

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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Notably, heat resistance increased as precipitation decreased, suggesting that factors related to water in the environment are more important in driving upper thermal limits than high temperature alone. For *Drosophila* species, in which behavioral thermal regulation is likely to play a key role in avoiding heat stress, we predict that low canopy-cover environments will select for higher heat resistance owing to less microclimatic heterogeneity and thus smaller scope for thermal refuges. Because decreased canopy cover in warm regions coincides with $P_{ANN} < 1,000$ mm (25, 26), we divided the species into low annual precipitation ($< 1,000$ mm) and high annual precipitation ($\geq 1,000$ mm) groups. A strong relationship between heat resistance and T_{max} was detected for species occupying dry environments ($< 1,000$ mm annual precipitation; $R^2 = 0.44$, slope = 0.30 °C/°C, $P = 0.001$), whereas there was no relationship for species occupying wet environments ($\geq 1,000$ mm annual precipitation; $R^2 = 0.008$, slope = 0.05 °C/°C, $P = 0.44$) (Fig. S2; species occupying environments $\geq 1,000$ mm of annual precipitation include both tropical and subtropical species).

Estimates of phylogenetic signal were moderate, with both Pagel's λ and Bloomberg's K significantly different from both 0 and 1 (Table 1). These results suggest that heat resistance is neither evolving completely in accordance with Brownian motion nor evolving free from phylogenetic associations. A third estimate of phylogenetic signal, the SLOUCH analysis, further confirmed these results, with moderate to high levels of phylogenetic signal detected (see $t_{1/2}$ estimate in Table 1). Using Moran's I we estimated phylogenetic signal at three taxonomic levels and found only a weak association at the subgenus level, with correlations increasing between the species group and species subgroup levels, indicating that heat resistance has arisen relatively late in the phylogeny (Fig. 1 and Table 1). In comparison with the observed range of heat resistance across species, the ancestral state was low to moderate heat resistance. This further supports the more recent evolution of heat resistance within the *Drosophila* phylogeny (Fig. 1). Importantly, highly heat-resistant phenotypes were tightly clustered, with only a few groups of related species having evolved a critical thermal maximum (CT_{max}) greater than 39 °C (Fig. 1). The *virilis* and *repleta* species group were most notable, with phenotypes > 1.5 °C higher than the mean heat resistance across all species (*virilis* $\bar{x} = 39.98$ °C ± 0.09 , *repleta* $\bar{x} = 40.06$ °C ± 0.19 , overall $\bar{x} = 38.32$ °C ± 0.15) (Fig. 1). These results suggest that high heat resistance has evolved rarely within drosophilids.

Table 1. Phylogenetic signal in CT_{max} estimated using alternative methods

Variable	λ	AIC _c	K	SLOUCH $t_{1/2}$ (trait)	Moran's I		
					SubG	SppG	SubSppG
CT_{max} ♀	0.69	288.94	0.36*	0.58 (0.27-∞)	0.05	0.33*	0.42*
	λ_0	315.47					
	λ_1	295.77					
CT_{max} ♂	0.64	291.86	0.35*	0.45 (0.22-∞)	0.08†	0.27*	0.40*
	λ_0	314.40					
	λ_1	299.74					

Estimates of phylogenetic signal for λ and K range from no phylogenetic signal with λ_0 and $K = 0$, to high phylogenetic signal with λ_1 and $K \geq 1$. The appropriate λ model is chosen by comparing the AIC_c with λ_0 or λ_1 . The SLOUCH phylogenetic $t_{1/2}$ estimates the association between phylogeny and heat resistance, where $t_{1/2} = 0$ reflects no phylogenetic signal in the trait of interest, and an increasing $t_{1/2}$ reflects a stronger relationship, with $t_{1/2} = 1$ reflecting strong phylogenetic signal. The two-unit support surface is shown in parentheses. Moran's I characterizes the level of phylogenetic signal across three taxonomic levels (SubG, subgenus; SppG, species group; and SubSppG, subspecies group) using a phylogenetic autocorrelation method.

*Significant at the 0.001 level.

†Significant at the 0.01.

Strong phylogenetic signal need not reflect evolutionary constraints but could alternatively be driven by similar selection pressures in similar environments occurring in spatial proximity (phylogenetic structured adaptation) (18). Here we tested for such spatial association by examining the relationship between levels of heat resistance and species' spatial proximity (distance in kilometers between the midpoint of each species' distribution), with the hypothesis that this relationship should be strong if closely related species share similar adaptations because they are closely associated spatially. A significant association between spatial parameters and heat resistance was found, but this explained $< 1\%$ of variation in heat resistance (females: $R^2 < 0.01$, slope = 2.30 km/°C, $P < 0.01$; males: $R^2 < 0.01$, slope = 2.45 km/°C, $P < 0.01$). An alternative analysis, testing qualitatively similar ideas, determines the degree to which heat resistance is structured across the phylogeny, estimating the covariance between phylogeny, heat resistance, and climatic variables as the phylogenetic $t_{1/2}$ ($t_{1/2}$ is equal to total tree height = 1, thus a $t_{1/2} > 1$ reflects a strong association between phylogeny and heat resistance) (Table 2, SLOUCH) (24). Strong to moderate phylogenetic signal (estimated from the relationship between phylogeny and CT_{max}) was detected for heat resistance ($t_{1/2} = 0.45$ – 0.58). With the inclusion of the predictor variables P_{ANN} and T_{max} , a weaker albeit still-strong association ($t_{1/2} = 0.38$ – 0.46) was found between heat resistance, predictors, and phylogeny, suggesting that most phylogenetic signal in heat resistance was related to phylogenetic inertia. These results coupled with only weak associations between heat resistance and spatial proximity suggest phylogenetic signal driven by phylogenetic inertia rather than phylogenetically structured adaptation.

One way to predict responses to climate change is to assess how close species upper thermal limits are to the current environment (safety margin: difference between heat exposure and heat resistance) (10). Both the species-level safety margins and distributional safety margin (safety margin plus 1 SD) were lowest for species occupying latitudinal environments between 10° and 23° , particularly within the northern hemisphere (Fig. 2 and Table S2). Moreover, we also found a significant polynomial relationship between latitude and T_{max} (northern hemisphere: $r^2 = 0.64$, $P < 0.01$; southern hemisphere: $r^2 = 0.41$, $P < 0.01$). Removal of outlier data points around the equator did not influence the strength of the polynomial relationship. Safety margins for species occupying northern latitudes were smaller than for those in the southern hemisphere (Fig. 2 and Table S2). The species closest to their safety margin (between latitudes 10° and 23°) represented species from both humid tropical and drier tropical environments (with $< 1,500$ mm annual precipitation).

Discussion

Given that drosophilids are ectothermic, it is perhaps surprising to find only a weak association between species' heat resistance and measures of environmental temperature across their range. This observation is in line with a previous comparative study on *Drosophila*, which also failed to find an overall association between heat resistance and latitude (4). Studies of intraspecific variation in *Drosophila* are also equivocal, with some finding significant associations between measures of heat resistance and climatic variables/latitude, whereas others have not (27). Consistent with evidence that *Drosophila* species from desert environments tend to be more heat resistant than mesic temperate species (28), we found a positive correlation between CT_{max} and environmental temperature in dry environments. The association between heat resistance and precipitation was such that temperature played a larger role in shaping heat resistance in dry environments ($< 1,000$ mm annual precipitation) and not at all in wet environments ($\geq 1,000$ mm annual precipitation). Thus, instead of temperature playing an overarching role, we found that precipitation was more important and that a combination of temperature and precipitation explained the most variation in this trait (Table S1).

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} + P_{ANN}	0.46 (0.24-∞)	42.23 ± 2.76	0.19 ± 0.10	42.66 ± 2.76	0.10 ± 0.05	0.10	291.18*
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	-1.89 ± 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} + P_{ANN}	0.38 (0.20-∞)	41.11 ± 2.72	0.24 ± 0.09	41.61 ± 2.80	0.14 ± 0.05	0.12	293.64*
T_{max} + P_{ANN} + P_{DRY}	0.44 (0.20-∞)	41.61 ± 2.76	0.27 ± 0.10	42.30 ± 2.87	0.16 ± 0.05	0.13	295.95
			-4.72 ± 1.70		-2.91 ± 0.98		
			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

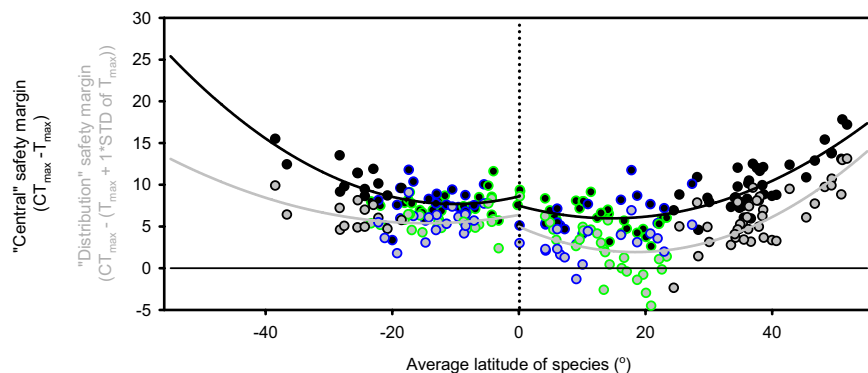


Fig. 2. Species and distribution safety margins. The “Central” species safety margins were calculated as the difference between heat tolerance (CT_{max}) and the average of maximal environmental temperature (T_{max}) (filled black circles). To include the species-specific populations that experience 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 $>23^\circ$ were deemed temperate, whereas tropical species ($<23^\circ$ 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 $T_{max} \times P_{ANN}$, and the analysis also provides a phylogenetically corrected r^2 value (21). For a more detailed description see ref. 20.

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