

# The interplay between thermal tolerance and life history is associated with the biogeography of *Drosophila* species

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## ABSTRACT

**Background:** Physiological tolerances are important determinants of the biogeography of species.

**Questions:** What is the relationship between thermal tolerance and the biogeographic origin of species? What are the relationships between thermal tolerance and life-history traits?

**Organisms:** Four *Drosophila* species, two from a tropical biogeographic area (*D. melanogaster* and *D. simulans*) and two from a temperate geographic zone in the Andes mountains (*D. pavani* and *D. gaucha*).

**Methods:** We assessed upper and lower lethal temperature. We used thermal limits to construct a thermal tolerance polygon that represents the total thermal range of each species after acclimation at different ambient temperatures. We also measured differences in life history (fertility and egg-to-adult viability) between species.

**Conclusions:** Both temperate species have broader thermal tolerance ranges than either tropical species. But temperate species have lower fitness at higher temperatures than tropical species, and both of them have low fitness at lower temperatures.

**Keywords:** acclimation, biogeography, *Drosophila*, life history, thermal tolerances polygons.

## INTRODUCTION

Physiological tolerances are important to understand the biogeography of species and populations (Chown and Gaston, 1999). However, the biological processes that explain physiological tolerances within a biogeographic framework are poorly understood (McNab, 2002). Thus, knowledge of physiological responses to climatic variables is necessary to predict how climate may affect the distribution of species and populations (Chown and Gaston, 1999).

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Theoretically, the principal mechanism behind the range of tolerance shown by an organism is acclimation, which plays an important role in several ectotherm species in adjusting their physiological limits. This ability gives them the potential to acquire a wider range of thermal tolerances (Bale *et al.*, 2000; Terblanche *et al.*, 2005; Slabber *et al.*, 2007). Thus, organisms can increase their survival in stressful environmental conditions by previous exposure to non-lethal conditions (Hoffmann, 1995). Also, a limited ability to adjust thermal limits to changes in environmental temperature is likely to affect species' responses to current climate change scenarios (Stillman, 2003; Calosi *et al.*, 2008). Several studies have demonstrated an increase in acclimation ability with latitude (Naya *et al.*, 2009; Bozinovic *et al.*, 2010). It has been suggested that interspecific comparative studies are of benefit to evaluate the adaptive value of acclimation (Doughty and Reznick, 2004). Unfortunately, the adaptive value of acclimation and tolerances is poorly understood. Also, as pointed out by Spicer and Gaston (1999), some of the reported variations in physiological traits between species may arise from comparisons of phylogenetically unrelated species, methodological differences, allometric problems, or specific environmental conditions experienced by species and populations.

In the present interspecific experimental study, we attempt to avoid these problems and examine the relationship between original climate of species and their thermal tolerance in *Drosophila* (*D. melanogaster*, *D. simulans*, *D. pavani*, and *D. gaucha*). We also consider the life-history consequences of the putative differences in their tolerances. In this vein, life history is commonly defined as a set of evolved strategies that more or less influence survival and reproductive success directly (Ricklefs and Wikelski, 2002). Thus, life-history traits are a critical component of an organism's capacity to colonize and survive in different environments (Lardies, 2003). Indeed, genetic variation in life-history traits is a requirement for geographic range expansion. The importance of understanding the link between life history and physiology has been reported previously (Zera and Harshman, 2001; Zera and Larsen, 2001; Ricklefs and Wikelski, 2002; Folguera *et al.*, 2010).

*Drosophila* species constitute a good model to test hypotheses in evolutionary physiological ecology because their phenotypic and genetic responses to environmental temperature are well understood (reviewed by Hoffmann, 2010). We used related species to minimize phylogenetic problems but with larger biogeographic differences. Indeed, *D. melanogaster* and *D. simulans* have a tropical origin and are cosmopolitan (Keller, 2007), whereas *D. pavani* and *D. gaucha* exhibit a comparatively smaller geographic range with an Andean high-altitude origin and they still inhabit their original range (Brncic, 1969, 1987; Budnik and Brncic, 1974). Nevertheless, these four species co-exist in nature where they exhibit similar life modes, food habits, and reproductive sites (Godoy-Herrera and Connolly, 2007). We measured upper and lower lethal limits in adult individuals of the four species acclimated to three temperatures and used these data to estimate their acclimation ability and their thermal tolerance ranges through construction of a thermal tolerance polygon. The area of the polygon expresses the temperature tolerance in units of °C<sup>2</sup>, and hence reflects the degree of eurythermicity of a species. The larger the area of the polygon, the wider the range in thermal tolerance. Thus, the thermal tolerance polygon is equivalent to Hutchinson's (1965) fundamental thermal niche of a species (Beitinger and Bennett, 2000). Temperature tolerance data portrayed as a polygon convey important information about the physiology and ecology of a species, and the shape of a polygon offers a clue to the role acclimation temperature plays in the temperature tolerance of a species (Beitinger and Bennett, 2000). We also measured life-history traits (fecundity and egg-to-adult viability) at three acclimation temperatures. In summary, we examined: (1) the relationship between thermal tolerances and the biogeographic origin of species; (2) the

impact of acclimation temperature on thermal limits and life-history traits among species; and (3) the relationships between interspecific differences in physiological thermal tolerance and species-specific life-history traits (i.e. fertility and egg-to-adult viability).

## METHODS

### Experimental individuals

We used the fourth generation of laboratory culture adults of the four species. Flies were reared at 24°C in 250-ml glass vials with Burdick (1954) culture medium until the fourth generation emerged.

### Lethal limits

We assessed lethal limits using the  $TL_{50}$  method (Fry *et al.*, 1942), defined as the temperature at which 50% of the individuals could not survive for a certain period. At each generation, we randomly collected 40 adult flies from the rearing vials and transferred them to fresh vials. After 3 days we removed the adults to prevent overlap between generations. To determine upper (ULT) and lower (LLT) lethal temperatures, 7 days after the fourth generation emerged we randomly assigned to each of three thermal treatments ten vials, each with 10 males and 10 females. We acclimated individuals for one week at 10°C, 20°C, and 30°C using a 12:12 h light/dark regime in climatic chambers. Next, we removed individuals from each group and randomly assigned them to upper and lower experimental temperature tests. To obtain  $TL_{50}$ , we transferred individuals from glass vials into plastic tubes and submerged them in a water bath with commercial antifreeze ( $\pm 0.5^\circ\text{C}$ ) at the set temperature for 30 min. We set upper and lower experimental temperatures with 2°C intervals according to Hoffmann (2010) *Drosophila* lethal limits. Thirty minutes after cold treatment and 15 min after heat treatment, we assessed survivorship. We considered an individual alive if it could stand on its legs after returning to room temperature. If an individual was unable to right itself at this time, we considered it dead.

When tests were performed to obtain ULT, we added a wet filter paper to each vial to avoid desiccation effects.

### Thermal tolerance range

We assessed thermal tolerance range of each species by calculating the total area of a thermal tolerance polygon that consists in a polygon of acceptable ambient temperatures for a species delimited by ULT and LLT evaluated under different acclimation conditions. It was constructed by plotting acclimation temperature versus lethal limits. Thermal tolerance area was calculated from the polygon and is reported in  $^\circ\text{C}^2$ . The larger the area, the wider the range in thermal tolerance of the species (Ford and Beiting, 2005; Calosi *et al.*, 2008).

### Life-history traits

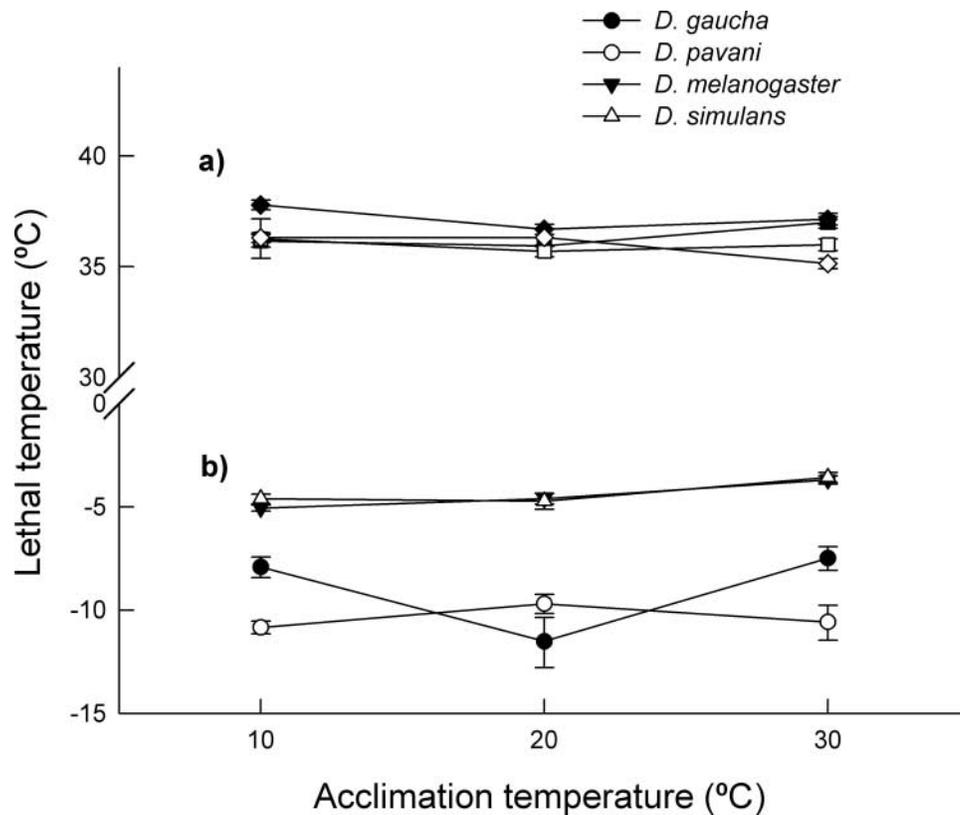
We tested the effect of temperature acclimation on life-history traits of the four species differing in biogeography. We estimated fecundity as the number of eggs laid per female and viability as the number of adults that emerged from a known number of eggs ( $n = 15$ ).

### Data analyses

We performed statistical analyses using the Statistica® (StatSoft 2001) statistical package for Windows. We used factorial ANOVA or ANCOVA with body mass as covariate for comparisons among fixed factors (species and acclimation temperatures). Before the analyses we tested assumptions of normality and constant variance using Shapiro-Wilks  $W$  and Levene tests. When required to satisfy the assumptions of normality for parametric statistical tests, data were  $\log_{10}$  transformed. Survivorship was plotted against exposition temperature and  $TL_{50}$  was obtained from the model using a probit analysis. Confidence intervals were estimated for each lethal temperature. Data are reported as arithmetic mean  $\pm$  1 standard deviation.

### RESULTS

Upper lethal limit was better conserved than lower lethal limit among species (Fig. 1). Indeed, none of the species was able to increment their ULT following acclimation ( $P = 0.284$ ; Table 1). Body mass did not affect ULT among species ( $P = 0.365$ ; Table 1).



**Fig. 1.** Effect of acclimation on (a) upper and (b) lower lethal temperature (mean  $\pm$  95% confidence intervals) in four species of *Drosophila* after acclimation at 10°C, 20°C, and 30°C: *Drosophila gaucha*, *D. pavani*, *D. melanogaster*, and *D. simulans*.

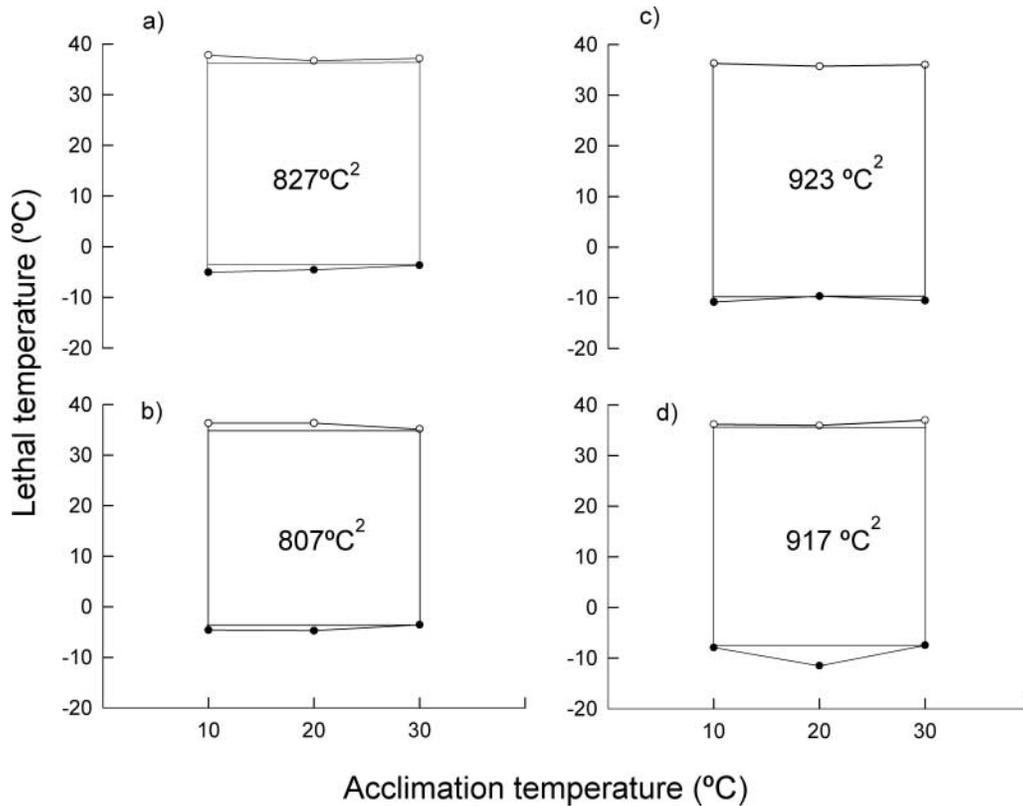
**Table 1.** Influence of species identity, body mass, and acclimation temperature on the upper and lower thermal limit in *Drosophila melanogaster*, *D. simulans*, *D. pavani*, and *D. gaucha*

Source	d.f.	SS	F-ratio	P
<b>Upper lethal limit</b>				
Species	3	2.994	9.082	<0.001
Body mass	1	0.091	0.830	0.365
Acclimation	2	0.281	1.280	0.284
Species × acclimation	6	2.405	3.648	0.003
<b>Lower lethal limit</b>				
Species	3	2.361	29.397	<0.001
Body mass	1	0.001	0.051	0.822
Acclimation	2	0.130	2.428	0.095
Species × acclimation	6	0.472	2.941	0.013

Nevertheless, responses to acclimation at the ULT differed among species, accounting for the significant interaction term in the model ( $P = 0.003$ ; Table 1).

Lower lethal limit was decoupled from ULT. *Drosophila gaucha* (LLT =  $-8.23^{\circ}\text{C}$ ) and *D. pavani* (LLT =  $-10.37^{\circ}\text{C}$ ) tolerated much lower temperatures than *D. melanogaster* (LLT =  $-4.47^{\circ}\text{C}$ ) and *D. simulans* (LLT =  $-4.30^{\circ}\text{C}$ ) (Fig. 1). As with ULT, individuals exposed to low temperatures did not respond to acclimation ( $P = 0.095$ ; Table 1) with the exception of *D. gaucha*, which was able to tolerate much lower temperatures when acclimated at  $20^{\circ}\text{C}$  (Fig. 1). There was a significant interaction between species and acclimation temperature, as in the case of ULT, and so responses to acclimation differed among species ( $P = 0.013$ ; Table 1). The two tropical species exhibited smaller total thermal tolerance polygons than the temperate species (Fig. 2). *Drosophila pavani* had the largest thermal tolerance polygon of all species ( $923^{\circ}\text{C}^2$ ), followed by *D. gaucha* ( $917^{\circ}\text{C}^2$ ). *Drosophila melanogaster* and *D. simulans* had smaller polygons of  $827^{\circ}\text{C}^2$  and  $807^{\circ}\text{C}^2$ , respectively.

Egg laying differed among species in response to acclimation ( $P < 0.001$ ; Table 2). Flies were able to lay eggs at all three acclimation temperatures. The highest number of eggs was recorded in flies acclimated to  $20^{\circ}\text{C}$  and the lowest number in flies acclimated to  $10^{\circ}\text{C}$ , with no differences among species, but when flies were acclimated at  $30^{\circ}\text{C}$  the two tropical species laid significantly more eggs than temperate species (Fig. 3). No species was viable at  $10^{\circ}\text{C}$ ; the highest egg-to-adult viability was observed when acclimated at  $20^{\circ}\text{C}$ . At  $30^{\circ}\text{C}$ , only *D. melanogaster* and *D. simulans* were viable (Fig. 4). The viability response to acclimation differed among species ( $P < 0.001$ ; Table 2). Finally, the relationship between life-history traits and physiological lethal limits is shown in Fig. 5. Upper panels relate life-history traits to lower lethal limits, while lower panels show the relationship between life-history traits and upper lethal limits. Egg laying and egg-to-adult viability exhibit a clear pattern related to LLT: (i) temperate species with high egg laying and egg-to-adult viability, (ii) tropical species with high egg laying and egg-to-adult viability, (iii) temperate species with low egg laying and egg-to-adult viability, and (iv) tropical species with low egg laying and egg-to-adult viability. Life-history responses to acclimation temperature in relation to ULT

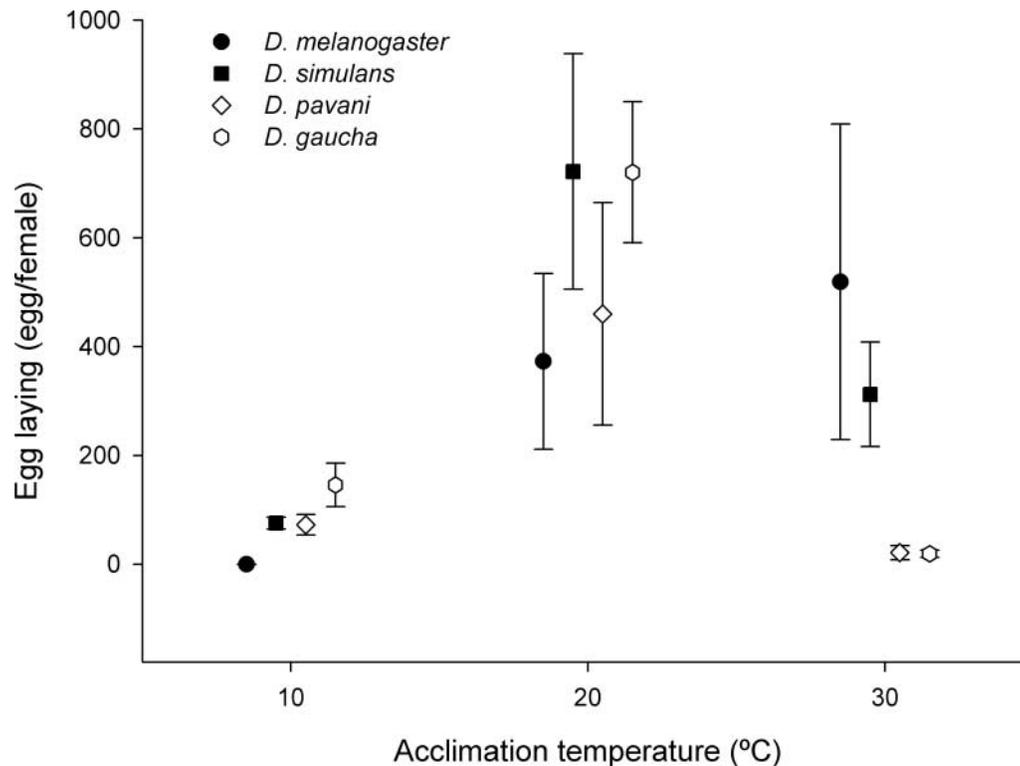


**Fig. 2.** Thermal tolerance polygons ( $^{\circ}\text{C}^2$ ) in (a) *Drosophila melanogaster*, (b) *D. simulans*, (c) *D. pavani*, and (d) *D. gaucha*. The apices of the total polygons are set by the upper and lower lethal limits of individuals acclimated at 10°C, 20°C, and 30°C.

**Table 2.** Influence of species identity and acclimation temperature on life-history traits in *Drosophila melanogaster*, *D. simulans*, *D. pavani*, and *D. gaucha*

Source	d.f.	SS	F-ratio	P
<b>Egg laying</b>				
Species	3	4.210	10.833	<0.001
Acclimation	2	9.130	35.240	<0.001
Species $\times$ acclimation	6	16.045	20.642	<0.001
<b>Viability</b>				
Species	3	0.54	11.63	<0.001
Acclimation	2	3.93	127.71	<0.001
Species $\times$ acclimation	6	0.74	7.96	<0.001

(panels b and d) were similar to those in panels (a) and (c), but subdivision into temperate and tropical species was not so evident because, as already explained (see Fig. 1), the upper lethal limit was conserved among species.



**Fig. 3.** Effect of temperature acclimation on egg laying in *D. melanogaster*, *D. simulans*, *D. pavani*, and *D. gaucha*.

## DISCUSSION

Explanations of species biogeography call for – among other ecological and evolutionary data – the intrinsic physiological properties of organisms, in order to predict their responses to environmental biotic and abiotic variables (Pither, 2003). In this paper, we experimentally assessed the relationship between thermal tolerances and the biogeographic origin of four *Drosophila* species of tropical and temperate origin; the effect of temperature acclimation on thermal limits and life-history traits among species; and finally, the relationships between interspecific differences in physiological thermal tolerance and species-specific life-history traits. Interestingly, our results showed a diversity of responses among characters to our experimental thermal treatments.

We found that among the *Drosophila* species studied, upper lethal limits were more conserved than lower lethal limits, supporting previous observations. Indeed, Addo-Bediako *et al.* (2000) compiled data on both upper and lower lethal limits in insects and found that variation in upper lethal limits is much less pronounced than variation in lower lethal limits. Although interspecific variation in upper limits was significant, it was much less than that in lower limits. These results suggest that physiological responses accounting for heat and cold tolerance are achieved by different underlying mechanisms (Chen *et al.*, 1990; Gaston and Chown, 1999; Chown and Nicolson, 2004). Such decoupling of upper and lower lethal limits has been

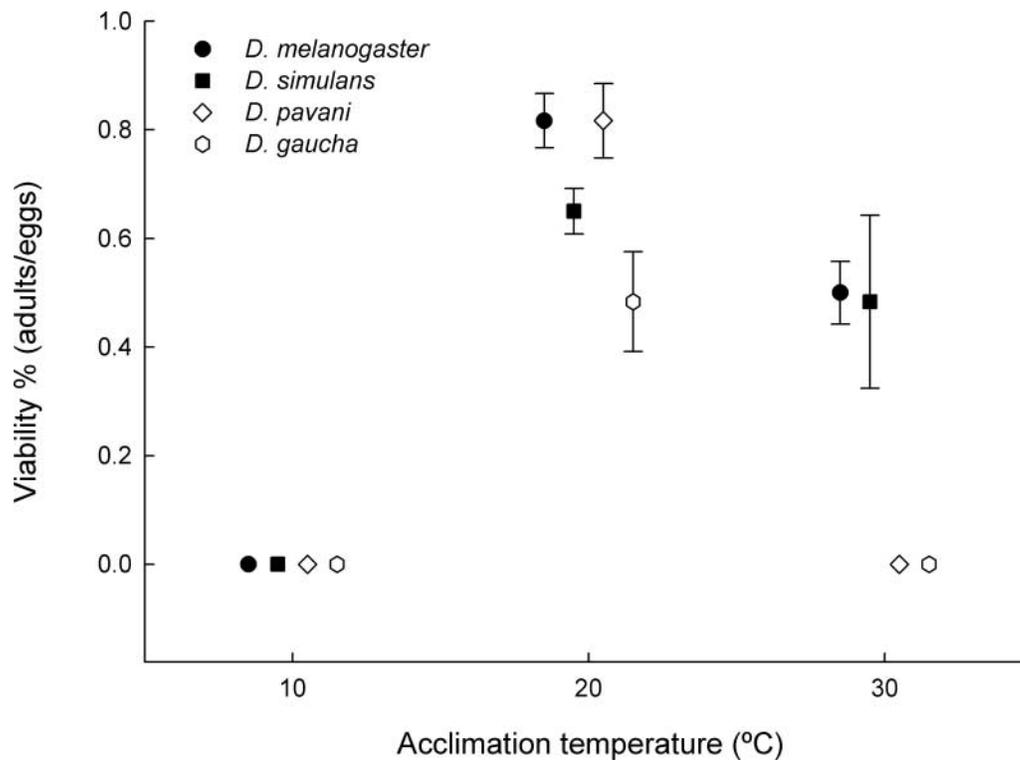
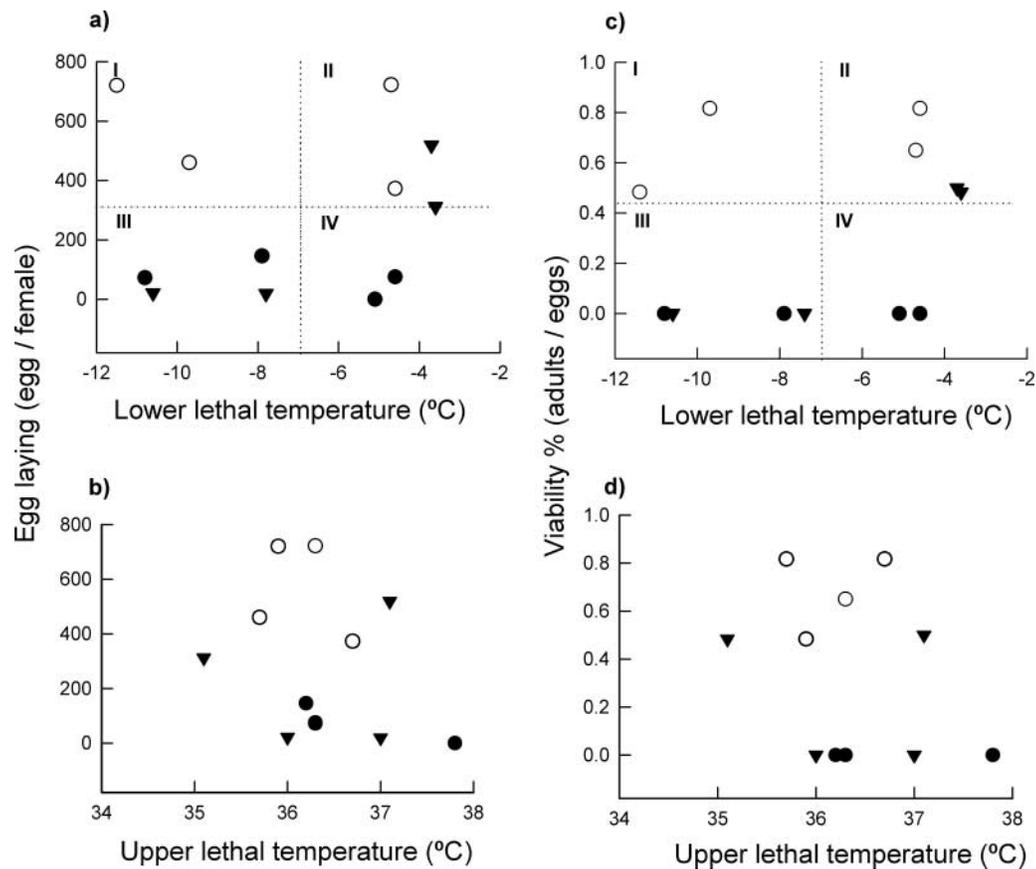


Fig. 4. Effect of temperature acclimation on egg-to-adult viability in *D. melanogaster*, *D. simulans*, *D. pavani*, and *D. gaucha*.

documented in several other species of insects (Chen *et al.*, 1990; Gaston and Chown, 1999; Addo-Bediako *et al.*, 2000; Hercus *et al.*, 2000; Kimura, 2004; Deere *et al.*, 2006; Ragland and Kingsolver, 2008). In contrast, lower thermal limits differed markedly between species.

Temperate species with an Andean origin (*D. gaucha* and *D. pavani*) tolerate much lower temperatures than tropical species (*D. melanogaster* and *D. simulans*), suggesting a tendency of species to retain ancestral ecological characteristics. Some kind of niche conservatism regarding lower thermal limits can be outlined (Wiens and Graham, 2005). Our results suggest that historical biogeography may be an important feature associated with the observed pattern of lower thermal limits. This process allows species to track favoured habitats rather than adapt to new ones (Harvey and Pagel, 1991; Webb *et al.*, 2002). Similarly, Kellermann *et al.* (2009) noted that tropical *Drosophila* species lack genetic variation for traits underlying cold tolerance, suggesting that species retain similar traits over long periods of time. This is particularly relevant in the current global climate change scenario. If species could simply adapt to changing conditions, then we would have little cause for concern. However, species that cannot adapt and cannot shift their geographic ranges may be at risk of extinction.

On the other hand, the two temperate species have broader physiological thermal ranges than the two tropical species, but the latter have larger distributions. One possible explanation is that the two widespread species in this study are also human commensals, so their range expansion may be associated with human activities. Another possible



**Fig. 5.** Relationship between life-history traits and lethal limits under three acclimation temperatures in *D. melanogaster*, *D. simulans*, *D. pavani*, and *D. gaucha*. Panels (a) and (c) have four divisions: temperate species – high egg laying (aI) and egg-to-adult viability (cI); tropical species – high egg laying (aII) and egg-to-adult viability (cII); temperate species – low egg laying (aIII) and egg-to-adult viability (cIII); tropical species – low egg laying (aIV) and egg-to-adult viability (cIV). These divisions are not so evident at upper lethal limits (panels b and d). ●, acclimation at 10°C; ○, acclimation at 20°C; ▼, acclimation at 30°C

explanation is that cold-adapted species have an associated fitness cost (see below). Thus, besides this apparent contradiction, the total polygon areas of all four species are greater than those of other insect species (Kimura, 2004; Deere *et al.*, 2006; Calosi *et al.*, 2008), and no acclimation responses were evident for both upper and lower limits, supporting Stillman's (2003) hypothesis of a trade-off between total thermal range size and ability to acclimate. The lack of acclimatory response suggests a limited plasticity in thermal limits. Thus, the inability to express a wider thermal range by means of physiological adjustments after a change in environmental temperature suggests that thermal tolerance levels in these flies can change only through adaptive evolution, in contrast to what has been proposed for some other insect species (Deere *et al.*, 2006; Terblanche *et al.*, 2006). A limited ability to adjust thermal limits to environmental temperature changes is likely to affect species responses under

current global warming (Stillman, 2003; Somero, 2005). In what at first might seem paradoxical, warm-adapted tropical congeners may be more threatened by increased temperatures than their temperate relatives. These differences arise from two factors: the proximity of current habitat temperatures to upper lethal temperatures and a limited ability to acclimate to increases in temperature. As Stillman (2003) and Tewksbury *et al.* (2008) predicted, tropical species would be most vulnerable to current warming because they have limited acclimatory ability and already live close to their upper thermal limits. The absence of acclimatory responses must be explained because plasticity has a cost (DeWitt *et al.*, 1998). Constitutive resistance to high temperatures may not be maintained in individuals that do not often experience such temperatures because there are costs associated with supporting a biochemistry associated with these conditions (DeWitt *et al.*, 1998; Chown and Nicolson, 2004). In this vein, Deutsch *et al.* (2008) integrated fitness curves describing the thermal tolerance of terrestrial insects of the world with the projected distribution of climate change for the next century on earth to estimate the impact of climate warming on Darwinian fitness. They showed that warming in the tropics, although relatively small in magnitude, is likely to have the most negative impact because tropical insects are relatively sensitive to temperature change and are currently living close to their optimal temperature. In contrast, species at higher latitudes have broader thermal tolerance and are living in climates that are currently cooler than their physiological optima, so that warming may even enhance their fitness.

When acclimated to 20°C, only *D. gaucha* showed a marked acclimatory response in the lower lethal limit. According to the beneficial acclimation hypothesis (Leroi *et al.*, 1994), individuals acclimated to 10°C (the lowest acclimation temperature) must show the lowest lethal limit. But in this case, when individuals were acclimated to 20°C, close to their rearing temperature, they performed better according to the predictions of the optimal developmental temperature hypothesis (Huey *et al.*, 1999). Cohet and David (1978) first proposed this hypothesis based on a study of the effects of developmental temperature on various life-history traits of *D. melanogaster*. They noted that flies reared at intermediate temperatures were vigorous and performed well in a variety of adult thermal environments. Most likely, development at such intermediate temperature is harmonious and results in adults with a well-integrated physiology that do well in diverse thermal environments. Similarly, fertility and egg-to-adult viability at different acclimation temperatures follow a similar pattern as predicted by the optimal developmental temperature hypothesis. All the species performed better when acclimated to 20°C, close to their rearing temperatures, similar to what has been reported for other insect species (Milano *et al.*, 2008; de Oliveira *et al.*, 2009).

Most studies involving thermal tolerance polygons to assess thermal range size of species have been conducted with fish. In a review of 21 fish species, Beitinger and Bennett (2000) concluded that the acclimation response of fish doubles polygon area compared with non-acclimated fish. The lower acclimation dependent area was nearly three times greater than the upper acclimation dependent area, suggesting that acclimation plays a larger role in tolerance of low rather than high temperatures. Indeed, the only species to show a low acclimation response had a large thermal range. Again, this example together with our own study supports Stillman's (2003) hypothesis of a trade-off between total range size and acclimation ability of thermal limits. As far as we know, this is the first study to use a thermal polygon to assess thermal range size in a terrestrial species. Indeed, only one other study (Calosi *et al.*, 2008) used a thermal polygon to assess thermal range size in insects. Finally, it seems that acclimation effects are typically stronger in aquatic than in terrestrial species, keeping with the prediction of strong acclimation responses in species from predictably

variable environments, but weaker responses in species from unpredictable environments (Deere *et al.*, 2006). These differences are in line with theoretical predictions of the conditions under which plasticity is most likely to develop (Kingsolver and Huey, 1998; Tufto, 2000; Berrigan and Scheiner, 2004).

Temperate species in our study did tolerate much lower temperatures than tropical species. But when we analysed the relationships between thermal limits and life-history traits, we observed that although temperate species could lay eggs in the lowest acclimation treatment, the eggs were non-viable. Something similar happens when we relate upper lethal limits to life-history traits. Although the upper thermal limits were similar among all species, temperate species laid eggs in the upper acclimation treatment that were non-viable, whereas the eggs of tropical species were viable under these conditions. These data suggest that these traits could be negatively genetically correlated. Indeed, as pointed out by Service *et al.* (1998) and Ohtsu *et al.* (1993), increased cold resistance could decrease fecundity. This trade-off might arise because both traits are related to lipid metabolism. Hoffmann and Parsons (1989) suggested that increased resistance to a range of stresses could be associated with a lowered metabolic rate, and this, in turn, is likely to decrease early fecundity. Traits that would allow range expansion may be pleiotropically linked to traits that reduce fitness. In fact, Jenkins and Hoffmann (1999) suggested that in the Australian *Drosophila serrata*, range expansion into cooler temperate regions is limited because evolution of increased cold resistance is associated with decreased fecundity. Similarly, Watson and Hoffmann (1996) found that increased resistance to cold in *D. melanogaster* and *D. simulans* leads to a reduction in fecundity, suggesting that variation in these traits is influenced by the same genes. This might explain the apparent contradiction that temperate species that have the biggest polygon areas do not have the largest geographic distributions. In this context, future studies might address the question: What would be the advantage of tolerating such extreme temperatures if fecundity and viability would not allow populations to persist in those extreme conditions? Life-history estimations need to be incorporated into physiological studies of thermal limits and tolerances (Ricklefs and Wikelski, 2002; Lardies, 2003; Folguera *et al.*, 2007) if we are to understand in a broad sense the way organisms respond physiologically to climate change in nature.

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