**Small differences in upper thermal limits and competition structure *Drosophila* distributions along a tropical elevational gradient**

**Running title:** Abiotic and biotic regulators of species distributions

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JC and OTL both contributed to the development of ideas. JC designed and conducted the experimental work. JC analyzed the results and led the writing of the manuscript. OTL contributed to the writing.

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**Abstract:**

Species turnover along temperature gradients (e.g. latitude, elevation) is a widespread phenomenon and provides valuable information on how ecological communities might reorganize as the climate warms. However, the precise response of species distributions to climate change will depend on the mechanistic causes of range limits. Evidence for the contribution of temperature to species distributions on tropical mountains is limited and the proximate causes (i.e. thermal tolerance, biotic competition) are rarely examined. We tested thermal tolerance and biotic competition as causes of species turnover in nine most-abundant species of *Drosophila* along elevational gradients in the Australian Wet Tropics. Contrary to our predictions, we found that differences in species’ upper thermal limits determined species composition in lowland areas. In contrast, upland community composition was driven by interspecific competition rather than tolerance to low temperatures. These results contribute to the discussion about the role of abiotic and biotic factors in structuring tropical communities and indicate that these tropical insects may be vulnerable to future warming wherever their distributions lie along elevational and other temperature gradients.

**Introduction**

Temperature is one of the fundamental environmental factors deciding species ranges and abundance (Hoffmann and Blows 1994). Insects are directly challenged by warming because their reproduction, survival, growth, and behaviour are dictated by the environmental temperature (Huey and Kingsolver 1989; Huey and Stevenson 1979). Insect populations are also indirectly influenced by temperature via the thermal responses of their biotic interactors (e.g. competitors and predators) (Gilman et al. 2010). Changes in insect distributions, species composition and phenology can have direct environmental and economic consequences (Deutsch et al. 2018; Logan, Régnière, and Powell 2003; Pecl et al. 2017).

Estimates of thermal tolerance made using laboratory measures or observed distributions have been used in species distribution models to evaluate species’ sensitivity to climate change (Kearney and Porter 2009). Literature suggests distinguishing views on the vulnerability of tropical biotas to global warming (Corlett 2011). Tropical species are suggested to live near their upper thermal limits (Deutsch et al. 2008; Diamond et al. 2012; Huey et al. 2009), and thus are expected to face particular difficulty adapting to projected warming (Bonebrake and Deutsch 2012; Deutsch et al. 2008; Kellermann et al. 2012). Narrow thermal ranges (Khaliq et al. 2014) lead to larger range-shift gaps of tropical species when following the latitudinal or elevational temperature gradient, increasing the risk of extinction and community disassembly (Colwell et al. 2008; Sheldon, Yang, and Tewksbury 2011).

However, it is uncertain whether thermal tolerance is *the* key predictor of distribution at warmer margins. The observation that species occupying cooler environments have similar upper thermal limits to tropical species casts doubt on the role of high temperatures in structuring tropical communities (Huey et al. 2009; MacLean et al. 2019; Nowrouzi et al. 2018; Overgaard, Kearney, and Hoffmann 2014). Instead, other abiotic factors (e.g. precipitation) or biotic interactions may be critical in determining range limits in the tropics (Engelbrecht et al. 2007; Jankowski et al. 2013a; Louthan, Doak, and Angert 2015). A commonly held belief is that tolerance to low temperatures sets cold boundaries while biotic interactions predominantly drive ecological limits at the warm boundaries (O’Brien et al. 2017). This asymmetrical role of temperature is supported by the smaller changes observed at species’ warm boundaries than cold boundaries in response to warming (Chen, Hill, Shiu, et al. 2011; Sunday, Bates, and Dulvy 2012), contradicting the gloomy predictions for tropical species in response to warming.

Despite the controversy and urgent nature of this research topic (Corlett 2012), a limited amount of empirical evidence comes from species-rich tropical systems (Feeley, Stroud, and Perez 2017), and most such studies only examine the correlational relationship between thermal tolerance and distribution (Cahill et al. 2014). It remains crucial to understand how laboratory-measured thermal traits, e.g. critical temperature and optimal temperature, are ecologically relevant under realistic climate conditions (Sinclair et al. 2016). Additionally, biotic interactions themselves are regulated by temperature. The sensitivity of a particular species to temperature may depend on the temperature responses of its interacting species (Gilman et al. 2010). Therefore, a significant correlation between tolerance and distribution does not rule out a role for biotic interaction. Likewise, the species of interest can still be sensitive to temperature change even when biotic interactions are the immediate cause of species composition. To understand the relationship between temperature and distribution, it is necessary, though difficult, to quantify thermal traits and temperature-dependent biotic interactions (Cahill et al. 2014). Such empirical studies will contribute to the theory unifying the long-separate concepts of environmental and biotic filters (HilleRisLambers et al. 2012). Additionally, it provides practical information for wildlife management and conservation when facing climate change.

To investigate the roles of thermal tolerances and biotic interactions in determining species’ range margins and structuring communities, we investigated the community of *Drosophila* flies occupying rainforest habitats in the Wet Tropics bioregion of Queensland, north-eastern Australia. Tropical mountains provide natural environmental gradients to test the sensitivity of tropical species to temperature (Corlett 2011), and our study sites are part of a region with high biodiversity value because of high levels of endemism in cool, moist upland refugia (Williams, Bolitho, and Fox 2003). Species composition from many taxa changes significantly along the elevation gradient on these tropical mountains (Williams et al. 2003). *Drosophila* flies are sensitive to temperature at the organismal level (Batista, Rocha, and Klaczko 2018), while their population sizes are additionally regulated by humidity, food availability, competition, and natural enemies (e.g. parasitoid wasps) (Fletcher 1973; Krebs and Barker 1991; Mitsui et al. 2007). At our study sites, methods have been established to quantify their field distribution (Jeffs et al. 2020), laboratory thermal performance (Hoffmann, Sørensen, and Loeschcke 2003) and competitive ability (Terry, Chen, and Lewis 2021). Thus, this system offers an ideal opportunity to investigate the roles of temperature and competition in determining warm and cold boundaries in the tropics.

We hypothesize that species turnover observed on this elevational gradient results from thermal constraints at cool, high elevations and competitive exclusion at warmer, lowland sites. Thus, we predict that cold tolerance will correlate with the upper limit of species’ distributions. In contrast, we predict that species that are less common at low elevations will be limited by competition with lowland-favoring species, and not necessarily because of lower heat tolerance. To test these hypotheses, we first documented species turnover along the elevational gradient. We then examined correlations between species distributions and multiple thermal tolerance traits. Thirdly, pairs of species with similar or different distribution types were placed in laboratory temperatures mimicking upland and lowland conditions to examine their competitive outcomes in both the short and long term. Counter to our original predictions, our results suggest that interspecific competition significantly reduces lowland-biased species at high elevations, whereas high temperature, regardless of competition, constrains upland *Drosophila* species at low elevations. Small differences in these species’ upper thermal limits implies the vulnerability of all species to future warming.

**Methods**

1. Study system

Field data were collected, and laboratory cultures were initiated, from rainforest sites spanning elevations of from 59 – 916 m at Paluma Range (S18° 59.031' E146° 14.096') and Kirrama Range (S18° 12.134' E145° 53.102'), Queensland, Australia. The overall *Drosophila* abundance experiences an annual cycle due to the combined effects of temperature and precipitation, with the density peaking during March to June.

*Drosophila* isofemale cultures were established in 2017 and 2018 from pupae collected from high- and low-elevation sites. Cultures had been maintained at 24°C at the Biology Centre, Czech Academy of Sciences since collection and transferred and maintained at 25°C at the Department of Zoology, University of Oxford, UK, since December 2018. All cultures and the following experiments were maintained under a 12h/12h light/dark cycle. Theywere maintained for approximately 15 to 30 non-overlapping generations in the Czech Republic and four to seven non-overlapping generations in Oxford before being used to culture mass bred lines (MBLs).

To revive genetic variation, we made MBLs of each *Drosophila* species by combining four isofemale lines (except for *D. pandora*, where only three isofemale lines were available). The four lines were selected from different mountains and different elevations if possible (detailed as provided in Supplementary Table 1). Each population cage was initiated using two independently-reared MBLs of the same species. Large populations were maintained at 25°C for more than four generations before measuring the thermal traits. Therefore, measurements should not have been influenced by maternal effect, acclimation, or isofemale line effects. The MBLs were maintained at 23°C from 2020–[end date?].

Nine tropical *Drosophila* species were included in laboratory measurements. *Drosophila* *rubida* was not included because it has a low reproduction rate and much longer development and sexually maturation time than other species, making it practically difficult to raise to a large number and synchronize with the other species. Isofemale lines of *D. pseudotakihashii* were contaminated before measuring the thermal traits. Therefore, its thermal traits were not measured. Another MBL made up by the only two *D. pseudotakihashii* isofemale lines was constructed and used in the competition experiment. *Drosophila* *melanogaster* does not occur naturally at the study sites. A laboratory strain (wild type, *Dah* strain) was measured for thermal performance together with the focal species as a benchmark for future comparisons.

1. Field distributions

*Field survey. Drosophila* pupae were sampled using bottle traps baited with fermented banana from 11th March – 12th April 2016 (Jeffs et al. 2020). Relative abundance was surveyed for three sites at elevation of 70m, 350-390m, and 730-880m on the two mountain ranges. In total, 182 pupae were sampled at each site and 716 pupae were successfully identified to species by DNA metabarcoding (Jeffs et al. 2020), with 86 – 134 pupae at each site. *Drosophila serrata* (1 individual) and *D. immigrans* (4 individuals) were excluded from the distribution analysis due to infrequent occurrence.

*Distribution analysis*. To calculate the abundance-weighted mean elevation (hIndex) of each species’ distribution, the location of each sample was assigned values of 0, 0.5, and 1 if it was collected at low-, middle- and high-elevation sites, respectively. In addition, intra-specific abundance patterns were assessed by logistic regression of occurrence with elevation. For each species, pupal identity was labeled as 1 if the pupa was identified as the focal species and 0 if it was identified as any other species. Their occurrences were fitted against elevation as a fixed effect and the transect as a random effect in a generalized linear mix-effect model (varying intercept, varying slope) using the *lmer* package (examples as shown in Supplementary Figure 1). Spearman’s rank test suggested these two ways of describing distribution were highly correlated (Figure 1b). Thus, hIndexes were used to represent the distribution patterns in the following analysis, as the confidence intervals of the regression coefficients are highly variable due to different numbers of present samples of each species.

It is important to note that the abundance patterns we focus on here is to compare the abundance of a species with itself along the elevation, rather than to compare the abundance of multiple species in a given location. With this definition, an upland-biased species may have a higher absolute value of population size than the lowland-biased species in the lowland.

1. Reproductive thermal performance

*Experimental measurements*: We exposed flies to temperatures ranging from 14°C to 32°C and measured how their reproductive success changed with temperature (Supplementary Figure 2). To prepare the adult flies for reproduction measurement, fly eggs collected from the population cage were reared at low density (less than 100 eggs per vial) at 25°C. Upon eclosion, adults that emerged within 12 hours were separated by sex to guarantee they remained unmated. Rearing of different species was started on different days to synchronize the first day of egg-laying of all species. Sexual maturation was monitored daily in two additional vials with five pairs of flies. Two days after the first observation of egg-laying in both vials, flies were subjected to measurements.

Two virgin females were paired with two virgin males in a vial containing 4ml *Drosophila* medium (weight/volume concentration: 8% corn flour, 4% yeast, 5% sugar, 1% agar, and 1.67% methyl-4-hydroxybenzoate.). Vials were randomly subjected to water baths set at one of seven constant temperatures (14°C, 17°C, 20°C, 23°C, 26°C, 29°C, 32°C). Vials were submerged in water baths. The water level was kept above the area that flies could freely move. The temperature and humidity of vials in each water bath were monitored in two additional empty tubes placed at the centre and the corner. The level of humidity was similar to field condition, ranging between 80% - 95%. The observed temperature showed ±0.5°C fluctuation around the mean temperature. The temperature in the centre of the water bath was 0.5°C higher than the temperature at the corner. The average of the central temperature was used as the corrected temperature in the analysis.

As fecundity changed through time and this trend of change was influenced by temperature (Supplementary Figure 3), offspring numbers were measured for the 1st – 2nd day and the 7th – 8th day and they were combined to reflect relative fecundity in early adult life. After eight-day exposure to temperature treatments, all flies were kept at 25°C for another four days to examine their recovery of reproduction. Surviving flies were recorded at the beginning and end of each period. Offspring produced during the test periods developed at the same temperature as their parents. The first emergence dates were recorded for different species in different temperatures. Vials were left for 5 – 7 days for all offspring to emerge, then they were frozen and counted later. For each species and each temperature treatment, eight replicates were evenly split between two blocks.

*Thermal performance curve*: A multi-level, non-linear piecewise model was fitted under the Bayesian framework using MCMC sampling within the *rstan* package in R. Total offspring numbers were calculated by combining the offspring numbers on day 1-2 and day 7-8. The average daily fecundity per female was calculated, then square root transformed to approximate a Gaussian distribution. Square root-transformed daily fecundity, *P(T)*, was modeled with the Briere2 function (Briere et al. 1999):

where *T* is the temperature, *RTmin* and *RTmax* are the minimum and maximum temperatures for the species to reproduce, *a* is a scaling factor and *b* is a shape factor of the curve. Values of each parameter shared the same normal distribution among the nine species, whose mean and variance were the super parameters in the multi-level model.  *P(T)* was modeled using a normal distribution with a temperature-dependent standard deviation. A normal distribution is not ideal to model the transformed count data, which are all positive. However, modeling offspring counts with Poisson, zero-inflated Poisson, negative binomial, lognormal distribution did not produce model convergence results, potentially due to the piecewise nature of the thermal performance functions. Assuming temperature dependence of the standard deviation generated better fitting than assuming the same standard deviation across temperature treatments, as judged by leave-one-out cross-validation. This was because when the temperature was equal or close to *RTmin* and *RTmax*, the standard deviation should be zero or close to zero. Models converged and we examined diagnostic plots to ensure assumptions were met (Supplementary Figure 4). Priors of parameters were sampled from a non-informative distribution. The values of *a* and *b* were bounded to be positive. The values of *RTmin* were bounded to be lower than 17°C and the values of *RTmax* were bounded between 26°C - 35°C according to prior knowledge. Medians of the posterior distributions were used as the parameter values to construct the thermal performance curve.

The model parameters were also estimated by maximum likelihood using the *bbmle* package in R. Total offspring numbers were modeled using a Poisson distribution. This method was not multi-level; therefore, the shapes of curves of different species varied more than when assuming shared distributions of model parameters. Besides, this method behaved poorly in estimating the uncertainty of the parameter estimation. Nevertheless, the ranks of the *RTmax* (Rho = 0.88, p = 0.003, Spearman’s rank test) and *RTmin* (Rho = 0.97, p = 0.00016, Spearman’s rank test) estimated by both methods are highly correlated.

*Regression analysis*: The posterior distributions of *RTmin*, *RTmax*, and *RTopt* (100 samples of each parameter of each species) were modeled by hIndex as the fixed effect and species identity as the random effect in the linear mixed-effects model with phylogenetic correction. Fecundity at 29°C and 17°C and recovered fecundity after 29°C and 14°C were used as direct measurements of their performance in the high and low temperatures. These offspring numbers were modeled by hIndex and experimental block as fix effects and species as a random effect in the generalized (family = “negative binomial”) linear mixed-effects model with phylogenetic correction. All the above regression analyses were conducted using the *brms* package in R. The correlation between medians of *RTmin* and *RTmax* was evaluated by Spearman’s rank test. *Drosophila melanogaster* and *D. simulans* were not included in the regression because their distribution patterns were unavailable from the field survey.

1. Thermal knockdown

*Experimental measurements*: Resistance to extreme cold temperature was measured by individuals’ knockdown time at 5°C and the time for recovery of mobility after a 30-minute exposure to 5°C. Constant temperature for cold stress is often chosen around 0°C (Gibert et al. 2001). As tropical species often have significantly weaker cold resistance (Gibert et al. 2001), 5°C was used instead to increase the variation among the tested species after pilot trials. Heat stress was chosen to be 40°C, which follows common practice for *Drosophila* species (Hoffmann et al. 2003) and is expected to capture the between-species variance in heat stress over a time scale which is convenient to measure (Jørgensen, Malte, and Overgaard 2019). After being knocked down by heat (40°C), most flies did not survive. In this case, only knockdown time was used to evaluate resistance to the extremely high temperature.

Virgin adult flies, which were siblings of those in the reproduction measurement, were kept in same-sex groups at 25°C for 9-10 days before measurement. Measurements were conducted for male and female flies separately. An observation rack was divided into nine (3x3) cells. Each cell was randomly assigned one of the nine *Drosophila* species and each cell held seven tubes of that species. One randomly-selected individual was placed in one empty flat-bottom 3ml tube. We repeated measurements for three experimental blocks, and the allocation of species to cells was redrawn for each block. In total, we measured 21 individuals per species per sex. During measurement, the observation rack was moved immediately into the incubator set at 5°C or 40°C. Every tube was examined by eye once every minute and flies that lost their ability to stand on their feet in that minute were recorded. After exposing to 5°C for 30 minutes, all flies were in a chill coma. The observation rack was moved to a 25°C room. Flies were left undisturbed and the duration of each individual fly regaining its ability to stand on its feet, decided by eye inspection, was recorded.

*Regression analysis*: The knockdown time by heat, knockdown time by cold and recovery time from cold of both sexes were first compared among species by ANOVA. When inter-specific variation was observed, they were modeled by hIndex, block, and cell position as fixed effects, and species as a random effect in linear mixed-effects model with phylogenetic correction using the *brms* package in R. *D. melanogaster* and *D. simulans* were not included in the regression because their distribution patterns were unavailable.

1. Short-term competition

*Experimental design*: Pairs of species were reared in the same vials for one generation to evaluate how the densities of intra-specific and inter-specific competitors influenced reproductive success in two temperature regimes representing lowland and upland environments. Five species were chosen as representative species for upland-biased, non-biased and lowland-biased distribution types. Each two-species combination (6 combinations in total) was measured at different founding densities in a factorial design: (4 pairs of species A, 2 pairs of species B), (4A, 4B), (4A, 8B), (2A, 4B) and (8A, 4B). We also included monocultures of each species of 2, 4, and 8 pairs. Each density and species combination was replicated ten times across two or three blocks staggered by two days (two blocks for the *pandora*-*pallidifrons* combination, which was conducted before the other five pairs; three blocks for the other five pairs).

Adults that were subjected to competition were reared from eggs collected from the population cage. They were reared under moderate density (300 – 500 per bottle) at their testing temperature regimes. After eclosion, individuals that emerged within the same 48 hours were kept together in mixed-sex containers. Two days after the first observation of egg-laying, adults of different sexes were separated and then they were used as the founders in 5ml-food vials the next day. Founders laid eggs in vials for two days before being discarded. Offspring of the founders experienced intra- and inter-specific competition over food and space in the vials. Offspring that successfully developed to adulthood were identified to species and counted. The above animal preparation and competition were conducted in incubators set at alternating temperature regimes mimicking day/night temperatures on February, immediately prior to our survey season, at upland (23°C /21°C) and lowland (28.5°C /24°C) (Supplementary figure 5).

*Competition models*: We used the Beverton-Holt model to describe the population growth of a single generation of flies on discrete and temporary resources:

where R0 is the generational reproduction rate and α represents intraspecific competition coefficients. β represents the interspecific competition coefficients, which define the equivalence between the two competing species. Their values and 90% credible intervals were estimated using the same Bayesian statistical method detailed in (Terry et al. 2021). The equilibrium state of each pair was predicted following (Hassell and Comins 1976).

1. Long-term competition

*Experimental design*: A lowland species, *D. pandora*, and an upland species, *D. pallidifrons*, were reared in monoculture and mixed-culture environments for multiple generations in lowland and upland temperature regimes to evaluate the long-term impact of temperature and competition on population size.

Four monocultures of each species and eight mixed-species cultures were maintained at each temperature regime for 13 weeks. Monocultures were started with ten pairs of individuals. Mixed-species cultures were started with ten pairs of individuals of each species. The starting density was very low compared to the equilibrium density. The populations were evenly divided into two blocks starting on different dates. Each population was maintained in a series of five bottles following Ayala’s type one system (Ayala, Gilpin, and Ehrenfeld 1973). At the start of each week, individuals surviving in the most recent bottle and individuals which were freshly emerged in the older four bottles were separately collected, photographed and transferred into a new bottle with fresh food. In this way, adult survival and reproduction were recorded separately. The total population size of each species was counted at the end of the experiment. To avoid pseudo-replication introduced by ‘incubator’ effects, the two incubators were switched between temperature regime every week, with their contents moved accordingly. Trays were shuffled inside the incubator every two days. Temperature and humidity were recorded and the temperature regimes were confirmed during and at the end of experiments.

*Data analysis*: The population sizes were modelled by a three-way interaction of temperature treatment, species identity and the presence/absence of competitors, and the population identity as the random effect in the generalized linear mix-effect model (family = “zero-inflated negative binomial”) using the *brms* package in R. To visualize the three-way interactive effects, the posterior estimates of the high-temperature effect were plot against zero for the two species with or without the presence of competitors; the posterior estimates of the effect of the competition were plotted against zero for the two species in two temperature regimes.

All statistics mentioned in the *Methods* section were performed with R statistical software (version 3.6.0 ). All analysis code is available in ??GITHUB or SUPP??.

**Results**

Figure 1a shows the absolute numbers of identified samples found at low-, medium- or high-elevation sites for each of the nine major *Drosophila* species (accounting for 99% of total samples). Distribution quantified using regression of occupancy against elevation and by weighted elevation (hIndex) showed consistent patterns (Figure 1b) regarding the elevational biases of species. *Drosophila bipectinate* and *D. pandora* were categorized as lowland-biased species with high confidence. *Drosophila pseudoananassae* was more likely to bias towards the lowland. *Drosophila rubida* and *D. sulfurigaster* showed no significant change with elevation. *Drosophila birchii* was most likely to bias towards the upland. *Drosophila pallidifrons* and *D. pseudotakahashii* were significantly more abundant at high elevations. The only six samples of *D. bunnanda* were all found at low elevation, which explained the large negative value of its regression coefficient (Coefficient = -69, not shown in figure 1b) and its large standard error (se = 21603). Another larger-scale study confirmed it as a lowland species (Schiffer and McEvey 2006).

Thermal performance curves of daily fecundity per female vary in the range, optimal temperature, peak fecundity, and shape factors among species (Figure 2; Table 1; See supplementary figure 6 for original data for each species).The temperature for optimal reproductive performance, *RTopt*, did not correlate with their distribution patterns (Coefficient = 0.17, 95% credible interval -2.74 – 3.17). There was no general trade-off between cold tolerance (*RTmin*) versus heat tolerance (*RTmax*) that correspond to their distribution types (Rho = -0.55, p = 0.13, Spearman’s rank test). For example, the lowland-biased species *D. bunnanda* has better heat tolerance and worse cold tolerance than its upland-biased relative, *D. birchii*. In contrast, *D. sulfurigaster* outperforms its upland-biased relative, *D. pallidifrons*, in the whole range of temperature.

Values of *RTmin* were not correlated with the species distribution patterns (Figure 3a. Coefficient = -0.24, 95% credible interval = -4.26 – 3.85). Similarly, upland-biased species did not show higher fecundity at the low temperature, 17°C (Figure 3b. Coefficient = -0.13, 95% credible interval -3.93 – 3.77). When exposed to acute sublethal low temperature (5°C), all seven tropical *Drosophila* species showed similarly weak resistance compared to *D. simulans* and *D. melanogaster* (Supplementary table 2 for Tukey multiple pairwise-comparisons). All species recovered their fecundity after eight-day exposure to 14°C. This recovered fecundity showed a minor but non-significant increase among upland species (Figure 3c. Coefficient = 0.34, 95% credible interval -0.52 – 1.18). It took longer for upland species to regain mobility after the chill coma (Figure 3d. Male: coefficient = 14.14 (-8.43 – 36.8); female: coefficient = 9.44 (-1.77 – 22.26)), presumably disadvantaged in the upland environment.

Regardless of the small variation of *RTmax* compared with *RTmin*, species whose distribution were biased towards lowland consistently had higher *RTmax* (Figure 3e. Coefficient = -3.34, 95% credible interval -5.42 – -1.17). Reproductive performance at 29°C also decreased with hIndex (Figure 3f. Coefficient = -5.68, 95% credible interval -9.08 – -2.11). After exposure to 29°C for eight days, neither of the two upland species could reproduce when transferred back to mild temperature. Four out of the five non-biased and lowland-biased species resumed reproduction (Figure 3g). Knockdown time at lethal high temperature (40°C) was lower among upland species (Figure 3h. Male: coefficient = -7.83 (-14.67 – -1.20); female: coefficient = -3.12 (-10.43 – 3.77)), indicating these species lose their mobility faster in high temperatures.

When raised in the laboratory environment mimicking the lowland sites, the reproductive success of the two lowland-biased species was the highest, followed by the widespread species, *D. sulfurigaster*. The two upland species could barely reproduce regardless of the presence of competitors (Figure 4a). When raised in the upland environment, all species could reproduce and sustain their populations. Lowland species were strongly affected by the density of *D. pallidifrons*, an upland species. While upland species were significantly less affected by lowland species, shown by lower interspecific competition coefficients, β (Figure 4a, Table 2). Competition under upland conditions was predicted to drive lowland species to exclusion (Table 2, SI 3abf). In the empirical test of the long-term competition between two representative upland and lowland species, high temperature drove the upland *D. pallidifrons* to extinction regardless of the starting species composition (monoculture versus mixed species culture). In contrast, the monoculture of the lowland *D. pandora* remained abundant in both temperatures when raised alone. Their populations were significantly reduced at the low temperature only when in the presence of *D. pallidifrons* (Figure 5).

**Discussion**

Our results run counter to the common assumption that cold boundaries to species’ ranges are constrained abiotically and reflect thermal niches, while biotic interactions define warm boundaries. We found that high temperatures experienced on a daily basis at lowland sites were sufficiently high to serve as an environmental filter determining the composition of *Drosophila* communities. In cooler environments at high elevations, lowland species were outcompeted by certain upland species whose distribution are confined to high elevations as a result of their intolerance to heat.

*1. Low variation in upper thermal limits*

Upper thermal limits show low levels of variation among species (Hoffmann 2010). Consistent with others (Goulet, Thompson, and Chapple 2017; Hangartner and Hoffmann 2016), our study showed that heat tolerance is a systematic trait manifested in the critical temperature, the fecundity at a sub-sterile temperature, the recovered fecundity and the locomotive response. Therefore, a modest difference in critical temperature may represent a distinct difference in overall performance when organisms are operating at real, variable temperature regimes.

Small thermal safety margins suggest a severe threat of biotic attrition in tropical lowlands (Colwell et al. 2008; Deutsch et al. 2008; Duarte et al. 2012). Discussion about biotic attrition faces great uncertainty. The thermal niches of tropical lowland species estimated by their extant distribution may be truncated because hotter areas are unavailable for species to occupy (Feeley and Silman 2010). Laboratory-measured critical temperatures are sensitive to experimental conditions, making it difficult to relate these temperatures to climatological means or maxima, and thus the threat of rising temperatures (Sinclair et al. 2016). This study benefits from a comparative approach with detailed quantification of species with different thermal traits and distribution. We first showed that upland species are already constrained by lowland temperature, and also showed the marginal difference (about one degree Celsius) in upper thermal limits between lowland species and upland species. Given the low evolutionary potential of heat tolerance (Hoffmann, Chown, and Clusella-Trullas 2013), lowland species are likely vulnerable to small amounts of warming at lowland sites. Thus, lowland biotic attrition and upland range contraction are likely to happen with future climate change, leading to cascading effects in lowland communities and threatening endemic upland species (Freeman et al. 2018).

*2. Controversy over the contributions of abiotic and biotic factors in determining distributions*

The idea of comparing the contributions of abiotic versus biotic factors in warm versus cold boundaries has roots to Charles Darwin but remains contested (Cahill et al. 2014; Hargreaves, Samis, and Eckert 2014). There is mixed evidence on the importance of heat tolerance in deciding warm boundaries (positive relationship: (Batista et al. 2018; Duarte et al. 2012; García-Robledo et al. 2016; Kellermann et al. 2012; Merrill et al. 2008); null relationship: (Gaston and Chown 1999; Huang and Tu 2008; Kimura 2004; Nowrouzi et al. 2018)). This discrepancy could arise from at least three reasons: geographic bias of research effort, the distinctive nature of mechanisms governing latitudinal versus elevational distribution, and methodological differences when measuring traits.

First, data available for synthetic studies is dominated by temperate latitudes in the northern hemisphere (Feeley et al. 2017) and focus on cold limits (Cahill et al. 2014). However, a recent synthesis of the global latitudinal trend shows that the difference in the contribution of biotic factors to warm versus cold boundaries disappears toward the equator (Paquette and Hargreaves 2021). Limited and mixed results from tropical systems cast great uncertainty on the importance of biotic and abiotic contribution in the tropics (Cahill et al. 2014). Second, mechanisms governing latitudinal or altitudinal distribution patterns could be very different, indicated by very different recorded responses to past warming (Chen, Hill, Ohlemüller, et al. 2011). Region-specific rules may emerge upon accumulating datasets from neglected locations (Duarte et al. 2012; Freeman et al. 2021). Comparative studies can shed light on the general relationships between biotic and abiotic factors (Amundrud and Srivastava 2020; Schemske et al. 2009; Srinivasan et al. 2018).

Methods of measuring thermal traits will also affect conclusions. (Overgaard et al. 2014) examined a similar subset of tropical rainforest *Drosophila* species and concluded that the sensitivity of their demographic traits to temperature was indistinguishable between widespread and tropical species and was thus a poor predictor of distribution. In this study, we showed that interspecific differences in the demographic response to heat correlates well with elevational distribution types. Our main differences in measuring demographic traits are that 1) we left eggs produced in the tested temperature to develop to adults to measure their parents’ fecundity, 2) adults are virgins before testing fecundity, 3) we combined two time periods after exposing to the tested temperature. In our experiment, individuals laid eggs at high temperatures while those eggs did not eventually hatch, presumably due to the sterilization of sperm at high temperature (Parratt et al. 2021). We also observed different speeds of senescence after exposure to different temperatures (supplementary figure xxx), which the three-day period of fecundity measurement in Overgaard’s study would not have captured. Nevertheless, both studies showed the conservativeness of physiological heat tolerance and suggested small safety thermal margins to warming in the future.

*3. Daily peak and extreme high temperature as the main abiotic filters*

Daily high temperature rather than mean contributes more to structuring distributions (Lynch et al. 2014). The mean temperature during the survey season in the lowlands is around 26°C, at which all the species are around their peak reproductive performance. In addition to the mean, upland and lowland sites significantly differ in the number of days and the daily duration that the temperature reached above a stressful level for *Drosophila* reproduction. A preliminary experiment done by JC (not shown) raising long-term populations in constant 20°C and 26°C showed that *D. pallidifrons* always out-numbered *D. pandora* in mixed-species culture, contrasting the poor performance of *D. pallidifrons* when raised in a 24°C /28.5°C scenario. Brief exposure to stressful thermal environments is suggested to have similar fitness costs to continuously stressful conditions (Saxon, O’Brien, and Bridle 2018). Therefore, it is essential to consider daily temperature variation and extreme temperature events when studying species distributions and making future projections (Kingsolver, Diamond, and Buckley 2013; Ma, Hoffmann, and Ma 2015).

*4. Thermal performance is context-dependent*

Our quantifications of thermal performance traits only focus on local populations and cannot reflect the plasticity and evolutionary response to temperature. Between-populations variations (Hoffmann, Anderson, and Hallas 2002), especially the adaptation to abiotic environments at distribution boundaries (Peterson, Doak, and Morris 2019), should not be neglected if studying distributions at a large spatial scale (e.g. latitudinal patterns). In our study, our scope is limited to distribution patterns within a mountain range. Mass bred lines are sourced from different elevations, if available, so that they reflect the average features of the local populations that have sufficient gene flow, which is the case in our study sites. Plasticity and evolutionary adaptation of tropical species are suggested to be very limited (MacLean et al. 2019; O’Brien et al. 2017).

Thermal tolerance interacts with precipitation (Bozinovic and Pörtner 2015; Kellermann et al. 2012), landscape features (e.g. open versus close canopy), diet, and larval conditions (Bubli, Imasheva, and Loeschcke 1998). Synergistic effects between multiple abiotic factors are widespread phenomena, and these effects on the focal species/trophic depend on the particular structure of the community (Beauchesne et al. 2021). Thermal traits measured in isolated laboratory conditions can never overcome this issue, limiting its predictive power. As an alternative solution, long-term, high-resolution data which capture the temporal variation of abundance and climate are especially valuable for understanding abiotic limits for field populations (Corlett 2011; Gade et al. 2020).

Last but not least, sensitivity to temperature depends on the biotic environment (Gilman et al. 2010). It is not only the demographics of the interacting species that are affected by temperature (Huey and Kingsolver 1989); interactions themselves may change with temperature due to behavioural change (Burnside et al. 2014). Our long-term experiment showed that the thermal response of population size to temperature depends on the competitive conditions, highlighting the difference between thermal performance curves of organisms and their population when considering biotic interactions (Davis et al. 1998). My experiment only examined pair-wise competition and found that the competitive effect of one particular upland species could drive the observed decrease of a lowland species at higher elevations. It did not rule out the contribution by other biotic interactions, for example, predation, parasitism and pathogen load (Jankowski et al. 2013b), and it did not explain the coexistence between some upland species pairs. Higher-order interactions, intransitivity and trophic interaction modifications are also likely to contribute to coexistence, while their response to temperature is even more elusive to quantify. Nevertheless, theoretical and empirical studies are needed to reveal how thermal sensitivity is dependent on their positions in and the structure of the ecological networks.

*5.Conclusions*

This study contributes to the growing literature demonstrating that the warmer margins of species’ distributions can be highly sensitive to warming (Wilson et al. 2005). Benefiting from the *Drosophila* system, we examined the roles of both abiotic (temperature) and biotic factors as direct determinants of species abundance along an environmental gradient. The generality of this relationship is essential to realistically estimate the magnitude of the impact of warming on tropical species. Our case study emphasizes environmental sorting by daily high temperature in a tropical lowland, highlighting the vulnerability of species to increasing temperatures and extremes given the limited evolutionary potential of upper thermal limits. The long-standing understanding of the relative contribution of abiotic and biotic factors in determining distribution needs to be updated with the recognition of regional differences and standardized methods to measure traits. The tropics host a broad suite of endemic species (Laurance et al. 2011) and tropical insects are essential to the delivery of key ecosystem functions of tropical forests (Crespo-Pérez et al. 2020). Predicting their thermal sensitivity to climate change is urgently important, and will require identifying proximate causes of current distributions and understanding the interactions between thermal tolerance and other environmental (abiotic and biotic) factors.

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**Figure 1. Distribution patterns.** a) Proportion of all samples found at the low-, middle- and high-elevation sites for the nine *Drosophila* species. b) Regression coefficients and hIndex give consistent descriptions of elevational distribution patterns. *Drosophila bunnanda* is not included in the figure because its regression coefficient and standard error are peculiarly large in absolute value due to its small sample size. Error bars show 90% confidence intervals.

Chart, bar chart

Description automatically generated

**Figure 2. The thermal performance curve of reproduction.** The numbers of adult offspring produced per female parent per day are fitted against temperatures. Line colours are ordered by the species’ distribution pattern, with upland-biased species represented by cold colours and lowland-biased species represented by warm colours.

Chart

Description automatically generated

**Figure 3. Reproductive and physiological thermal tolerance of the seven tropical *Drosophila* species examined.** Species are ordered by their distribution patterns, with lowland-biased species on the left and upland-biased species on the right. Cold tolerance is represented by *RTmin* (a), fecundity at 17°C (b), recovered fecundity after 14°C (c) and recovery time after chill coma (d). Hot tolerance is represented by *RTmax* (e), fecundity at 29°C (f), recovered fecundity after 29°C (g) and knockdown time by high temperature (h).

Diagram, engineering drawing

Description automatically generated

**Figure 4. The inter-specific competitive effect of the competing species on the focal species in upland and lowland temperature regimes.** Each line shows the fecundity of the focal species when its founder number is kept at four pairs while changing the number of competing species. Colours indicate the identities of the focal species in the tested pairs. The pair names, e.g. BIP\_PST, are structured with the focal species in the front and the competing species behind. The shaded area indicates the 90% credible interval of the fitted values of fecundity by the Beverton-Holt model of pairwise species competition. PST is short for *D. pseudotakahashii*, PAL for *D. pallidifrons*, SUL for *D. sulfurigaster*, BIP for *D. bipectinata*, PAN for *D. pandora*.

Chart

Description automatically generated

**Figure 5. The effects of temperature and inter-specific competition on the population sizes of *D. pallidifrons* and *D. pandora*.** a) The ending population sizes of *D. pallidifrons* and *D. pandora* whichwere initiated in monoculture or mixed-species culture in the cold (upland) and hot (lowland) temperature regimes. b) the posterior distribution of the effect of high temperature when the indicated species were maintained alone (single) or with the other species (mix). c) the posterior distribution of the effect of competition when the indicated species were maintained in upland and lowland temperature regimes.

Diagram

Description automatically generated

**Table 1 Estimated parameters of thermal performance functions and their 90% credible intervals (ci90) of the nine species.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **species** | **a** | **a.ci90** | **b** | **b.ci90** | **RTmin** | **RTmin.ci90** | **RTmax** | **RTmax.ci90** |
| *D. bipectinata* | 0.0046 | 0.0031 - 0.0060 | 1.27 | 1.02 - 1.55 | 15.28 | 14.54 - 15.87 | 30.44 | 30.08 - 31.05 |
| *D. birchii* | 0.0034 | 0.0022 - 0.0056 | 1.17 | 0.95 - 1.56 | 13.45 | 13.07 - 13.77 | 29.27 | 28.12 - 29.81 |
| *D. bunnanda* | 0.0016 | 0.0012 - 0.0025 | 0.88 | 0.81 - 1.06 | 14.57 | 14.10 - 15.18 | 31.21 | 30.60 - 31.78 |
| *D. melanogaster* | 0.0037 | 0.0032 - 0.0042 | 1.72 | 1.48 - 2.03 | 8.29 | 6.89 - 9.37 | 32.13 | 32.03 - 32.28 |
| *D. pallidifrons* | 0.0073 | 0.0056 - 0.0098 | 1.74 | 1.37 - 2.38 | 16.23 | 15.54 - 16.75 | 29.06 | 28.19 - 29.38 |
| *D. pandora* | 0.0052 | 0.0037 - 0.0065 | 1.26 | 1.03 - 1.51 | 15.25 | 14.58 - 15.80 | 30.13 | 29.87 - 30.58 |
| *D. pseudoananassae* | 0.0053 | 0.0035 - 0.0071 | 1.68 | 1.24 - 2.32 | 15.06 | 14.17 - 15.89 | 29.22 | 28.38 - 29.77 |
| *D. simulans* | 0.0035 | 0.0027 - 0.0046 | 1.69 | 1.36 - 2.21 | 8.51 | 6.94 - 9.64 | 31.09 | 30.39 - 31.78 |
| *D. sulfurigaster* | 0.0040 | 0.0028 - 0.0051 | 1.27 | 1.03 - 1.52 | 14.37 | 13.91 - 14.96 | 30.11 | 29.84 - 30.63 |

**Table 2. Fitted values of the parameters of the competition and their predicted equilibrium states.** R0 is the reproductive rate. is the intra-specific competition coefficient. is the inter-specific competition coefficients. ci90 represents the 90% credible intervals of each parameter. The equilibrium states of the focal species are inferred from (Hassell and Comins 1976).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Temperature** | **Focal species** | **R0** | **R0.ci90** |  | **.ci90** | **Competitor** |  | **.ci90** | **Equilibrium state of the focal species** |
| Upland | BIP | 11.36 | 8.06-15.21 | 0.05 | 0.02-0.09 | PAL | 2.26 | 1.4-4.08 | excluded |
| PAN | 0.99 | 0.47-1.95 | excluded |
| PST | 0.47 | 0.12-1.09 | stable coexistence |
| SUL | 2.95 | 1.91-5.25 | excluded |
| PAL | 27.94 | 19.52-38.66 | 0.42 | 0.27-0.64 | BIP | 0.3 | 0.15-0.49 | dominant |
| PAN | 0.32 | 0.14-0.52 | dominant |
| SUL | 1.22 | 0.9-1.62 | unstable coexistence |
| PAN | 13.68 | 10.4-17.85 | 0.07 | 0.04-0.12 | BIP | 0.74 | 0.33-1.36 | dominant |
| PAL | 3.41 | 2.26-5.59 | excluded |
| PST | 6.27 | 3.4-10.66 | 0.08 | 0.03-0.19 | BIP | 0.79 | 0.35-1.76 | stable coexistence |
| SUL | 20.96 | 14.27-31.13 | 0.25 | 0.14-0.44 | BIP | 0.41 | 0.19-0.67 | dominant |
| PAL | 1.05 | 0.71-1.53 | unstable coexistence |
| Lowland | BIP | 15.35 | 12.51-19.05 | 0.07 | 0.05-0.11 | PAL | 0.29 | 0.07-0.63 | dominant |
| PAN | 0.87 | 0.54-1.35 | stable coexistence |
| PST | 0.31 | 0.07-0.63 | dominant |
| SUL | 1.35 | 0.93-2 | excluded |
| PAL | 0.99 | 0.19-2.37 | 0.12 | 0.02-0.46 | BIP | 6.81 | 2.98-22.27 | die out |
| PAN | 2.99 | 1.52-8.93 | die out |
| SUL | 3.98 | 1.77-12.83 | die out |
| PAN | 17.18 | 14.2-21.24 | 0.09 | 0.06-0.14 | BIP | 0.27 | 0.08-0.51 | stable coexistence |
| PAL | 0.11 | 0.01-0.29 | dominant |
| PST | 0 | \ | \ | \ | BIP | \ | \ | die out |
| SUL | 13.65 | 10.26-19.03 | 0.15 | 0.09-0.24 | BIP | 0.37 | 0.18-0.62 | dominant |
| PAL | 0.04 | 0-0.14 | dominant |