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Title: Temperatures that sterilise males better match global species distributions than lethal temperatures

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Abstract

Attempts to link physiological thermal tolerance to global species distributions have relied on lethal temperature limits, yet many organisms lose fertility at sublethal temperatures. Here we show that, across 43 Drosophila species, global distributions better match male-sterilising temperatures than lethal temperatures. This suggests that species distributions may be determined by thermal limits to reproduction, not survival, meaning we may be underestimating the impacts of climate change for many organisms.

Main Text:

To preserve biodiversity, we urgently need to understand the physiological, behavioral and evolutionary factors that underpin species' thermal distributions[1]. Laboratory-derived estimates of the highest temperatures at which an organism can survive (critical thermal limits/CTL) provide measures of species' thermal tolerances. Linking CTLs to current distributions has enabled better modelling of future species distributions under climate change scenarios[2], likely to be vital for prioritizing conservation efforts[3] and effectively managing invasive species[4].

Despite CTLs being measured in artificial laboratory conditions, they correlate reasonably well with species' macroecological distributions[5,6] and have been used to estimate species' capacity to tolerate temperature increases across their current range; their 'thermal safety margins'[5,7]. However, CTLs can be higher than the temperatures that cause seasonal population declines in nature[8]. Some of this discrepancy has been attributed to methodological shortcomings[9-11], but could also be due to organisms becoming infertile at sub-lethal temperatures[12]. Sub-lethal temperatures cause losses in fertility in plants[13], insects[14-16], fish[17], corals[18], birds[19] and mammals, including humans[20]. If the temperatures that cause infertility (thermal fertility limits/TFLs) are often lower than CTLs, we may both be generally underestimating organisms' vulnerability to climate change, and misidentifying which organisms are most at risk. If TFLs correlate with natural distributions better than CTLs, incorporating TFLs into models of climate change impacts may improve accuracy.

We recorded three measures of upper thermal limits in adult males from 43 species of *Drosophila*. To compare fertility and survival limits under identical heat-stress conditions, we

exposed flies to a 4-hour static heat stress at a range of temperatures from benign through to lethal (Supplementary Table 1). From these data we estimated both the temperature that is lethal to 80% of individuals (LT80), and the temperature at which 80% of surviving males are sterilized (TFL80). Measuring thermal traits under static temperature stress rather than slowly increasing temperatures (i.e. ramping) has received criticism[21]. However, ramping assays require an immediate observable response, such as flies losing coordinated motor function. Unfortunately, sterilization is not immediately observable, so we use static temperatures and assay fertility through subsequent matings. We score fertility at two time points: (i) cumulatively over 1-6 days post-heat, to capture any immediate sterilizing effect of heat, and (ii) 7-days after heat-stress to capture any recovery of fertility or delayed sterility. To compare our estimates of TFL80 and LT80 with a measure of lethal temperature under ramping thermal stress, we also assayed the CT_{MAX} of each species. This is the temperature at which males lose coordinated motor function under gradually increasing temperatures. CT_{MAX} is commonly used to predict species' sensitivity to thermal stress associated with climate change[3,5,7].

We found that 11 of 43 species experience an 80% loss in fertility at cooler-than-lethal temperatures immediately following heat-stress (Extended Data Figure 1). Interestingly, rather than seeing a recovery of fertility over time, the impact of high temperatures on fertility was more pronounced 7-days post heat stress (Figure 2A). Using this delayed measure of fertility, 44% of species (19/43) showed fertility loss at cooler-than-lethal temperatures. The difference between lethal and fertility limits ranged from 0°C to 4.3°C (mean of all species = 1.15 ± 0.22°C), and LT80 and TFL80 predict dramatically different rankings of species' robustness to high temperature (Figure 2B). All three thermal limits significantly, positively correlate with each other (Supplementary Table 2). Despite deriving from different types of heat-stress, the

correlation coefficient between CT_{MAX} and LT80 is larger than that between TFL80 and either measure of lethal temperature. Relatively low correlations between survival (measured as CT_{MAX} under dynamic conditions or LT80 under static stress) and fertility (measured under static heat stress) suggests they are distinct phenomena, and measuring both may be important for understanding species responses to thermal stress.

Our data confirm that fertility loss at sub-lethal temperatures is common in *Drosophila*, suggesting that lethal limits alone may overestimate the thermal tolerance of many species. However, the key question is whether TFLs are linked to organisms' distributions in nature. To test this, we integrated existing distribution data of each sampled *Drosophila* species with global climate data. From this we estimated the mean maximum air temperatures species are likely to encounter in natural populations. Our measurement of CT_{MAX} significantly predicted mean maximum environmental air temperature (PGLS: $t_{40} = 2.647$, $P = 0.012$), albeit this relationship negatively interacts with annual rainfall (PGLS: $t_{40} = -2.077$, $P = 0.044$, $_{adj}R^2 = 0.186$, $_{partial}R^2 = 0.336$). LT80 also significantly predicted mean maximum environmental temperature (PGLS: $t_{40} = 3.360$, $P = 0.002$) to a similar extent ($adjR^2 = 0.197$, $_{partial}R^2 = 0.337$). However, the relationship between TFL80 and mean environmental maximum temperature was stronger, both when TFL80 was measured immediately following heat-stress (PGLS: $t_{40} = 4.225$, $P < 0.001$, $_{adj}R^2 = 0.286$, $_{partial}R^2 = 0.401$) and 7 days later (PGLS: $t_{40} = 5.014$, $P < 0.001$, $_{adj}R^2 = 0.365$, $_{partial}R^2 = 0.455$). Comparing all best–fit models, TFL80 measured 7-days after heat shock most strongly predicted mean maximum air temperatures in species' environments, explaining 36.5% to 45.5% of the variation (Supplementary Table 3). Based on $_{adj}R^2$, TFL improves accuracy by 85.3% and 95.8% compared to CT_{MAX} and LT80 respectively. Based on partial R^2, which account for non-independence in residuals from phylogenetic models[22], TFL80

provides a 35.1% and 35.5% improvement over LT80 and CT_{MAX} (Supplementary Table 3). TFLs also outperformed lethal measures when we used a more conservative 50% threshold for LT and TFL estimates (Supplementary Table 4). These analyses suggest that TFLs and species distributions are strongly linked in nature, and that fertility losses due to high temperature may be an important determinant of where species occur.

Thermal safety margins (the difference between an organism's thermal limit and the maximum temperature it faces in nature) can be used to predict vulnerability to climate change[7]. TFLs produce significantly smaller safety margins than CTLs (Extended Data Figure 2 & Table 5). We illustrate the potential implications of TFL-based safety margins with distribution models of *Drosophila flavomontana,* which has one of the largest differences between LT and TFL estimates, a well-documented distribution not associated with urban areas or farms, and a well understood habitat ecology. Safety margins based on TFL80 predict a 17.9% reduction in habitable landscape compared to an identical LT80-based model under current climate conditions (Figure 2A). The disparity between predictions based on sterility and lethality grew to 48.0% by the year 2080 under moderately optimistic future climate forecasts (ICCP-AR5 RCP 4.5, Figure 2B), and to 58.9% under pessimistic climate change scenarios (ICCP-AR5 RCP 8.5, Figure 2C). TFL-based models also predict that by 2080 the available habitat for *D. flavomontana* will have reduced by 42.3% and 62.9% under RCP4.5 and RCP8.5 respectively.

How to most accurately measure thermal limits to predict how species will respond to climate change is currently being debated[8,9,11,21]. Tolerance landscape measures of lethal temperatures, which integrate the intensity and duration of heat stress, have been proposed

as superior alternatives to point-estimate methods such as CT_{MAX}[8,10]. Here, we step back from this methodological debate and show the importance of identifying and measuring the correct thermally sensitive traits in the first instance. High throughput point-estimates such as we use here for TFL allow cross-species comparison of thermal sensitivity. Importantly, this reveals contrasting patterns of inter-specific variation in survival and fertility, of which fertility loss better matches variation in species natural thermal habitat. Exploration of the physiological, genetic, behavioral and ecological mechanisms that underly thermal fertility limits will now be an important step towards linking temperature-driven sterility with species' responses to climate change.

If our data for *Drosophila* can be extrapolated to other organisms, then male fertility losses at high temperatures may be common, occurring at substantially lower temperatures than lethality. The limited data on fertility at extreme temperatures supports this, with high temperature losses in male fertility observed in diverse organisms[12], including some high temperature adapted species. For instance, the zebra finch, a desert-dwelling organism with naturally high body temperature and good thermoregulation, shows substantial damage to sperm at temperatures it regularly experiences in nature[19]. Behavioral thermoregulation could potentially reduce the impact of high temperatures on fertility in nature. However, while studies have found that *Drosophila* are able to behaviorally thermoregulate in the lab[23], some evidence suggests that behavioral preferences for cooler microclimates such as leaf litter, shade, or higher altitudes do not necessarily translate into natural settings[24]. Further, many species are able to survive high temperature periods by aestivating as adults, eggs or pupae. This may explain why our data predict negative thermal safety margins for some species.

Despite these potential mechanisms, we still find that species' distributions are predicted by thermal fertility limits.

Our work emphasizes that temperature-driven fertility losses may be a major threat to biodiversity during climate change. We urgently need to understand the range of organisms likely to suffer thermal fertility losses in nature, and the traits that predict vulnerability. However, we currently do not understand the physiology underlying variation in TFLs between species, nor the selective forces that created this variation. Ultimately, we need to know whether evolution for higher TFLs will allow species to adapt to a warming environment.

Data Availability: All novel data underlying the analyses and figures presented in this paper are available from Dryad at https://doi.org/10.5061/dryad.f4qrfj6tt.

Code Availability: Analyses R code are available upon request from the corresponding authors.

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Author Contributions: The study was conceived by TP, AB, RS, AH & SP. The experimental methodology and data collection were developed and conducted by SP, BW, NW. Data from existing open sources were curated by SP & SM. Statistical analysis and visualization was conducted by SP, AM & SM. The manuscript was drafted by SP, TP, AB, RS & AH. All authors reviewed and commented on the final version of the manuscript.

Competing Interests Statement:

The authors have no competing interest to declare.

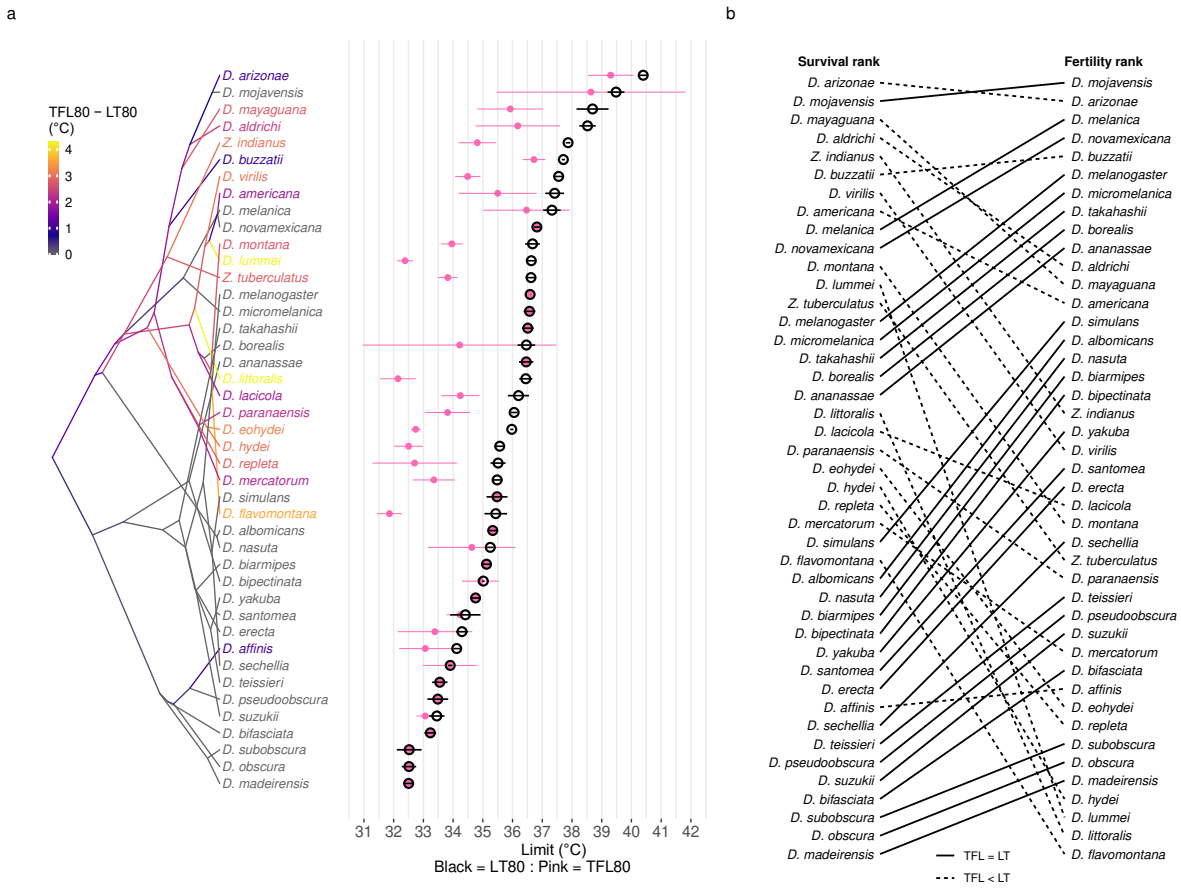
Figure 1: 80% lethal temperatures (LT80) and 80% sterilising temperatures (TFL80) for 43 species of *Drosophila*. Species ranked by LT80 from highest tolerance (top) to lowest (bottom). a) Upper lethal temperature (LT80, black circles) and upper thermal fertility limits (TFL80 measured 7-days after heat stress, pink points) of all 43 species. Pale pink bar links estimates form the same species. 19 of 43 species show significantly lower thermal fertility limits than lethal limits. 95% CI are shown as error bars for both measures, differences between a species' TFL80 and LT80 considered to be significant if these bars do not overlap. Axis phylogeny branches coloured by the difference between species' LT80 and TFL80 measured 7-days post heat stress. Yellower colours indicate larger differences, species with no significant difference indicated in grey. b) Relative ranking of species by each thermal

tolerance measure. Dashed lines indicate species with significantly lower TFL80 than LT80. For fertility measured immediately following heat-stress see Extended Data Figure 1.

Methods:

We assayed three metrics of upper thermal limits in sexually mature males from 43 species of *Drosophila*: Lethal Temperature (LT), Thermal Fertility Limit (TFL) and Maximum Critical Temperature (CT_{MAX}). We measured LT and TFL under static temperature conditions by exposing flies to four-hour temperature pulses and recording survival and fertility. Using static temperatures to measure thermal tolerances has received criticism[21]. However, in *Drosophila* fertility is internal and has no directly observable marker indicating a male has become sterile, rendering ramping methods impossible. Measuring LT under static temperatures allows us to directly compare measures of fertility loss and lethality under identical conditions. Following heat treatment, males were transferred to fresh vials and allocated to floating racks in pre-heated waterbaths set to a range of temperatures (Supplementary Table 1). Males were heated for 4 hours between ~10am - ~2pm and then returned to temperature-controlled rooms at the species' benign temperature. We scored survival of males the next morning to account for immediate recovery or delayed death. Surviving males were aspirated into separate vials containing 3-4 sexually mature virgin females. Males were kept in these vials at their benign temperature to mate freely for 6 days, then transferred to a second vial with 1-2 more virgin females and allowed to mate for 24 hours. This allowed us to score fertility at two time points to capture any recovery or delayed sterilization. Vials were scored as 'fertile' by the presence of larvae or larval tracks. We used dose-response models to estimate the temperatures that kill and sterilize 80% of males; LT80 and TFL80 respectively. We only allow TFL80 to be lower than or equal to the species' LT80 and we only consider a species' TFL to be

265 statistically lower than its LT if the 95% confidence intervals of these two point-estimates do
266 not overlap. We also measured upper critical limits of our 43 *Drosophila* species under
267 ramping heat conditions (CT_{MAX}). Individual sexually mature males were exposed to
268 temperature increments of 0.1°C/min and the temperature at which flies collapsed for 30
269 seconds and did not right themselves after tapping the vial was recorded. We explored the
270 correlations between LT80, TFL80 and CT_{MAX} using multiple phylogenetically controlled
271 approaches (supplementary methods).
272
273 We tested how well LT80, TFL80 and CT_{MAX} explained interspecific variation in the mean
274 maximum air temperature species experience in nature. We obtained species distributions
275 from Taxodros.ch and integrated these coordinates with the mean maximum air temperature
276 between the years 1970-2000 form the WorldClim V2 database (Tmax hereafter). We used
277 phylogenetically controlled models to fit each physiological limit as a predictor of Tmax. We
278 compare the adjusted and partial likelihood-based R^2 of each model.
279
280 We predicted future range contraction using TFL and LT for *Drosophila flavomontana*. We used
281 MaxEnt modelling to predict *D. flavomontana's* putative current range based on ecological
282 parameters at its known occurrence in Taxodros. We then constrained this area by matching
283 both LT80 and TFL80 to the maximum annual temperature experienced across this range. We
284 then forecast this to future moderately optimistic (RCP4.5) and pessimistic (RCP8.5) climate
285 change scenarios.



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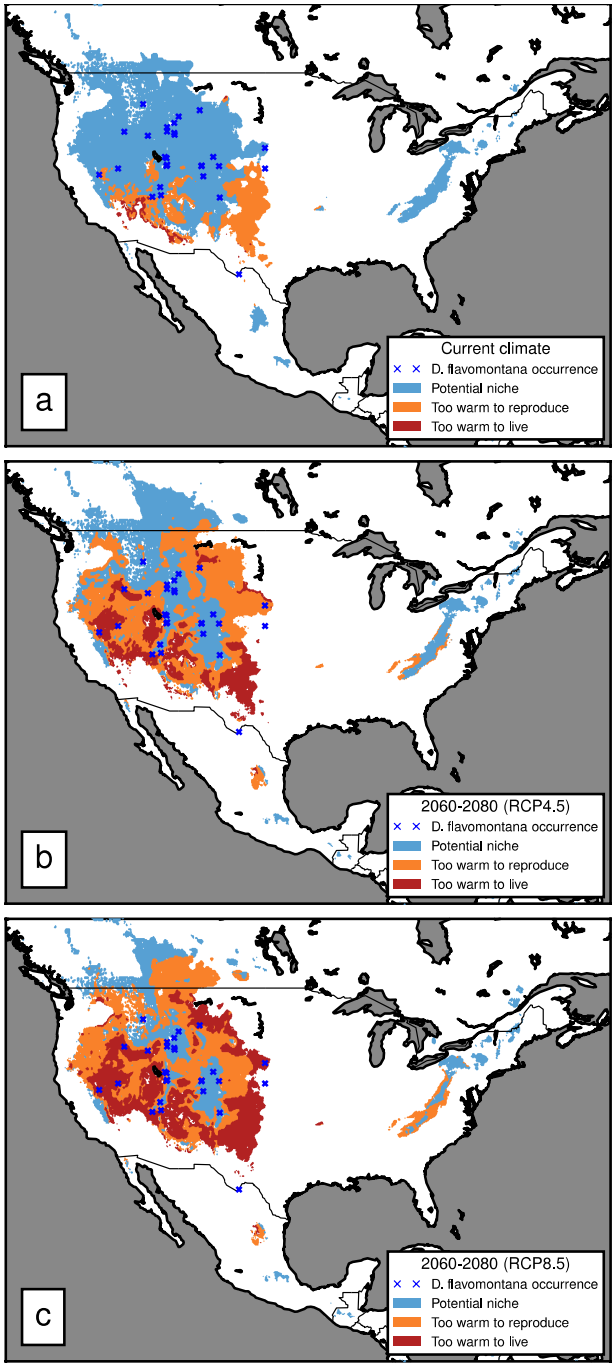

Figure 2: Potential current and future habitat range of *Drosophila flavomontana* (LT80 = 35.4°C, TFL80 = 31.9°C). A) current and B & C) possible future climate scenarios (B = RCP4.5 'moderately optimistic', C = RCP8.5 'pessimistic', predicted for 2060 - 2080). Colored areas in each panel represent suitable habitat range predicted by a model that excludes maximum temperature. Red areas show regions where maximum summer temperatures exceed LT80.

305 Orange areas show regions where maximum summertime temperatures exceed TFL80. Blue

306 regions are areas where limits for *D. flavomontana* are not exceeded all year.

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