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Genetic variation in heat-stress tolerance among South American *Drosophila* populations

Lindsey C. Fallis · Juan Jose Fanara · Theodore J. Morgan

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Abstract Spatial or temporal differences in environmental variables, such as temperature, are ubiquitous in nature and impose stress on organisms. This is especially true for organisms that are isothermal with the environment, such as insects. Understanding the means by which insects respond to temperature and how they will react to novel changes in environmental temperature is important for understanding the adaptive capacity of populations and to predict future trajectories of evolutionary change. The organismal response to heat has been identified as an important environmental variable for insects that can dramatically influence life history characters and geographic range. In the current study we surveyed the amount of variation in heat tolerance among *Drosophila melanogaster* populations collected at diverse sites along a latitudinal gradient in Argentina (24°–38°S). This is the first study to quantify heat tolerance in South American populations and our work demonstrates that most of the populations surveyed have abundant within-population phenotypic variation, while still exhibiting significant variation among populations. The one exception was the most heat tolerant population that comes from a climate exhibiting the warmest annual mean temperature. All together our results suggest there is abundant genetic variation for heat-tolerance phenotypes within and among natural populations of *Drosophila* and this variation has likely been shaped by environmental temperature.

Keywords Heat survival · Thermotolerance · Temperature stress resistance

L. C. Fallis · T. J. Morgan (✉)
The Division of Biology, The Ecological Genomics Institute, Kansas State University, 116 Ackert Hall, Manhattan, KS 66506, USA
e-mail: tjmorgan@ksu.edu

J. J. Fanara
Departamento de Ecología, Genética y Evolución, FCEN UBAI, Buenos Aires, Argentina

Introduction

Nearly all organisms live in heterogeneous environments, which vary in biotic and abiotic factors on both spatial and temporal scales. One environmental factor that dramatically influences phenotypic evolution is the whole-organism response to temperature (Umina et al. 2005; Rashkovetsky et al. 2006; Reusch and Wood 2007; Zhen and Ungerer 2008). Temperature is a critical environmental parameter and thermal variation has significant effects on local adaptation (Anderson et al. 2003) and can limit species distributions (Clarke 1996) in nature. This is especially true for organisms that are isothermal with their environment, such as insects. Variation in temperature (Cossins and Bowler 1987; Leather et al. 1993; Clarke 1996) imposes stress and directly influences physiology, behavior, and fitness (Hoffmann and Parsons 1991; Gilchrist and Huey 1999; Gibert et al. 2001; David et al. 2003; Hoffmann et al. 2003a, b; Rohmer et al. 2004). Thus, for species to thrive across a range of thermal environments populations must contain either sufficient genetic variation to allow phenotypic adaptation across generations, the capacity to respond plastically to environmental variation, or some combination of both genetic and plastic responses (Hoffmann and Parsons 1991; Ayrinhac et al. 2004; Hoffmann et al. 2005; Hoffmann and Willi 2008).

A comprehensive understanding of genetic variation that underlies differences in thermotolerance phenotypes is critically important in light of a rapidly changing global

climate. The future climate is projected to have higher global average temperatures, but also an uneven distribution of temperature changes and a greater frequency of extreme thermal events (IPCC 2007). Thus, organisms will have to cope with an increased probability of extreme weather events including novel high temperatures across seasons (Jentsch et al. 2007). The ability of animal populations to survive these thermal shifts in the long term rests on how much genetic variation they currently harbor (Potvin and Tousignant 1996; Hoffmann and Willi 2008). Quantifying how genetic variation partitioned itself within and among populations in response to natural temperature gradients will help predict the evolutionary responses to a changing global climate.

Drosophila melanogaster is a cosmopolitan species that has been extremely successful in adapting to a wide range of thermal environments in nature (David and Capy 1988; Ayrinhac et al. 2004). *Drosophila* has been widely used in studies of thermotolerance (Hoffmann et al. 2003a, b)revealing in some cases patterns of thermal variation consistent with clinal variation (Davidson 1990; Karan and David 2000; Gibert and Huey 2001; Gibert et al. 2001; Hoffmann et al. 2002; Ayrinhac et al. 2004; Rashkovetsky et al. 2006) and in other cases simply population differentiation (Parsons 1977; Stanley and Parsons 1981; Hoffmann and Watson 1993; Bubliy et al. 2002; Hoffmann et al. 2005; Rako et al. 2007). Each of these studies has documented clinal and/or population variation and attempted to link this variation with changes in environmental parameters, which co-vary with latitude. Together these studies demonstrate how natural variation has been shaped by adaptation to local environments. Although these studies provide a compelling description of thermal variation, all of these studies have been performed on North American, European, Australian or African populations. To date no study has quantified thermal variation among populations in South America.

In this study we quantified phenotypic variation in heat-tolerance phenotypes within and among six natural populations of *D. melanogaster* sampled from an environmental gradient in Argentina (24°–38°S). We used an isofemale line approach that allows us to accurately estimate the standing variation within and among populations (David et al. 2005). We find that most of the populations surveyed have abundant within-population phenotypic variation, while still exhibiting significant variation among populations.

Materials and methods

Drosophila stocks

Gravid females were collected from six populations in central Argentina described previously (Lavagnino et al. 2008; Folguera et al. 2008). Flies were collected by net sweeping over fermented banana baits at six locations along a north to south latitudinal gradient ranging from approximately 24°–38° south latitude in Argentina (Fig. 1). Populations were named for the nearby city or the providence where the sampling location was positioned (i.e., Guemes, Jachal, Chilecito, Lavalle, Uspallata, and Neuquén). Geographical locations, latitude, longitude, altitude and climatological data (http://www.smn.gov.ar/) for each population are presented in Table 1. Ten isofemale lines were created from single wild-caught females from each population and inbred via full-sib mating for 10 generations. All lines have been maintained in the laboratory since February 2004 on standard cornmeal-agar-molasses medium sprinkled with live yeast to stimulate oviposition. Flies were maintained from egg to adult at 25°C and on a light/dark cycle of 12 h.

Heat survivorship profiles

Heat tolerance profiles were measured for each line within each population using a percent survival after heat-stress assay (Morgan and Mackay 2006). Heat tolerance was measured on mated, adult flies (5–7 day old). Flies were anesthetized using light CO_2 and were sorted in single-sex groups of 20 individuals in standard vials containing 5 ml of cornmeal-agar-molasses medium. The experimental assay was performed at least 24 h later to allow flies to recover from the effect of CO_2. On the day of the heat stress exposure, flies from each replicate vial were transferred without anesthesia into vials without food and

Fig. 1 Geographic locations of the Argentinean populations used in this study: *A* Guemes, *B* Chilecito, *C* Jachal, *D* Uspallata, *E* Lavalle, and *F* Neuquén

Table 1 Collection sites and selected climatological data for the six populations of *Drosophila melanogaster* in Argentina (http://www.smn.gov.ar/)

Population	Latitude	Altitude (m)	Temperature (°C)			Mean rainfall (mm)	Mean humidity (%)	Isofemale lines (n)
			Mean annual	Max. monthly high mean	Min. monthly low mean			
Guemes	24°41′S	695	16.58	27.5	3.4	69.73	73.83	10
Chilecito	29°10′S	1,043	17.25	31.6	2.1	15.75	59.66	10
Jachal	30°12′S	1,238	16.45	31.6	0.9	11.84	54.25	9
Uspallata	32°35′S	1,915	11.61	27.9	−3.7	12.75	51.45	10
Lavalle	32°50′S	647	15.93	30.2	3.2	22.53	58.75	10
Neuquén	38°57′S	260	14.74	31.7	−0.1	15.23	52.08	10

'Maximum/minimum monthly high/low mean' refers to an average highest/lowest temperature across all months

placed at 38°C (±0.5°C) for 60, 90, 120, 150, 180, and 210 min. After heat-stress exposure, flies were immediately transferred to fresh vials containing 5 ml of standard cornmeal-agar-molasses medium and returned to 25°C and 60% humidity for 24 h. After 24 h, the percentage of surviving flies per vial was recorded for each sex, line, and exposure time, generating a heat tolerance profile for each line and sex (Fig. 2). A fly was considered a survivor if it could move when the vial was gently tapped. Four replicate assays were performed per line, sex, and exposure time resulting in a slightly unbalanced design consisting of 48,000 flies in total.

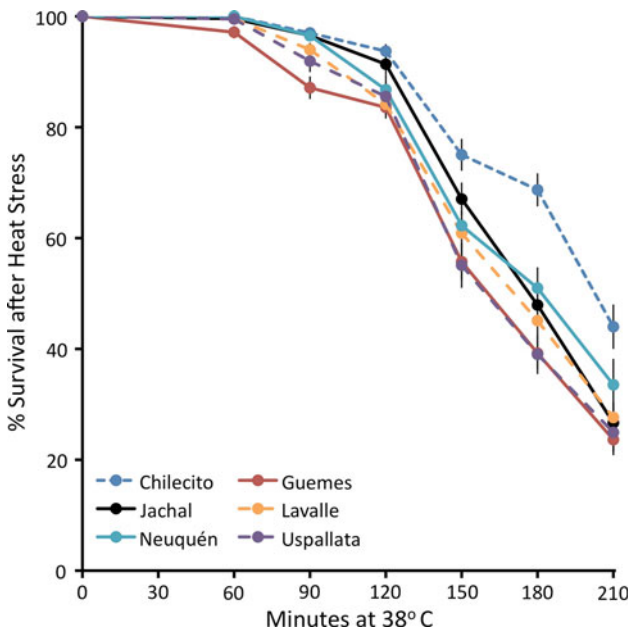

Fig. 2 Population-specific heat survival curves for the six natural populations. The *x*-axis denotes the length of the heat exposure (0–210) in minutes, while the *y*-axis is the mean percent survival of each population averaged across *lines* and *sexes*. *Error bars* denote plus or minus one standard error

Statistical analysis

Analysis of phenotypic variation

We used a series of mixed model analyses of variance (ANOVA) to determine the sources of variation in heat tolerance within and among populations. The initial full model was:

$$y = \mu + P + L(P) + S + T + S \times P + S \times L(P) + T \times P + T \times L(P) + S \times T \times P + S \times T \times L(P) + \varepsilon.$$

where y is exposure-time-, population-, line-, and sex-specific heat survivorship percentages, μ is the overall mean, while P, S, and T are the fixed effects of population, sex and heat exposure time, respectively. $L(P)$ is the random effects of line nested within population and vial nested within the population, line, sex and exposure time, and ε is error. In this study, the terms of primary interest are population, line nested within population, and the interaction of these terms with sex and exposure time as these terms test for local phenotypic differentiation among populations, significant genetic variation within populations, and sex or treatment specific effects of populations or lines nested within populations. To further dissect the population and line-nested-within-population terms, reduced models were used, which separated the data by population and/or exposure time. The reduced analyses separated by population tested for significant genetic differences among lines within each population (i.e. significant within population genetic variation). While the reduced analyses separated by exposure time simplified the analyses, the significant differences among populations were generally consistent across exposure times (Fig. 2; Table 2). For the reduced analyses at a single exposure, we used the survival data from the 180-min exposure time. We used this time point because the mean survival across all six populations is closest to 50%, thus giving us maximum power to detect variation on the percent survivorship scale. ANOVAs and variance component

Table 2 Percentage of the total phenotypic variance within populations explained by among line differences, sex-specific line differences, and residual error

	Population					
	Guemes	Chilecito	Jachal	Lavalle	Uspallata	Neuquén
Line	8.92*	14.97^{NS}	27.38***	56.72****	49.28****	24.28****
Line × sex	10.80^{NS}	0.00^{NS}	11.27^{NS}	0.00^{NS}	0.34^{NS}	25.42^{NS}
Error	80.28	85.03	61.36	43.28	50.38	50.30
H^2	0.197	0.150	0.386	0.567	0.496	0.497

Broad-sense heritablities (H^2) were calculated as $H^2 = \sigma^2_G/\sigma^2_P$, where $\sigma^2_G = \sigma^2_L + \sigma^2_{L\times S}$and $\sigma^2_P = \sigma^2_L + \sigma^2_{L\times S} + \sigma^2_\varepsilon$

^{NS} $P > 0.05$; * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$

calculations were performed using the PROC GLM implemented in SAS 9.2 (SAS Institute 2009). Population specific broad-sense heritabilities (H^2) were calculated as in Morgan and Mackay (2006) where $H^2 = \sigma^2_G/\sigma^2_P$, where $\sigma^2_G = \sigma^2_L + \sigma^2_{L\times S}$ and $\sigma^2_P = \sigma^2_L + \sigma^2_{L\times S} + \sigma^2_\varepsilon$.

Associations between environmental and heat survival variation

To test if variation in environmental or geographic factors is associated with variation in survival after heat stress, we used a stepwise forward–backward selection model implemented in PROC REG in SAS 9.2 (SAS Institute 2009). This approach tests for associations between the line-specific mean survivorships and the geographic and/or climatological data, by evaluating the significance of each geographic or climatological factor (Table 1) on survival after heat stress.

Results

Phenotypic variation within and among populations

We observed significant variation among populations (Fig. 2; $F_{5,56} = 2.99$, $P = 0.0184$) and among lines within populations (Fig. 3; $F_{53,77} = 3.74$, $P < 0.0001$) across all exposure times. The largest amount of variation in survival after heat stress among populations was observed at the 180-min exposure time. At this time point populations were the most divergent in their ability to survive heat exposure, with highly significant differences among populations ($F_{5,54} = 3.70$, $P = 0.0060$) and differences among lines within populations ($F_{53,53} = 4.01$, $P < 0.0001$). This significant population effect was driven primarily by the Chilecito population which had an elevated mean survival after heat stress score (68.67 ± 2.98%) relative to the five other populations that were not significantly different from one another (Fig. 2).

In addition to significant phenotypic differentiation in heat survival among populations, there was also significant genetic variation among replicate lines within each population (Fig. 3) for five of the six populations assayed in the study (Table 2). Population specific broad-sense heritabilities ranged from 0.150 for Chilecito to 0.567 for Lavalle (Table 2). The single population that did not have significant variation among lines ($F_{9,78} = 1.79$, $P = 0.0838$) and had the smallest broad sense heritability was Chilecito. Chilecito was also responsible for the significant population effect in the global analysis across all populations (Figs. 2, 4).

Associations between environmental and heat survival variation

A single environmental factor, the maximum monthly average high temperature in each population, was positively associated with variation in survival after heat stress among the six populations (Fig. 4; $\beta = 4.064$; $P = 0.0046$). This significant association was driven primarily by the Chilecito population, which had both an extreme monthly average high temperature (31.6°C) and high average survival after heat stress (68.69 ± 2.98%).

Discussion

In this paper we quantified genetic variation in heat tolerance within and among six populations of *D. melanogaster* collected along a latitudinal transect in Argentina. The goal of this study was to quantify the standing levels of genetic variation and thus the general ability of populations to adaptively respond to changes in their climate. We found highly significant variation in mean heat tolerance levels within population and significant variation among populations. The majority of populations exhibited significant variation among lines within each population, suggesting that although five of the six populations are not significantly different at the level of the mean survival after heat

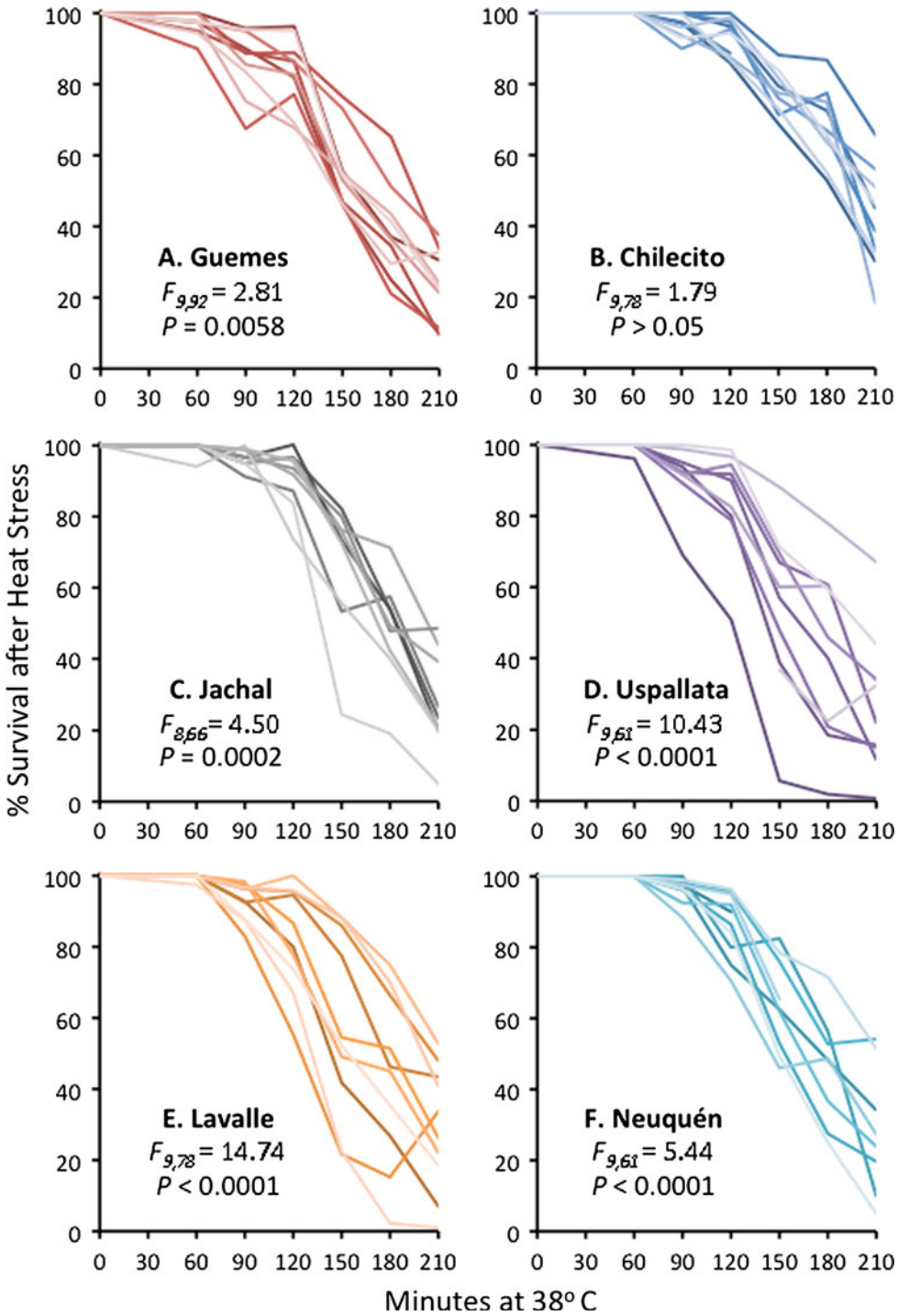

Fig. 3 Line-specific heat survival curves for the six natural populations. Each subpanel (**A**–**F**) is for each of the six populations: **A** Guemes, **B** Chilecito, **C** Jachal, **D** Lavalle, **E** Uspallata, and **F** Neuquén. The *x*-axis denotes the length of the heat exposure (0–210) in minutes, while the *y*-axis is the mean percent survival of each population averaged across lines and sexes. *F* statistics and *P* values are for population specific analyses testing for significant variation among lines at the 180-min exposure time

stress, each population still contains significant genetic variation for heat tolerance. We identified a predicted association between maximum monthly average high temperature and the level of heat tolerance, where populations that encounter the warmest temperatures have the greatest amount of heat tolerance (Fig. 4). That said the Chilecito population drives this association. The combination of reduced variation within Chilecito and significant variation between Chilecito and the other populations provided a compelling pattern that may suggest a response to the extreme monthly average high temperature at this site.

Most surveyed populations of *Drosophila* have variation both within and between them for many traits. Documentation of clinal variation has previously been shown in body size (Gilchrist and Partridge 1999), egg size (Azevedo et al. 1996), cold tolerance (Karan and David 2000; Gibert and Huey 2001; Gibert et al. 2001; Hoffmann et al.

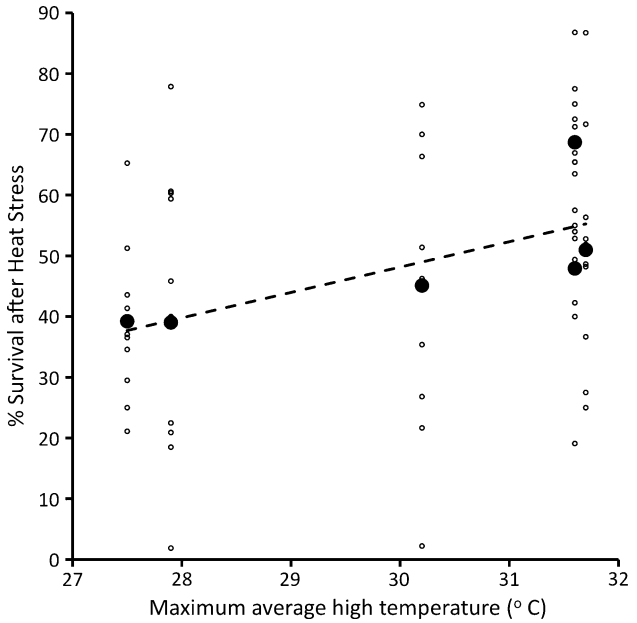

Fig. 4 Association between maximum monthly average high temperature and mean percent survival after heat stress. The *x*-axis is the maximum monthly average high temperature for each of the six populations in degrees Centigrade. While the *y*-axis is the percent survival after 180-min exposure to 38°C (±0.5°C). The small *open circles* are line means and the large *closed circles* are population means

2002; Ayrinhac et al. 2004), and heat tolerance (Rashkovetsky et al. 2006). Populations from many different locations and from several *Drosophila* species have been surveyed and in general, populations often harbor high levels of phenotypic and genetic variation in heat tolerance phenotypes with heritabilities ranging from 0.03 to 0.5 (Jenkins and Hoffmann 1994; Loeschcke and Krebs 1996; Loeschcke et al. 1997). In this study, our heritability estimates (Table 2) were all at the high end of this range (0.150 in Chilecito to 0.567 in Lavalle). These estimates should be treated with some caution because each population contains a maximum of ten lines, which is a small sample size for precise estimates of quantitative genetic parameters. As expected the broad-sense heritability estimates are high in five of the six populations due to the large amounts of within population (among line) phenotypic variation (Table 2).

The lack of phenotypic variation in population Chilecito is curious and can be explained by several possibilities. These include, a recent bottleneck event has reduced variation, the effective population size of Chilecito is small, thus allowing drift to eliminate allelic variation, or genetic variation could have been reduced during adaptation to the environment. The latter explanation may be most reasonable because *Drosophila* populations are continuous over the gradient sampled. In addition, a recent study has found reduced measures of narrow-sense heritability for heat shock in tropical Australian *Drosophila* species (Mitchell and Hoffmann 2010), most likely due to previous local adaptation to constant, high thermal environments. Low narrow-sense heritability is often used as a measure of potential evolvability or potential future local adaptation. Our data suggest, a similar occurrence in the Chilecito population where local adaptation may have reduced genetic variation within this particular population and may limit response to future thermal changes.

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References

Anderson A, Collinge J, Hoffmann AA, Kellett M, McKechnie SW (2003) Thermal tolerance trade-offs associated with the right arm of chromosome 3 and marked by the *hsr-omega* gene in *Drosophila melanogaster*. Heredity 90:195–202

Ayrinhac A, Debat V, Gibert P, Kister A, Legout H, Moreteau B, Vergillino R, David J (2004) Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. Funct Ecol 18:700–706

Azevedo RBR, French V, Partridge L (1996) Thermal evolution of egg size in *Drosophila melanogaster*. Evolution 50:2338–2345

Bubliy OA, Riihimaa A, Norry FM, Loeschcke V (2002) Variation in resistance and acclimation to low temperature stress among three geographical strains of *Drosophila melanogaster*. J Therm Biol 27:237–344

Clarke A (1996) Climate change and animal distributions. In: Johnston IA, Bennett AF (eds) Animals and temperature: phenotypic and evolutionary adaptation. Cambridge University Press, Cambridge, pp 377–407

Cossins AR, Bowler K (1987) Temperature biology of animals. Chapman & Hall, New York

David JR, Capy P (1988) Genetic variation of *Drosophila melanogaster* natural populations. Trends Genet 4:106–111

David JR, Gibert P, Moreteau B, Gilchrist GW, Huey RB (2003) The fly that came in from the cold: geographic variation of recovery time from low-temperature exposure in *Drosophila subobscura*. Funct Ecol 17:425–430

David JR, Gibert P, Legout H, Petavy G, Capy P, Moreteau B (2005) Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations. Heredity 94:3–12

Davidson JK (1990) Nonparallel geographic patterns for tolerance to cold and desiccation in *Drosophila melanogaster* and *Drosophila simulans*. Aust J Zool 38:155–161

Folguera G, Cebalos S, Spezzi L, Fanara JJ, Hasson E (2008) Clinal variation in developmental time and viability, and the response to thermal treatments in two species of *Drosophila*. Biol J Linn Soc 95:233–245

Gibert P, Huey RB (2001) Chill-coma temperature in *Drosophila*: effects of developmental temperature, latitude, and phylogeny. Physiol Biochem Zool 74:429–434

Gibert P, Moreteau B, Petavy G, Karan D, David JR (2001) Chill-coma tolerance, a major climatic adaptation among *Drosophila* species. Evolution 55:1063–1068

Gilchrist GW, Huey RB (1999) The direct response of *Drosophila melanogaster* to selection on knockdown temperature. Heredity 83:15–29

Gilchrist AS, Partridge L (1999) A comparison of the genetic basis of wing size divergence in three parallel body size clines of *Drosophila melanogaster*. Genetics 153:1775–1787

Hoffmann AA, Parsons PA (1991) Evolutionary genetics of environmental stress. Oxford University Press, Oxford

Hoffmann AA, Watson M (1993) Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. Am Nat 142:93–113

Hoffmann AA, Willi Y (2008) Detecting genetic responses to environmental change. Nat Rev Genet 9:421–432

Hoffmann AA, Anderson AR, Hallas R (2002) Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. Ecol Lett 5:614–618

Hoffmann AA, Sorensen JG, Loeschcke V (2003a) Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. J Therm Biol 28:175–216

Hoffmann AA, Hallas RJ, Dean JA, Schiffer M (2003b) Low potential for climatic stress adaptation in rainforest *Drosophila* species. Science 301:100–102

Hoffmann AA, Shirriffs J, Scott M (2005) Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. Funct Ecol 19:222–227

IPCC Report (2007) Climate change 2007: physical science basis. Summary for policy makers approved at the 10th session of Working Group I at the IPCC. IPCC, Paris, Feb 2007

Jenkins NL, Hoffmann AA (1994) Genetic and maternal variation for heat resistance in *Drosophila* from the field. Genetics 137:783–789

Jentsch A, Kreyling J, Beierkuhnlein C (2007) A new generation of climate-change experiments: events, not trends. Front Ecol Environ 5:365–374

Karan D, David JR (2000) Cold tolerance in *Drosophila*: adaptive variations revealed by the analysis of starvation survival reaction norms. J Therm Biol 25:345–351

Lavagnino J, Anholt RRH, Fanara JJ (2008) Variation in genetic architecture of olfactory behavior among wild-derived populations of *Drosophila melanogaster*. J Evol Biol 21:988–996

Leather S, Walter K, Bale J (1993) The ecology of insects overwintering. Cambridge University Press, Cambridge

Loeschcke V, Krebs RA (1996) Selection for heat-shock resistance in larval and in adult *Drosophila buzzattii*: comparing direct and indirect responses. Evolution 50:2354–2359

Loeschcke V, Krebs RA, Dahlgaard J, Michalak P (1997) High-temperature stress and the evolution of thermal resistance in *Drosophila*. EXS 83:175–190

Mitchell KA, Hoffmann AA (2010) Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. Funct Ecol 24:694–700

Morgan TJ, Mackay TFC (2006) Quantitative trait loci for thermotolerance phenotypes in *Drosophila melanogaster*. Heredity 96:232–242

Parsons PA (1977) Resistance to cold temperature stress in populations of *Drosophila melanogaster* and *Drosophila simulans*. Aust J Zool 25:693–698

Potvin C, Tousignant D (1996) Evolutionary consequences of stimulated global change: genetic adaptation or adaptive phenotypic plasticity. Oecologia 108:683–693

Rako L, Blacket MJ, McKechnie W, Hoffmann AA (2007) Candidate genes and thermal phenotypes: identifying ecological important genetic variation for thermotolerance in the Australia *Drosophila melanogaster* cline. Mol Ecol 16:2948–2957

Rashkovetsky E, Iliadi K, Michalak P, Lupu A, Nevo E, Feder ME, Korol A (2006) Adaptive differentiation of thermotolerance in *Drosophila* along a microclimatic gradient. Heredity 96:353–359

Reusch TBH, Wood TE (2007) Molecular ecology of global change. Mol Ecol 16:3973–3992

Rohmer C, David JR, Moreteau B, Joly D (2004) Heat induced male sterility in *Drosophila melanogaster*: adaptive genetic variations among geographic populations and role of the Y chromosome. J Exp Biol 207:2735–2742

SAS Institute (2009) SAS/STAT 9.2 user's guide, 2nd edn. SAS Institute, Cary

Stanley SM, Parsons PA (1981) The response of the cosmopolitan species, *Drosophila melanogaster*, to ecological gradients. Proc Ecol Soc Aust 11:121–130

Umina PA, Weeks AR, Kearney MR, McKechnie SW, Hoffmann AA (2005) A rapid shift in a classic clinal pattern in *Drosophila* reflecting climate change. Science 308:691–693

Zhen Y, Ungerer MC (2008) Clinal variation in freezing tolerance among natural accessions of *Arabidopsis thaliana*. New Phytol 177:419–427