

Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically

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Upper thermal limits vary less than lower limits among related species of terrestrial ectotherms. This pattern may reflect weak or uniform selection on upper limits, or alternatively tight evolutionary constraints. We investigated this issue in 94 *Drosophila* species from diverse climates and reared in a common environment to control for plastic effects that may confound species comparisons. We found substantial variation in upper thermal limits among species, negatively correlated with annual precipitation at the central point of their distribution and also with the interaction between precipitation and maximum temperature, showing that heat resistance is an important determinant of *Drosophila* species distributions. Species from hot and relatively dry regions had higher resistance, whereas resistance was uncorrelated with temperature in wetter regions. Using a suite of analyses we showed that phylogenetic signal in heat resistance reflects phylogenetic inertia rather than common selection pressures. Current species distributions are therefore more likely to reflect environmental sorting of lineages rather than local adaptation. Similar to previous studies, thermal safety margins were small at low latitudes, with safety margins smallest for species occupying both humid and dry tropical environments. Thus, species from a range of environments are likely to be at risk owing to climate change. Together these findings suggest that this group of insects is unlikely to buffer global change effects through marked evolutionary changes, highlighting the importance of facilitating range shifts for maintaining biodiversity.

niche conservatism | stress resistance | thermal adaptation | evolutionary history | space

Temperatures are expected to rise across the globe over the coming decades and centuries (1), and many studies suggest the potential for range shifts/reductions in many species (2). When assessing the likely impact of temperature changes on species survival, an implicit assumption is that the thermal environment shapes resistance to temperature extremes and thus dictates species range limits. Nevertheless, few studies have directly tested for such links between physiological upper thermal limits of ectothermic species and temperature conditions within their geographic range (3, 4). A related, little-investigated key point for predicting species range responses to climate change is whether upper thermal limits are modifiable through plastic and/or evolutionary responses (5, 6). Ideally, evolutionary and plastic responses within and across generations, including short-term hardening and acclimation, should be separated, as through a common garden approach whereby species are kept under controlled laboratory conditions (7, 8). Without controlling for plastic responses, it is not possible to distinguish adaptive evolutionary responses, and species may erroneously seem close to their upper thermal thresholds (9–12), biasing extinction risk estimates.

Furthermore, by examining the evolution of upper thermal limits (heat resistance) across a known phylogeny, it is possible to determine when trait limits related to evolutionary history have arisen, which will aid in predicting potential evolutionary response

to climate change (13, 14). In the past phylogenetic analyses have mainly aimed to control for the effects of phylogeny (4, 15, 16); however, studies are now emerging that examine phylogenetic signal with the purpose of determining the role of adaptation vs. constraints (17–20).

In the present study we undertake a large and rigorous evaluation of heat resistance (estimated as critical thermal maxima) in 94 *Drosophila* species. We examine the link between climate conditions within species ranges and heat resistance as well as safety margins, and assess the relative contribution of phylogenetic inertia and common selection pressures in resistance variation using recently developed approaches (18, 21). Related species may exhibit similar heat resistance (phylogenetic signal) owing to either evolutionary phylogenetic constraints (phylogenetic inertia) or spatial proximity; the latter may result in species being similar owing to common selection regimes (13, 17, 18, 21). We further examined the question of which species groups are likely to be more threatened by global climate change. Both mean temperatures and temperature extremes are expected to increase under the prevailing climate change scenarios (1), but the impact of these changes on species performances and distributions is still unclear. Previous studies comparing upper thermal limits of species provided conflicting geographical differences in thermal safety margins, with some finding these to be smaller for species from tropical regions (10, 22, 23) and others finding species from temperate or dry environments to be closer to their thermal maxima (11, 16, 24).

Results

The positive association between heat resistance and average and maximal temperature was generally weak ($R^2 < 0.05$; Table S1; nonphylogenetic analysis), whereas heat resistance was more strongly negatively associated with annual precipitation (P_{ANN}) ($R^2 = 0.18–0.20$) (Fig. S1 and Table S1). Combining temperature and precipitation into the variable drying power of air improved this relationship only slightly ($R^2 = 0.21–0.22$) (Table S1). Using a multiple regression approach, a stronger association with climate emerged. The best multiple predictor model included P_{ANN} and maximum temperature of the warmest month (T_{max}) for females ($R^2 = 0.29$, $P < 0.01$) and P_{ANN} , T_{max} , and precipitation of the driest month (P_{DRY}) ($R^2 = 0.32$, $P < 0.01$) for males (Table S1).

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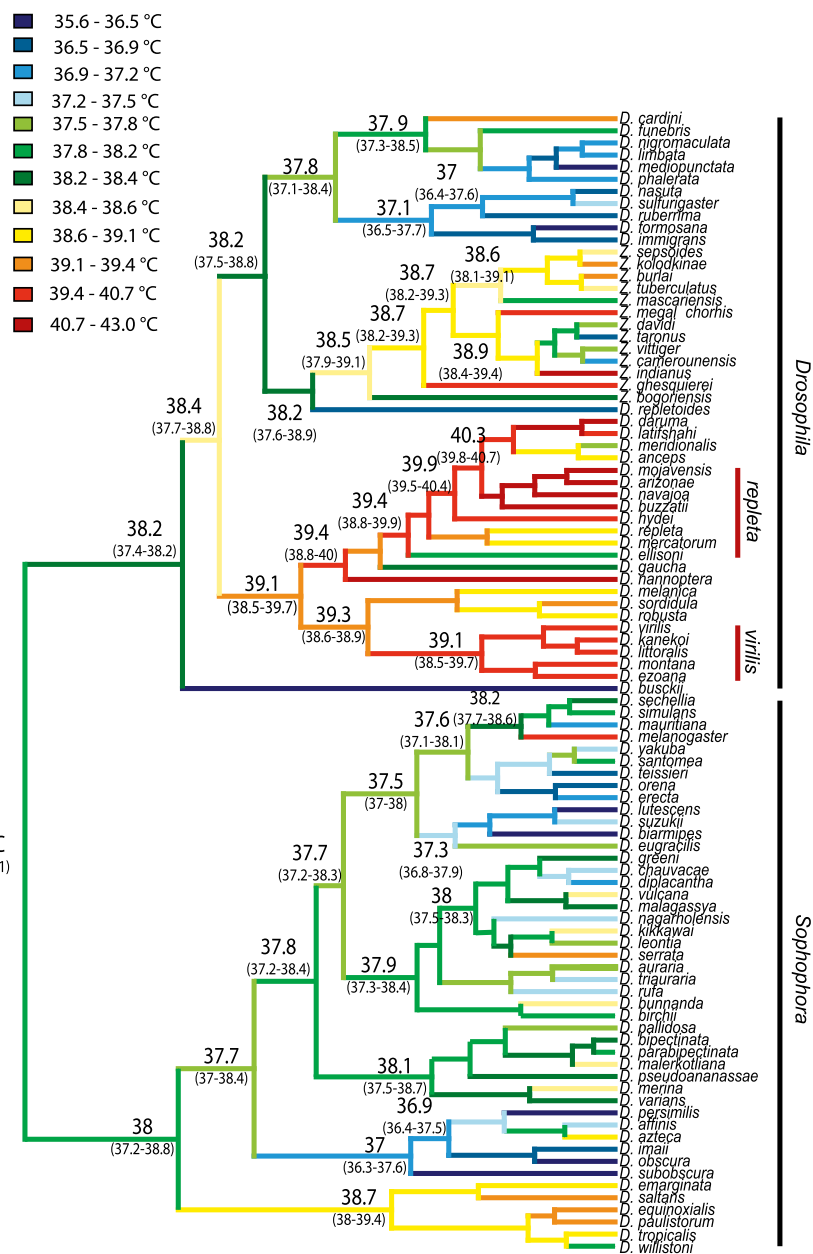


Fig. 1. Phylogenetic hypothesis for 94 *Drosophila* species. CT_{max} was mapped onto the phylogeny using ancestral trait reconstruction via maximum likelihood; the 95% confidence intervals are shown in parenthesis. Branches were color-coded according to their likelihood states; color groups were determined by dividing all 94 species into 12 equal-sized groups.

Although the exact reason for this relationship is unclear, it may reflect the extent of canopy cover being linked to the amount of precipitation (25, 26). It is unlikely that small insects like drosophilids use evaporative cooling in any significant way, but humid environments are likely to give rise to a more diverse array of microclimates where flies might seek shelter when temperatures are high (i.e., by streams, under leaves) (29, 30). In drier regions it will be more challenging for the flies to find a suitable microhabitat during warm days; as a consequence they may be unable to behaviorally thermo-regulate, relying on innate heat resistance (31, 32). Thus, it would seem that warm and dry environments select for increased heat tolerance in ectotherms like *Drosophila*. In lizard species there is also an association between optimal body temperature and both the temperature and annual precipitation of a particular location (11, 16).

We detected moderate to strong phylogenetic signal for heat resistance. This was further supported by the ancestral reconstruction showing that highly heat resistant phenotypes have evolved rarely across the *Drosophila* phylogeny and within specific species groups (Fig. 1). There was little evidence for spatial associations driving common adaptations in closely related species; instead, phylogenetic signal in heat resistance was related to phylogenetic inertia (Table 2). Hence, drosophilid species have only limited evolutionary capacity to evolve and alter upper thermal limits. In line with this finding, recent studies have suggested that the ability of terrestrial insect species to evolve higher heat resistance may be limited; there is limited variance in maximal heat resistance (3), and experimental studies suggest low evolutionary potential for different estimates of resistance (33, 34). This may indicate that substantial changes at the molecular level are

Table 2. Phylogenetic association in the relationship between CT_{max} and T_{max} and P_{ANN}

Trait and predictor	$t_{1/2}$	Optimal regression		Evolutionary regression		r^2	AIC
		Intercept	Slope	Intercept	Slope		
CT _{max} : ♀	0.58 (0.27-∞)	38.15 ± 0.57	—	—	—	—	295.70
T _{max}	0.51 (0.25-∞)	35.97 ± 1.46	0.17 ± 0.11	35.98 ± 1.47	0.08 ± 0.05	0.03	295.53
P _{ANN}	0.55 (0.25-∞)	44.19 ± 2.46	-4.46 ± 1.79	44.47 ± 2.56	-2.01 ± 0.81	0.06	292.35
T _{max}	0.46 (0.24-∞)	42.23 ± 2.76	0.19 ± 0.10	42.66 ± 2.76	0.10 ± 0.05	0.10	291.18*
+ P _{ANN}			-4.50 ± 1.60		-2.41 ± 0.82		
CT _{max} : ♂	0.45 (0.22-∞)	38.13 ± 0.43					300.12
T _{max}	0.45 (0.22-∞)	34.89 ± 1.47	0.23 ± 0.10	34.89 ± 1.47	0.11 ± 0.05	0.05	297.57
P _{ANN}	0.40 (0.22-∞)	43.81 ± 2.59	-3.45 ± 1.56	44.05 ± 2.67	-1.8 9 ± 0.84	0.05	297.92
P _{DRY}	0.40 (0.20-∞)	38.44 ± 0.45	-0.01 ± 0.01	38.44 ± 0.45	-0.008 ± 0.01	0.02	300.65
T _{max}	0.38 (0.20-∞)	41.11 ± 2.72	0.24 ± 0.09	41.61 ± 2.80	0.14 ± 0.05	0.12	293.64*
+ P _{ANN}			-3.98 ± 1.48		-2.38 ± 0.84		
T _{max}	0.44 (0.20-∞)	41.61 ± 2.76	0.27 ± 0.10	42.30 ± 2.87	0.16 ± 0.05	0.13	295.95
+ P _{ANN}			-4.72 ± 1.70		-2.91 ± 0.98		
+ P _{DRY}			0.01 ± 0.01		0.01 ± 0.01		

The SLOUCH method estimates the degree of phylogenetic inertia in heat resistance. The phylogenetic $t_{1/2}$ (in units of tree height, i.e., 1) and the two-unit support surface (in parentheses) are shown. The optimal regression describes the relationship between heat resistance and the predictor variables without the influence of ancestry (phylogeny), whereas the evolutionary regression controls for any phylogenetic association. The r^2 value describes the variance in heat resistance while controlling for phylogeny.

*Model with the best AIC.

required to alter upper thermal limits, such as by duplications of heat shock genes (35). In contrast, there seems to be more variation among ectotherms for lower thermal limits (36). The results for both cold and heat resistance are indicative of environmental sorting, with species moving into environments where they are preadapted, rather than having selection shape resistance levels. However, the ability of flies to avoid heat stress through behavioral responses (37) was not considered here.

By computing safety margins, recent research has suggested that species from low tropical latitudes are at greater risk from climate change (10, 11, 22, 23). In the present study, when maximal temperatures were considered, species occupying low latitudinal environments had the smallest safety margins, particularly for species occupying latitudes from 10° to 23° in the northern hemisphere. Similar to Sunday et al. (22), northern hemisphere species were closer to their thermal limits than those from the southern hemisphere (Fig. 2 and Table S2). When dividing species belonging to humid tropical and dry tropical environments according to annual precipitation ($\geq 1,500$ mm annual precipitation = humid tropical), the smallest safety margins were found for species

occupying both wet and dry environments, highlighting that species not from humid tropical environments will also be at risk (Fig. 2) (11, 16).

In this study we did not examine plastic effects but instead controlled for phenotypic plasticity to reveal the genetic component of heat resistance. In *Drosophila* the time to knockdown during a heat exposure has been shown to respond to both developmental acclimation and short-term hardening treatments (5, 38). However, a recent study found that developmental acclimation and short-term plastic responses increased upper thermal limits by less than 1 °C across both tropical and temperate *Drosophila* species (39). This finding suggests that plastic responses may be small and vary little across species when measured as a tolerance temperature rather than a tolerance time. A low capacity to respond plastically to temperature changes combined with a high level of phylogenetic inertia suggest that most species within the *Drosophila* phylogeny are unlikely to increase their upper thermal limits via plastic and/or evolutionary responses.

Our results could be influenced by inbreeding and laboratory adaptation because many species tested were represented by

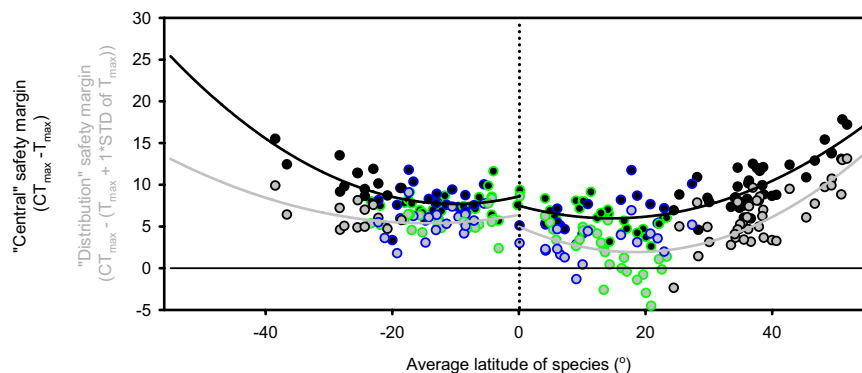


Fig. 2. Species and distribution safety margins. The “Central” species safety margins were calculated as the difference between heat tolerance (CT_{max}) and the average of maximal environmental temperature (T_{max}) (filled black circles). To include the species-specific populations that experience more severe heat stress we also calculated the “distribution” safety margins using the assumption that these populations experience a T_{max} equivalent to average + SD of T_{max} . Species present on both hemispheres are represented for the northern and southern hemisphere. The species are separated [temperate (black edge), humid tropical (green edge), and dry tropical (blue edge)] according to their average environmental habitat (see text).

strains from stock centers. Inbreeding effects on CT_{max} tend to be minor across *Drosophila* species (40), whereas the impact of laboratory adaptation is less certain (41, 42). Nevertheless, in our opinion these confounding effects will be minor relative to heat resistance variation found between species.

CT_{max} as estimated in the present study may be confounded by effects of desiccation and starvation (assays are performed without food or moisture for ~3 h) as discussed recently in the literature (43, 44). This might inflate estimates of CT_{max} particularly for highly desiccation/starvation-resistant species, but recent experiments on *Drosophila melanogaster* have found the impact of desiccation and starvation stress to be negligible with respect to CT_{max} when using heat exposures similar to the one used here (45). Furthermore, Mitchell et al. (33) have shown that static and ramping estimates of heat resistance correlate strongly across tropical and widespread *Drosophila*. Although a number of factors may influence the results of this study, we consider these effects to be small and unlikely to influence the conclusions of this study.

This study highlights three points. First, there is a high level of phylogenetic inertia in heat resistance. Second, in relatively drier environments there is a correlation between species resistance and T_{max} , suggesting that upper thermal tolerance limits (measured under environmentally controlled conditions) can influence species range limits. Finally, drosophilid species across a range of environments, not only humid tropical species, are living in environments where maximal environmental temperature approaches tolerance limits; with limited evolutionary potential this raises concerns about the ability of species to counter climate change (3, 16). Thus, although studies of genetic variation and geographic variability in heat resistance point to some evolutionary adaptive potential (27, 33), this may not be enough under current Intergovernmental Panel on Climate Change scenarios.

Materials and Methods

Sources and Maintenance of Experimental Animals. *Drosophila* stocks were obtained from five sources and maintained at 200 individuals for a minimum of two generations before testing. All flies were reared and tested in the same laboratory, under identical conditions. For a more detailed explanation of stocks and experimental methods, see ref. 20. Briefly, flies were reared at 20 °C at a 12-h/12-h light/dark cycle and maintained on an oat-based medium (Leeds); some species, however, required the addition of *Opuntia* cactus and banana to medium. Experimental flies were controlled for larval density and age effects and prepared for trait measurements (20). For each species we examined 10 flies per sex unless otherwise stated. Although the number of individuals measured for heat resistance was low, the variance was small for both females and males (Table S3). Heat resistance was estimated as CT_{max} under gradual heating. Individuals were placed into empty 5-mL glass vials and placed into a water bath set at 20 °C. The temperature was gradually increased at 0.1 °C/min, and the temperature at which a fly lost the ability to move, after tapping on the vial, was scored as CT_{max} .

Estimations of Climatic Variables. The distribution of each species was collated from the taxodros Web site, which provides global positioning system coordinates of published *Drosophila* collections (<http://www.taxodros.uzh.ch/>). Duplicate records in terms of species, latitude, and longitude (with one-decimal precision) were removed. Climatic variables from the WorldClim data set (www.worldclim.org) (46) were extracted to these coordinates. Five temperature and precipitation variables thought to be related to heat resistance were chosen: (i) annual mean temperature and (ii) absolute maximal temperature of the warmest month (T_{max}) are the most likely candidates for shaping the evolution of heat resistance, whereas (iii) absolute minimum temperature of the coldest month (T_{min}) was chosen because there may be a tradeoff between cold and heat resistance. Finally, precipitation related variables (iv) annual precipitation (P_{ANN}) and (v) precipitation of the driest month (P_{DRY}) have also been linked to upper thermal limits (16). Average values were calculated from the extracted climatic data for each species. We included a sixth variable that combined precipitation and temperature into the metric, drying power of air (dpa), in which the water saturation deficit between animal and environment is estimated using temperature and precipitation variables (20). All geographic information system (GIS) operations were performed in ArcGIS 10 (ESRI).

To examine whether spatial sampling bias existed in our climatic averages, we created an approximate 100 × 100-km grid (in Behrmann equal area projection). For all occurrence records within the same grid cell, we calculated the median of the climatic values. The grid cell medians were then used to calculate average climatic data for each species. A regression of the species average climatic values using the median values against the species average climatic values, as opposed to using all observations, demonstrated a high association ($r^2 > 0.95$), suggesting minor sampling bias, and thus analyses are based on climate data from all observations.

To assess current proximity of a species to stressful environmental temperatures, we estimated safety margins for the center point of a species distribution (central safety margin), calculated as the difference in heat resistance (CT_{max}) from the average of the absolute maximal temperature of the environment (T_{max}). To obtain the proximity of the “threatened” populations (distribution safety margins) we used the assumption that such populations experience higher heat stress, and we therefore estimated safety margins by comparing CT_{max} of the species to the average + 1 SD of maximal environmental temperature for this species. Thus, the estimated distribution of safety margins takes into account that, because averages of T_{max} were generated from many observations for some species, local populations will experience T_{max} considerably higher than the average for this species. To investigate safety margins across species groups sharing similar environments, we divided species relative to the average latitude, temperature, and precipitation of species. Species having a latitude >23° were deemed temperate, whereas tropical species (<23° latitude) were split into dry tropical ($P_{ANN} < 1,500$ mm) or humid/wet tropical ($P_{ANN} \geq 1,500$ mm).

Construction of Phylogeny. We combined previously published phylogenies to generate a *Drosophila* phylogeny incorporating all 94 species, with the basis of the phylogeny taken from ref. 47; see ref. 20 for details.

Mapping Traits and Ecological Variables onto the Phylogeny. Phylogenetic signal was estimated using Pagel's λ , Bloomberg's K , and Moran's I (48–50). Pagel's λ was estimated for the residuals with a comparison of the Akaike information criteria (AIC) to determine the best model, with λ_0 (H_0 = no phylogenetic signal) and λ_1 (H_a = phylogenetic signal). A value of K not significantly different from 0 indicates the absence of phylogenetic signal, whereas $K = 1$ indicates that the trait is evolving under a Brownian motion model of evolution with significant phylogenetic signal. These and other methods, unless otherwise stated, were implemented in R using the ape and picante packages (51–53). Moran's I computes the phylogenetic autocorrelation of the data at different taxonomic levels and was estimated using the ape package (48). Here we used the taxonomic divisions from the taxodros Web site (www.taxodros.uzh.ch/), dividing species into three levels: subgenus, species group, and species subgroup (20).

Trait Analyses. Nonphylogenetic least-squares regression was applied to determine the association between heat resistance and ecological variables using the Poptools add-in for Microsoft Excel (54). Initially, the relationship between heat resistance and climatic variables was estimated singularly with the lm() function in R. To examine a multiple predictor model, variables with the lowest AIC were then added sequentially, the initial model having the climatic variable with the lowest AIC. Climatic variables with the lowest AIC were also the least autocorrelated. Consequently, of the seven climatic variables considered, the multiple predictor models included P_{ANN} , T_{max} , and P_{DRY} . Because dpa was calculated from both precipitation and temperature, this variable was not included in the multiple predictor model. Ancestral trait reconstruction was used to examine how heat resistance has evolved across the phylogeny, based on maximum likelihood methods on continuous characters with the ace function in the ape package (51). Phylogenetic analyses are only presented on environmental variables explaining the largest proportion of variation in resistance.

Phylogenetic Signal: Adaptation vs. Inertia. The relationship between spatial patterns and heat resistance was estimated by regressing distance matrices of heat resistance onto spatial relatedness. Distance matrices were calculated for heat resistance using the dist() function in R (based on Euclidean distances). Spatial distance matrices were generated by initially calculating the longitude and latitude midpoint of a species distribution, and using the fossil package a distance matrix in kilometers was calculated between each species midpoint (55).

The SLOUCH v1.1 program models the evolution of traits according to an Ornstein-Uhlenbeck process (21). The speed at which phylogenetic covariances decay with phylogenetic distance, the phylogenetic $t_{1/2}$, provides an estimate of the level of phylogenetic signal within a dataset. Increasing values of $t_{1/2}$

suggest an increasing association between the trait and phylogeny, with $t_{1/2} = 0$ no association and $t_{1/2} > 1$ a high phylogenetic signal (this value depends on the height of a phylogenetic tree; here the total tree height = 1). Initially regressions are fitted with only the trait values to estimate phylogenetic signal within the data. The inclusion of the predictor variables (T_{\max} and P_{ANN}) allows for estimation of phylogenetic inertia. This method also permits inclusion of multiple predictors such as $T_{\max} \times P_{\text{ANN}}$, and the analysis also provides a phylogenetically corrected r^2 value (21). For a more detailed description see ref. 20.

- Intergovernmental Panel on Climate Change (2007) *Climate Change 2007: Synthesis Report* (Intergovernmental Panel on Climate Change, Geneva) p 52.
- Thomas CD, et al. (2004) Extinction risk from climate change. *Nature* 427:145–148.
- Addo-Bediako A, Chown SL, Gaston KJ (2000) Thermal tolerance, climatic variability and latitude. *Proc R Soc B Biol Sci* 267:739–745.
- Kimura MT (2004) Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia* 140:442–449.
- Hoffmann AA, Sørensen JG, Loeschke V (2003) Adaptation of *Drosophila* to temperature extremes: Bringing together quantitative and molecular approaches. *J Therm Biol* 28:175–216.
- Fischer K, et al. (2010) Environmental effects on temperature stress resistance in the tropical butterfly *Bicyclus anynana*. *PLoS ONE* 5:e15284.
- Jenkins NL, Hoffmann AA (1994) Genetic and maternal variation for heat resistance in *Drosophila* from the field. *Genetics* 137:783–789.
- Garland T, Adolph SC (1991) Physiological differentiation of vertebrate populations. *Annu Rev Ecol Syst* 22:193–228.
- Stillman JH, Somero GN (2000) A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: Influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiol Biochem Zool* 73:200–208.
- Deutsch CA, et al. (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proc Natl Acad Sci USA* 105:6668–6672.
- Huey RB, et al. (2009) Why tropical forest lizards are vulnerable to climate warming. *Proc R Soc B Biol Sci* 276:1939–1948.
- Duarte H, et al. (2012) Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Glob Change Biol* 18:412–421.
- Kellermann V, van Heerwaarden B, Sgrò CM, Hoffmann AA (2009) Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. *Science* 325:1244–1246.
- Wiens JJ, et al. (2010) Niche conservatism as an emerging principle in ecology and conservation biology. *Ecol Lett* 13:1310–1324.
- Nyamukondiwa C, Terblanche JS, Marshall KE, Sinclair BJ (2011) Basal cold but not heat tolerance constrains plasticity among *Drosophila* species (Diptera: Drosophilidae). *J Evol Biol* 24:1927–1938.
- Clusella-Trullas S, Blackburn TM, Chown SL (2011) Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *Am Nat* 177:738–751.
- Cooper N, Freckleton RP, Jetz W (2011) Phylogenetic conservatism of environmental niches in mammals. *Proc R Soc B Biol Sci* 278:2384–2391.
- Freckleton RP, Jetz W (2009) Space versus phylogeny: Disentangling phylogenetic and spatial signals in comparative data. *Proc R Soc B Biol Sci* 276:21–30.
- Willis CG, Ruhfel B, Primack RB, Miller-Rushing AJ, Davis CC (2008) Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proc Natl Acad Sci USA* 105:17029–17033.
- Kellermann V, et al. Phylogenetic constraints in key functional traits behind species' climate niches: Patterns of desiccation and cold resistance across 95 *Drosophila* species. *Evolution*. 10.1111/j.1558-5646.2012.01685.x.
- Hansen TF, Pienaar J, Orzack SH (2008) A comparative method for studying adaptation to a randomly evolving environment. *Evolution* 62:1965–1977.
- Sunday JM, Bates AE, Dulvy NK (2011) Global analysis of thermal tolerance and latitude in ectotherms. *Proc R Soc B Biol Sci* 278:1823–1830.
- Diamond SE, et al. (2012) Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Glob Change Biol* 18:448–456.
- Bonebrake TC, Mastrandrea MD (2010) Tolerance adaptation and precipitation changes complicate latitudinal patterns of climate change impacts. *Proc Natl Acad Sci USA* 107:12581–12586.
- Greve M, Lykke AM, Blach-Overgaard A, Svenning JC (2011) Environmental and anthropogenic determinants of vegetation distribution across Africa. *Glob Ecol Biogeogr* 20:661–674.
- Hutley LB, et al. (2011) A sub-continental scale living laboratory: Spatial patterns of savanna vegetation over a rainfall gradient in northern Australia. *Agric For Meteorol* 151:1417–1428.
- Sgrò CM, et al. (2010) A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia. *J Evol Biol* 23:2484–2493.
- Stratman R, Markow TA (1998) Resistance to thermal stress in desert *Drosophila*. *Funct Ecol* 12:965–970.
- Willmer PG (1982) Microclimate and the environmental physiology of insects. *Adv Insect Physiol* 16:1–57.
- Pincebourde S, Woods HA (2012) Climate uncertainty on leaf surfaces: The biophysics of leaf microclimates and their consequences for leaf-dwelling organisms. *Funct Ecol* 26:844–853.
- Parsons PA (1978) Habitat selection and evolutionary strategies in *Drosophila*: An invited address. *Behav Genet* 8:511–526.
- Huey RB, Pascual M (2009) Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. *Ecology* 90:1715–1720.
- 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.
- Gilchrist GW, Huey RB (1999) The direct response of *Drosophila melanogaster* to selection on knockdown temperature. *Heredity (Edinb)* 83:15–29.
- Bettencourt BR, Feder ME (2001) Hsp70 duplication in the *Drosophila melanogaster* species group: How and when did two become five? *Mol Biol Evol* 18:1272–1282.
- Chown SL, Addo-Bediako A, Gaston KJ (2002) Physiological variation in insects: Large-scale patterns and their implications. *Comp Biochem Physiol B Biochem Mol Biol* 131:587–602.
- Marais E, Chown SL (2008) Beneficial acclimation and the Bogert effect. *Ecol Lett* 11:1027–1036.
- Levins R (1969) Thermal acclimation and heat resistance in *Drosophila* species. *Am Nat* 103:483.
- Overgaard J, Kristensen TN, Mitchell KA, Hoffmann AA (2011) Thermal tolerance in widespread and tropical *Drosophila* species: Does phenotypic plasticity increase with latitude? *Am Nat* 178(Suppl 1):S80–S96.
- Kristensen TN, et al. (2011) No inbreeding depression for low temperature developmental acclimation across multiple *Drosophila* species. *Evolution* 65:3195–3201.
- Griffiths JA, Schiffer M, Hoffmann AA (2005) Clinal variation and laboratory adaptation in the rainforest species *Drosophila birchii* for stress resistance, wing size, wing shape and development time. *J Evol Biol* 18:213–222.
- Cavicchi S, Guerra D, Latorre V, Huey RB (1995) Chromosomal analysis of heat shock tolerance in *Drosophila melanogaster* evolving at different temperatures in the laboratory. *Evolution* 49:676–684.
- Terblanche JS, et al. (2011) Ecologically relevant measures of tolerance to potentially lethal temperatures. *J Exp Biol* 214:3713–3725.
- Rezende EL, Tejedo M, Santos M (2011) Estimating the adaptive potential of critical thermal limits: Methodological problems and evolutionary implications. *Funct Ecol* 25:111–121.
- Overgaard J, Kristensen TN, Sørensen JG (2012) Validity of thermal ramping assays used to assess thermal tolerance in arthropods. *PLoS ONE* 7:e32758.
- Hijmans RJ, et al. (2005) Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978.
- van der Linde K, Houle D, Spicer GS, Stepan SJ (2010) A supermatrix-based molecular phylogeny of the family Drosophilidae. *Genet Res (Camb)* 92:25–38.
- Gittleman JL, Kot M (1990) Adaptation statistics and a null model for estimating phylogenetic effects. *Syst Zool* 39:227–241.
- Pagel M (1999) Inferring the historical patterns of biological evolution. *Nature* 401:877–884.
- Blomberg SP, Garland T, Jr., Ives AR (2003) Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* 57:717–745.
- Paradis E, Claude J, Strimmer K (2004) APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289–290.
- Kembel SW, et al. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:1463–1464.
- Team RDC (2011) The R environment for statistical computing and graphics. Version 2.6.2. <http://www.r-project.org>. Accessed March 1, 2010.
- Hood GM (2010) PopTools version 3.2.3. <http://www.poptools.org>. Accessed March 1, 2010.
- Vavrek MJ (2011) fossil: Palaeoecological and palaeogeographical analysis tools. *Palaeontol Electronica* 14:1T.