

Research



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# Evolution and plasticity of thermal performance: an analysis of variation in thermal tolerance and fitness in 22 *Drosophila* species

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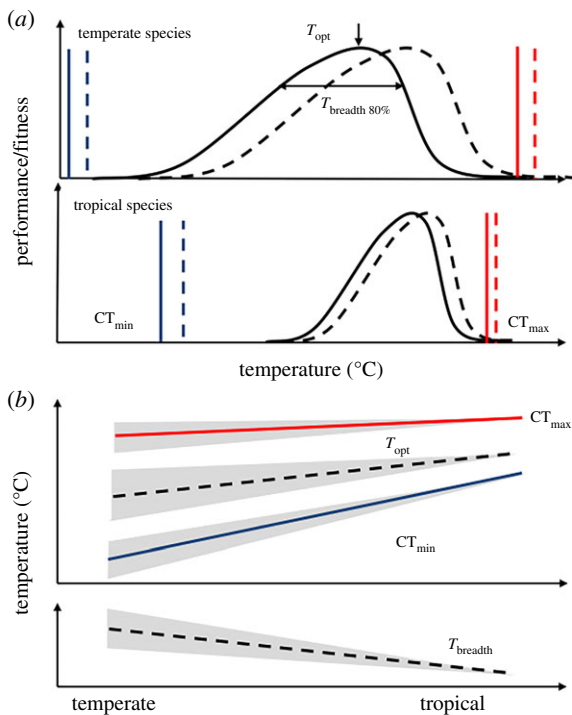
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The thermal biology of ectotherms is often used to infer species' responses to changes in temperature. It is often proposed that temperate species are more cold-tolerant, less heat-tolerant, more plastic, have broader thermal performance curves (TPCs) and lower optimal temperatures when compared to tropical species. However, relatively little empirical work has provided support for this using large interspecific studies. In the present study, we measure thermal tolerance limits and thermal performance in 22 species of *Drosophila* that developed under common conditions. Specifically, we measure thermal tolerance ( $CT_{min}$  and  $CT_{max}$ ) as well as the fitness components viability, developmental speed and fecundity at seven temperatures to construct TPCs for each of these species. For 10 of the species, we also measure thermal tolerance and thermal performance following developmental acclimation to three additional temperatures. Using these data, we test several fundamental hypotheses about the evolution and plasticity of heat and cold resistance and thermal performance. We find that cold tolerance ( $CT_{min}$ ) varied between the species according to the environmental temperature in the habitat from which they originated. These data support the idea that the evolution of cold tolerance has allowed species to persist in colder environments. However, contrary to expectation, we find that optimal temperature ( $T_{opt}$ ) and the breadth of thermal performance ( $T_{breadth}$ ) are similar in temperate, widespread and tropical species and we also find that the plasticity of TPCs was constrained. We suggest that the temperature range for optimal thermal performance is either fixed or under selection by the more similar temperatures that prevail during growing seasons. As a consequence, we find that  $T_{opt}$  and  $T_{breadth}$  are of limited value for predicting past, present and future distributions of species.

This article is part of the theme issue 'Physiological diversity, biodiversity patterns and global climate change: testing key hypotheses involving temperature and oxygen'.

## 1. Introduction

Temperature directly affects many biological processes, from enzymatic reactions to population growth [1–3], making environmental temperature an important abiotic determinant of fitness for most organisms [4]. The thermal biology of animals is often characterized from either thermal tolerance (the ability to survive short-term exposure to extreme temperatures) or thermal performance (quantified by measuring fitness-related traits over a range of temperatures) [4–6]. Thermal performance and thermal tolerance have both been used to



**Figure 1.** (a) Thermal performance curves (TPCs) can be characterized by an optimal temperature ( $T_{opt}$ ) and a thermal breadth ( $T_{breadth}$ —defined here as the range where performance is above 80% of optimal). Thermal performance is also restricted within upper and lower tolerance limits ( $CT_{max}$  and  $CT_{min}$ , respectively) beyond which behaviour ceases. Thermal adaptation and/or acclimation can potentially shift the TPC and tolerance limits (dashed lines in (a), grey area in (b)) but the evolutionary and ecological patterns of such shifts have rarely been examined in a systematic and comparative manner. (b) Changes in  $CT_{max}$  that result from either acclimation or adaptation to an environmental temperature typically have no or a weak positive slope. In the case of  $CT_{min}$ , a strong positive slope is expected. Few studies have systematically investigated the role of acclimation/adaptation for  $T_{opt}$  or  $T_{breadth}$  but theory predicts that  $T_{opt}$  should have a slope such that animals adapted, or acclimated (degree of plasticity represented as grey area around the slope) to warmer environments should have higher thermal optima ( $T_{opt}$ ) and a lower thermal breadth ( $T_{breadth}$ ). (Online version in colour.)

predict patterns of species distribution and responses to environmental change [7–11]. However, the usefulness of these measures requires an understanding of the evolution and plasticity of thermal traits as well as a critical evaluation of how these traits vary among and within species.

It is often assumed that thermal tolerance and performance will vary predictably with the environment of terrestrial ectotherms [4,5,12]. As illustrated in figure 1a, temperate species that experience a greater range of temperatures throughout the year are predicted to tolerate colder temperatures, have a lower optimal temperature ( $T_{opt}$ ) and to maintain performance across a wider range of temperatures (a broader performance breadth,  $T_{breadth}$ ), compared to tropical species [4,13,14]. Empirical evidence for these predictions comes mainly from studies on tolerance of acute exposure to heat and cold stress [9,15], where temperature tolerance is measured by observing cessation of neuromuscular control and/or the onset of death [16]. These approaches have facilitated direct comparison across taxa and geographical ranges and large comparative studies and meta-analyses show that species tend to increase cold tolerance with distance from the equator [9,17,18]. Interestingly, upper thermal limits vary

less across latitudes, which is consistent with less latitudinal variability in maximal environmental temperature [15,19,20] (figure 1b). Additionally, studies on ectotherms have found that tolerance of and performance at high temperatures are evolutionarily constrained in many species [19,21–23].

Thermal performance is a broad term that can be measured on many traits including metabolism, locomotion, growth rate and fecundity [6,24–28]. Because of the variety of traits and methods used to assess thermal performance, there are few directly comparable studies that can be used to investigate broad-scale patterns of adaptation in performance in terms of either thermal optima ( $T_{opt}$ ) or thermal breadth ( $T_{breadth}$ ) [6,29]. Moreover, it is often time- and resource-consuming to generate the data across the many experimental temperatures needed to generate high-quality thermal performance estimates—especially if more than one trait or one species is considered. The dearth of comparable studies may also reflect the practical challenges related to the generation of robust thermal performance curve (TPC) data. Despite little empirical evidence, it is generally assumed that  $T_{opt}$  and  $T_{breadth}$  will follow theoretically adaptive patterns across latitudinal and environmental gradients (figure 1).

It is often suggested that species from highly fluctuating thermal environments (temperate and sub-tropical species) exhibit greater plasticity than species from stable environments [4,12,30,31]. Species from temperate environments are therefore assumed to have larger acclimation responses that should also be reflected in more plastic TPCs (figure 1). Tests of this hypothesis are, however, mostly limited to tolerance traits ( $CT_{min}/CT_{max}$ ) and comparative studies often fail to find marked evolutionary differences in plasticity across latitudinal gradients ([32–37] but see [38,39]). Despite the lack of comparative data on TPCs of ectotherms, textbook examples often depict temperate species as having greater plasticity in addition to broad TPCs and lower optimal temperatures [4,40,41] (figure 1). These patterns are based largely on theoretical work predicting that environmental variation will drive the evolution of thermal acclimation to seasonal environments [4,12,13,42,43]. On the other hand, there are many traits linked to fitness and population growth (such as reproduction, growth and developmental success) that are only relevant for parts of the yearly cycle, particularly in species that occur farther from the equator. Since summer maximal temperatures vary less across latitude, it is possible that such performance traits vary less along the temperate to tropical axis [44].

In the present study, we address these questions by measuring thermal tolerance limits and thermal performance of several fitness components (developmental viability, development speed and adult fecundity) at seven test temperatures in 22 species of *Drosophila* reared at a common temperature. For 10 of these species, we also measure thermal limits and thermal performance following developmental acclimation to three additional developmental temperatures. Using the interspecific data, we are able to test three *a priori* assumptions: that tropical species from stable warm climates (i) are less cold tolerant; (ii) have a higher  $T_{opt}$ ; and (iii) have a lower  $T_{breadth}$  compared to temperate species from colder and more variable thermal environments (compare patterns in figure 1a,b). Furthermore, by examining intraspecific patterns of plasticity within species reared at different temperatures, we examine the plasticity of TPCs and test if temperate species from variable environments are more

plastic in thermal tolerance or thermal performance traits (grey shaded area in figure 1b).

## 2. Material and methods

### (a) Experimental protocol

Thermal traits were measured in 22 species of *Drosophila* through assessment of thermal tolerance ( $CT_{\min}$  and  $CT_{\max}$ ) as well as thermal performance in life-history traits (using data of three fitness traits: egg-laying capacity, egg-to-adult viability and developmental speed). The 22 species span the *Drosophila* phylogeny and include tropical, temperate, widespread, cold-adapted and xeric species (electronic supplementary material, table S1). Flies used in this experiment originated from several laboratory stocks (electronic supplementary material, table S1) but we have recently shown that there is no significant or systematic difference in thermal tolerance and life-history traits investigated in flies recently collected in the field and laboratory stocks, respectively [45]. To obtain data, we reared 15 species under common garden conditions: density-controlled, 19°C, 12 L:12D, on standard fly media [46]. Data for an additional 10 species were extracted from a previous study [7] with almost identical rearing conditions (electronic supplementary material, table S1). Three of the species were included in both the new dataset and the dataset from Overgaard *et al.* [7]. Collectively, these data allowed us to explore evolutionary and ecological patterns in thermal tolerance and in thermal performance optima and breadth.

To investigate if/how thermal acclimation affects thermal tolerance and thermal performance, we explored the role of developmental thermal acclimation for 10 of the species (*Drosophila birchii*, *D. immigrans*, *D. lutescens*, *D. melanogaster*, *D. mercatorum*, *D. montana*, *D. mojavensis*, *D. simulans*, *D. subobscura* and *D. yakuba*), highlighted in electronic supplementary material, table S1). Each of these species was reared at 15, 19, 23 and 27°C prior to assessments of thermal performance (fecundity, viability and developmental speed) or thermal tolerance ( $CT_{\min}$  and  $CT_{\max}$ ). Because *D. subobscura* and *D. montana* originate from cooler climates, they were acclimated to 11, 15, 19 and 23°C, respectively.

### (b) Rearing of experimental animals

To produce experimental flies, we allowed parental flies to oviposit on medium and subsequently placed the eggs in vials with 7 ml standard fly medium at a density of 40 eggs/vial (typically we set up 15 vials (600 eggs), per species/acclimation group). Upon emergence, the adult flies were transferred to fresh food bottles and tipped every third day until eggs and first instar larvae were observed in the bottle. The adults were then deemed to be reproductively mature and subsequently used for experimentation to estimate critical thermal limits ( $CT_{\min}$  or  $CT_{\max}$ ) or to test developmental speed, viability and fecundity.

### (c) Thermal performance (fitness traits)

*Viability and developmental speed* were measured by transferring eggs laid by each species/acclimation combination to vials with 7 ml standard fly food (20 eggs per vial) and then placing the vials at one of the seven test temperatures (11, 15, 19, 23, 27, 31 and 33°C; figure 2). A minimum of five replicates per species/acclimation combination were placed at each of the test temperatures. The vials were scored daily for the number of adults that emerged to assess developmental speed (calculated as 1/time to emergence). The proportion of adults that emerged was used to determine egg-to-adult viability at each temperature (figure 2).

*Fecundity* was measured by placing a single mated pair of adult flies (sorted with less than 5 min of CO<sub>2</sub>, 48 h prior to

experimentation) into empty 35 ml plastic vials containing a small spoon filled with 1 ml of standard fly medium. For each of the test temperatures, we used a minimum of seven and a maximum of 15 replicates per species/acclimation combination. Flies were allowed to lay eggs for 48 h. After 24 h, spoons were replaced to prevent the medium from drying out at high temperatures and flies were assessed for survival daily. In cases where one member of the pair was dead, the replicate was removed from the final analysis.

### (d) Thermal tolerance ( $CT_{\min}/CT_{\max}$ )

Twenty mated females from each species/acclimation combination were placed in individual 5 ml glass vials with lids. The vials were then submerged in a circulating water/glycol bath at 20°C and exposed to a slow temperature-ramp down or up (rate of 0.1°C per minute) for assessment of critical thermal minimum and maximum, respectively. The temperature at which all movement ceased was recorded as the tolerance limit ( $CT_{\min}$  or  $CT_{\max}$ ) for that individual.

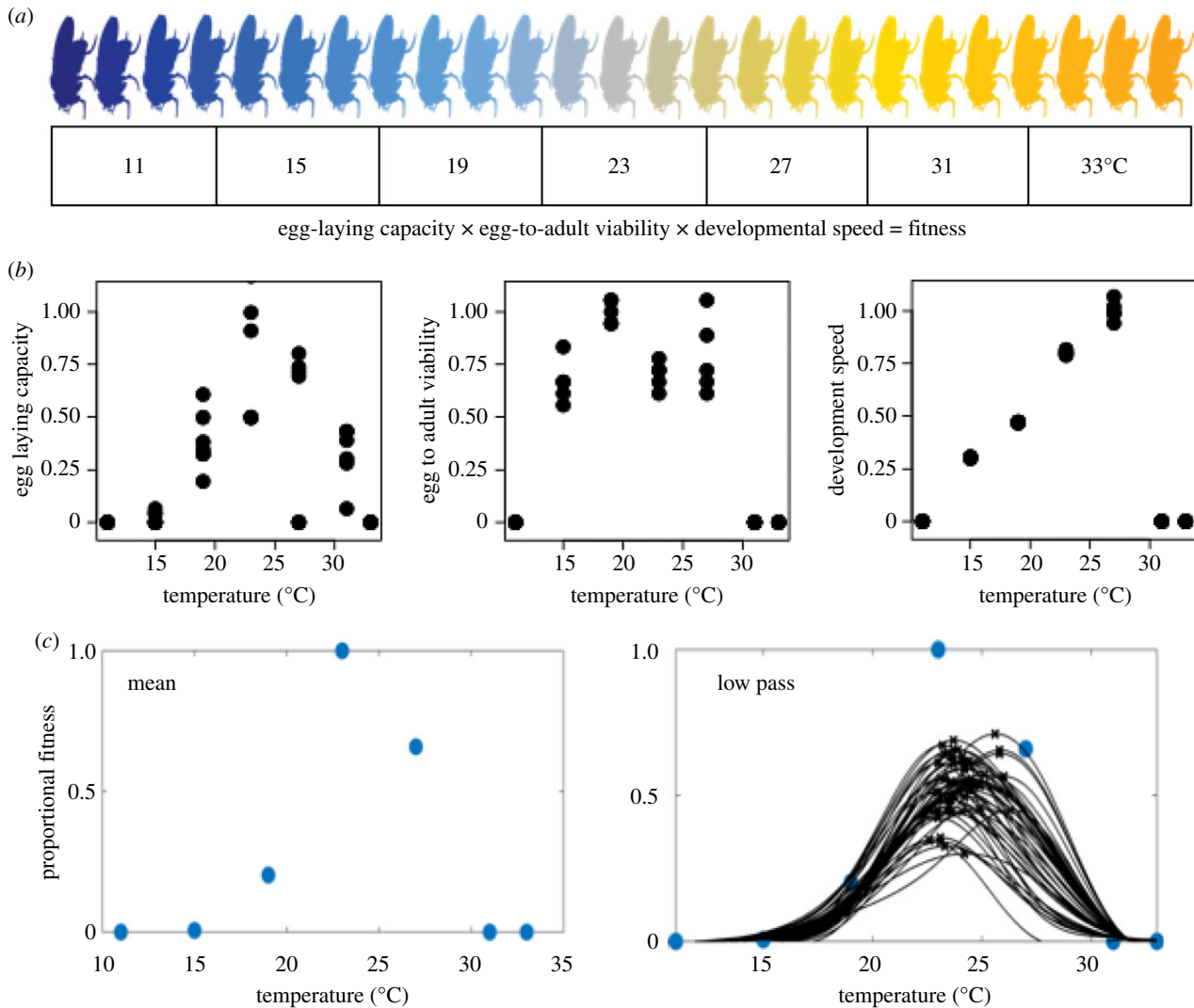
### (e) Environmental data

We used published environmental values calculated from the mean environmental conditions experienced across the distribution range of a given species (electronic supplementary material, table S1 and [17] for details). For the analysis, we considered the following environmental traits: latitude, mean annual temperature, the temperature of the warmest quarter, the temperature of the coldest quarter, precipitation in the wettest quarter and precipitation in the driest quarter as these have previously been identified as relevant to thermal tolerance in ectotherms.

### (f) Data analysis

Egg-to-adult viability, developmental speed and fecundity were measured at each of seven test temperatures (11, 15, 19, 23, 27, 31 and 33°C), after which each fitness component was standardized to the mean maximal value possible within a given species/acclimation temperature combination (i.e. if the highest mean viability across the seven test temperatures was 80%, then viability of the six other test temperatures was normalized to this value). In this way, all trait values were between 0 and 1 for each of the three traits. Using these normalized trait values, we computed a composite fitness measure by taking the product of all three (see [7] for details). Using this estimate of composite fitness, we fit the data to estimate the optimal temperature ( $T_{\text{opt}}$ ) and the breadth of thermal performance ( $T_{\text{breadth}}$ ), which represents the temperature interval in which composite fitness is greater than 80% of  $T_{\text{opt}}$  (see figures 1 and 2 and electronic supplementary material, methods for further details).

For our estimate of  $T_{\text{opt}}$  and  $T_{\text{breadth}}$ , we took the normalized trait values for each of the test temperatures and up-sampled the data to a 0.1°C spacing, using simple linear interpolation. We then smoothed the result with a zero-phase low pass finite impulse response (FIR) filter (implemented in MATLAB 2016a, The MathWorks, Inc., Mass., USA). The result was a smooth estimate of the data, sampled at 4°C intervals and then optimally up-sampled to yield a frequency resolution of 0.1°C. This low pass fit entailed no *a priori* assumptions on the underlying shape of the data and comparisons to Gaussian and polynomial fits were qualitatively similar (see electronic supplementary material, figures S1 and S2). In order to obtain an estimate of variance in our estimates of  $T_{\text{opt}}$  and  $T_{\text{breadth}}$  and to mitigate effects of spurious data within and between species/acclimation temperature treatments, we performed this analysis repeatedly by bootstrapping the data in MATLAB, thereby subsampling the data 50 times. Specifically, we made each of the 50 estimates by randomly sampling half (rounded up—i.e. five replicates would



**Figure 2.** (a) For each species (and acclimation temperature), thermal performance was measured three ways across seven test temperatures (11, 15, 19, 23, 27, 31 and 33°C): as egg-to-adult viability, developmental speed (1/developmental time in days) and fecundity measured as egg-laying. Each of these trait measurements was replicated 5 to 15 times. Each replicate was standardized to the mean maximal value of a given trait across the seven test temperatures. We generated a proxy for fitness by taking the product of each of these traits. Specifically, we subsample replicates from each trait at each test temperature (see S2f). (c) Using these data, we fit a TPC with the low pass function and estimated  $T_{\text{opt}}$  and  $T_{\text{breadth}}$  (defined as the temperature range where the fitness estimate is above 80% of the maximum value). We repeated this procedure 50 times by randomly subsampling the data. This subsampling procedure mitigates the effects of spurious variability within and between species/acclimation temperatures and enables us to obtain estimates of variability in our  $T_{\text{opt}}$  and  $T_{\text{breadth}}$  estimates. (Online version in colour.)

be sampled three times) of the available replicates per trait per test temperature (electronic supplementary material, figure S2).

### (g) Adaptation in thermal performance and thermal tolerance

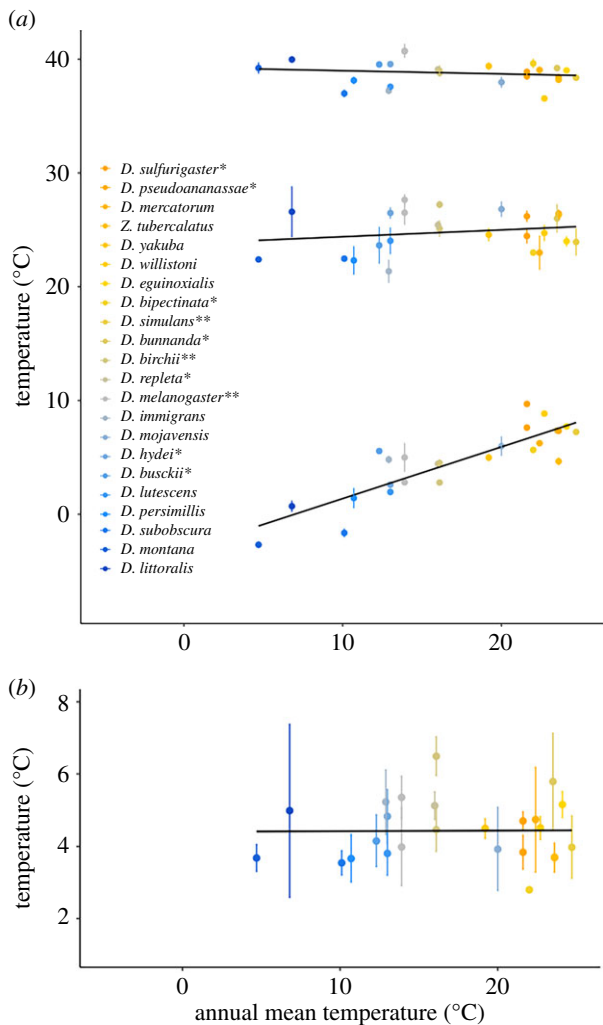
With 22 species distributed across the *Drosophila* phylogeny that differed in their distribution and environmental sensitivities, we were able to investigate the relationship between thermal tolerance/performance and the environmental conditions experienced by different species in nature. To test for evolved differences across species, we analysed the data from animals reared at a common temperature (19°C) using a generalized linear mixed model approach with either  $CT_{\text{min}}$ ,  $CT_{\text{max}}$ ,  $T_{\text{opt}}$  or  $T_{\text{breadth}}$  as the response variable. We then performed formal model selection using all of the environmental variables (electronic supplementary material, table S3) as predictor variables. We treated species as a random effect. For thermal performance, we nested species within resampling iteration to account for pseudo-replication generated by resampling the data. These analyses were performed in R (v. 3.3.3) using the nlme package [47]. We did not find a significant phylogenetic signal in our data (see

electronic supplementary material, methods) and thus did not include phylogenetic corrections in our analysis.

The composite fitness measure reported here is based on measurements where the egg/larvae are exposed chronically to the test temperature in the assessment of egg-to-adult viability and developmental speed. Such chronic treatments do, in themselves, represent an acclimation treatment. Therefore, we performed a similar analysis of  $T_{\text{opt}}$  by fitting a performance curve using only the data of egg-laying capacity, which represents a more acute measure of thermal performance. This analysis resulted in qualitatively similar patterns to the analysis based on composite fitness (compare electronic supplementary material, tables S3 and S4 and compare figure 3 and electronic supplementary material, figure S5).

### (h) Acclimation of thermal performance and thermal tolerance

For 10 species, we investigated if/how developmental acclimation affected  $CT_{\text{min}}$ ,  $CT_{\text{max}}$ ,  $T_{\text{opt}}$  and  $T_{\text{breadth}}$ . For this analysis, we used a generalized linear mixed model approach with either  $CT_{\text{min}}$ ,  $CT_{\text{max}}$ ,  $T_{\text{opt}}$  or  $T_{\text{breadth}}$  as the response variable and



**Figure 3.** (a) The thermal limits ( $CT_{\min}$  and  $CT_{\max}$ ) and the calculated thermal optimum ( $T_{\text{opt}}$ ) for all 22 species reared at 19/20°C as a function of annual mean temperature, showing that only  $CT_{\min}$  exhibits a strong correlation to environmental temperature. The colour of each symbol corresponds to the mean absolute latitude of that species' range (orange for low and blue for high latitude). \* indicates species included from [7] and \*\* indicates species included in both studies. *Z. tubercalatus*, *Zaprionus tubercalatus*. (b) The calculated breadth of thermal performance ( $T_{\text{breadth}}$ ) as a function of annual mean temperature shows no relationship with annual mean temperature. (Online version in colour.)

with species as a random effect. We nested species and acclimation within bootstrap iteration to account for pseudo-replication generated by resampling the data. We then performed formal model selection using acclimation and all of the relevant environmental variables (electronic supplementary material, table S3) as predictor variables. Additionally, we performed a similar analysis of  $T_{\text{opt}}$  using only the data of egg-laying capacity, which revealed more short-term effects of developmental acclimation temperature (electronic supplementary material, table S4 and figure S6).

### 3. Results

#### (a) Adaptation in thermal performance and thermal tolerance

Thermal performance was quantified as both the temperature where the highest performance was obtained ( $T_{\text{opt}}$ ; figure 3a) and as the temperature range where high performance (>80% of  $T_{\text{opt}}$ ) was maintained ( $T_{\text{breadth}}$ ; figure 3b). Model

selection suggested that mean temperature of the warmest quarter explained the highest amount of observed variance in  $T_{\text{opt}}$  ( $F_{1,1690} = 0.034$ ,  $p = 0.17$ ) but the interspecific variance in  $T_{\text{opt}}$  was not significantly explained by any of the climatic variables (electronic supplementary material, table S3).  $T_{\text{breadth}}$  exhibited more variation within species than  $T_{\text{opt}}$ , and similar to  $T_{\text{opt}}$ , we failed to find significant relations with any of the climatic variables. The environmental variable that explained the highest amount of variance in  $T_{\text{breadth}}$  was precipitation in the driest quarter ( $F_{1,1690} = 1.77$ ,  $p = 0.09$ ).

For thermal tolerance, model selection showed that  $CT_{\min}$  increased significantly with the mean temperature of the coldest quarter ( $F_{1,296} = 32.97$ ,  $p = 0.0007$ ) and the explanatory power of the model was not improved by the inclusion of any of the other climatic variables (electronic supplementary material, table S3).  $CT_{\max}$  showed no relationship to any of the climatic variables. The best model explaining variance in  $CT_{\max}$  included the mean temperature of the warmest quarter as the sole predictor variable, but this was not significant ( $F_{1,313} = 0.03$ ,  $p = 0.86$ ). For all traits, we found that annual mean temperature represented the second-best model (for ease of comparison, we have chosen to plot all the traits against annual mean temperature in figure 3, electronic supplementary material, table S3).

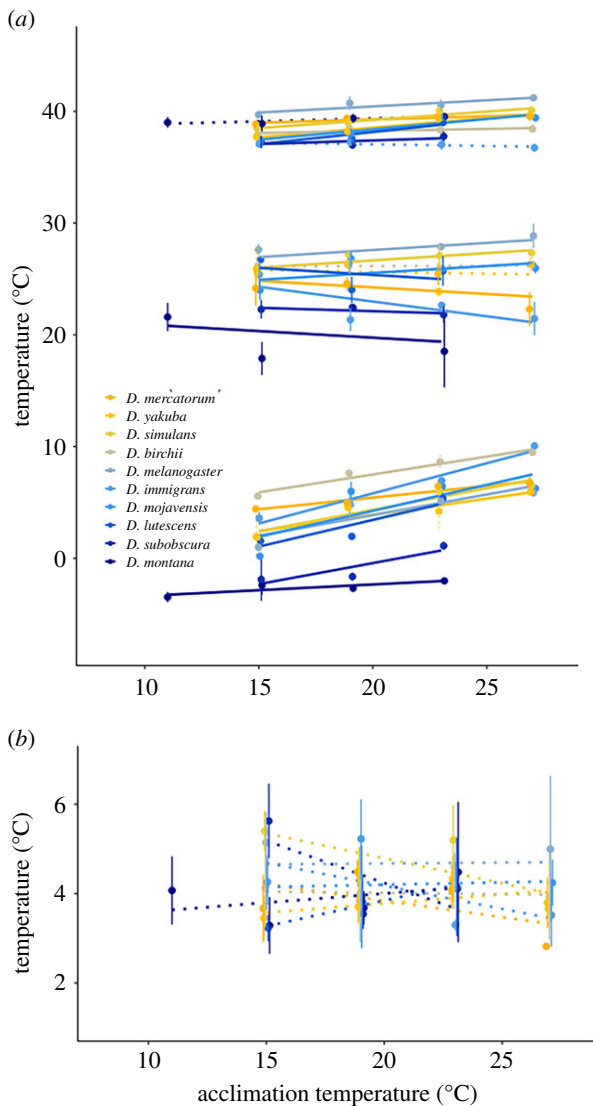
#### (b) Acclimation of thermal performance and thermal tolerance

We considered developmental acclimation in 10 of the investigated species and did not find  $T_{\text{opt}}$  to be correlated to acclimation temperature ( $F_{1,1690} = 0.47$ ,  $p = 0.49$ , figure 4a). There was, however, a positive association between  $T_{\text{opt}}$  and mean temperature of the warmest quarter in this smaller subset of species ( $F_{1,1690} = 10.21$ ,  $p = 0.01$ , table 1).  $T_{\text{breadth}}$  decreased significantly with acclimation temperature, suggesting that exposure to higher temperatures serves to decrease thermal breadth ( $F_{1,1690} = 23.97$ ,  $p < 0.001$ , figure 4b). However, this pattern in  $T_{\text{breadth}}$  was not clear at the single species level as none of the 10 species showed a significant directional response in  $T_{\text{breadth}}$  (figure 4b).

For thermal tolerance traits, we found that  $CT_{\min}$  increased with both acclimation temperature ( $F_{1,824} = 1326.54$ ,  $p < 0.001$ ) and mean temperature of the coldest quarter ( $F_{1,824} = 8.51$ ,  $p = 0.019$ ). The effect of acclimation temperature on  $CT_{\max}$  was smaller but still significant ( $F_{1,872} = 347.56$ ,  $p < 0.001$ ). Additionally, we analysed if there was any relation between environmental variables and acclimation for any of the four traits ( $T_{\text{opt}}$ ,  $T_{\text{breadth}}$ ,  $CT_{\min}$  or  $CT_{\max}$ ). Acclimation potential was calculated from the slope of the linear fits of trait values against acclimation temperature, where steep slopes indicate high plasticity. When acclimation potentials were analysed across species we found no relationship with the environmental origin, i.e. species from cold (temperate) climates did not have higher plasticity than those originating from warm (tropical) climates (electronic supplementary material, figure S4).

### 4. Discussion

How organisms cope with thermal variation and extreme temperature exposure is a central research topic in ecophysiology and evolutionary biology. Insects such as *Drosophila*



**Figure 4.** (a) The thermal limits (CT<sub>min</sub> and CT<sub>max</sub>) and the calculated thermal optima (T<sub>opt</sub>) for the 10 species reared at additional temperatures shown as a function of rearing temperature. The colour corresponds to the mean absolute latitude of that species' range (orange for low and blue for high latitude), with solid lines representing a significant slope and dashed lines representing a non-significant slope. (b) The calculated breadth of thermal performance (T<sub>breadth</sub>) as a function of acclimation temperature.

spp. are often used as models and different measures of performance at different temperatures are used to explore adaptation, explain patterns of distribution and predict responses to climate change [7,9,11,19,48]. Measures obtained from such studies on thermal tolerance and performance include the ability to survive extreme temperatures (CT<sub>min</sub> or CT<sub>max</sub>), as well as physiological and behavioural measures of different life-history traits (characterized by T<sub>opt</sub> and T<sub>breadth</sub>). Numerous comparative studies have tested for an association between temperature tolerance and species' distributions. These studies typically provide support for the association between cold tolerance and species distributions (and a weak association with heat tolerance) [9,15,17–19,49]. The relationship between thermal performance measures (T<sub>opt</sub> and T<sub>breadth</sub>) and species distribution is less apparent. This is partially owing to the lack of large comparative datasets where the thermal performance of different species is examined under comparable conditions. Nevertheless, theory predicts that TPCs should differ

considerably between tropical and temperate species, as outlined in figure 1 [4,5,12,13]. To test these predictions, the present study examined patterns of thermal performance and tolerance in 22 *Drosophila* species using a common garden design. Specifically, we tested the hypotheses that T<sub>opt</sub>, CT<sub>min</sub> and CT<sub>max</sub> are lower in species inhabiting cooler climates while thermal tolerance curves are broader (increased T<sub>breadth</sub>) in cold-adapted species. Further, we tested the hypothesis that developmental thermal acclimation can shift T<sub>opt</sub>, T<sub>breadth</sub>, CT<sub>min</sub> and CT<sub>max</sub> and that high latitude species from cooler climates are characterized by larger plasticity of these traits as such species have adapted to greater variability in environmental temperatures.

We find, in accordance with several earlier studies of ectotherms, that upper thermal limits (CT<sub>max</sub>) are not significantly correlated with the average environmental temperature of the species' origin, but that cold tolerance (CT<sub>min</sub>) is strongly correlated with the environmental temperatures that characterize the species' geographical ranges [15,18,19,49,50]. Earlier studies including *ca* 100 species of *Drosophila* also failed to find a simple association between heat tolerance and annual mean temperature [19], but previous studies, including Kellermann *et al.* [19], do reveal a relationship between tolerance and the warmest environmental temperatures in *Drosophila* living in dry habitats. It is therefore possible that the inclusion of more xeric species in the present study would have resulted in a similar finding [19,51,52]. We observed considerably larger variation in CT<sub>min</sub> between species and CT<sub>min</sub> was strongly correlated to temperature (table 1, figure 2a). This result is consistent with several earlier studies of insects in general, and *Drosophila* specifically, which all find a strong relationship between cold tolerance and distribution range of species [9,15,18,53]. Thus, tropical species from relatively stable and warm thermal environments are considerably less cold tolerant than their temperate congeners.

Thermal performance is inherently more complex to measure and analyse than thermal limits, partly because it is difficult to identify which traits are the most appropriate to explore when assessing the relation between temperature and fitness (or population growth potential) (reviewed in [6]). There are relatively few large-scale empirical studies of TPCs because of their time-consuming nature, but directly comparable datasets are necessary in order to test theoretical expectations of thermal performance. The present study is, to our knowledge, the largest common garden examination of intraspecific patterns in T<sub>opt</sub> or T<sub>breadth</sub> in insects. We chose to measure and combine three aspects of fitness: rate of egg production, egg-to-adult viability and developmental speed. Each of these traits can be argued to capture major aspects of fitness (population growth potential) and by using the product of the three traits we aimed to provide a composite trait related to species fitness [7]. The use of fitness-related traits, and our composite trait in particular, offers a direct measure of population growth potential in contrast to traits such as locomotor performance or feeding rate that are connected to fitness more indirectly (i.e. feeding and locomotion are included in traits such as developmental rate and reproduction, but this is not necessarily true the other way round). There was variation within and among species in both T<sub>opt</sub> and T<sub>breadth</sub>, suggesting that these trait parameters do evolve; however, this variation was not correlated to any of the climatic or phylogenetic variables

**Table 1.** Summary of mixed effects models for the evolution and acclimation of all four traits. The models with the lowest AIC values are shown in italics, additional models are the second best and directly relate to the annual mean temperatures as depicted in figures 3 and 4. AMT, annual mean temperature.

model	est	s.e.	F-value	p-value
for evolution of thermal traits				
best models $T_{opt}$				
<i>optimum ~ mean temp of the warmest</i>	0.155	0.108	2.038	0.167
optimum ~ AMT	0.060	0.062	0.939	0.343
best models $T_{breadth}$				
<i>Tb ~ precip dry</i>	0.021	0.012	3.117	0.0907
Tb ~ AMT	0.002	0.029	0.004	0.9506
best models $CT_{min}$				
<i><math>CT_{min}</math> ~ mean temp of the coldest</i>	0.284	0.049	32.970	0.0001
$CT_{min}$ ~ AMT	0.430	0.065	43.778	<0.0001
best models $CT_{max}$				
<i><math>CT_{max}</math> ~ mean temp of the warmest</i>	-0.015	0.080	0.034	0.8576
$CT_{max}$ ~ AMT	0.007	0.045	0.022	0.8839
for plasticity of thermal traits				
best models $T_{opt}$				
optimum ~ acclitemp + mean temp of the warmest				
acclitemp	-0.006	0.008	0.467	0.4944
mean temp of the warmest	0.533	0.167	10.206	0.0127
optimum ~ acclitemp	-0.006	0.008	0.492	0.483
best models $T_{breadth}$				
<i>optimum ~ acclitemp</i>	-0.024	0.005	23.972	<0.0001
best models $CT_{min}$				
$CT_{min}$ ~ AccTemp + mean temp of the coldest				
AccTemp	0.314	0.009	1325.018	<0.0001
mean temp of the coldest	0.240	0.082	8.512	0.019
$CT_{min}$ ~ AccTemp + AMT				
AccTemp	0.314	0.009	1326.229	<0.0001
AMT	0.372	0.108	11.909	0.009
best models $CT_{max}$				
<i><math>CT_{max}</math> ~ AccTemp</i>	0.092	0.005	347.670	<0.0001

investigated (table 1, figure 3a; electronic supplementary material, table S2). Importantly, we do not find that temperate species have a lower  $T_{opt}$  than tropical species, and we do not observe increased  $T_{breadth}$  in temperate or widespread species as compared to tropical or restricted species. These results are in sharp contrast to the widely accepted theoretical construct which suggests that there are marked and directional patterns in both TPC shape, TPC temperature range and TPC plasticity among ectotherms (figure 1, [4,13,14]). The lack of empirical evidence for directional patterns in TPCs in the present study raises the question of whether these 'expected' patterns are valid representations of TPC's from species with marked differences in environmental origin. This was also questioned in two recent meta-analyses by Sørensen *et al.* [29] and Tüzün & Stoks [54], who explored inter- and intraspecific patterns of thermal performance among insects and other ectothermic animals. Both of these studies found some evidence of positive correlations between  $T_{opt}$  and environmental temperature, but in both cases, this

relation was weak and only explained a small fraction of the variance in  $T_{opt}$  observed among and within species (see also discussion below).

To investigate if plasticity in TPCs is related to rearing temperature, we examined the plasticity of TPCs in 10 of the 22 species with the hypothesis that cold acclimation would shift the TPC ( $T_{opt}$  and  $T_{breadth}$ ) to lower temperatures and with the additional expectation that temperate species are more plastic than tropical species from stable thermal environments. We found no support for either of these hypotheses. Developmental temperature shifted cold tolerance limits ( $CT_{min}$ ) by approximately 0.4°C per °C of acclimation, whereas heat tolerance limits ( $CT_{max}$ ) shifted by approximately 0.1°C per °C of change in acclimation temperature. This is consistent with previous reports of acclimation responses in thermal tolerance limits of *Drosophila* [34,37,55]. In contrast to the thermal tolerance limits, we observed no clear change in  $T_{opt}$  with acclimation (table 1). There was seemingly an overall significant decrease

in  $T_{\text{breadth}}$  with increasing acclimation temperature, but this was very small (0.24°C shrinking of  $T_{\text{breadth}}$  for a 10°C shift in acclimation temperature, table 1) and not significant for any of the 10 species when analysed individually. Furthermore, there was no relationship between the degree of plasticity in either tolerance ( $CT_{\text{min}}$  and  $CT_{\text{max}}$ ) or performance ( $T_{\text{opt}}$  and  $T_{\text{breadth}}$ ) traits when the species were ranked according to annual mean temperature (figure 4a; electronic supplementary material, figure S4). This lack of pattern in plasticity is consistent both when analysing our measure of composite fitness (that includes long-term exposure to the test temperatures) and when using fecundity alone (electronic supplementary material, figure S5). Accordingly, we do not find any support suggesting that temperate species have higher plasticity than tropical species. Our finding of similar plasticity among species from the temperate and tropical origin is also consistent with earlier reports from *Drosophila* [34,37,55,56] and is emerging as a general pattern in ectothermic animals [35], although some have found a weak but positive association between plasticity and latitude in a large meta-analysis of ectotherms [36]. Thus, we conclude that our empirical findings offer no support for the idea that distance from the equator, degree of seasonality or thermal safety margin (calculated as the difference between upper thermal limit and maximum environmental temperature) correlate with the degree of plasticity, as has been suggested elsewhere [12,13,57].

Our results contradict expectations as neither  $CT_{\text{max}}$  TPCs nor plasticity of TPCs vary with latitude or thermal characteristics of origin in ways predicted from theoretical studies. This lack of empirical support raises the question of how this discrepancy can be explained. One possible explanation could relate to a putative evolutionary ‘limit’, or at least an inertial hurdle, to the evolution of  $CT_{\text{max}}$  and  $T_{\text{opt}}$  [58–60]. Specifically, a lack of genetic variation may prevent adaptation and plasticity of physiological tolerance traits [61–64]. A second reason might be owing to behavioural thermoregulation causing species from different latitudes to experience more similar thermal regimes as they may show a similar preference for particular microhabitats (i.e. seeking cooler refuges in warm environments and warm refuges in cool environments) [62]. It is also possible that the theory of TPCs is valid, but that we are simply analysing the wrong ‘fitness-related traits’, which compromises our conclusions. Traits related to growth and reproduction are generally more important during the summer/growth season [44], when temperatures differ less between temperate and tropical species. The species investigated in our study, for example, may experience quite similar selection pressures

when it comes to the thermal performance of development and reproduction. By contrast, we do actually find marked differences in tolerance traits ( $CT_{\text{min}}$ ) that separate the species according to the environmental origin. Perhaps this is also true for other forms of tolerance traits (i.e. immune function, desiccation tolerance, starvation tolerance at low/high temperature). It is not possible for us to discern if the theoretical patterns of TPCs are ‘wrong’ or if we are simply measuring the ‘wrong’ traits. We note, however, that most other fitness curves are also based on growth-related traits and we argue that analysis of such fitness curves should be used cautiously in modelling as they are unlikely to reveal how past, current and future climates can influence species distribution.

The present study represents the largest common garden examination of thermal tolerance and performance in insects to our knowledge. The results support the idea that the thermal tolerance limits (primarily  $CT_{\text{min}}$ ) have evolved in response to extreme environmental conditions and that they act to limit range expansion. Moreover, those differences are reflected in species distribution, while we find no clear patterns related to optimal thermal performance. This leads us to conclude that TPCs are not particularly useful for predicting current or future species distributions. Specifically, there was no association with latitude or climatic characteristics of species distributions in thermal performance ( $T_{\text{opt}}$  and  $T_{\text{breadth}}$ ) based on population growth parameters. The absence of clear shifts along environmental gradients in thermal performance among *Drosophila* species calls to question if this is also the case for other ectothermic animals and when testing thermal performance in the field. We argue that there should be a continued search for thermal performance traits that do differ markedly between species from different environmental origin. Identifying such traits will reveal great insights into the evolutionary adaptations that have allowed some species to occur new habitats.

**Data accessibility.** Data (trait data and climatic data files) available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.1t38n12> [65].

**Authors' Contributions.** Data were collected and analyzed by H.J.M. K.B. fitted and compared performance curves. V.K. led the phylogenetic analysis. All authors contributed to writing and editing and approved the final manuscript.

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