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

**Distribution patterns of tropical rainforest Drosophila explained by small differences in upper thermal limits at warm-edge and competition at cool-edge**

**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.

**Funding**:

This work was supported by NERC grant NE/N010221/1 to OTL and a tuition grant from the China Scholarship Council to JC.

**Acknowledgements**:

We thank the laboratories of Jan Hrček (Czech Academy of Sciences) and Megan Higgie (James Cook University, Townsville) for their assistance in establishing *Drosophila* laboratory cultures. Jan Hrček, Chia-Hua Lue, Nick Pardikes, Mélanie Thierry and the Oxford Fly group provided valuable advice and shared facilities. We thank Chris Terry for advice on data analysis and comments on the manuscript.

**Abstract:**

Species turnover with elevation is a widespread phenomenon and provides valuable information on how and why ecological communities might reorganize as the climate warms. Tropical mountains typically have pronounced thermal gradients and intense species interactions, providing a testing ground for investigating whether thermal tolerances and biotic interactions are the proximate factors determining species’ distributions. We investigated thermal tolerances and interspecific competition as causes of species turnover in the nine most abundant species of *Drosophila* along elevational gradients in the Australian Wet Tropics. Upper thermal limits varied less among species than lower thermal limits; nonetheless, at low elevation sites these small differences determined the composition of communities through environmental sorting. In contrast, community composition at cool, high elevations was driven by interspecific competition rather than tolerance to low temperatures. These results run counter to common assumptions about the role of abiotic and biotic factors in structuring tropical communities, and indicate that tropical insects may be vulnerable to future warming wherever their distributions lie along elevational and other temperature gradients.

**Introduction**

Temperature is a critical factors determining species’ ranges and abundances (Hoffmann and Blows 1994). Ecothermic organisms such as insects are particularly sensitive to thermal conditions, which dictate their reproduction, survival, growth, and behaviour (Huey and Kingsolver 1989; Huey and Stevenson 1979). Insect populations are also influenced indirectly by temperature via the thermal responses of other species with which they interact, such as competitors and predators (Gilman et al. 2010). Changes in insect distributions, species composition and phenology arising from changes to temperatures can have direct environmental and economic consequences (Deutsch et al. 2018; Logan, Régnière, and Powell 2003; Pecl et al. 2017).

Estimates of thermal tolerances based on laboratory assays or species’ distributions have been used in species distribution models to evaluate species’ sensitivity to climate change (Kearney and Porter 2009). There are contrasting views on the vulnerability of tropical biotas to global warming (Corlett 2011). Tropical species live near to their upper thermal limits (Deutsch et al. 2008; Diamond et al. 2012; Huey et al. 2009), and so may not be able to tolerate or adapt to the projected warming (Bonebrake and Deutsch 2012; Deutsch et al. 2008; Kellermann et al. 2012). The narrow thermal ranges of tropical insects (Khaliq et al. 2014) also mean that they will need to undertake relatively large latitudinal or elevational range-shifts to track their climate envelopes, 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 distributions 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. 2013; Louthan, Doak, and Angert 2015). It is commonly assumed that tolerance to low temperatures set cold boundaries while biotic interactions predominantly drive ecological limits at the warm boundaries (O’Brien et al. 2017; Paquette and Hargreaves 2021). 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).

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 response 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 focused on 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). Individual *Drosophila* flies are sensitive to temperature (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 (Hangartner et al. 2015; Jeffs et al. 2021), 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 tolerances will correlate with the changes of species abundance toward high elevations. In contrast, we predict that species that have reduced abundance at low elevations will be limited by competition with lowland-biased species, and not necessarily because of lower heat tolerance. To test these hypotheses, we first investigated species turnover patterns along the elevational gradients. We then examined correlations between species distributions and multiple thermal tolerance traits. Finally, 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.

**Methods**

1. Species distributions along elevational gradients

*Field survey.* Field data were collected from rainforest sites at Paluma Range (S18° 59.031' E146° 14.096') and Kirrama Range (S18° 12.134' E145° 53.102'), Queensland, Australia. Abundances of *Drosophila* at these sites peak during the period March to June. *Drosophila* pupae were sampled using bottle traps baited with fermented banana from 11th March – 12th April 2016 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. 2021), 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 relative abundance patterns were assessed by logistic regression of occurrence with elevation. For each individual of 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. These occurrences were fitted against elevation as a fixed effect and transect as a random effect in a generalized linear mixed-effect model (family = “binomial”, varying intercepts and slopes) using the *lmer* package (examples as shown in Supplementary Figure 1a).

Species with significant increasing likelihood to be found at lowlands (negative regression coefficients) were defined as lowland-biased species. Species with significant increasing likelihood to be found at uplands (positive regression coefficients) were defined as upland-biased species. Those whose confidence intervals of regression coefficients in the above analysis were centred around zero were defined as elevation-generalist species. Regression coefficients and hIndexs were highly correlated (Supplementary Figure 1b).

1. Maintenance of laboratory culture

*Drosophila* isofemale cultures were established in 2017 and 2018 from adults 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 were transferred to the Department of Zoology, University of Oxford, UK, in December 2018, where they were maintained at 25°C. Cultures and 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-present.

Nine tropical *Drosophila* species were included in laboratory measurements (species are listed in Supplementary Table 1). Of the available species, only *Drosophila* *rubida* was not included because it was difficult to raise in large numbers and to synchronize with the other species. Isofemale lines of *D. pseudotakihashii* were contaminated by another species before measuring the thermal traits. Therefore, its thermal traits were not measured. A new MBL originated from the two available *D. pseudotakihashii* isofemale lines was constructed and used in the competition experiment. *Drosophila* *melanogaster* does not occur naturally at the study sites but a laboratory strain (wild type, *Dah* strain) was measured for thermal performance together with the focal species, as a benchmark for future comparisons.

1. Reproductive thermal performance

*Experimental measurements*: We exposed flies to temperatures ranging from 14°C to 32°C and measured how their reproductive success changes with temperature (Supplementary Figure 2). To prepare the adults 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 that they were 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 the following 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%. High-temperature water baths had higher humidity than low-temperature ones. The observed temperature showed ±0.5°C fluctuation around the mean. The temperature in the centre of the water bath was 0.5°C higher than at the corner. The former 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 averaged 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 making up to a total of 1512 vials examined.

*Thermal performance curve*: A multi-level, non-linear piecewise model was fitted to describe how reproductive success changed with temperature for the nine *Drosophila* species tested. The average daily fecundity per female was square root transformed, then modeled with the Briere2 function (Briere et al. 1999):

where *i* is the index of *Drosophila* species, *T* is the temperature, *Pi(T)* is the reproductive performance of species *i* in temperature *T*, *RTmini*and *RTmaxi* are the minimum and maximum temperatures for the species *i* to reproduce, *ai* is a scaling factor and *bi* is a shape factor of the curve. Values of *RTmini* of the nine species were modeled by a Gaussian distribution whose mean, , and variance, , were the hyper parameters in the multi-level model. The same applied to *RTmaxi*, *ai*, and *bi*. *P(T)* was modeled assuming a Gaussian distribution. A Gaussian distribution is not ideal to model the transformed count data, which are all positive. However, modeling the original counts of offspring with Poisson, zero-inflated Poisson, negative binomial, lognormal distribution did not adequately converge, potentially due to the piecewise nature of the thermal performance functions. The standard deviations of the Gaussian distribution of *P(T)* were assumed different in different temperature treatments and followed a Gaussian distribution, . It generated better fitting than assuming the same standard deviation of *P(T)* 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*, *P(T)* was close to zero and its standard deviation was also small. Models converged and performance was acceptable in diagnostic plots (Supplementary figure 4). The prior distributions of the hyper parameters , , , , and are Gaussian distribution with reasonable means (15**°**C, 30**°**C, 0, 0, 0) and relatively large standard variations (10, 10, 1, 10, 10). The prior distribution of the hyper parameters , , , , and is inverse-gamma(0.001, 0.001), which is a commonly used non-informative distribution for priors of variance. The values of *ai* and *bi* were bounded to be positive. The values of *RTmini*were bounded between 0°C to 17°C and the values of *RTmaxi* were bounded between 26°C to 35°C based on prior knowledge of the range of temperatures under which Australian *Drosophila* can reproduce. The multi-level model was fitted Medians of the posterior distributions were used as the parameter values to construct the thermal performance curve. Optimal temperature for peak reproduction was directly calculated known parameters:

*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 phylogenetic mixed-effects linear model which uses the taxonomic relationships of the species to define the covariance among species. 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 as knockdown time for each individual at 5°C and the time for recovery of mobility after a 30-minute exposure to 5°C. The constant temperature chosen for cold stress studies is often 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 (siblings of those used for reproduction measurements) were kept in same-sex groups at 25°C for 9-10 days before knockdown assays. Assays 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 held seven flat-bottomed 3ml glass vials, each with a randomly-selected individual of the allocated species.



One set of observations on such a set-up represents a single block. 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 were measured. During measurement, the observation rack was moved immediately into the incubator pre-set at 5°C or 40°C. Every tube was visually examined 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 time taken until each fly regained its ability to stand on its feet, decided by visual 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 identity as a random effect in linear mixed-effects model with phylogenetic correction using the *brms* package in R. The species identity was included as random effect in order to avoid pseudo-replication due to repeated measurements of the same species. *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 lowland and upland temperature regimes. Animal preparation and competition experiments were conducted in incubators set at alternating temperatures mimicking day/night temperatures on February of the study sites (Australian summer), immediately prior to our survey season, at upland (23°C /21°C) and lowland (28.5°C /24°C) (Supplementary figure 5). Five species were chosen as representative species for upland-biased, elevation-generalist and lowland-biased distribution type,as defined XXXXX. Each two-species combination (lowland species x upland species: *D. bipectinata* x *D. pallidifrons*, *D. bipectinate* x *D. pseudotakahashii*, *D.pandora* x *D. pallidifrons*; lowland species x lowland species: *D. bipectinate* X *D.pandora*; lowland species x elevation-generalist: *D. bipectinate* x *D. sulfurigaster*; upland species x upland species: *D. pallidifrons* X *D. sulfurigaster*) 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 *D. pandora*-*D. 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.

*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  is a constant defining the form of the density-dependence relationship. *β* represents the interspecific competition coefficient of the competitor species to the focal species, which define the equivalence between the two competing species. Offspring numbers of the focal species were modeled assuming a negative binomial error distribution, under a Bayesian framework using MCMC sampling within the *rstan* package in R (see more details in in Terry et al. (2021). The medians of the posterior distribution were used as the parameter values to infer the equilibrium state of each pair 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, totalling 24 cultures. 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 culture ID as the random effect in the generalized linear mixed-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 4.0.3 (R Core Team, 2020). All analysis code is available in GitHub.

**Results**

*1.Field distributions*

Figure 1 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 (Supplemental Figure 1b) regarding the elevational biases of species. *Drosophila bipectinata* 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).

*2.Thermal performance curves*

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*, across the temperature range.

*3.Cold tolerance*

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.

*4.Heat tolerance*

Regardless of the small variation of *RTmax* (standard deviation = 2.79) compared with *RTmin* (standard deviation = 0.98), 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.

*5.Interspecific competition*

When raised in the laboratory environment mimicking the warmer, lowland sites, the reproductive success of the two lowland-biased species was the highest, followed by the elevation-generalist species, *D. sulfurigaster*. The two upland species could barely reproduce regardless of the presence of competitors (Figure 4). When raised in the cooler, 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 4, 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-species *D. pallidifrons* to extinction regardless of the starting species composition (monoculture versus mixed species culture). In contrast, the monoculture of the lowland-species *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**

Counter to expectation, 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. Thus, our results do not support the common assumption that cold boundaries to species’ ranges are constrained abiotically and reflect thermal niches, while biotic interactions, such as interspecific competition in our case, 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). 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, consistent with other studies (Goulet, Thompson, and Chapple 2017; Hangartner and Hoffmann 2016). 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 high-temperatures, and also showed the marginal difference (about one degree Celsius) in upper thermal limits that lowland species are higher than upland species. Given the low evolutionary potential of the heat tolerance (Hoffmann, Chown, and Clusella-Trullas 2013), the lowland species are likely vulnerable to small amount 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 factors versus biotic factors in warm versus cold boundaries has roots to Charles Darwin but remains contested until now (Cahill et al. 2014; Hargreaves, Samis, and Eckert 2014). There is especially 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 elevational 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 were indistinguishable between widespread and tropical species and was thus a poor predictor of distribution. In this study, we showed that the interspecific differences in the demographic response to heat correlates well with the 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 3), which the three-day period of fecundity measurement in Overgaard’s study would not have captured. Nevertheless, both our study and that of Overgaard et al. showed the conservativeness of physiological heat tolerance and suggested small safety thermal margins to warming in the future.

(O’Brien et al. 2017) they observed birchii’s reproductive success (in isolated cage) increase (and reach a plateau) with PC1 (mainly contributed by temperature, but also humidity) in Paluma, while abundance decrease in low elevation. Thus they argue that biotic environment controls the field abundance of birchii. My result expect birchii to be already constrained by heat at lowland .

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

It has been shown that daily high temperature rather than mean contributes more to structuring distributions (Lynch et al. 2014). The mean temperature during the survey season at lowland 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 distribution 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 captures the temporal variation of abundance and climate are especially valuable for understanding abiotic limits for field populations (Corlett 2011; Gade et al. 2020).

The 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 (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). Our experiment only examined pair-wise competition and found that the competitive effect of one particular upland species could drive the observed decrease of 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. 2013), 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 a tractable *Drosophila* system, we examined in depth 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 the environmental sorting by the daily high temperature at 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 difference and standardized methods to measure traits. The tropics host a broad suite of endemic species (Laurance et al. 2011). 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.** Proportion of all samples found at the low (yellow)-, medium (gree)- and high (blue)-elevation sites for the nine *Drosophila* species. The grey numbers inside the bars show the absolute counts of samples of the corresponding species and sites.

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). Graph (a) and (e) show the medians (round dot) and 90% credible intervals of the posterior distribution of the estimated parameters. Boxplots in (b-d, f-h) show the minimum, 25th percentile, median, 75th percentile, maximum and potential outliers. In graph (d) and (h), female data was colored by grey and male data was colored by white.

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 = *D. pseudotakahashii*, PAL = *D. pallidifrons*, SUL = *D. sulfurigaster*, BIP = *D. bipectinata*, PAN = *D. pandora*.

Chart

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**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 (coefficient of temperature) when the indicated species were maintained alone (single) or with the other species (mix). c) the posterior distribution of the effect of competition (coefficient 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 the predicted equilibrium states between the indicated pairwise competition.** R0 is the reproductive rate. is a constant defining the form of the density-dependence relationship. is the inter-specific competition coefficient. 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 | unable to establish |
| PAN | 2.99 | 1.52-8.93 | unable to establish |
| SUL | 3.98 | 1.77-12.83 | unable to establish |
| 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 | \ | \ | unable to establish |
| 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 |