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PHYLOGENETIC CONSTRAINTS IN KEY FUNCTIONAL TRAITS BEHIND SPECIES' CLIMATE NICHES: PATTERNS OF DESICCATION AND COLD RESISTANCE ACROSS 95 *DROSOPHILA* SPECIES

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Species distributions are often constrained by climatic tolerances that are ultimately determined by evolutionary history and/or adaptive capacity, but these factors have rarely been partitioned. Here, we experimentally determined two key climatic niche traits (desiccation and cold resistance) for 92–95 *Drosophila* species and assessed their importance for geographic distributions, while controlling for acclimation, phylogeny, and spatial autocorrelation. Employing an array of phylogenetic analyses, we documented moderate-to-strong phylogenetic signal in both desiccation and cold resistance. Desiccation and cold resistance were clearly linked to species distributions because significant associations between traits and climatic variables persisted even after controlling for phylogeny. We used different methods to untangle whether phylogenetic signal reflected phylogenetically related species adapted to similar environments or alternatively phylogenetic inertia. For desiccation resistance, weak phylogenetic inertia was detected; ancestral trait reconstruction, however, revealed a deep divergence that could be traced back to the genus level. Despite drosophilids' high evolutionary potential related to short generation times and high population sizes, cold resistance was found to have a moderate-to-high level of phylogenetic inertia, suggesting that evolutionary responses are likely to be slow. Together these findings suggest species distributions are governed by evolutionarily conservative climate responses, with limited scope for rapid adaptive responses to future climate change.

KEY WORDS: **Ancestral trait reconstruction, evolutionary history, niche conservatism, phylogenetic signal, species distribution, stress resistance.**

Access to water and permissive environmental temperatures are fundamental requirements for the survival of all species. However, most species have evolved some ability to endure drought or stressful temperatures, and the ability to tolerate these adverse conditions plays a central role in defining the fundamental niche of species (Addo-Bediako et al. 2000; Chown and Terblanche 2007). Many species have, through evolutionary time, experienced changes in local water availability and temperature and such changes are expected to accelerate as global climate change develops (IPCC 2007; Wiens et al. 2009). The degree to which these

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changes will influence species distributions depends on the combination of existing tolerance capacity, presence of genetic variation in key traits that convey resistance (Hoffmann et al. 2003a; Kearney et al. 2009; Kellermann et al. 2009), and the extent to which distributions are limited by nonclimatic factors such as dispersal and biotic interactions (Skov and Svenning 2004; Kissling et al. 2010).

Predicting species responses to environmental change requires an understanding of how climatic factors shape current species distributions (Helmuth et al. 2005; Portner and Farrell 2008; Somero 2010) as well as an assessment of the degree to which these constraints are influenced by evolutionary processes (Kellermann et al. 2009; Mitchell and Hoffmann 2010). With respect to the latter, the ability of species to adapt to changing environments may to some degree be constrained by their evolutionary history. Comparative studies have increasingly incorporated quantitative analysis of phylogenetic effects in a bid to understand how historical/current adaptations may provide insight into the potential for future adaptation of species (Blomberg and Garland 2002; Willis et al. 2008; Labra et al. 2009). These studies have often aimed to control for evolutionary history with the purpose to highlight the effect of adaptation to specific environments.

Closely related species may display similar phenotypes for other reasons than phylogenetic relatedness per se primarily because they are likely to be found in similar geographic locations and thus in similar environments, leading to parallel adaptive responses and environmental effects on traits (Losos 2008; Freckleton and Jetz 2009). Although strong phylogenetic signal may indicate phylogenetic conservatism under some circumstances (Cooper et al. 2010), this should only be assumed once the effects of spatial autocorrelation in the environment experienced by related species have been accounted for (Losos 2008). Distinguishing between the causes of phylogenetic signal is therefore crucial when making predictions about the extent to which species responses to environmental changes such as those expected under climate change might be phylogenetically constrained.

In the present study, we experimentally determined desiccation and cold resistance, in over 90 *Drosophila* species, within a phylogenetic framework to establish the relative role of evolutionary and environmental factors in shaping species distributions. To distinguish between phenotypic plasticity (acclimation) and genetic effects, all species were reared in a common environment. Resistance traits were mapped onto the phylogeny through ancestral trait reconstruction and phylogenetic signal was evaluated using a broad array of analytical methods (Cooper et al. 2010). In addition, we determined the degree of niche conservatism by assessing whether closely related species are similar because they have evolved under similar environments (adaptive) or because they seek out similar environments related to phylogenetic inertia (species sorting).

Materials and Methods

SOURCES AND MAINTENANCE OF EXPERIMENTAL ANIMALS

Drosophila species used in the present study originated from five major sources (field collections from Denmark and Australia, collections kept in Professor Jean R. David's laboratory at the CNRS (Gif-sur-Yvette, France), the *Drosophila* Species Stock Center, San Diego, USA and the EHIME University *Drosophila* Stock Center, Koyoto, Japan. Due to the large number of species used (95 species for cold and 92 species for desiccation), it was impossible to get hold of stocks with large effective population sizes that were collected recently from the wild. We only succeeded to do this with a subset of species ($n = 19$). For a complete list of species and their respective collection and source information, please see Table S1.

Prior to testing, the flies were maintained for a minimum of two generations in our laboratory with population sizes of approximately 200 individuals. Flies were maintained on an oat-based medium (Leeds) at 20°C on a 12:12 light cycle. However, a number of cactophilic species had special dietary requirements and these species were therefore maintained on a banana *Opuntia* medium using the recipe from The *Drosophila* Species Stock Center, San Diego (URL: https://stockcenter.ucsd.edu/info/food_banana_Opuntia.php).

ESTIMATIONS OF CLIMATIC VARIABLES

Species-specific information of natural distribution was collated from the taxodros website (http://www.taxodros.uzh.ch/). This website has compiled geographical information (GPS coordinates) from published studies for a wide number of *Drosophila* species. From this database, 1–3513 observations were obtained per species (average 189 observations per 95 species). However, to reduce the risk of including erroneous data (which were found) on geographical distribution, we removed datapoints that were more than 3 standard deviations from the average latitudinal distance from the equator. For each of the locations, global climate data were obtained from the WorldClim data set (www.worldclim.org) (Hijmans et al. 2005). We chose six temperature and precipitation variables thought to be related to cold and drought resistance: (1) annual mean temperature (AMT), (2) absolute maximum temperature of the warmest month (T_{max}), (3) absolute minimum temperature of the coldest month (T_{min}), (4) annual precipitation (P_{ANN}), (5) precipitation of the wettest month (P_{WET}), (6) precipitation of the driest month (P_{DRY}). We also combined estimates of precipitation and temperature to examine an additional metric:

drying power of air (this was calculated for all GIS locations as (1-(Precipitation$_{location}$−Precipitation$_{min}$)/(Precipitation$_{max}$−Precipitation$_{min}$))× exp$^{(20.386-5132/Temperature_{location})}$, where temperature of the location (Temperature$_{location}$) is in Kelvin. The former part of this expression rates the humidity of the air assuming that the driest environment (Precipitation$_{min}$) has a relative humidity (RH) close to 0% and the wettest environment (Precipitation$_{max}$) a RH close to 100%. This relative measure of humidity is then multiplied by the latter part of the expression that returns the maximal drying power of the air at a given temperature. Climatic variables for each species were estimated as the average values for each climatic variable across all localities for each species (see Table S2). All GIS operations were performed in ArcGIS 9.3 (ESRI, Redlands, CA).

EXPERIMENTAL PROTOCOL

For all species, both sexes were tested for cold and desiccation resistance. To avoid influence of developmental density, approximately 40 pairs of parental flies from all species were allowed to lay eggs on medium-filled spoons spread with live yeast to stimulate oviposition. From these spoons, we collected 25 eggs into each of 10 vials for larval development at 20°C. Following eclosion, flies were collected over a 2-day period such that the difference in age within species was ±1 day. As development time differed between the species, the age difference between species at the time of testing was typically ±3 days. Two days prior to the experiments, flies were briefly anesthetized with CO_2 and the sexes were separated. Flies were between 7 and 12 days of age at the time of assessment for cold and desiccation resistance.

For all species, we assayed 10 flies of each sex and each trait unless otherwise stated (see Table S3). During each experimental round, we examined all stress resistance traits for a single species but as a consequence of the large number of species tested, we performed a total of six rounds of experiments. A number of species were assessed in more than one round to control for possible block effects and the final experimental round included at least one species from each experimental round representing the full range of tolerances that existed for the different traits.

Temperature in nature tends to change gradually and here we investigated the critical thermal minimum (CT_{min}) under gradual cooling. CT_{min} was scored by placing individual flies in empty 5-mL glass vials subsequently submerged in a water bath, containing glycol, preset at 20°C. The temperature of the water bath was then gradually decreased from 20°C at a rate of 0.1°C/min and the CT_{min} was recorded as the temperature at which the flies were knocked down and had lost the ability to move any body part.

Desiccation was scored as the time to death following exposure to extreme desiccation at 20°C by placing individual flies into 5-mL vials covered with gauze in a glass tank containing silica gel (RH < 5%). Desiccation resistance was scored hourly by recording flies that had succumbed to desiccation, post 36 h, flies were scored every 2 h.

PHYLOGENY CONSTRUCTION

There is currently no phylogeny that encompasses all the *Drosophila* species examined in this study. The most comprehensive phylogenetic study to date includes 58 species that were also examined in this study (van der Linde et al. 2010). To incorporate all species examined, we constructed a composite phylogeny by incorporating previously published phylogenies with the 58 species from the van der Linde et al. (2010) study (Table S1). Estimates of branch lengths (genetic distances) for the remaining species were taken from other published phylogenies and branch lengths were standardized to the van der Linde et al. (2010) study. If branch lengths for sister species were unknown, we used the smallest genetic distance found between two sister species.

The *Drosophila* phylogeny can be divided into four subgenera. The subgenus *Sophophora*, comprises the *melanogaster, obscura, willstoni*, and *saltans* species groups; The subgenus *Drosophila*, includes the species groups *funebris*, *guttifera, melanica, mesophragmatica, nannoptera*, *polychaeta, quinaria, robusta, tripunctata, immigrans, virilis, repleta*, and *tumidtarsus*; The *Zaprionus* subgenus includes the *armatus* and *inermis* species groups and finally the subgenus *Dorsilopha* of which the present study only included one member (*Drosophila busckii*). As this subgenus was only represented by one member, we excluded this from any subgenus comparisons. Furthermore, because the subgenus *Zaprionus* shares a recent divergence from the subgenus *Drosophila*, we combined the subgenus *Zaprionus* and *Drosophila* (named the subgenus *Drosophila* from here on) (Fig. 1).

PHYLOGENETIC SIGNAL

To determine whether phylogeny influenced the observed patterns between traits and their associated climatic variables, we initially tested for the presence of phylogenetic signal prior to fitting a phylogenetic model. In the present study, we employed a suite of analytical approaches to analyze phylogenetic signal. Although these analytical methods are related in their aims, their different assumptions may potentially lead to different conclusions and we therefore based our conclusions on the consensus findings (Cooper et al. 2010). The first two methods for estimating phylogenetic signal, Pagel's λ and Bloomberg's K, are based on a model of trait evolution via Brownian motion (BM) (Lynch 1991; Blomberg et al. 2003). These methods (and all others unless stated otherwise) were implemented in R (R Devolopment Core Team 2011), in this case using the picante package (Kembel et al. 2010).

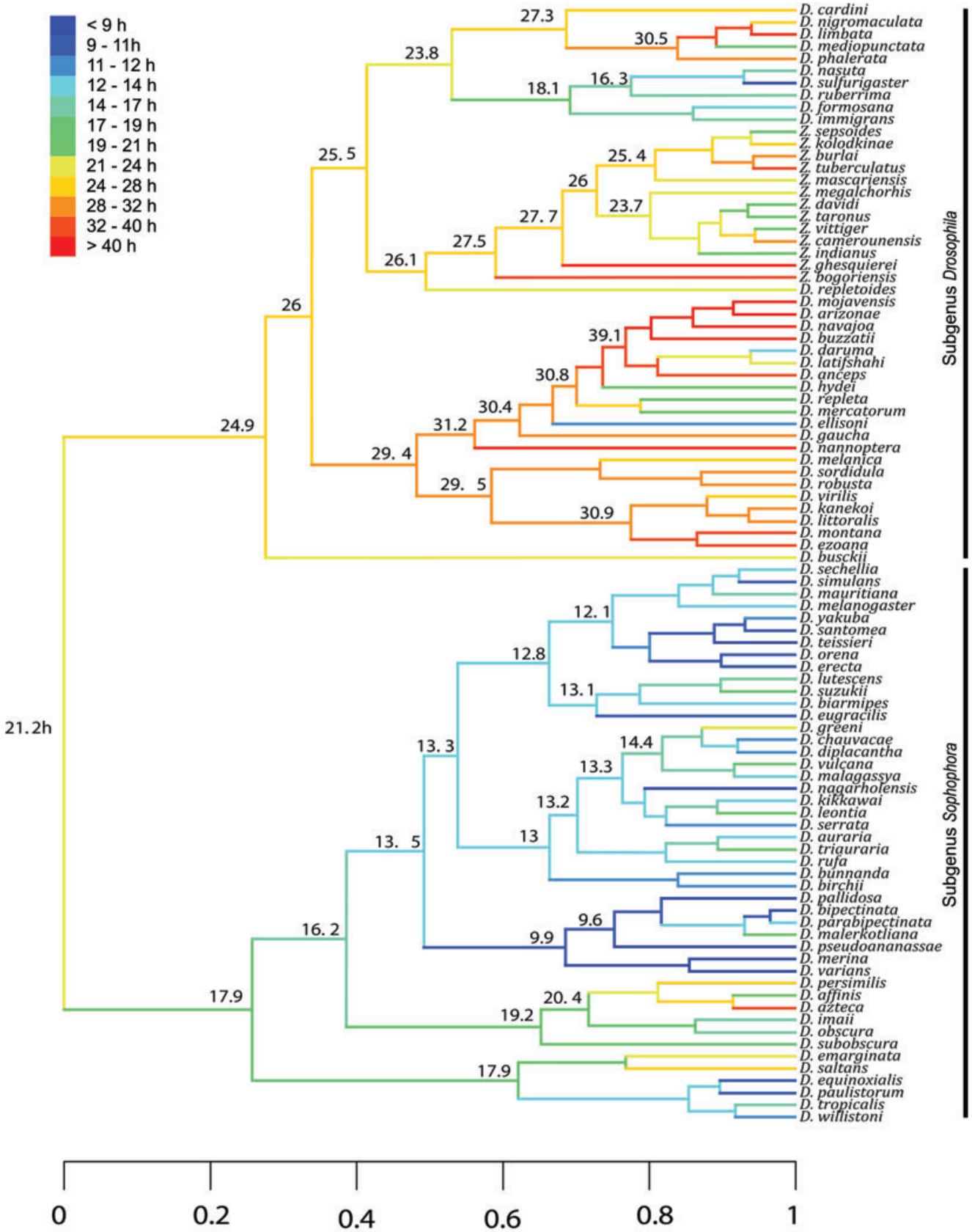

Figure 1. Continued on next page.

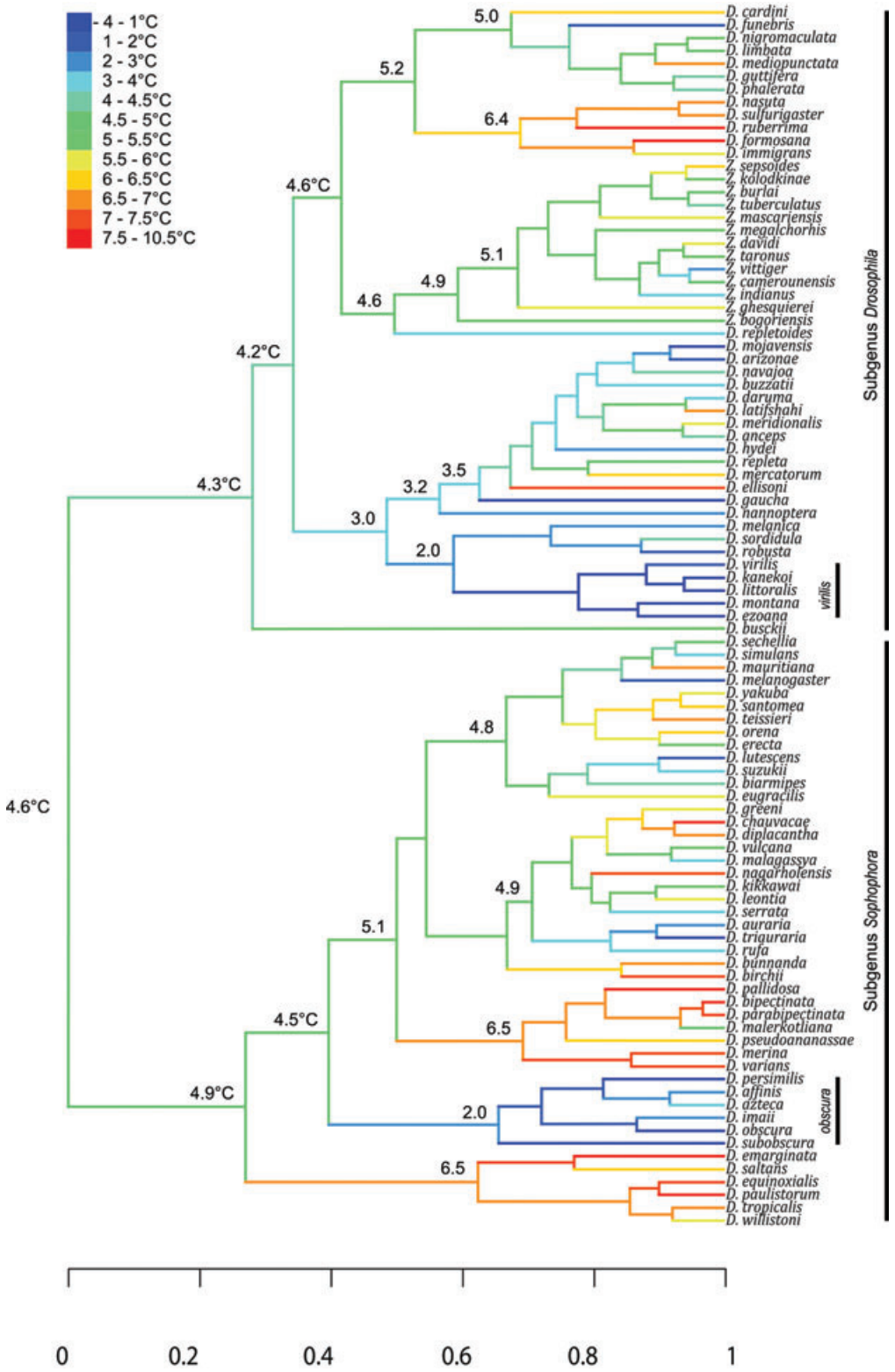

Figure 1. Phylogenetic hypothesis for the 92–95 *Drosophila* species examined in this study, with branch lengths reflecting standardized time. Values shown next to the branches are from the ancestral trait reconstruction calculated using maximum likelihood methods for continuous traits. To illustrate trends in the evolution of cold and desiccation resistance, species were divided equally into 12 arbitrary groups and color-coded depending on resistance level (only female data shown). For specific values for the individual species, refer to Table S3. (a) Desiccation resistance (h = hours survival in dry air) b) Cold resistance (temperature in °C at which individuals were knocked down).

Table 1. **Summary of phylogenetic signal using different comparative methods for the traits desiccation (Des) and cold resistance (CT_{min}). λ and *K* range from no phylogenetic signal with λ and $K = 0$ to high phylogenetic signal with $\lambda = 1$, $K \geq 1$. For λ, the estimate with the best corrected Akaike Information Criterion (AICc) is given in bold. Phylogenetic signal based on the SLOUCH $t_{1/2}$ can be interpreted as follows: a $t_{1/2} > 0$ reflects an increasing association between the phylogeny and the trait ($t_{1/2}$ has the same units as the phylogeny). Moran's *I* provides an estimate of the autocorrelation found within a dataset at three taxonomic levels: subgenus (SubG), species group (SppG), and subspecies group (SubSppG).**

					Morans *I*		
	λ	AICc	*K*	SLOUCH $t_{1/2(trait)}$[1]	*SubG*	SppG	SubSppG
Des: ♀	0.17	**692.43**	0.23**	0.24 (0.12–0.55)	0.14**	0.25**	0.36**
		λ_0 696.32					
		λ_1 725.09					
Des: ♂	0.19	**704.11**	0.24**	0.30 (0.15–0.59)	0.21**	0.27**	0.48**
		λ_0 710.12					
		λ_1 733.60					
CT_{min}: ♀	0.76	**347.41**	0.38**	0.88 (0.33–∞)	−0.05	0.56**	0.47**
		λ_0 367.12					
		λ_1 358.23					
CT_{min}: ♂	0.83	**335.41**	0.42**	2.40 (0.37–∞)	−0.07	0.60**	0.55**
		λ_0 362.78					
		λ_1 344.66					

***$P < 0.05$ and **$P < 0.01$**

[1]For a further description of these values and full parameter estimates, see Table 3.

Pagel's λ was estimated for the residuals with significance tested by comparing the AIC_c ratios of the estimated λ with $\lambda = 0$ (H_0 = no phylogenetic signal) and $\lambda = 1$ (H_a = phylogenetic signal). The *K*-statistic can be divided into four scenarios: (1) *K* not significantly different from 0 suggests no phylogenetic signal, (2) $K = 1$ indicates the trait is evolving under a BM model with clear phylogenetic signal, (3) *K* greater than 1 suggests traits are more similar than would be expected under BM, and (4) *K* less than 1 (but > 0) indicates traits are less similar than expected under BM which may be linked to either convergent evolution of unrelated species or measurement error (Blomberg et al. 2003). Phylogenetic signal was also estimated using the SLOUCH package that models traits as evolving under an Ornstein–Uhlenbeck process (Hansen et al. 2008). This was done by fitting a regression with only the trait variables (i.e., CT_{min}/Desiccation) (for a more detailed description of the method see below). An alternative estimate of phylogenetic signal, Moran's *I*, was also estimated using the ape package (Paradis et al. 2004). This measure makes no underlying assumptions as to the mode of evolution within traits but computes the phylogenetic autocorrelation in the data at different taxonomic levels, providing an indication of how phylogenetic signature changes across the phylogeny (Gittleman and Kot 1990). Estimates of Moran's *I* rely on accurate taxonomic information that may be arbitrary when taxonomic divisions are made on genetic distances alone. However, the taxonomic groups in *Drosophila* are well established and we divided species to three levels (subgenus, species group, and species subgroup) based on the taxonomic divisions used by the taxodros website (Table S3).

TRAIT ANALYSES

The association between traits and the climatic variables was determined by standard least squares linear regressions using the Poptools add in for Microsoft Excel (Hood 2010). To avoid multicollinearity among climatic variables, the environmental variable that explained most variation was used for all further analyses. When investigating the correlation of trait resistance against P_{ANN}, we log-transformed P_{ANN} under the assumption that the log-transformed data are more representative of absolute differences in humidity than the nontransformed precipitation data (i.e, a difference in precipitation from 100 to 200 mm annually is more significant to environmental humidity than a difference between 4100 and 4200 mm annually). To examine the effects of inbreeding/laboratory adaptation that may be present within a dataset of this nature, we compared the full dataset to a subset of species (19) that were considered to be outbred and tested within 2 years of collection from the field.

Using the geiger package, we reconstructed ancestral states for both traits using a maximum likelihood approach for continuous characters, based on a BM model (Harmon et al. 2008). To examine the proportion of trait variation that could be entirely

attributed to climatic variables, we controlled for phylogenetic effects using two independent methods of analysis. This was done by computing phylogenetic independent contrasts (PIC) and the SLOUCH model (SLOUCH, described below). These approaches were calculated in ape and SLOUCH (Hansen et al. 2008), respectively.

PHYLOGENETIC SIGNAL: ADAPTATION VERSUS INERTIA

To distinguish whether spatial proximity drives the relationship between phylogeny and traits, we examined the relationship between traits and space by comparing distance matrices for these two variables with a linear regression analysis (Legendre et al. 1994). Here, we generated distance matrices for traits and spatial proximity to determine the relationship between these matrices and evaluate the extent to which spatial proximity influenced patterns of trait resistance. Distance matrices (based on Euclidean distances) were calculated for traits using the dist() function in R, spatial distance matrices were calculated from the average longitude and latitude data for each species using the fossil package with the earth.dist function generating a matrix of distances in kilometres (Vavrek 2011).

The SLOUCH v1.1 program (http://freshpond.org/software/SLOUCH/) models the evolution of traits based on an Ornstein–Uhlenbeck process (Hansen et al. 2008). Unlike traditional BM models, traits are modeled as evolving toward a primary optimum. Estimates of phylogenetic signal are given by the phylogenetic half-life ($t_{1/2}$) that is a measure of the influence of the ancestral trait on the ability of the present day trait to evolve toward the optimum. Thus, $t_{1/2}$ is a measure of the speed at which phylogenetic covariances decay with phylogenetic distances. When $t_{1/2}$ is close to 0, the trait is evolving freely without any influence from the ancestral trait, whereas increasing values of $t_{1/2}$ reflect an increasing effect of the ancestral trait on the present-day trait, with a $t_{1/2} = \infty$ reflecting the trait is evolving under BM. Initially, a regression model is fitted without predictor variables (climatic variables), which provides an estimate of the phylogenetic signal within a dataset. By comparing a model without and with the inclusion of predictor variables, we can determine how much of the phylogenetic signal can be attributed to phylogenetic inertia. No reduction in the $t_{1/2}$ on the inclusion of the predictor variables suggests that phylogenetic signal within the data can entirely be contributed to phylogenetic inertia (the value of $t_{1/2}$will depend on the total length of a given tree from root to tip). The program also provides a phylogenetically corrected r^2 giving an estimation of the association between the predictor and trait variables following correction for phylogeny (for a detailed explanation and implementation of the model, see Hansen et al. 2008 and Labra et al. 2009).

Results

PHYLOGENETIC PATTERNS AND ANCESTRAL TRAIT RECONSTRUCTION

We observed clear differences in stress resistance between the two major subgenera of the *Drosophila* phylogeny *Sophophora* and *Drosophila*. The *Drosophila* group displayed more resistant phenotypes with respect to cold and particularly desiccation stress, with the average desiccation resistance within the *Drosophila* group being twofold higher (*Drosophila*: 28.79 $\pm$ 2.56, *Sophophora*: 13.88 $\pm$ 0.84 h of survival in dry air) (Fig. 1a). The difference in average cold resistance was smaller with cold resistant phenotypes found in both groups (*Drosophila:* 4.03°C $\pm$ 0.34, *Sophophora*: 5.10°C $\pm$ 0.35) (Fig. 1b).

Phylogenetic signal was detected for both desiccation and cold resistance irrespective of the method implemented, with patterns of phylogenetic signal similar for females and males across traits (Table 1). The degree of phylogenetic signal differed between the two traits. Phylogenetic signal for desiccation resistance was low suggesting a weak association with phylogeny, with low values for both λ and K (Table 1). The observed $t_{1/2}$ for females and males (0.24–0.30) also indicated moderate phylogenetic signal, while the significant estimates of Moran's I, detected at all taxonomic levels suggested that the evolution of desiccation resistance was closely tied to the phylogeny (Table 1). This result was also inferred from the marked differences in mean resistance between the *Drosophila* and *Sophophora* subgenera (Fig. 1a). Phylogenetic signal for cold resistance was stronger than for desiccation resistance, with all three estimates of phylogenetic signal higher (λ, $t_{1/2}$, and K). Estimates of K and $\lambda < 1$ suggested cold resistance was not evolving strictly under a BM model of evolution (for λ compare AIC_c scores across different models), which indicated an incomplete association between cold resistance and phylogeny that was less than would predict through BM. The $t_{1/2}$ differed between females and males with a stronger association for males suggesting the trait was evolving close to a BM model of evolution, albeit the $t_{1/2}$ for females also suggested a strong association between trait and phylogeny. However, the apparent differences between females and males were not statistically significant with the support regions clearly overlapping. Moran's I for cold resistance suggested an association between traits and phylogeny, although phylogenetic signal was not detected at the subgenus level, which indicated that phylogenetic associations related to the evolution of cold resistance have arisen more recently in evolutionary history than for desiccation resistance.

The ancestral trait reconstruction showed that the derived state for both desiccation and cold stress was an intermediate phenotype. The evolution of desiccation-intolerant phenotypes occurred rapidly within the *Sophophora* group following the split between the *Drosophila* and *Sophophora* subgenus (Fig. 1a).

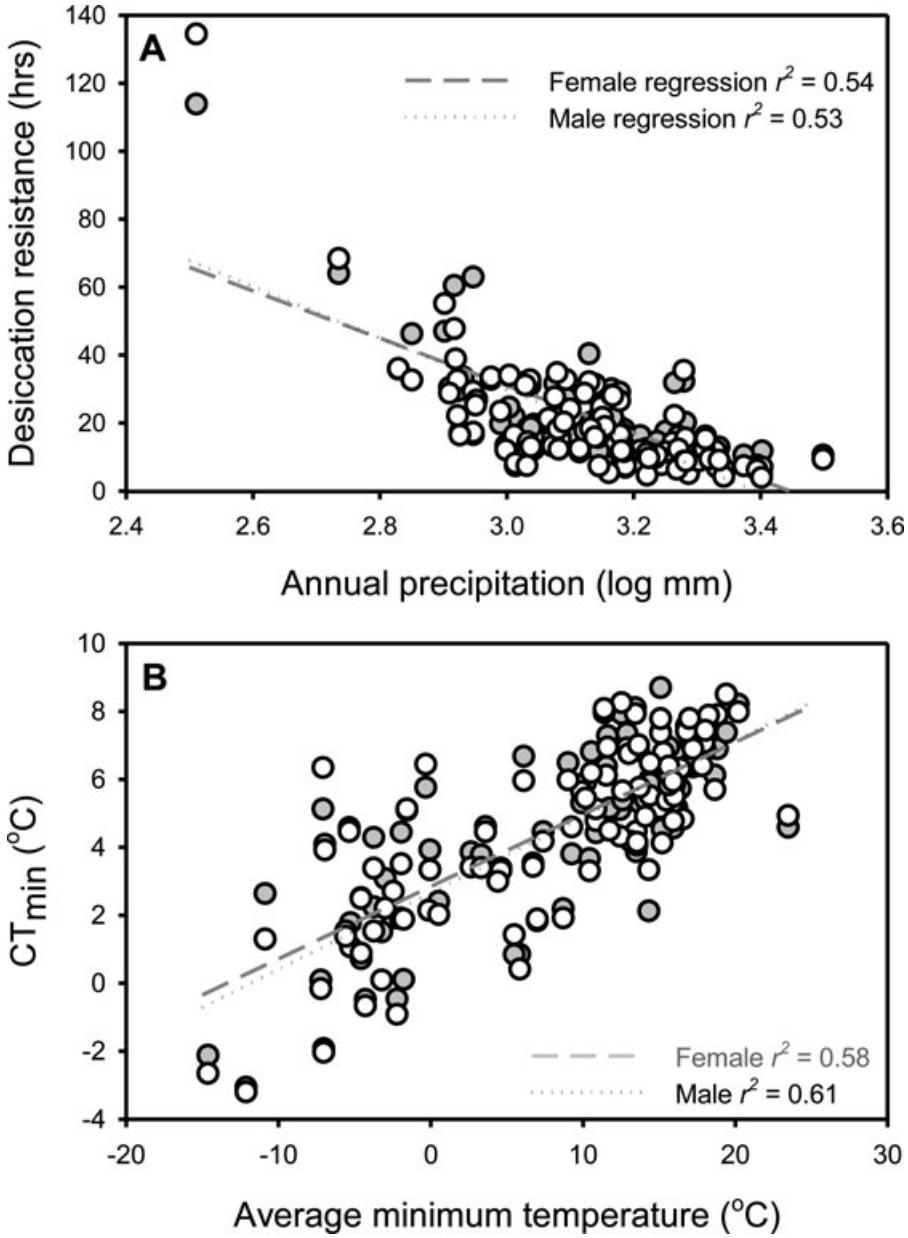

Figure 2. **(a) The association between desiccation resistance and average annual precipitation (P_{ANN}), (b) the association between cold resistance (CT_{min}) and average minimum temperature (T_{min}). Female data points are shaded in gray whereas male data points are white. Data in the figures reflect the original raw data (nonphylogenetic regression).**

These results were in accordance with the Morans *I* analysis suggesting phylogenetic signal at all three taxonomic levels. Since the divergence of the *Drosophila* and *Sophophora* subgenera, resistance to desiccation stress has slowly decreased within the *Sophophora* subgenus, but increased in the *Drosophila* subgenus. Desiccation-resistant phenotypes appeared to have evolved very rarely within the *Sophophora* group, with only a single transition to a highly desiccation-resistant phenotype (*Drosophila azteca*). Conversely, it seems that the evolution of desiccation-resistant phenotypes has been gained and lost a number of times within the *Drosophil*a group (Fig. 1a). Despite striking differences in desiccation resistance between the two subgenera, indicative of a strong phylogenetic effect, the evolution of desiccation resistance across the phylogeny appeared tightly linked to climatic parameters (Fig. 2a).

In contrast to desiccation resistance, cold-resistant phenotypes had evolved a number of times across the phylogeny irrespective of the subgenus. Evolution of high cold resistance nevertheless appeared mostly restricted to two species groups (*virilis* within the *Drosophila* subgenus and *obscura* within the *Sophophora* subgenus) (Fig. 1b). In both cases, the divergence of these groups occurred early in the phylogeny, giving rise to a

higher phylogenetic signal (Moran's I) at the species group level in comparison to the species subgroup level (Table 1).

CLIMATIC PATTERNS AND PHYLOGENETIC SIGNAL

A number of climatic variables were found to associate strongly with desiccation resistance, but the strongest relationship was found with average P_{ANN} (females: $r^2 = 0.54$, males: $r^2 = 0.53$) (Fig. 2a, Table 2) (nonphylogenetic regressions). The drying power of air was also considered because this metric includes contributions of both temperature and humidity to the saturation deficit experienced by an organism. However, P_{ANN} explained a larger proportion of the variation in this trait and was therefore considered the most important environmental correlate. Cold resistance also correlated with a number of climatic variables, with more than 58% of the interspecific variation being explained by average T_{min} (females: $r^2 = 0.58$, males: $r^2 = 0.61$) (Fig. 2b, Table 2) (nonphylogenetic regressions).

To quantify the effect of inbreeding and/or laboratory adaptation, we examined the relationship between traits and climatic variables in 19 lines that were considered to be outbred. Overall, we found similar relationships for the "outbred" subset. A slightly stronger relationship was found for the male outbred data for desiccation resistance (females: $r^2 = 0.52$, slope $= -63.86$ h/log mm, $P < 0.001$, males: $r^2 = 0.58$, slope $= -63.59$ h/log mm, $P < 0.001$) and for cold tolerance, we also found a slightly stronger relationship between traits and climatic variables for the outbred subset (females: $r^2 = 0.72$, slope $= 0.32\ ^\circ$C/$^\circ$C, $P < 0.001$, males: $r^2 = 0.78$, slope $= 0.34\ ^\circ$C/$^\circ$C, $P < 0.001$).

With evidence for phylogenetic signal in the two resistance traits, we used two methods to examine if the association between traits and climatic variables persisted after controlling for phylogeny. The two different methods produced similar results, with significant associations remaining present between traits and their main climatic correlates (as identified above) also after controlling for phylogeny (Table 2). Thus, following phylogenetic correction, P_{ANN} explained between 23% and 45% of the variation in desiccation resistance for females and males, whereas CT_{min} explained 33–43% of the variation in cold resistance for females and males. The proportion of trait variation that could be explained by climatic variables depended on the method used to correct for phylogeny with PIC consistently producing weaker relationships (Table 2).

ADAPTATION VERSUS PHYLOGENETIC INERTIA

Phylogenetic signal can arise due to phylogenetic inertia or because closely related species share similar selection pressures because (1) closely related species tend to occur in spatial proximity due to dispersal limitation, and (2) there is spatial autocorrelation in environmental (climatic) conditions, leading to related species being subject to similar selective pressures. We controlled for environmental variation by rearing species under common conditions and used two different methods to clarify if patterns were due to inertia or common selection pressures. One method was based on the notion that if spatial proximity is the main driver of the association between traits and phylogeny-related species that share common adaptations should also be spatially related (Freckleton and Jetz 2009). An alternative method executed in the SLOUCH package jointly estimates the degree of phylogenetic inertia and adaptation within a dataset (Hansen et al. 2008).

Using a distance matrices regression approach, we determined the relationship between spatial proximity and trait resistance. For each trait and in both females and males, we found a significant effect of space; however, significance was driven by large sample size as spatial proximity had only very weak explanatory power (desiccation tolerance, females and males: $r^2 = 0.02$, slope < 0.01 h/km, $P < 0.01$; cold tolerance, females and males: $r^2 < 0.01$, slope $< 0.01\ ^\circ$C/km, $P < 0.01$). Thus, species with similar trait values only exhibited a very weak tendency to occur in proximity. If we take spatial proximity as a proxy for common selection pressures (Freckleton and Jetz 2009), our results suggest patterns of trait resistance across the phylogeny are not driven by common selection pressures and consequently phylogenetically structured adaptation. Under a scenario in which common selection pressures drive phylogenetic signal, we would expect to see phylogenetically closely related species sharing similar resistance values and having similar distributions.

The SLOUCH analysis was used to estimate the degree of phylogenetic association between trait variables (desiccation or cold resistance) and climatic variables (P_{ANN} or CT_{min}). Estimates of phylogenetic signal were moderate for desiccation resistance and high for cold resistance for both females and males (Table 2). The inclusion of climatic variables (P_{ANN}) for desiccation resistance halved the $t_{1/2}$ (Table 3). Taken together, these results suggested that the presence of moderate phylogenetic signal was partly attributed to phylogenetic structured adaptation (i.e., that closely related species have evolved similar adaptations due to common selection pressures) and partly phylogenetic inertia.

The phylogenetic signal for cold resistance was stronger than for desiccation, particularly for males. We observed no reduction in $t_{1/2}$ for the males after the inclusion of T_{min} as the predictor variable, suggesting that this trait was evolving close to a BM model. However, with the 2 unit support surface ranging from $\sim$0.3 to ∞, it is difficult to say whether phylogenetic inertia within this trait is moderate or high. A smaller $t_{1/2}$ was detected for females, suggesting a moderate level of phylogenetic inertia within this trait; however, the support regions for both females and males overlapped. Despite a moderate-to-high level of phylogenetic inertia, T_{min} could still explain a large proportion of the observed variation for cold resistance as seen in the high phylogenetically corrected r^2 in both sexes (16–20% reduction in the uncorrected r^2).

Table 2. Summary of r^2 for the association between traits and climatic variables following correction for phylogeny using three independent methods.

	Desiccation (h)				Cold (°C)			
	♀		♂		♀		♂	
	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Slope
Standard	0.54**	−69.14	0.53**	−75.98	0.58**	0.21	0.61**	0.22
SLOUCH	0.45	−58.16	0.41	−61.08	0.43	0.20	0.39	0.19
PIC	0.25**	−43.62	0.23**	−44.13	0.33**	0.17	0.38**	0.18

*$P < 0.05$ and **$P < 0.001$

Table 3. Summary of results from the SLOUCH analysis fitting the regression of desiccation and cold resistance without and with climatic variables. This method estimated the phylogenetic half-life ($t_{1/2}$) (in units of tree height), the 2 unit support surface shown in parentheses, and the intercept and slope of the optimal and evolutionary regression as well as the phylogenetically corrected r^2. The model with the best Akaike Information Criterion (AICc) is shown in bold.

Trait and predictor	$t_{1/2}$	Optimal regression		Evolutionary regression		r^2	AICc
		Intercept	Slope	Intercept	Slope		
Des: ♀	0.24 (0.12–0.55)	21.36 ± 2.52	–			–	738.06
P_{ANN}	0.11 (0.04–0.24)	198.63 ± 21.43	66.96 ± 8.09	200.88 ± 21.55	−58.16 ± 6.97	0.43	**700.46**
Des: ♂	0.30 (0.15–0.59)	19.91 ± 3.72	–		–	–	741.49
P_{ANN}	0.13 (0.06–0.25)	207.94 ± 24.89	70.33 ± 9.29	210.68 ± 25.06	−61.08 ± 8.00	0.39	**711.11**
CT_{min}: ♀	0.88 (0.33–∞)	4.57 ± 1.05	–	–		–	398.72
T_{min}	0.34 (0.15–∞)	3.07 ± 0.50	0.35 ± 0.03	3.04 ± 0.45	0.20 ± 0.21	0.45	**351.80**
CT_{min}: ♂	2.40 (0.37–∞)	4.38 ± 0.92	–			–	390.40
T_{min}	2.80(0.32–∞)	3.15 ± 0.52	1.63 ± 0.15	3.13 ± 0.53	0.19 ± 0.02		**338.92**

The SLOUCH method assumes that traits are evolving toward an optimum, with the optimal regression describing the relationship between traits and climatic variables without the influence of ancestry, and the evolutionary regression representing the optimal regression with inclusion of phylogenetic correction. A comparison of the evolutionary and optimal regression slopes for cold resistance showed that the slope for males was steeper under the optimal regression (Table 3) suggesting they are further away from their optima than females, although the evolutionary regression could still explain a similar level of variation across the two sexes. This suggested a moderate correlation between phylogeny and trait variables, which may slow evolutionary responses, although T_{min} was also an important driver of trait evolution.

Discussion

Using a defined phylogenetic group expected to have high evolutionary potential due to short generation times and high population sizes, the present study provides a comprehensive assessment of how physiological capacities to tolerate cold temperatures and dehydration are linked to species distributions. By examining 92–95 *Drosophila* species under controlled environmental conditions, we showed how physiological capacities to endure cold and dry environments have evolved to allow some species within this family to expand from their ancestral niche and thereby change their distribution. Nevertheless, we also showed how these traits were influenced to a varying degree by phylogenetic relationships. The associations between desiccation and cold resistance and climate range characteristics found here demonstrated that these climatic niche traits were closely linked to the geographic distribution of *Drosophila* species. This finding thereby supports previous comparative studies both within *Drosophila* and for other insect groups (Parsons 1982; Karen et al. 1998; Addo-Bediako et al. 2000; Gibert et al. 2001; Kellermann et al. 2009; Strachan et al. 2011).

The analysis revealed weak-to-moderate levels of phylogenetic signal for desiccation resistance and stronger phylogenetic signal for cold resistance. Phylogenetic associations related to the evolution of desiccation resistance acted at higher taxonomic levels than for cold resistance with phylogenetic signal in this trait detected at all taxonomic levels including the subgenus level (Table 1). This finding was illustrated by the ancestral trait reconstruction analysis (Fig. 1a) showing differences in mean resistance between the *Drosophila* and *Sophophora* subgenera. Thus, there has been an early split in the evolution of high/low desiccation

resistance between these two subgenera, with higher desiccation resistance likely to be the derived state. The *Drosophila* subgenus, which includes highly desiccation-tolerant desert species, may have a greater potential to adapt to increasingly arid environments. In contrast, desiccation resistance has rapidly been lost in the *Sophophora* subgenus, suggesting contrasting evolutionary trajectories. Inherent differences between drosophilid groups have also been shown for other temperature-related traits, while studies on insects in general also suggest much of the variation in desiccation resistance can be partitioned at higher taxonomic levels (Addo-Bediako et al. 2000; Chown et al. 2002; Matzkin et al. 2009; Strachan et al. 2011). In contrast, cold-resistant species were found across the two subgenera, suggesting that the evolution of cold resistance has evolved repeatedly and more recently in evolutionary time. High phylogenetic signal at the species group level was likely to be driven by the resistant *obscura* and *virilis* species groups, whereas very few species outside these groups had evolved high cold resistance (Fig. 1b, Table 1).

Comparative *Drosophila* studies dealing with phylogenetic patterns of climatic resistance have generally focused on the presence/absence of phylogenetic signal rather than understanding the reasons underlying such signals (van Herrewege and David 1997; Gibert et al. 2001; Matzkin et al. 2009; Dillon et al. 2010; Strachan et al. 2011). Thus, related species may be similar because they inherit their ancestral niche by moving across space and settling into environmentally similar, but potentially distant locations (species sorting). Alternatively, species may be similar as a result of similar selection pressures and limited dispersal from the ancestral area (Cooper et al. 2011). Under the former scenario, which is indicative of phylogenetic inertia, no clear relationship between resistance and spatial parameters is expected and this was the pattern observed for both cold and desiccation resistance. Thus, we found only a weak effect of spatial proximity between closely related species, suggesting that the presence of phylogenetic signal, particularly for cold resistance, was related to phylogenetic inertia rather than spatially autocorrelated adaptive processes. The SLOUCH analyses led to similar conclusions, as the $t_{1/2}$ for models including the predictor variables was generally only slightly smaller than the $t_{1/2}$ without the predictor variables. For desiccation resistance, only a weak-to-moderate association with phylogeny was found, which could be contributed to both inertia and adaptation. In contrast, moderate-to-strong phylogenetic inertia was detected for cold resistance; however, due to wide support surfaces, it was difficult to conclude whether these effects were moderate or strong, particularly in relation to the male data. These results suggest that desiccation resistance evolves rapidly and free from phylogenetic restrictions. However, the clear division between the *Sophophora* and *Drosophila* group observed with the ancestral trait reconstruction suggests there may be inherent differences in resistance between *Drosophila* species that may be difficult to overcome. For cold resistance, there is clearly more evidence of strong phylogenetic associations that may limit/slow evolutionary responses. The data also suggest that the presence of phylogenetic signal for cold resistance is related to phylogenetic inertia rather than common selection pressures structured across the phylogeny. Together these results suggest that current species distributions reflect a combination of environmental adaptation, which is perhaps at a slower pace for cold resistance compared to desiccation resistance, and species moving into new localities with environments to which they are preadapted.

There are a number of different experimental factors that may influence the observed results of this large-scale study. The use of single population estimates to reflect species estimates, the use of stocks maintained in the laboratory for many generations, and finally, the methods in which we estimated cold resistance may contribute to noise within the dataset. Under ideal circumstances, intra- as well as interspecific comparison would provide an important perspective into the variation available for selection within species (Gaston et al. 2009). Nevertheless, we believe that the variation within species is unlikely to exceed that between species. This has been demonstrated on a smaller scale in a *Drosophila* comparative study (Kimura 2004). Estimates of trait resistance will also be influenced by both laboratory adaptation and inbreeding that were likely to be extensive in the current dataset as many species were obtained from stock centres. However, this effect will be small in interspecies comparisons in which once again the variation between species is likely to be larger than the effects of inbreeding and laboratory adaptation as recently shown for inbred *Drosophila* (Kristensen et al. 2011). To further explore this, we examined 19 species in our dataset, which were considered outbred and found a similar relationship between traits and climatic variables, albeit a stronger relationship for cold resistance was found in the outbred species (14–17% increase in r^2). Finally, there has been some debate in the recent literature as to the use of ecologically relevant ramping methods, and whether estimates of CT_{min} may be confounded by starvation and desiccation stress as a consequence of assay length (<4 h) (Rezende et al. 2011). There is however empirical evidence showing that the effects of both starvation and desiccation stress do not influence estimates of cold tolerance in the assays used here (Terblanche et al. 2011; Overgaard et al. 2012).

There is currently great interest in predicting the likely susceptibility of different species and species groups to future climate change (Pearman et al. 2008; Hawkins and DeVries 2009; Buckley et al. 2010), with the literature highlighting the likely susceptibility of mid- and low-latitude species to increasing temperatures arising from climate change (Deutsch et al. 2008; Clusella-Trullas et al. 2011). The present results point to likely limits in the evolutionary responses of some species groups to increasingly arid conditions, which may develop regionally as a consequence

of altered rainfall patterns under climate change, and indirectly as a consequence of changes in vegetation cover and increasing temperature. The presence of constraints in some tropical *Drosophila* species has previously been attributed to a low evolutionary potential as reflected by a low level of heritable variation (Hoffmann et al. 2003b; Kellermann et al. 2009). Here, we suggest that a limited evolutionary potential may lie deep within the *Drosophila* phylogeny rather than only being limited to tropical species. A recent study has also shown that acclimation responses to temperature are similar across widespread and tropical *Drosophila* species (Overgaard et al. 2011). Together these findings indicate that the challenges induced by climate change may be of similar magnitude for widespread/temperate *Drosophila* species and not a problem primarily restricted to tropical species (Deutsch et al. 2008; Kellermann et al. 2009; Dillon et al. 2010). At the mechanistic/molecular level, an inability to adapt to climatic extremes may reflect the expansion and contraction of particular gene families due to processes like gene duplication and DNA decay, but further research is required both at the physiological and molecular level to determine what underlies constraints within these traits (McBride 2007; Hoffmann 2010).

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Supporting Information

The following supporting information is available for this article:

Table S1. List of species, the country of collection and year, the laboratory they originated from, and whether we can define them as being a mass bred or stock centre population.

Table S2. The means, variances and number of records for latitude and environmental variables: annual mean temperature (AMT), minimum temperature (T_{MIN}), maximum temperature (T_{MAX}), annual precipitation (P_{ANN}), precipitation of the wettest month (P_{WET}), and precipitation of the driest month (P_{DRY}).

Table S3. Taxanomic division of species into subgenus, species group, and species subgroup.

Supporting Information may be found in the online version of this article.

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