors irreversible plasticity when environmental conditions vary primarily among generations (Gabriel and Lynch, 1992). Alternatively, selection favors reversible plasticity when conditions vary gradually and predictably within generations (Gabriel, 2005). At high latitudes, seasonal and diel changes in temperature should impose strong selection pressure for irreversible or reversible plasticity, depending on the generation time of a species. Because shorter generations lead to more variation among generations, short-lived species should possess a high degree of irreversible plasticity at high latitudes, assuming that developmental or genetic constraints do not prevent adaptation (Cooper et al., 2010).

Comparative and experimental studies of thermal plasticity have provided mixed support for this prediction (Angilletta, 2009). In some cases, populations that experience more environmental variation exhibit a greater degree of plasticity. For example, populations of frogs (*Rana temporaria*) that experienced higher variation in hydric conditions among generations exhibited greater plasticity in developmental rate (Lind et al., 2011). Similarly, populations of flies (*Drosophila melanogaster*) that experimentally evolved at fluctuating temperatures exhibited a greater degree of cellular plasticity than did populations that evolved at constant temperatures (Cooper et al., in press). Temperate populations of *D. serrata* have higher plasticity for some wing morphologies (but not for developmental rate) than

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do tropical conspecifics (Liefting et al., 2009). Interestingly, for many other species of plants and animals, the degree of thermal plasticity appears unrelated to the thermal conditions in which populations have evolved (reviewed by Angilletta, 2009). For example, temperate and subtropical populations of *D. melanogaster* had equal capacities to acclimate their thermal optima for fecundity when reared under several thermal treatments (Cooper et al., 2010). Similarly, flies from temperate environments did not adjust their heat and cold tolerance more readily than did flies from tropical environments (Ayrinhac et al., 2004; Hoffmann et al., 2005; Overgaard et al., 2011). Therefore, additional comparisons of thermal plasticity within geographically widespread species are needed to identify general patterns.

In this study, we compared the plasticity of thermal tolerances between populations of *D. melanogaster*, one from a subtropical environment (Miami, Florida, USA) and the other from a more temperate environment (Marlton, New Jersey, USA). Previous modeling indicates that air temperatures vary nearly twice as much among generations in Marlton than they do in Miami (Cooper et al., 2010). We used records of air temperatures from these sites to design ecologically relevant thermal treatments. After allowing genotypes from both populations to develop in these treatments, we compared their heat and cold tolerances at adulthood. Our results complement those from previous studies, indicating that the degree of plasticity during development does not differ between populations from high and low latitudes.

2. Methods

2.1. Collection and establishment of isofemale lines

The flies used in this experiment were collected from Marlton, New Jersey, USA (39.89°N, 74.92°W), and Miami, Florida, USA (25.82°N, 80.28°W) in August of 2008. Traps were scattered throughout a 10 km radius at each site to ensure adequate sampling. Females were placed in separate vials and were shipped overnight to Indiana State University. Once in the lab, lines were maintained at 21 °C with a 12:12 light cycle. Two generations of virgin full-sib mating was performed within each line to rapidly isogenize the genomes and prevent adaptation to the laboratory. Male offspring were examined for species designation. Thereafter, flies from each isofemale line were transferred every three weeks to fresh vials with a standard medium. This experiment was conducted with flies from the 14th generation in the lab.

2.2. Thermal treatments

We reared flies from all isofemale lines in three thermal treatments (Fig. 1): (1) a relatively warm, constant environment (25 °C), which approximates the stable conditions of Miami, (2) a relatively warm, stochastic environment (25 ± 4 °C [SD]), which approximates summer conditions in New Jersey, (3) a relatively cool, stochastic environment (18 ± 4 °C [SD]), which approximates spring or autumn conditions in New Jersey. These treatments provided realistic thermal cues for acclimation. Flies in each treatment experienced a 12:12 light cycle.

Flies from each isofemale line developed in each of the thermal treatments. First, virgin males and females from each line were paired in vials containing fresh medium. After five days, females were transferred individually to a new vial and provided a drop of active yeast to stimulate oviposition. After three more days, we began a series of daily transfers to acquire a vial of eggs for each treatment. Each day, females were given 24 h at 21 °C to oviposit in a fresh vial. After each 24 h egg laying period, each vial was randomly assigned to one of the three treatments. A similar number of vials from each population were placed into each thermal treatment on all three days. In total, 8 and 7 isofemale lines were included from New Jersey and Florida, respectively. As flies emerged from the vials in each treatment, we transferred them to new vials to keep track of their age. For most isofemale lines, measurements of heat and cold tolerance were conducted on five males and five females. The flies used in our experiment were between 2 and 4 day of age.

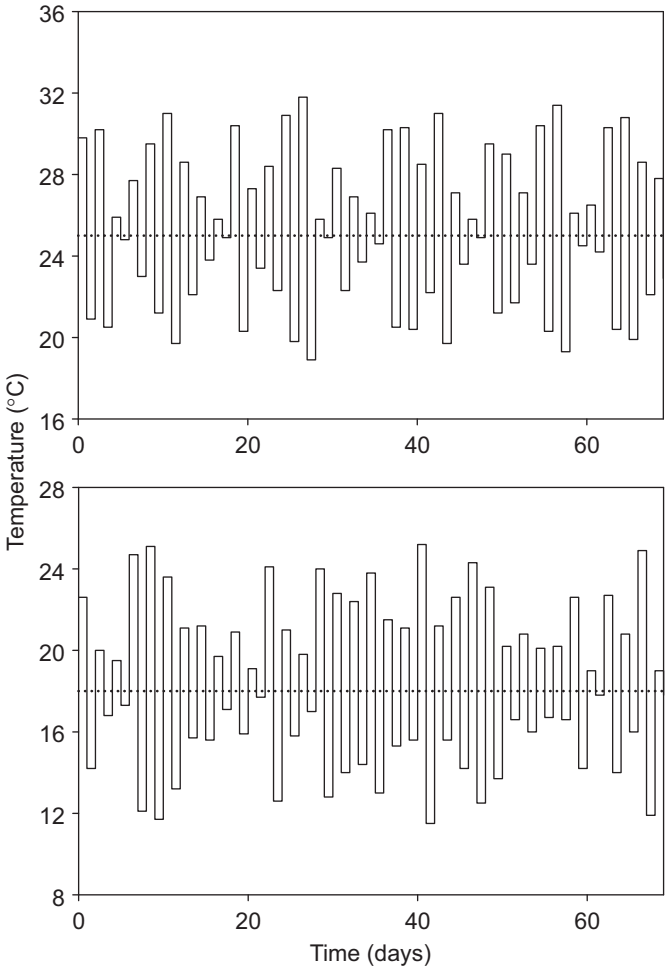

Fig. 1. We used different minimal and maximal temperatures to create stochastic environments with mean temperatures of 25 °C (top panel) and 18 °C (bottom panel). These treatments were based on air temperatures recorded near the sites of collection (Stations 28229 and 83163, Williams et al. 2006).

2.3. Heat tolerance

We estimated heat tolerance as the time required for flies to lose mobility at a stressful temperature, usually referred to as knockdown time (Huey et al., 1992; Cooper et al., 2008). Flies were anesthetized and transferred to clean glass vials (10 ml). After giving the anesthetized flies 90 min to recover, the vials were submerged in a water bath set at 41 °C. We recorded the time between submerging the flies and observing the knockdown of each individual (Blumstein and Daniel, 2007). Knockdown was defined as the point at which a fly was unable to move. To be sure that immobility reflected an inability to move, rather than unwillingness to move, we rotated the vials periodically to encourage movement. Because flies were assayed in successive trials, each trial included a similar number of individuals from each thermal treatment. To maximize our ability to detect and record recovery, no more than ten flies were assayed at a time.

2.4. Cold tolerance

We estimated cold tolerance as the time required to recover from exposure to 0 °C, usually referred to as chill-coma recovery (Gibert and Huey, 2001). Flies were anesthetized and transferred to Petri dishes (35 × 10 mm). Anesthetized flies were given 90 min to recover to minimize an effect on chill-coma recovery time (Nilson et al., 2006). The Petri dishes were entombed in ice, causing the air temperature within each dish to fall to 0 °C within 5 min. After 30 min, the dishes were removed from the ice and the flies were transferred to sheets of paper in a room maintained at 23 ± 0.5 °C. Using a small brush, we positioned each fly in the center of a printed circle (diameter=20 mm). A clear plastic container was placed over each fly to prevent escape following recovery. We used software to record the time between the removal of flies from the ice and the recovery of each individual (Blumstein and Daniel, 2007). Recovery was scored when a fly assumed an upright position (Ayrinhac et al., 2004; Gibert et al., 2001; Hoffmann et al., 2002).

Because flies were assayed in successive trials, each trial included a similar number of individuals from each thermal treatment. Petri dishes containing flies from different thermal treatments were chilled together, and the positions of these dishes were rotated between trials. To maximize our ability to detect and record recovery, no more than ten flies were assayed at a time.

2.5. Statistical analyses

We used Cox proportional hazards models to estimate the effects of population, developmental environment, and sex on knockdown time and chill-coma recovery. This nonparametric approach for comparing thermal tolerances requires fewer assumptions about the distribution of residuals than do parametric approaches (Crawley, 2007). Because the responses of flies from the same isofemale line were likely correlated, we also included a robust sandwich estimator of the variance attributable to this factor. To account for the skewed distributions of recovery times, we report median values and ranges for comparisons of expected times among groups. Models were fit using the survival library of the R Statistical Package (Therneau and Lumley, 2009).

3. Results

Contrary to our prediction, populations of flies from NJ and FL did not differ in the degree of plasticity of heat and cold tolerances (Tables 1 and 2, respectively). In general, flies that had developed in a warm environment (either 25C or 25S) tolerated heat better than did flies that had developed in the cool environment (Fig. 2). Likewise, flies that had developed in the cool environment (18S) recovered from chill-coma faster than did flies that had developed in a warm environment (25S, Fig. 3). Plasticities of heat tolerance were similar between flies that developed at constant and fluctuating temperatures, but the plasticity of cold tolerance depended on the variance as well as the mean of temperature; specifically, chill-coma recovery was longer for flies that developed in the warm fluctuating environment than it was for flies that developed in the warm, constant environment (Fig. 3). Developmental effects on chill-coma recovery were unaffected by sex; however, the magnitudes of the effect on knockdown time differed slightly between males and females (compare the two plots in Fig. 2). Median times for knockdown resistance and chill-coma recovery are listed in Tables 3 and 4, respectively.

4. Discussion

We predicted that flies from New Jersey, which experience considerable thermal variation among generations, would have a greater degree of plasticity of thermal tolerances than would flies from Florida, which experience nearly half the amount of variance in temperature among generations (Cooper et al., 2010).

Table 2
Parameters of the Cox proportional hazards model of the effects of population, developmental environment, and sex on chill-coma recovery. The model compares the rate of recovery for flies with a particular combination of characteristics to the rate for a baseline combination (e.g., females from FL acclimated to a stochastic environment with a mean of 18 °C). Thermal treatments are 18 ± 4 °C (18S), 25 ± 0 °C (25C), and 25 ± 4 °C (25S).

Effect	Coefficient	Robust SE	Z	P
Females from FL at 18S	—	—	—	—
Population NJ	0.372	0.297	1.251	0.211
Treatment 25C	−0.555	0.442	−1.257	0.209
Treatment 25S	−0.856	0.270	−3.170	0.002
Sex male	0.179	0.270	0.664	0.507
Population NJ × treatment 25C	−0.340	0.618	−0.549	0.582
Population NJ × treatment 25S	0.003	0.381	0.008	0.994
Population NJ × sex male	−0.072	0.433	−0.168	0.867
Treatment 25C × sex male	−0.399	0.368	−1.084	0.278
Treatment 25S × sex male	−0.137	0.308	−0.446	0.656
Population NJ × Treatment 25C × sex male	0.420	0.491	0.855	0.392
Population NJ × treatment 25S × sex male	−0.134	0.511	−0.261	0.794

Table 1
Parameters of the Cox proportional hazards model of the effects of population, developmental environment, and sex on knockdown time. The model compares the rate of knockdown for flies with a particular combination of characteristics to the rate for a baseline combination (e.g., females from FL acclimated to a stochastic environment with a mean of 18 °C). Thermal treatments are 18 ± 4 °C (18S), 25 ± 0 °C (25C), and 25 ± 4 °C (25S).

Effect	Coefficient	Robust SE	Z	P
Females from FL at 18S	–	–	–	–
Population NJ	0.093	0.286	0.327	0.744
Treatment 25C	−1.070	0.254	−4.211	< 0.001
Treatment 25S	−1.477	0.519	−2.847	0.004
Sex male	0.200	0.098	2.030	0.042
Population NJ × treatment 25C	−0.663	0.329	−2.013	0.044
Population NJ × treatment 25S	−0.412	0.598	−0.689	0.491
Population NJ × sex male	0.250	0.158	1.584	0.113
Treatment 25C × sex male	−0.694	0.426	−1.629	0.103
Treatment 25S × sex male	0.545	0.204	2.677	0.007
Population NJ × treatment 25C × sex male	−0.113	0.538	−0.209	0.834
Population NJ × treatment 25S × sex male	−0.800	0.306	−2.613	0.009

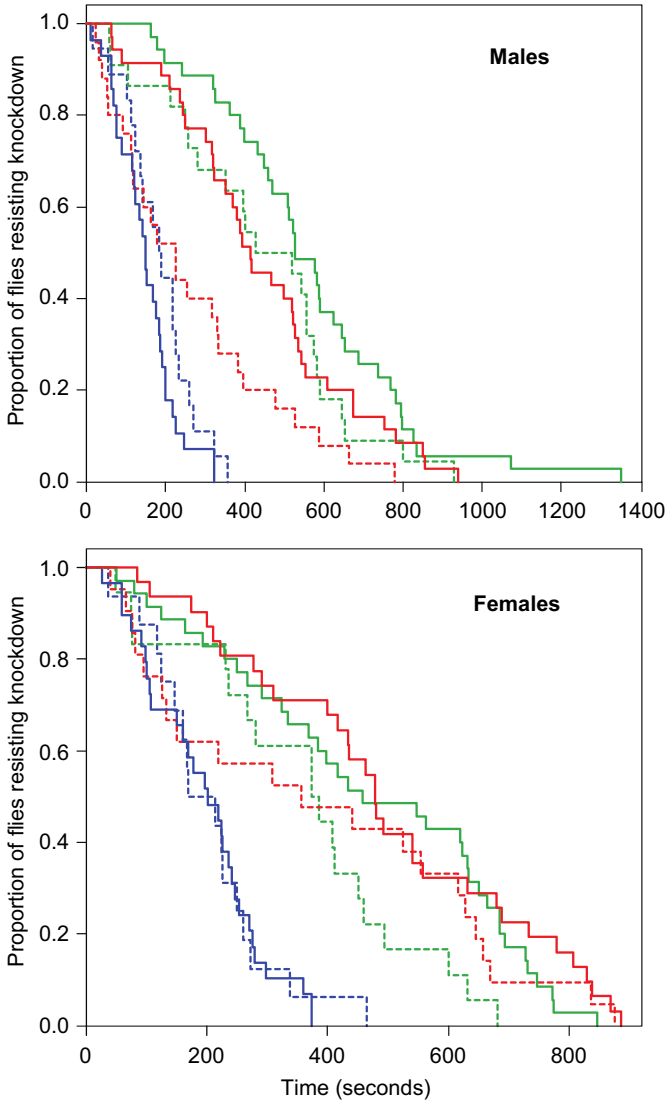

Fig. 2. Flies acclimated to high temperatures resisted knockdown longer than did flies acclimated to low temperatures. Knockdown times for flies acclimated to 18 ± 4 °C, 25 ± 0 °C, and 25 ± 4 °C are depicted by blue, green and red lines, respectively. Knockdown times for flies from NJ and FL flies are depicted by solid and dashed lines, respectively (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

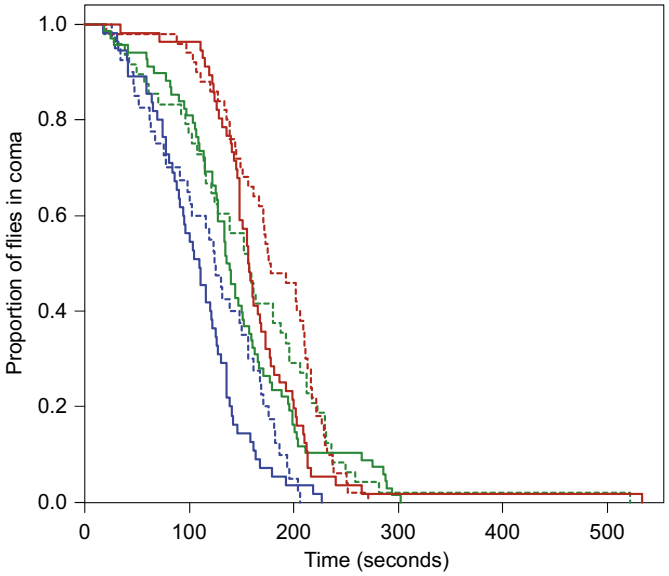

Fig. 3. Flies acclimated to low temperatures recovered from chill coma faster than did flies acclimated to high temperatures. Chill-coma times for flies acclimated to 18 ± 4 °C, 25 ± 0 °C, and 25 ± 4 °C are depicted by blue, green and red lines, respectively. Chill-coma times for flies from NJ and FL flies are depicted by solid and dashed lines, respectively (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 3
Median durations required for flies (*Drosophila melanogaster*) to resist knockdown after prolonged exposure to different thermal treatments. Thermal treatments were 18 ± 4 °C (18S), 25 ± 0 °C (25C), and 25 ± 4 °C (25S).

	Thermal treatments		
	18S	25C	25S
Florida			
Males	186.5 (16–356)	473 (58–929)	224 (22–780)
Females	191.5 (36–464)	379.5 (48–681)	356 (40–877)
New Jersey			
Males	148.5 (11–323)	528 (161–1350)	413 (63–938)
Females	201 (26–374)	457 (50–847)	479 (84–886)

Table 4
Median durations required for flies (*Drosophila melanogaster*) to recover from chill-coma after prolonged exposure to different thermal treatments. Thermal treatments were 18 ± 4 °C (18S), 25 ± 0 °C (25C), and 25 ± 4 °C (25S).

	Thermal treatments		
	18S	25C	25S
Florida			
Males	116 (41–204)	180 (42–282)	176 (88–251)
Females	139 (26–206)	139 (18–522)	202 (21–271)
New Jersey			
Males	104 (31–218)	140 (18–294)	156.5 (71–533)
Females	113.5 (17–227)	133 (25–302)	160.5 (34–265)

In contrast to our prediction, genotypes from both populations displayed similar responses to development in warm and cool environments. This result accords with those of other studies that compared the acclimation capacities of populations from different environments, including studies of *D. melanogaster*. For instance, genotypes of *D. melanogaster* from either northern or southern Australia were able to adjust their thermal tolerances according to simulated conditions of summer and winter (Hoffmann et al., 2005). Studies of other species of animals and plants have also failed to establish a relationship between latitude and the degree of plasticity (reviewed by Angilletta, 2009). Still, comparative studies suggest that selection favors some baseline level of plasticity in most populations.

The plasticity of knockdown time in *D. melanogaster* could reflect starvation and desiccation tolerances more than heat tolerance, Rezende et al. (2011) estimated that as much as 10% of stored energy and 30% of body water would be lost during a prolonged assay of knockdown temperature (>2 h); an even greater loss would be expected during an assay of knockdown time, since flies are exposed to a high temperature for the duration of the assay. If development in a warmer environment leads to a greater store of energy or water (or a lower rate of metabolism or dehydration), one should expect that flies raised at a high temperature would resist knockdown longer than would flies raised at a low temperature. This artifact might even obscure variation in heat tolerance among populations. Our assays of knockdown time were completed in very short durations (10–20 min), making this potential artifact an unlikely explanation

for the patterns that we observed. The short duration of exposure also made rapid acclimation, or heat hardening (Lee et al., 1987) an unlikely problem. Thus, we interpret our findings in light of evolutionary mechanisms that could maintain similar capacities for the acclimation of thermal tolerances in flies from high and low latitudes.

Extensive gene flow among populations could explain the maintenance of plasticity throughout the range of *D. melanogaster*. Dispersal among environments that differ in thermal characteristics would generate selection pressure on alleles that confer plasticity because offspring would likely experience different thermal conditions than their parents did (Gabriel and Lynch, 1992). Extensive gene flow likely occurs between populations of *D. melanogaster* on the same continent (Kennington et al., 2003) and even among continents (Singh and Rhomberg, 1987). Given the occurrence of gene flow, our intuition could fail us if we rely solely on temporal variation in environmental temperature to assess the selective advantage of plasticity (Cooper et al., 2010). Future efforts to quantify amounts of gene flow among thermal environments will increase our ability to predict divergence in degrees of plasticity.

Genetic correlations between developmental and reversible plasticity could also maintain developmental plasticity in populations that experience little variation among generations. *Drosophila melanogaster* experiences significant diel variation in temperature during larval development (Feder et al., 1997). Importantly, this diel variation in temperature is associated with a diel cycle of thermal tolerance in *D. buzzatti* (Sorenson and Loeschcke, 2002). Such variation likely involves expression of heat-shock proteins (Feder and Hofmann, 1999) and remodeling of cellular membranes (Hazel, 1995; Montooth et al., 2006; Overgaard et al., 2008; Cooper et al., in press). If the same mechanisms underlie acclimation to diel and seasonal variations in temperature, selection pressure on pleiotropic alleles involved in the diel response could also maintain the degree of a developmental response. Studies of the genetic correlation between reversible and irreversible plasticity would help to evaluate this hypothesis.

Only a few other researchers have examined developmental responses to fluctuating temperatures. In each case, related individuals were raised at either constant temperatures or fluctuating temperatures with the same or a similar mean. In *D. melanogaster*, thermal fluctuations during development enhanced the recovery of flies from heat coma but did not affect recovery from chill coma (Bozinovic et al., 2011). In another study, thermal fluctuations did not significantly affect the heat resistance of flies; however, chill-coma recovery time was slightly longer for flies that had developed under thermal fluctuations than for flies that had developed at a constant temperature (Hoffmann et al., 2005). Similarly, fluctuating temperatures caused zebrafish to develop with a greater heat tolerance than did constant temperatures (Schaefer and Ryan, 2006). Other studies have compared thermal tolerances of animals that had developed at either stochastic (fluctuating) or predictable (linear) declines in temperature. In crickets, a stochastic decline in temperature led to better recovery from chill coma than did a predictable decline (Niehaus et al., 2011). In isopods, no effect of thermal fluctuations was observed during cooling (Schuler et al., 2011). This mixture of patterns underscores the need to gain a better understanding of the physiological mechanisms underlying plastic responses to temperature.

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