Thermal Tolerance in Widespread and Tropical *Drosophila* Species: Does Phenotypic Plasticity Increase with Latitude?

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ABSTRACT: The distribution of insects can often be related to variation in their response to thermal extremes, which in turn may reflect differences in plastic responses or innate variation in resistance. Species with widespread distributions are expected to have evolved higher levels of plasticity than those from restricted tropical areas. This study compares adult thermal limits across five widespread species and five restricted tropical species of *Drosophila* from eastern Australia and investigates how these limits are affected by developmental acclimation and hardening after controlling for environmental variation and phylogeny. Irrespective of acclimation, cold resistance was higher in the widespread species. Developmental cold acclimation simulating temperate conditions extended cold limits by 2°–4°C, whereas developmental heat acclimation under simulated tropical conditions increased upper thermal limits by <1°C. The response to adult heat-hardening was weak, whereas widespread species tended to have a larger cold-hardening response that increased cold tolerance by 2°–5°C. These patterns persisted after phylogenetic correction and when flies were reared under high and low constant temperatures. The results do not support the hypothesis that widely distributed species have larger phenotypic plasticity for thermal tolerance limits, and *Drosophila* species distributions are therefore more closely linked to differences in innate thermal tolerance limits.

Introduction

Temperature affects virtually all biochemical and physiological processes, and consequently environmental temperature is one of the most important environmental factors dictating survival and dispersal of living organisms (Stevens 1989; Chown and Terblanche 2007; Angilletta 2009). This property applies particularly to small ectothermic animals with low thermal inertia (Stevenson 1985) that limits their ability to maintain operative temperature by means of physiological and behavioral thermoregulation when faced with stressful temperature. However, nu-

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merous examples have shown how animals display physiological adaptations that allow them to exploit specific thermal niches with high, low, or variable temperatures (Hazel and Prosser 1974; Cossins and Bowler 1987; Hochachka and Somero 2002; Angilletta 2009). These adaptations include both high inherent thermal tolerances and the capacity for changing the phenotype through adaptive phenotypic plasticity, which can aid in overcoming seasonal or daily exposure to thermal stress.

Ectotherms that occupy heterogeneous thermal environments are hypothesized to have evolved the physiological or behavioral capacity to optimize performance in variable thermal environments (Janzen 1967; Levins 1968; Cossins and Bowler 1987; Ghalambor et al. 2006; Chown and Terblanche 2007; Angilletta 2009). In arthropods, the scope between upper and lower thermal tolerance limits is larger for species that live at high latitudes than for low-latitude tropical species (Goto and Kimura 1998; Addo-Bediako et al. 2000; Calosi et al. 2010). This difference in scope is the result of a relatively conserved heat tolerance across latitude, whereas temperate and polar arthropods generally have much higher cold tolerance than do low-latitude species (Addo-Bediako et al. 2000). The broader fundamental niche of animals living in variable thermal environments is proposed to be at least partly attributable to greater thermal plasticity (Janzen 1967; Levins 1968; Hoffmann and Watson 1993; Ghalambor et al. 2006; Chown and Terblanche 2007).

A number of models exist to describe the evolution of the optimal thermal performance curve linking environmental temperature with fitness (Angilletta 2009; Kingsolver 2009). Although different models highlight either within- or among-generation variation as driving forces for broad tolerance, it is predicted that seasonal and diurnal temperature variation in temperate environments promotes wider tolerance breadths for widespread species than for restricted specialist species (Levins 1968; Lynch and Gabriel 1987; Gilchrist 1995; Bennett and Lenski

1999). Furthermore, animals from variable environments are expected to display a higher degree of phenotypic plasticity because the environmental cues are reliable, whereas plasticity in stable environments is lost or limited because it may incur costs (Levins 1968; Ghalambor et al. 2006). This strategy has also been characterized as "use it or loose it," whereas the generalist strategy has been described as "jack of all trades, but master of none" (Huey and Hetz 1984).

As pointed out by Ghalambor et al. (2006) and Angilletta (2009), few studies have directly investigated the relationship between thermal environmental variability, distribution range, and phenotypic plasticity of thermal tolerance limits using experimental designs in which acclimation and phylogenetic effects are controlled (but see Calosi et al. 2010). In this study, we address this question in a common garden experiment that compares the thermal tolerance of five widespread and five tropical species of eastern Australian *Drosophila* while controlling for the effects of phylogeny. These 10 species are all acclimated to temperatures reflecting temperate and tropical thermal conditions in the laboratory. The tropical species studied here inhabit humid rain forests with a relatively constant climate, limited seasonal and daily variation in temperature, and regular access to shade. In contrast, the widespread species are distributed across temperate as well as tropical environments and experience seasonal changes as well as considerably higher levels of diurnal fluctuations, particularly in exposed areas.

Thermal tolerance limits can be assessed in many ways, ranging from estimates of survival to more functional and physiological characteristics, such as limits for stupor or coma (Klok and Chown 2001, 2003), sterility (Chakir et al. 2002), or optimal aerobic scope (Pörtner 2002). For insects exposed to seasonally extreme temperatures, survival may be the key criterion for assessing the fundamental niche (Zachariassen 1985; Lee 1991; Addo-Bediako et al. 2000; Chown and Terblanche 2007). However, insects such as *Drosophila* do only rarely encounter long-term exposure to extreme temperatures, and they may be more limited by their ability to maintain performance under fluctuating thermal conditions. *Drosophila* species, for example, lose their ability to move at low temperature well before their lethal limit is reached, because even moderate cooling can impair their ability to maintain muscular and nervous function (Goller and Erch 1990; Hosler et al. 2000; Kostal 2004). In this study, we assessed thermal tolerance limits using a method modified from Huey et al. (1992) in which we measured the temperature at which individual flies became comatose as a consequence of cold or heat exposure. Specifically, we assessed thermal limits following long-term developmental acclimation as well as the effect of gradual hardening on upper and lower thermal limits

of widespread and tropical species. This provides estimates of both innate thermal resistance and the ability to modify thermal tolerance during chronic and gradual exposure to temperature extremes.

The data are used to empirically test several hypotheses proposed in the literature. (i) Widespread species are endowed with a wider thermal scope than are tropical species (Stevens 1989; Addo-Bediako et al. 2000). (ii) This wider temperature scope of widespread species is attributable in part to a larger acclimation response (Levins 1968; Hoffmann and Parsons 1991; Ghalambor et al. 2006; Angilletta 2009). (iii) Plastic responses are greater for lower thermal limits than for upper thermal limits (Addo-Bediako et al. 2000; Klok and Chown 2003; Chown and Terblanche 2007). (iv) The effects of gradually changing temperatures are more beneficial and less deleterious for widespread species than for tropical species (Terblanche et al. 2007; Chown et al. 2009).

Material and Methods

Maintenance and Collection of Flies

Ten *Drosophila* species were collected from December 2007 through April 2008 at different locations along the eastern coast of Australia. Five species are tropical specialists mostly restricted to the Australasian region and rain forests in northern Queensland, Australia, and Papua New Guinea. The other five are widespread species whose range incorporates temperate and tropical regions of Australia and elsewhere. A list of species and collection sites, general descriptions of the preferred habitat type, and latitudinal distribution are found in table 1. Because only one population of each species was assessed, we are not able to investigate intraspecific variation in thermal responses (e.g., Hoffmann et al. 2002). However, we assume that response variation among species is larger than variation within species (cf. Gibert and Huey 2001; Klok and Chown 2003; Hoffmann et al. 2003*a*). Information on the most southern latitudinal range of the Australian populations was based on personal observation from several years of collection along the Australian east coast and the TaxoDros Web site (http://www.taxodros.uzh.ch), with the latitudinal range of species assumed to cover the range between the southernmost and northernmost latitudes where they have been recorded. The average latitude of a species was taken as the average latitudinal distance from 0° where the species have been observed globally. For each species, inseminated field females were placed singly into vials for one generation at 25° ± 1°C with constant light to generate isofemale lines. From 18–22 of these isofemale lines, mass bred populations were established and maintained for at least 10 generations before experiments started. Mainte-

Table 1: Species list, collection site, and latitudinal distribution of the 10 *Drosophila* species used for comparative estimates of plasticity of thermal tolerance

Species	Location (date) of collection	Habitat type	Maximal southern latitude in Australia (°)	Latitudinal range (°)	Average latitude (° ± SD)
D. bipectinata	Gordonvale, Qld (February 2008)	Forest	−19.1	65.62	17.79 ± 8.28
D. birchii	Lake Eacham, Qld (April 2008)	Forest	−22.8	33.37	18.15 ± 5.94
D. bunnanda	Kirrama, Qld (April 2008)	Forest	−19.4	7.68	16.02 ± 2.06
D. pseudoananassae	Gordonvale, Qld (February 2008)	Forest	−19.1	67.4	13.04 ± 6.93
D. sulfurigaster	Gordonvale, Qld (February 2008)	Forest	−27.3	67.8	16.44 ± 9.91
D. busckii	Townsville, Qld (April 2008)	Open land	−38	119.31	38.65 ± 10.58
D. hydei	Melbourne, Vic (December 2007)	Open land	−38	112.32	37.25 ± 12.19
D. melanogaster	Melbourne, Vic (December 2007)	Open land	−43.2	129.4	35.25 ± 12.47
D. repleta	Townsville, Qld (April 2008)	Open land	−38	101.75	32.44 ± 13.09
D. simulans	Melbourne, Vic (December 2007)	Forest	−41.5	118.51	29.98 ± 12.89

Note: Habitat type and maximum southern limit are based on personal observations from several years of collection along the eastern coast of Australia. Average latitude and latitudinal range (maximal and minimal latitude) are based on worldwide registrations entered in the taxodros database available at http://www.taxodros.uzh.ch. Qld = Queensland; Vic = Victoria.

nance for several generations under controlled laboratory conditions is a necessity when using a common garden approach, but this obviously entails a risk of acclimation and adaptation to laboratory conditions. Populations were maintained at a minimum size of 1,000 adults in three 500-mL bottles with 40 mL of cornmeal-yeast-dextrose medium (300–500 flies per bottle).

Acclimation Treatments and Rearing of Experimental Animals

To examine the influence of developmental acclimation on resistance to thermal extremes, all 10 species were reared at four distinct acclimation regimes in the laboratory. The regimes (fig. 1) were designed to reflect temperate and tropical thermal conditions with and without thermal fluctuations. The thermal fluctuation regimes consisted of a daily cycle with 6 h spent at the minimum and maximum temperatures separated by a gradual increase or decrease in temperature over the intervening 6-h period. The acclimation regimes were as follows: (i) constant temperate (19°C); (ii) constant tropical (27°C); (iii) fluctuating temperate (13°–25°C); and (iv) fluctuating tropical (25°–29°C). All acclimation treatments involved constant light to limit any confounding factors of light cycle in the experiments.

Eggs from each species were collected into vials with 10 mL of food (50 eggs per vial) and placed in cabinets under the temperature regimes described above. Eggs from the species were set up at different times to synchronize adult emergence within each acclimation treatment so that adults eclosed at the same time. After eclosion, flies were aged at their respective acclimation regimes (∼50 adults of mixed sex per vial with 10 mL of food). Two days before

the experiments, the flies were briefly (<3 min) sexed under CO_2 anesthesia so that thermal responses of 3–8-day-old mated females could be tested. These short exposures to CO_2 are unlikely to affect performance (Nilson et al. 2006).

Knockdown Temperature under Acute Heat and Cold Thermal Stress Exposure

Acute thermal tolerance limits were assessed by placing individual flies into 5-mL glass vials and stressing five individuals in a water bath preset to temperatures aimed at giving approximately 50% knockdown after 5 min of exposure (fig. 2A). This approach was repeated for 4–8 exposure temperatures (i.e., a total of 20–40 flies per species per acclimation treatment were tested at high and low temperature) to produce a relationship between the proportion of flies standing and exposure temperature. Linear regression provided a reasonable fit to the data (average R value = 0.87; see fig. A1), and linear regression was then used to estimate the temperature at which 50% of the flies were knocked down, including 95% confidence intervals of this estimate. This measure of acute thermal tolerance was obtained for both the upper (CT_{max_acute}) and lower (CT_{min_acute}) limits for all species from the four acclimation regimes.

Ramping Knockdown Temperature Assays

Temperature in nature tends to change gradually, which results in the potential accumulation of thermal stress. However, this gradual change may also allow animals time to respond by means of physiological acclimation. The outcome of these opposing effects will ultimately be de-
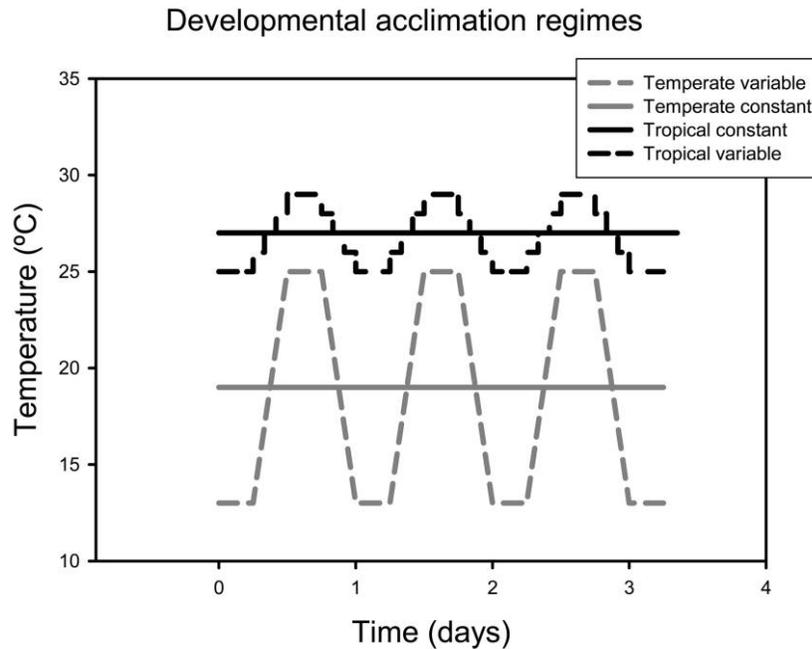
Developmental acclimation regimes

Figure 1: Acclimation regimes. All species were acclimated under four separate acclimation regimes representing (i) constant temperate conditions (19°C); (ii) constant tropical conditions (27°C); (iii) fluctuating temperate conditions with daily cycles of 6 h at 13°C, 6 h of gradual heating to 25°C, 6 h at 25°C, and 6 h of gradual cooling to 13°C; and (iv) fluctuating tropical conditions with daily cycles of 6 h at 25°C, 6 h of gradual heating to 29°C, 6 h at 29°C, and 6 h of cooling to 25°C.

termined by the rate of temperature change and the species in question (Kelty and Lee 2001; Overgaard et al. 2006; Terblanche et al. 2007; Chown et al. 2009; Mitchell and Hoffmann 2010). Here, we investigated the critical thermal maxima (CT_{max}) and minima (CT_{min}) under gradual heating and cooling using an ecologically relevant rate of temperature change of 0.1°C/min. The CT_{max} was scored by placing individual flies in empty 5-mL glass vials that were subsequently submerged in a water bath preset at 23°C. The temperature of the water bath was then gradually increased from 23° to 43°C at a rate of 0.1°C/min, and the critical thermal maximum (CT_{max}) was recorded as the temperature at which the flies were knocked down by heat and became unable to move any body part (fig. 2*B*). A similar approach was used to assess CT_{min} (fig. 2*C*). Here, we recorded CT_{min} while gradually cooling the flies from 23° to 0°C at a rate of 0.1°C/min. Subsequently, the flies were maintained at 0°C for 1 h before temperatures were gradually raised again to 43°C at a rate of 0.1°C/min. During the heating period, we scored the temperature at which the flies regained the ability to stand after being knocked down by the low temperatures ($T_{recovery}$), and as the heating progressed, we also measured the critical thermal maximum ($CT_{max\_after\_cold}$) as the temperature at which the flies were knocked down (and unable to move any body part)

by heat. An estimate of the thermal scope was obtained by subtracting the CT_{min} from the $CT_{max\_after\_cold}$, because this measure quantifies the ability to deal with heat following cold exposure and therefore estimates an animal's response to fluctuating conditions. For all ramping knockdown tests, we aimed at testing 20 flies from each species and each acclimation group (see table A1 for specific numbers for replicates).

Assessment of Adult Plasticity

The role of both developmental acclimation and short-term hardening on thermal tolerance limits in the 10 species was examined. The effect of developmental acclimation was assessed by comparing the thermal limits of flies that developed under different conditions, whereas the effect of hardening and cumulative damage was assessed by comparing the acute measure of thermal tolerance with that measured under gradually changing temperatures (i.e., $CT_{max} - CT_{max\_acute}$). We observed that damage accumulation during heating was typically larger than the putative benefits of heat hardening during this period, so that $CT_{max\_acute} > CT_{max}$. In contrast, for cold exposures we generally saw benefits of gradual cooling, so that $CT_{min\_acute} > CT_{min}$. Consequently, a small negative value
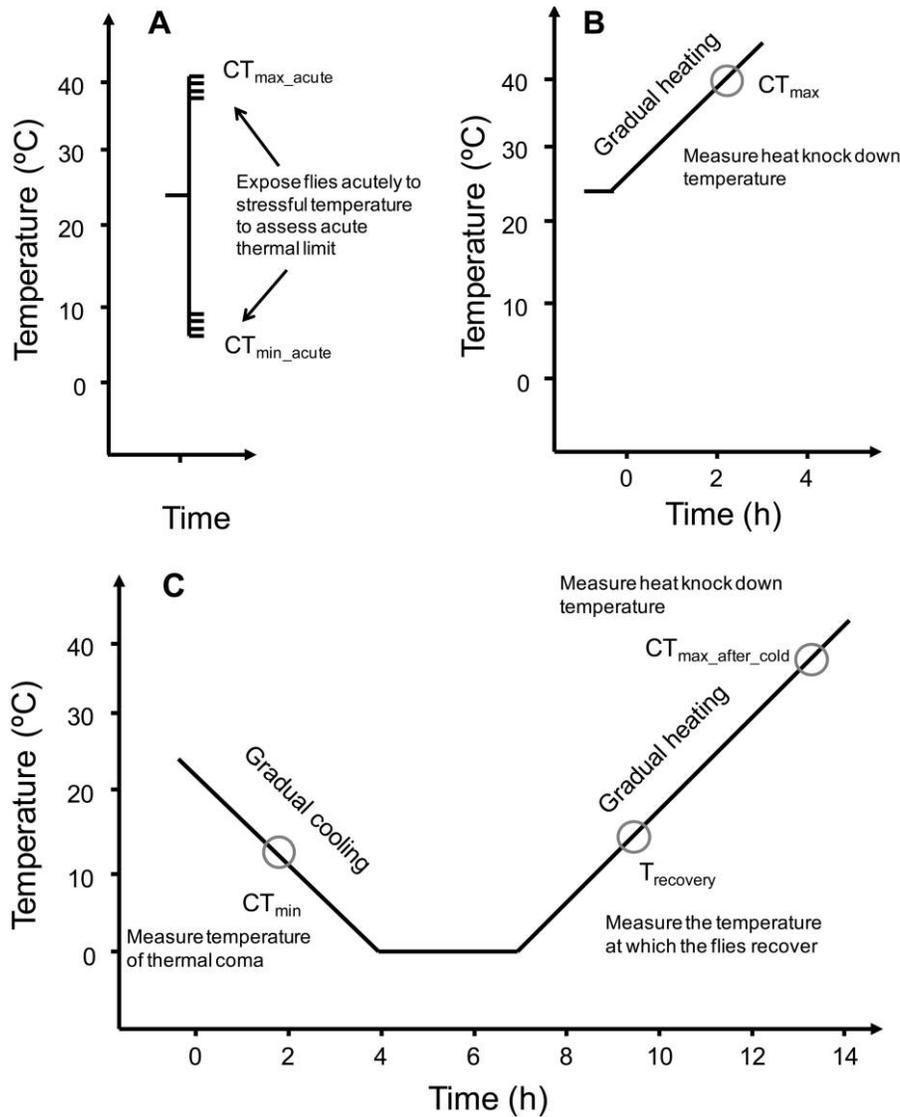
Figure 2: Graphical depiction of experimental protocol to test thermal tolerance traits of five restricted tropical and five widespread species of *Drosophila*. Acute tolerance was tested by estimating the proportion of flies standing after acute exposure to stressful temperatures for 5 min (*A*) or by using ramping assays that allow for physiological acclimation during the exposure to stressful temperatures (*B* and *C*). Gray circles indicate points assessed in the different assays. CT_{max} = critical thermal maximum; CT_{max_acute} = critical thermal maximum with acute exposure; $CT_{max\_after\_cold}$ = critical thermal maximum after cold exposure; CT_{min} = critical thermal minimum; CT_{min_acute} = critical thermal minimum with acute exposure; $T_{recovery}$ = temperature at which flies recover from thermal coma.

of $CT_{max} - CT_{max\_acute}$ and a large positive value of $CT_{min\_acute} - CT_{min}$ is interpreted as an indication of beneficial hardening or low damage.

Statistical Analysis

The tropically restricted species group and widespread species group differed considerably in their distribution, with widespread species ranging as far south as 38°–43.2°S,

whereas the southern limit of tropical species was between 19.1° and 27.3°S. The associations between the southern species distribution and thermal tolerance or hardening capacity were analyzed using linear regression, with the southernmost distribution latitude as the independent variable. Acclimation responses were assessed as the difference between either the tolerance levels of constant 27°C versus constant 19°C regimes or the difference between flies acclimated to variable "tropical" versus variable "temperate"

regimes. Hardening or damage effects were assessed from the average difference between the critical limits of flies exposed to acute or ramped exposure. Significant correlations between the southern latitude and thermal tolerance or plasticity of thermal tolerance are interpreted as indicative of differences between species or species groups relating to their distributions. All values are presented as mean ± SEM unless otherwise stated. Phylogenetically independent contrasts (PICs) were used to examine the influence of phylogenetic relatedness on these correlations. Following Kellermann et al. (2009) and Mitchell and Hoffmann (2010), a phylogenetic tree was constructed using nucleotide sequences for two mitochondrial (COII and ND5) and two nuclear genes (ADH and HB9) for all 10 species. The branch lengths from this tree were then used to conduct phylogenetically independent contrasts to assess the influence of phylogenetic relatedness on all correlations performed. This was done with the PDAP: PDTree software (ver. 1.14; Midford et al. 2005) for Mesquite (ver. 2.6; Maddison and Maddison 2009) following Garland et al. (1992).

Results

Thermal Tolerance of Adult Drosophilids

The aim of this study is to characterize putative differences in thermal tolerance between widespread and restricted tropical species of *Drosophila* with respect to absolute limits and particularly in terms of differences in acclimatory (plastic) induced changes in thermal limits. Our assessments of thermal scope and plasticity in these groups are outlined below, and species means and variances are presented in table A1. Because our five widespread species are distributed at lower southern latitudes than our five tropical species, we interpreted significant associations between maximal southern latitude and thermal tolerance as an indication of a difference in thermal resistance between species groups. Using maximal southern latitude as independent values, we performed regressions of thermal parameters with and without PICs to examine the influence of phylogenetic relatedness on these correlations and generally found weak phylogenetic signals influencing overall patterns. One trait and acclimation treatment (CT_{min}, tropical acclimation) exhibited significant lack of fit to branch lengths following examination of the absolute values of the PICs against their standard deviation (Garland et al. 1992). This was corrected by an exponential transformation of the branch lengths.

Knockdown Temperature under Acute Heat and Cold Temperature Stress Exposure

The maximum temperature at which flies could maintain function during acute exposure was significantly affected by both species distributions and acclimation regime. Widespread species had significantly higher levels of acute heat tolerance, as reflected by a significant negative correlation between maximal southern latitude and acute heat tolerance (fig. 3A), and this effect was also highly significant after phylogenetic correction. Acclimation at high temperature regimes involving either constant 27°C or variable tropical conditions improved acute heat tolerance by 0.7°–0.8°C, but this acclimatory response was similar between tropical and widespread species, with no significant relationship between latitudinal distribution and acclimation response (fig. 3B).

The minimum temperature at which flies can maintain function during acute exposure was significantly affected by both latitudinal distribution and acclimation regime (fig. 4A), and this was also highly significant when phylogeny was included in the analysis. Here, the difference between species groups was larger than in the case of heat, because widespread species, on average, had a CT_{min_acute} that was 2.9°C lower than that of tropical species. Acclimation to low temperature improved the tolerance to acute cold stress by 2°–2.5°C, with tropical species showing marginally larger acclimation responses. This was reflected in a positive relationship between latitudinal distribution and the effect of acclimation (fig. 4A). However, this positive relationship was only significant when flies from constant acclimation regimes were compared and was no longer significant when phylogenic signal was included in the analysis. In the case of both acute cold and heat knockdown, there was no difference between the observed effects of acclimation at variable versus constant temperatures.

Knockdown Temperatures Using Ramping Assays

Heat tolerance during gradual exposure was similar across species and developmental acclimation regimes (fig. 3C, 3D). Thus there was no significant correlation between CT_{max} and latitudinal distribution (fig. 3C), and widespread species were generally unaffected by developmental acclimation, whereas there was a trend towards a slight increase in CT_{max} for tropical species, with CT_{max} being 0.6°–0.7°C higher in warm-acclimated flies (fig. 3D). However, the relationship between distribution and acclimation response was not significant, which suggests that any difference in the plastic responses of the species groups was small. The hardening or damage response was similar irrespective of whether flies were exposed to constant or fluctuating regimes (fig. 3D).
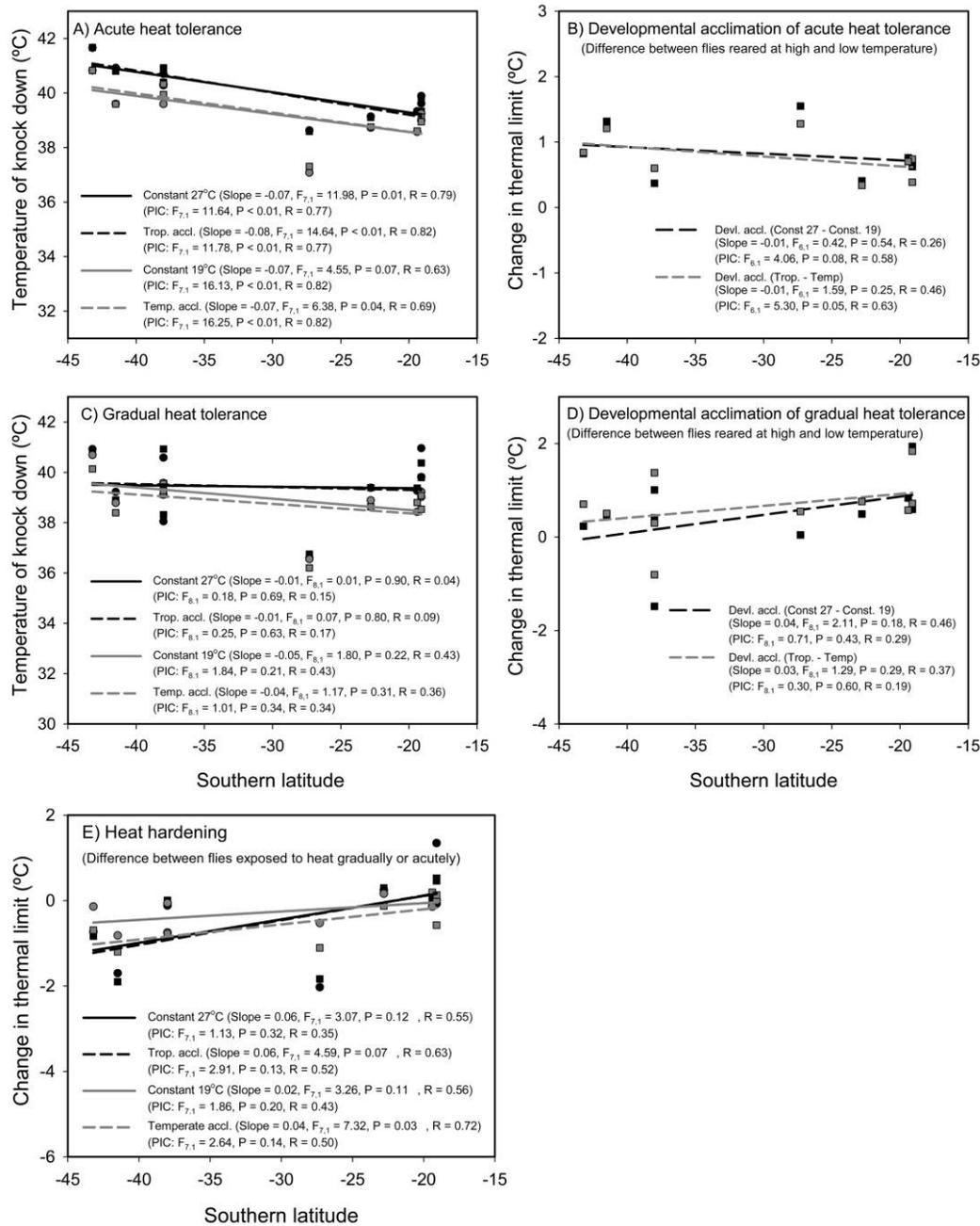
Figure 3: Measures of upper thermal limits for five widespread *Drosophila* species and five restricted tropical *Drosophila* species acclimated to 27°C (dark circles), variable tropical conditions (dark squares), 19°C (gray circles), or variable temperate conditions (gray squares). Data are plotted against the maximal southern latitudinal distribution in Australia. *A*, Thermal limits assessed using acute exposure to high temperature; *B*, effect of acclimation calculated from the difference in average estimates of gradual thermal tolerance between cold- and warm-acclimated flies. Here we specifically compare flies acclimated to 27°C versus 19°C and flies acclimated to fluctuating tropical versus fluctuating temperate conditions. *C*, Thermal limits assessed using gradual exposure to high temperature; *D*, effect of acclimation calculated from the difference in average estimates of gradual thermal tolerance between cold- and warm-acclimated flies; *E*, average heat-hardening ability assessed from the difference in acute thermal tolerance and tolerance during gradual exposure to high temperature.
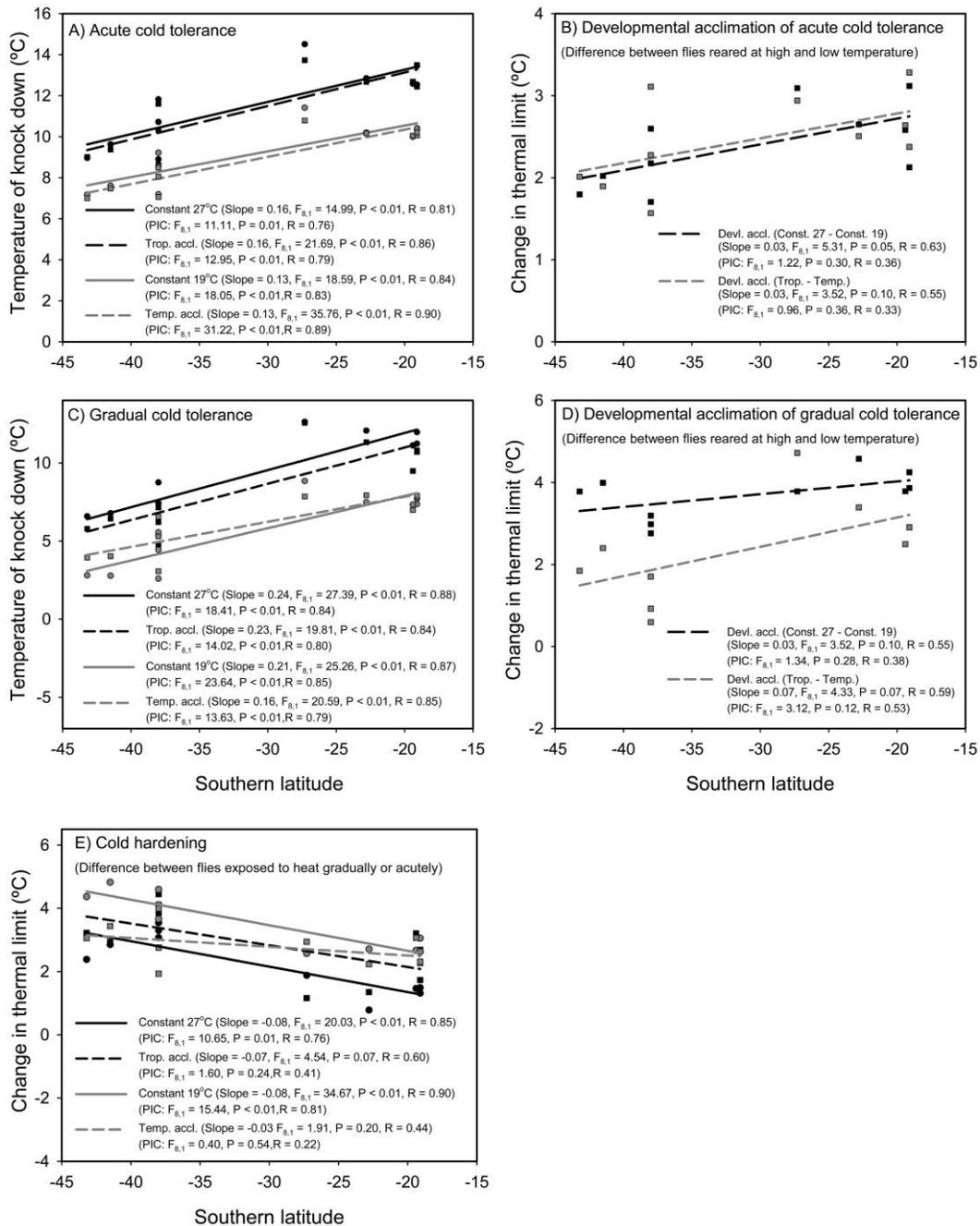
Figure 4: Measures of lower thermal limits for five widespread and five tropical-restricted *Drosophila* species acclimated to 27°C (dark circles), variable tropical conditions (dark squares), 19°C (gray circles), or variable temperate conditions (gray squares). Data are plotted against the maximal southern latitudinal distribution in Australia. *A*, Thermal limits assessed using acute exposure to low temperature; *B*, effect of acclimation calculated from the difference in average estimates of acute thermal tolerance between cold- and warm-acclimated flies. Here we specifically compare flies acclimated to 27°C versus 19°C and flies acclimated to fluctuating tropical versus fluctuating temperate conditions. *C*, Thermal limits assessed using gradual exposure to low temperature; *D*, effect of acclimation calculated from the difference in average estimates of gradual thermal tolerance between cold- and warm-acclimated flies; *E*, average cold-hardening ability assessed from the difference in acute thermal tolerance and tolerance during gradual exposure to low temperature.
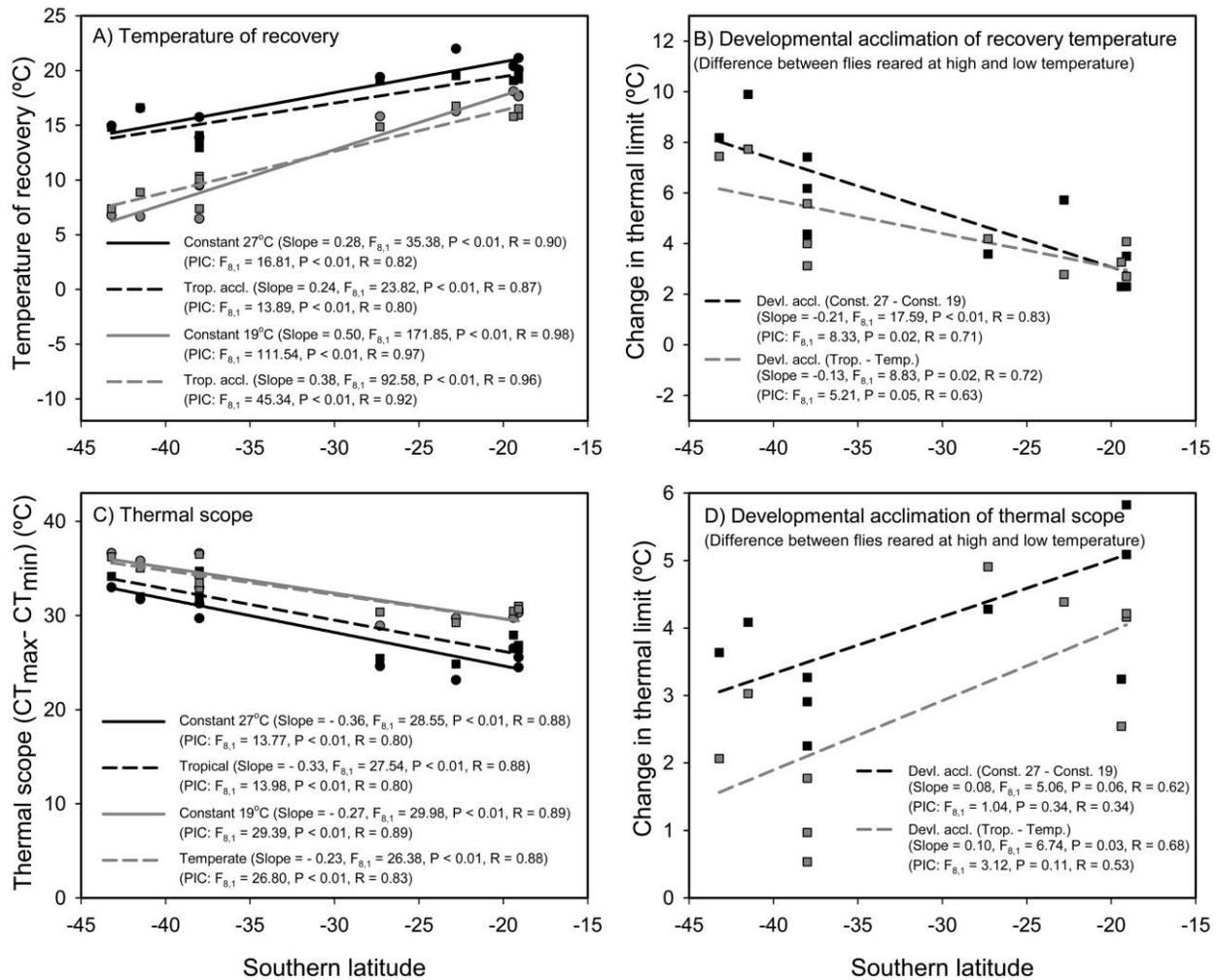
Figure 5: Measures of cold recovery and thermal scope for five widespread *Drosophila* species and five tropical-restricted *Drosophila* species acclimated to 27°C (dark circles), variable tropical conditions (dark squares), 19°C (gray circles), or variable temperate conditions (gray squares). Data are plotted against the maximal southern latitudinal distribution in Australia. *A*, Temperature of recovery after cold exposure; *B*, effect of acclimation on cold recovery calculated from the difference in average estimates of cold recovery between cold- and warm-acclimated flies. Here we specifically compare flies acclimated to 27°C versus 19°C and flies acclimated to fluctuating tropical versus fluctuating temperate conditions. *C*, Thermal scope assessed from the difference between cold knockdown temperature during gradual cooling and subsequent assessment of heat knockdown temperature during gradual heating following the cold exposure; *D*, effect of acclimation calculated from the difference in average estimates of thermal scope between cold- and warm-acclimated flies.

The CT_{min} recorded during gradual exposure was always much lower in widespread species (fig. 4*C*), and this result was not biased by phylogenetic signal. Cold acclimation always resulted in a considerable improvement of cold tolerance, and there was a tendency for acclimation at lower temperatures to affect tropical species more (3°–4°C improvement of tolerance) than widespread species (2°–3.5°C improvement) (fig. 4*D*). However, this was not significant after correction for phylogeny.

Widespread species recovered after cold treatment at temperatures 3°–7°C lower than temperatures at which tropical species recovered (fig. 5*A*), and this marked difference was not affected by phylogenetic signal. Cold acclimation markedly improved cold recovery of all species (fig. 5*B*), but the effect of acclimation for cold recovery was larger in widespread species where cold recovery was improved by 4°–10°C, whereas the improvement in tropical species was 2°–6°C (fig. 5*D*). The plastic response for recovery is, however, difficult to interpret, because chill coma recovery is markedly influenced by knockdown time

(Gibert et al. 2001), and in our test knockdown time decreased when cold resistance increased, which should by itself give faster recovery (fig. 2C).

Thermal scope was tested in the same individual flies by estimating maximum tolerance after cold exposure (fig. 2C) and using the difference between CT_{min} and $CT_{max\_after\_cold}$ as a measure of scope. Primarily as a result of the higher cold tolerance of widespread species, thermal scope was larger in widespread species (fig. 5C). Moreover, because cold acclimation depressed CT_{min}, whereas heat acclimation had little effect on CT_{max}, it follows that thermal scope was highest in cold-acclimated flies (fig. 5C). Tropical species tended to show a slightly larger acclimation response with regard to thermal scope (0.5°–6°C improvement with cold acclimation vs. 2°–4°C improvement in widespread species), but when plotted against latitudinal distribution, we found no significant relationship between southern latitude limits and thermal scope when phylogeny was included in the analysis (fig. 5D).

Assessment of Hardening Ability and Damage

In addition to the effects of long-term acclimation, thermal resistance is also influenced by hardening and damage during ramping. This was assessed by comparing thermal tolerance during acute exposure with thermal tolerance during gradual heating and cooling.

As seen in figure 3E, gradual exposure of adult flies to high temperature caused a small decrease in heat tolerance, because maximum tolerance was generally lower during ramping than during acute exposure. The ability to heat-harden and limit damage tended to be larger in tropical species, because these were generally less affected by gradual heating than were widespread species (fig. 3E). However, this effect was only significant for flies from the temperate acclimation group, and it was no longer significant when phylogeny was included in the analysis (fig. 3E).

Exposure to gradual cooling markedly improved cold tolerance relative to acute exposure (fig. 4E). This rapid cold-hardening response differed between acclimation groups, with a stronger response particularly in the flies acclimated to 19°C. Moreover, widespread species showed a greater ability to rapidly cold harden than did tropical species when the flies had developed in constant environments. The tendency for widespread species to have a larger cold-hardening response persisted in flies from the two fluctuating acclimation regimes, but here it was no longer significant (fig. 4E).

Discussion

Recent studies of ectothermic animals have highlighted how functional assessments of thermal performance in terms of performance curves (Deutsch et al. 2008), maintenance of cardiac function and aerobic scope (Pörtner 2002; Pörtner and Knust 2007; Somero 2010), permissive operational temperatures (Huey et al. 2010) or limits for neuromuscular performance (Huey et al. 1992; Klok and Chown 2001; Terblanche et al. 2007) can be used to describe species distribution in relation to climate and climate change scenarios. Thermal boundaries are, however, often flexible if animals are allowed to respond through acclimation, and this forms an important component when evaluating the thermal sensitivity of populations (Stillman 2003; Calosi et al. 2008; Somero 2010).

In our experimental protocol we exposed flies to both constant and variable acclimation regimes with similar mean temperatures. Exposure to developmental variability did not further increase the thermal breadth of the phenotypes under either warm (average of 27°C) or cool (average of 19°C) conditions (figs. 3–5). For the tropical group, there was even a slight decrease in cold tolerance in the variable regime, which resulted in a smaller thermal scope and smaller effect of developmental cold acclimation (figs. 4C, 5C). It is possible that the recurring exposure to high temperature (29°C) in this group resulted in a more sensitive phenotype, although most other indices suggested that this group had tolerance similar to that of the flies exposed to constant 27°C.

This study investigated functional thermal tolerance by assessing the thermal boundaries between which animals were able to maintain some form of coordinated neuromuscular function rather than focusing on absolute lethal limits. It is likely that our estimates of heat tolerance are close to lethal limits (Hoffmann et al. 2003a; Cooper et al. 2008), whereas our estimates of cold tolerance may be far from lethal limits, because drosophilids can survive and recover from extended periods of cold coma (Sinclair and Roberts 2005 and references therein). The thermal limits reported here are therefore beyond those where normal activity is maintained, but for cold in particular the limits are much narrower than the limits estimated for cold survival (cf. Goto and Kimura 1998; Gibert and Huey 2001; Kristensen et al. 2008). As a consequence, our assessments of functional thermal limits focus more on capacity adaptation for cold and resistance adaptation for heat as defined by Precht et al. (1973). Nonetheless, preliminary analyses (data not shown) indicate that our measures of thermal tolerance are predictive for individual species distributions. For drosophilids, it is often the adult stage that overwinters (Gibert et al. 2001; Kimura 2004 and references therein), and along the east Australian coast, air temperatures below 7°C are rare at latitudes less than 27°S, whereas minimal temperatures below 2°C are rare at latitudes around 40°S (Australian Bureau of Meteorology: http://www.bom.gov.au). This suggests that the

lower thermal limits of the cold-acclimated phenotypes reported in this study are rarely surpassed by environmental temperatures (cf. fig. 4C). Similarly, air temperatures above 40°C are rare along the entire east coast, which suggests that species in this study are restricted by thermal boundaries for sustained neuromuscular performance (cf. fig. 3C).

Thermal Tolerance and Thermal Scope

Maximum yearly temperature is fairly uniform across the entire eastern Australian coast, whereas minimum temperature decreases with increasing latitude (Hoffmann 2010). This implies that seasonal variation in temperature also increases with latitude and that the ectothermic animals with wide latitudinal distribution must be endowed with broad innate thermal tolerance and/or have the ability to alter their tolerance in the face of environmental temperature variation (Levins 1968; Stevens 1989; Hoffmann and Parsons 1991; Addo-Bediako et al. 2000; Ghalambor et al. 2006; Angilletta 2009). Using a comparative approach, Addo-Bediako et al. (2000) demonstrated a clear increase in the thermal scope of survivorship with increasing latitude. Similar trends are also often seen within arthropod families, such as porcelain crabs (Stillman 2003), water beetles (Calosi 2010), aphids (Hazell et al. 2010), and drosophilids (Goto and Kimura 1998; Kimura 1988, 2004) as well as in many other ectotherms (Janzen 1967; Brattstrom 1970; Ghalambor et al. 2006).

Our study confirms this pattern for functional limits with regard to innate thermal tolerance, because we found that cold tolerance was markedly higher (CT_{min} was lower) in widespread species regardless of acclimation treatment (fig. 4A and 4C). This resulted in larger thermal scope for the widespread species because there seems to be less interspecific variance in heat tolerance in *Drosophila* species (this study and Kimura 1988, 2004). The widespread species used in our study are not only found at high latitudes but also have much broader distributions, as attested to by the highly significant correlation between the most southern latitude and latitudinal range ($R = -0.89$; $P < .001$; table 1). Our results therefore also support Rapoport's rule (Stevens 1989), which states that high-latitude species have wider distributions linked to their broader tolerance to environmental variables. Nevertheless, range-restricted species do exist in temperate environments and could have been included to distinguish whether differences in thermal tolerances are related to range size or to adaptation to more temperate environments (see Brattstrom 1970 and Ghalambor et al. 2006 for discussion). However, given the relatively small differences in plasticity found between species groups in our study, we conclude that neither range size nor range position selects for increased phenotypic plasticity in thermotolerance in drosophilids (see below).

Acclimation Responses in Widespread and Tropical Species

Widespread *Drosophila* species are assumed to experience more thermal variation both within and among generations. This is predicted to result in larger acclimation responses during both long-term (developmental) and short-term (hardening) exposure. This was proposed by Levins (1968), but in his seminal work, it was only supported by circumstantial empirical evidence involving qualitative observations of widespread and tropical *Drosophila* species. Many studies have addressed putative interspecific or intraspecific estimates of plasticity of thermal tolerance with varying outcomes (Spicer and Gaston 1999; Chown and Terblanche 2007; Angilletta 2009). However, both Ghalambor et al. (2006) and Angilletta (2009) emphasized that targeted and phylogenetically corrected data sets testing this hypothesis are missing. To remedy this, Calosi et al. (2010) recently investigated the relationship between the plasticity of thermal survival limits and distribution range in water beetles, and our study is the first to use a common garden approach with phylogenetically corrected data to investigate levels of plasticity for functional performance traits in insects.

In a previous study of intraspecific variation in acclimation responses, Hoffmann and Watson (1993) found no evidence for differences in plastic responses with respect to heat and cold tolerance between tropical and temperate populations of *Drosophila simulans* and *Drosophila melanogaster*. They suggested that such differences might instead be found at the interspecific level, but in our study, we do not find conclusive support for this hypothesis. Thus, the tropical species showed similar or marginally larger responses to developmental cold and heat acclimation and heat hardening, compared with the widespread species. However, the response to cold hardening was larger in widespread species (below), and these species also showed a larger developmental acclimation response in the ability to recover after cold. Given these patterns, we conclude that, at least for drosophilids, developmental plasticity is fairly similar in widespread generalist and tropical specialist species, and differences between these groups mainly reflect innate thermal tolerance of functional performance. This supports similar findings of comprehensive studies of water beetles (Calosi et al. 2010) and amphibians (Brattstrom 1970) and contrasts with patterns proposed by Janzen (1967) and Levins (1968) that have lacked experimental verification (see Ghalambor et al. 2006 for discussion). Our study included both chronic and acute acclimation responses, but it is possible that our acclimation protocols did not exhaust or optimize the acclimation po-

tential of our animals. Thus, because acclimation responses are not linear (Terblanche et al. 2006), it is possible that widespread species could have developed at lower temperatures, which could further increase their tolerance. Moreover, it is also possible that larger effects of acclimation would have been observed with the use of other assays; for instance, Kristensen et al. (2008) demonstrated how field performance tests differentiated between acclimation groups that showed similar laboratory estimates of tolerance. Additional studies using a similar approach on other families and orders are needed to establish whether our findings constitute a general pattern for generalist and specialist species (but see Calosi et al. 2010).

Heat Hardening and Implications for Sensitivity to Climate Change

Widespread species had higher acute heat tolerance than did tropical species (fig. 3*A*), but this difference largely disappeared when we tested maximum heat tolerance using a ramping assay (fig. 3*C*). Thus, heat hardening during gradual heating may be more beneficial and/or less detrimental for tropical species than for widespread species. Interestingly, a similar pattern exists between southern (temperate) populations of *D. melanogaster* from the eastern coast of Australia that have a reduced hardening ability when compared with tropical populations (Sgrò et al. 2010). Together, these findings suggest that species or populations at high latitudes may be selected for irregular and extreme heat exposures, whereas low-latitude species are selected for countering regular exposures to high temperatures (Chown and Terblanche 2007; Sgrò et al. 2010). Nonetheless, the differences between species were small, and we found no significant correlation between latitudinal distribution and heat-hardening capacity, which is also consistent with relatively constant estimates of plasticity of heat knockdown time across eight *Drosophila* species (Kellett et al. 2005) and the limited interspecific variance in innate heat tolerance in *Drosophila* species (our study and Kimura 1988, 2004).

There is growing evidence that global warming is changing the species distribution of ectothermic animals (Parmesan and Yohe 2003; Perry et al. 2005; Deutsch et al. 2008), and it has been suggested that the ability to alter thermal resistance through adaptation or acclimation is a key factor for coping with changing thermal conditions (Stillman 2003; Wang and Overgaard 2007; Calosi et al. 2008, 2010; Franklin and Seebacher 2009; Somero 2010). Some restricted tropical species of *Drosophila* seem to have low evolutionary potential for adapting to changes in precipitation and temperature patterns (Hoffmann et al. 2003*b*; Kellermann et al. 2009). Furthermore, recent studies have suggested low levels of heritable genetic variation

for heat resistance traits in both tropical and temperate populations of *Drosophila* using ecologically relevant ramping assays (Mitchell and Hoffmann 2010). Thus, many species may rely on phenotypic plasticity rather than evolutionary shifts when countering stresses associated with climate change. On the basis of data from different species of porcelain crabs, Stillman (2003) found that a negative association existed between maximum thermal tolerance and acclimation potential, whereas Calosi et al. (2008) found the opposite relationship for water beetles. Nonetheless, both of these studies only showed small shifts in CT_{max} through acclimation (less than 2°C increase in tolerance), which is unlikely to be sufficient for long-term survival when dealing with future climate change scenarios (Solomon et al. 2007). In concordance with our study, developmental acclimation only increased acute heat tolerance by 1°C. Together, these findings suggest that neither adaptation nor acclimation will substantially alter heat tolerance and allow species to cope with heat stresses associated with global warming (Stillman 2003; Wang and Overgaard 2007; Calosi et al. 2008; Franklin and Seebacher 2009; Somero 2010; Mitchell and Hoffmann 2010).

Cold-Hardening Responses

Cold tolerance is often much more plastic with respect to both developmental acclimation and cold hardening than is heat tolerance. Our hypothesis that widespread species showed larger acclimation responses was not confirmed with respect to developmental acclimation (fig. 4*B* and 4*D*). However, we did find that the effects of rapid cold hardening through gradual cooling were more marked in the widespread species, probably enabling them to counter the rapid daily thermal fluctuations experienced at high latitudes (fig. 4*E*; Kelty and Lee 2001; Overgaard and Sørensen 2008). Gradual exposure to cold markedly decreases critical minimal temperatures in widespread drosophilids and temperate flesh flies, whereas tropical flesh flies showed a smaller response (Chen et al. 1990; Kelty and Lee 2001). Similarly, tropical tsetse flies succumbed earlier under gradual cooling through accumulated cold damage (Terblanche et al. 2007), and soil-dwelling springtail species that experience a low level of diurnal environmental variability have smaller rapid cold-hardening response than do surface-living species (Bahrndorff et al. 2009). These differences in hardening response are consistent with the model of Lynch and Gabriel (1987), which predicts that within-generation variability promotes broad thermal tolerance, although their model concerned niche breadth in general and not the evolution of plasticity of niche breadth.

Concluding Remarks

This study found clear differences between widespread species and tropically restricted species with respect to their thermal tolerance. The results support Rapoport's rule predicting that temperate species at high latitudes have a broad thermal niche, compared with more restricted thermal specialists in the tropics. The broader fundamental niche was mainly attributable to a higher innate cold tolerance in widespread species, because the overall levels of phenotypic plasticity were similar between species groups. Tropical species exhibited slightly larger developmental cold acclimation and heat-hardening responses, whereas widespread species exhibited a stronger cold-hardening response. These results do not support the hypothesis that widely distributed species have larger phenotypic plasticity for thermal tolerance levels. Together with the limited potential for heat acclimation, we propose that neither widespread nor restricted species are likely to benefit markedly from heat acclimation under the warmer conditions that are expected with continued climate change.

APPENDIX

Supporting Data
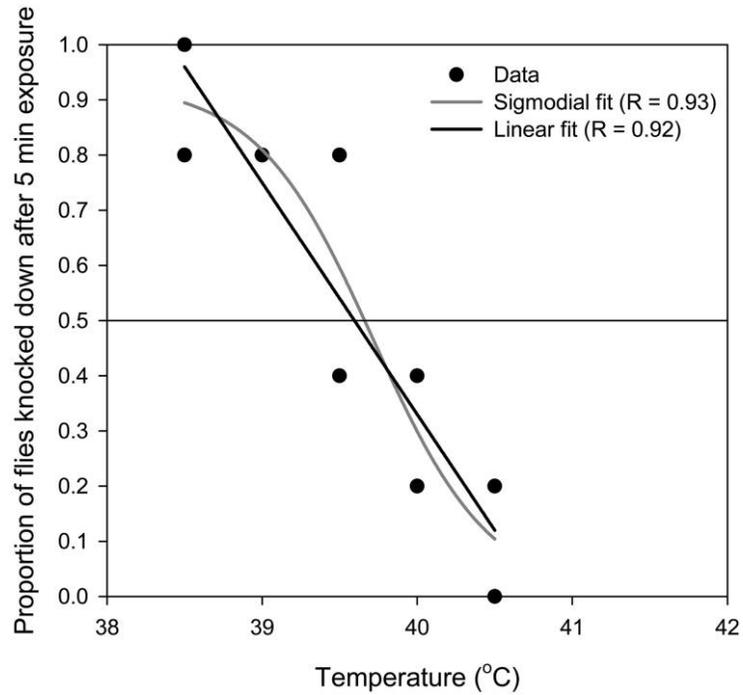
Figure A1: The temperature of knockdown of 50% of the flies was obtained by exposing samples of flies to a range of temperatures close to the knockdown thermal limit. This was done at both high and low temperatures. From these data, we estimated the temperature at which 50% of flies would be knocked down by fitting the data with use of linear regression. As seen in the example, linear and sigmodial relationships produce very similar estimates of 50% knockdown. There are too few data points to determine which of these relationships is most appropriate for estimating 50% knockdown, but in any case, estimates are similar, and using either technique does not affect our conclusions.

Table A1: Thermal knockdown limits of widespread and tropical *Drosophila* species acclimated to temperate or tropical conditions

Species	27°C constant					
	$Acute_{min}$	$Ramp_{min}$	$Acute_{max}$	$Ramp_{max}$	$Ramp_{recovery}$	Scope
Widespread:						
D. bipectinata	12.54 (11.67–13.49)	11.23 ± .23 (20)	39.89 (39.30–40.49)	39.81 ± .30 (19)	20.10 ± .68 (20)	25.56 ± .47 (20)
D. birchii	12.85 (12.21–13.52)	12.07 ± .23 (14)	39.14 (38.37–39.92)	39.38 ± .46 (10)	22.00 ± .49 (13)	23.16 ± .43 (14)
D. bunnanda	12.58 (12.03–13.16)	11.12 ± .28 (20)	39.33 (38.77–39.90)	39.27 ± .16 (20)	20.40 ± .39 (19)	26.51 ± .40 (19)
D. pseudoananassae	13.46 (12.77–14.19)	11.97 ± .29 (20)	39.62 (38.97–40.27)	40.96 ± .37 (20)	21.15 ± .38 (17)	24.48 ± .50 (18)
D. sulfurigaster	14.51 (13.00–16.19)	12.63 ± .17 (21)	38.63 (38.15–39.12)	36.60 ± .12 (20)	19.40 ± 1.03 (20)	24.62 ± .23 (20)
Tropical:						
D. busckii	11.82 (11.04–12.65)	8.75 ± .43 (20)	...	38.05 ± .30 (20)	13.88 ± .39 (20)	29.71 ± .45 (20)
D. hydei	8.90 (8.33–9.51)	5.36 ± .24 (21)	40.70 (40.16–41.26)	40.58 ± .38 (19)	13.89 ± .55 (21)	34.36 ± .34 (19)
D. melanogaster	8.97 (8.36–9.63)	6.59 ± .24 (20)	41.65 (41.17–42.15)	40.92 ± .32 (20)	14.96 ± .35 (20)	32.99 ± .24 (16)
D. repleta	10.72 (9.82–11.71)	7.43 ± .32 (20)	40.28 (39.34–41.23)	39.53 ± .40 (20)	15.75 ± .70 (20)	31.27 ± .36 (20)
D. simulans	9.63 (9.18–10.11)	6.78 ± .15 (19)	40.92 (40.30–41.55)	39.22 ± .26 (20)	16.56 ± .51 (18)	31.72 ± .24 (18)

Species	25°–29°C fluctuating					
	$Acute_{min}$	$Ramp_{min}$	$Acute_{max}$	$Ramp_{max}$	$Ramp_{recovery}$	Scope
Widespread:						
D. bipectinata	12.44 (11.34–13.66)	10.71 ± .26 (19)	39.85 (38.92–40.80)	40.37 ± .28 (20)	20.01 ± .33 (19)	26.84 ± .59 (16)
D. birchii	12.67 (12.12–13.25)	11.33 ± .21 (19)	39.10 (38.43–39.79)	39.40 ± .46 (10)	19.54 ± .65 (18)	24.86 ± .46 (19)
D. bunnanda	12.70 (11.98–13.46)	9.49 ± .38 (20)	39.31 (38.51–40.13)	39.38 ± .22 (20)	19.08 ± .34 (19)	27.93 ± .48 (19)
D. pseudoananassae	13.50 (12.84–14.20)	10.81 ± .23 (19)	39.33 (38.77–39.90)	39.79 ± .31 (20)	19.23 ± .42 (18)	26.47 ± .44 (16)
D. sulfurigaster	13.73 (13.08–14.42)	12.57 ± .20 (20)	38.59 (38.06–39.12)	36.75 ± .10 (20)	19.06 ± .84 (17)	25.46 ± .41 (18)
Tropical:						
D. busckii	11.60 (11.11–12.11)	7.15 ± .30 (20)	...	38.32 ± .27 (19)	13.46 ± .43 (20)	31.92 ± .34 (19)
D. hydei	8.63 (7.88–9.45)	4.78 ± .17 (18)	40.92 (40.30–41.55)	40.93 ± .37 (20)	12.96 ± .54 (20)	34.72 ± .20 (20)
D. melanogaster	9.01 (8.34–9.74)	5.79 ± .24 (20)	41.67 (40.99–42.37)	40.84 ± .29 (20)	14.82 ± .53 (20)	34.16 ± .28 (20)
D. repleta	10.33 (9.26–11.54)	6.22 ± .23 (20)	40.39 (39.51–41.30)	39.57 ± .31 (20)	14.08 ± .56 (21)	32.95 ± .27 (20)
D. simulans	9.37 (8.70–10.10)	6.44 ± .14 (19)	40.80 (40.08–41.54)	38.90 ± .16 (20)	16.62 ± .44 (19)	32.01 ± .27 (18)

Species	19°C constant					
	$Acute_{min}$	$Ramp_{min}$	$Acute_{max}$	$Ramp_{max}$	$Ramp_{recovery}$	Scope
Widespread:						
D. bipectinata	10.42 (9.81–11.06)	7.37 ± .15 (20)	39.25 (38.65–39.86)	39.22 ± .30 (20)	17.79 ± .43 (20)	30.65 ± .18 (20)
D. birchii	10.20 (9.48–10.96)	7.49 ± .19 (18)	38.73 (38.20–39.26)	38.89 ± .37 (18)	16.27 ± .36 (20)	29.72 ± .36 (16)
D. bunnanda	10.00 (9.34–10.70)	7.34 ± .20 (20)	38.57 (38.16–39.00)	38.43 ± .26 (20)	18.10 ± .29 (20)	29.75 ± .36 (20)
D. pseudoananassae	10.34 (9.75–10.97)	7.72 ± .12 (20)	38.99 (38.44–39.55)	39.02 ± .32 (19)	17.65 ± .41 (20)	30.31 ± .25 (19)
D. sulfurigaster	11.42 (10.46–12.45)	8.85 ± .10 (20)	37.08 (36.52–37.65)	36.55 ± .11 (20)	15.81 ± .47 (19)	28.90 ± .19 (19)
Tropical:						
D. busckii	9.22 (8.57–9.92)	5.56 ± .32 (20)	39.59 (39.07–40.13)	39.54 ± .44 (20)	9.50 ± .51 (20)	32.98 ± .37 (18)
D. hydei	7.19 (6.40–8.08)	2.60 ± .15 (19)	40.33 (39.81–40.86)	39.57 ± .30 (17)	6.47 ± .70 (20)	36.61 ± .19 (17)
D. melanogaster	7.17 (6.66–7.72)	2.81 ± .21 (19)	40.83 (40.27–41.40)	40.69 ± .29 (20)	6.78 ± .44 (20)	36.63 ± .23 (16)
D. repleta	8.55 (7.80–9.36)	4.44 ± .29 (19)	...	39.11 ± .56 (14)	9.57 ± .24 (21)	34.18 ± .36 (19)
D. simulans	7.61 (7.02–8.24)	2.79 ± .21 (20)	39.60 (38.50–40.74)	38.79 ± .28 (20)	6.66 ± .30 (19)	35.81 ± .24 (20)

Species	13°–25°C fluctuating					
	$Acute_{min}$	$Ramp_{min}$	$Acute_{max}$	$Ramp_{max}$	$Ramp_{recovery}$	Scope
Widespread:						
D. bipectinata	10.07 (9.55–10.61)	7.80 ± .26 (20)	39.11 (38.18–40.07)	38.53 ± .18 (19)	15.92 ± .52 (20)	31.01 ± .31 (20)
D. birchii	10.17 (9.61–10.75)	7.93 ± .22 (20)	38.76 (38.09–39.44)	38.63 ± .30 (1 8)	16.75 ± .38 (20)	29.25 ± .45 (20)
D. bunnanda	10.06 (9.15–11.05)	6.99 ± .30 (21)	38.61 (38.14–39.08)	38.80 ± .21 (20)	15.81 ± .42 (21)	30.49 ± .47 (20)
D. pseudoananassae	10.22 (9.77–10.69)	7.91 ± .10 (20)	38.95 (37.89–40.03)	39.08 ± .23 (20)	16.52 ± .34 (20)	30.68 ± .31 (19)
D. sulfurigaster	10.79 (9.93–11.72)	7.85 ± .36 (20)	37.31 (36.56–38.09)	36.20 ± .11 (18)	14.86 ± .35 (20)	30.36 ± .40 (19)
Tropical:						
D. busckii	8.49 (7.73–9.26)	6.56 ± .12 (20)	39.94 (39.29–40.60)	39.12 ± .28 (18)	10.34 ± .66 (20)	32.89 ± .30 (20)
D. hydei	7.06 (6.12–8.14)	3.07 ± .18 (20)	40.32 (39.71–40.94)	39.55 ± .25 (18)	7.38 ± .40 (20)	36.49 ± .27 (20)
D. melanogaster	7.00 (6.35–7.72)	3.95 ± .30 (20)	40.83 (40.27–41.40)	40.14 ± .14 (20)	7.37 ± .43 (20)	36.23 ± .40 (17)
D. repleta	8.06 (7.58–8.56)	5.30 ± .12 (20)	...	39.27 ± .40 (20)	10.08 ± .38 (20)	33.48 ± .51 (11)
D. simulans	7.48 (6.74–8.29)	4.04 ± .16 (20)	39.60 (38.79–40.41)	38.40 ± .11 (20)	8.88 ± .59 (20)	35.04 ± .20 (18)

Note: The data set is divided into the four acclimation regimes, and within each regime species are divided into tropical and widespread categories. $Acute_{max}$ = mean temperature at which 50% of flies have fallen upon acute heat exposure (95% CI); $acute_{min}$ = mean temperature at which 50% of flies have fallen upon acute cold exposure (95% CI); $ramp_{max}$ = median temperature at which the flies fall when ramped at 0.1°C/min ± standard error (no. flies) with the ramp test started at 23°C and no prior cold stress; $ramp_{min}$ = median temperature at which the flies fall when ramped at 0.1°C/min ± standard error (no. flies); $ramp_{recovery}$ = median temperature at which the flies recover ± standard error (no. flies); scope = $ramp_{max\ (after\ cold)}$ − $ramp_{min}$.

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