**Factors determining distributions of rainforest *Drosophila* shift from interspecific competition to high temperature with decreasing elevation**

**Running title:** Biotic and abiotic effects on distributions

**Authors**:

Jinlin Chen1\*

ORCID number: 0000-0002-8435-0897

Email: chen.jin.lin@hotmail.com

Owen T. Lewis1\*

ORCID number: 0000-0001-7935-6111

Email: owen.lewis@zoo.ox.ac.uk

1. Department of Zoology, University of Oxford

\*Co-corresponding authors

**Author Contributions**:

JC and OTL both contributed to the development of ideas. JC designed and conducted the experimental work. JC analysed 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.

**Conflict of Interest**:

There is no conflict of interest to declare.

**Data Accessibility:**

All original data are achieved on Zenedo (10.5281/zenodo.6400611) and code of analysis are available on GitHub (<https://github.com/Jinlinc/DistributionRegulators.git>).

**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, Mélanie Thierry, Eleanor O’Brien, Mukhlish Jamal Musa Holle and Benjamin Van Doren for advice on data analysis and comments on the manuscript.

**Abstract:**

Species turnover with elevation is a widespread phenomenon and provides valuable information on why and how 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 the relationship between thermal tolerances and biotic interactions as 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 communities along thermal gradients, and indicate that tropical insects may be vulnerable to future warming wherever their distributions lie along elevational and other temperature gradients.

**Keywords:**

Species distribution, climate change, *Drosophila*, tropics, thermal tolerance, thermal performance curve, biotic competition, coexistence.

**Introduction**

Temperature has a fundamental impact on the reproduction, survival, growth, and behaviour of organisms (Huey and Kingsolver 1989; Huey and Stevenson 1979), strongly influencing species’ ranges and abundances (Hoffmann and Blows 1994; Wilson et al. 2005). As a result, estimates of thermal tolerances based on laboratory assays or species’ distributions have been used widely to evaluate species’ sensitivity to climate change (Deutsch et al. 2008; Kearney and Porter 2009). Tropical ecosystems comprise many species that live close to their upper thermal limits (Deutsch et al. 2008; Diamond et al. 2012; Huey et al. 2009), and which may not tolerate or adapt to warmer temperatures (Bonebrake and Deutsch 2012; 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 as the climate warms, increasing the risk of extinction and community disassembly (Colwell et al. 2008; Sheldon, Yang, and Tewksbury 2011).

Despite high relevance for understanding species’ responses to climate change in tropical ecosystems (Corlett 2012), it remains questioned whether temperature is the major proximate factor setting the position of the ‘warm’ (low-latitude or low-elevation) limits of species’ distributions. Global analyses found smaller distribution shifts in response to warming at warm range limits than at cool range limits (Chen, Hill, Shiu, et al. 2011; Sunday, Bates, and Dulvy 2012), and some laboratory assays have found similar upper thermal limits for tropical and temperate species (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; Louthan, Doak, and Angert 2015).

A common expectation, first expressed by Darwin, is that tolerance to low temperatures will set cool range limits, while biotic interactions will more often set warm range limits (O’Brien et al. 2017; Paquette and Hargreaves 2021). However, a recent analysis of data for 654 taxa found that biotic factors became less important in determining warm limits towards the tropics, while abiotic factors remained consistently important across latitudes (Paquette and Hargreaves 2021), highlighting the regional differences in the relative roles of abiotic and biotic factors on local thermal gradients.

There remains limited empirical evidence on the proximate factors setting range limits in species-rich tropical communities (Feeley, Stroud, and Perez 2017; Jankowski et al. 2013), and most such studies examine only the correlation between thermal traits (such as critical temperatures and optimal temperatures) and species’ distributions (Cahill et al. 2014). A significant correlation between tolerance and distribution does not rule out a role for biotic interactions, which are themselves sensitive to temperature (Gilman et al. 2010). Additionally, the abundance and distribution of a species may still be sensitive to temperature change, even when biotic interaction is the proximate factor determining species composition. To understand the relationship between temperature and distribution, studies quantifying both thermal traits and temperature-dependent biotic interactions are required (Cahill et al. 2014). Such studies can help unify the long-separate concepts of environmental and biotic filters (HilleRisLambers et al. 2012), as well as providing practical information to inform conservation efforts in the face of climate change.

To investigate the roles of thermal tolerances and biotic interactions in determining species’ range limits and structuring communities, we focused on the community of *Drosophila* flies occupying mountain rainforest habitats in north-eastern Australia. These and other tropical mountains provide natural environmental gradients to test the sensitivity of species to temperature (Corlett 2011), with pronounced changes in species composition with elevation for many taxa (Williams, Bolitho, and Fox 2003). We hypothesized that species turnover along the elevational gradient would result from thermal constraints at cool, high elevation sites, but from competitive exclusion at warmer, lowland sites. Thus, we predicted that cold-tolerant species would become more abundant at higher elevations. We also predicted that species with reduced abundance at low elevations would be those limited by competition with species with lowland-biased distributions, and not necessarily those with lower heat tolerance. To test these predictions, we first examined correlations between species’ distributions and multiple thermal tolerance traits. We then used laboratory experiments mimicking upland and lowland thermal conditions to examine short-term and long-term competitive outcomes for pairs of species with similar or different distribution types.

**Methods**

1. Species distributions along elevational gradients

*Field survey.* Field data on *Drosophila* communities 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. These sites lie within the Wet Tropics bioregion which has high levels of endemism associated with cool and moist upland refugia (Williams et al. 2003). 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 (subsequently referred to as low-, mid- and high-elevation, respectively) on the two mountains. 182 pupae were sampled at each site and 716 pupae in total were successfully identified to species by DNA metabarcoding (Jeffs et al. 2021), with 86 – 134 pupae at each site. Two infrequent species, *Drosophila serrata* (1 sample) and *D. immigrans* (4 samples), were excluded from analyses.

*Distribution analysis*. Two methods were used to assess trends in relative abundance with elevation for individual species. First, the probability of detecting a particular *Drosophila* species among other co-occurring *Drosophila* species was modeled as a function of *elevation*, *transect* and their interaction in a generalized linear model (family = “binomial”) using the *stats* package in R. For each species, the response variable was 1 if the pupa was identified as the focal species and 0 if it was identified as any other species. Second, since none of the species had a bimodal distribution with elevation, abundance-weighted mean elevation (*hIndex*) was used as a simple alternative method to quantify distributions. The location of each sample was assigned a value of 0, 0.5, or 1 if it was collected at low-, mid- and high-elevation sites, respectively. *hIndex* was calculated for each species by averaging these values for samples from both transects. Correspondence between the two measures was tested by Spearman’s rank correlation test between the coefficient value for *elevation* in the regression and *hIndex* across species.

1. Maintenance of laboratory culture

*Drosophila* isofemale cultures were established in 2017 and 2018 from adults collected from high- and low-elevation sites. Cultures were maintained initially at 24°C at the Biology Centre, Czech Academy of Sciences, and then transferred to the Department of Zoology, University of Oxford, UK, in December 2018, where they were maintained at 25°C. Cultures and all experiments mentioned below were maintained under a 12h/12h light/dark cycle. There were approximately 15 to 30 non-overlapping generations in the Czech Republic and four to seven non-overlapping generations in Oxford before cultures were used to establish 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 information in Supplementary Table 1). Each mass bred population 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. The MBLs were maintained at 25°C until 2020 and at 23°C thereafter. In this way, measurements should not have been influenced by maternal effects, acclimation, or isofemale line effects.

Nine tropical *Drosophila* species (listed in Supplementary Table 1) were included in laboratory thermal trait measurements. Of the available species, only *D.* *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 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 changed with temperature (procedures are detailed in Supplementary Figure 1A). To generate the adults flies to initiate the experiment, fly eggs collected from the population cage were reared at low density (<100 eggs per vial) at 25°C. Emerging adults were separated by sex within 12 hours to guarantee that they were unmated. Rearing was started on different days for different species to synchronize the first day of egg-laying. Two additional vials with five pairs of flies were monitored daily for sexual maturation, indicated by egg laying. Two days after the first observation of egg-laying in both vials, two virgin females and two virgin males were paired in a new 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 assigned to water baths set at one of seven constant temperatures (14°C, 17°C, 20°C, 23°C, 26°C, 29°C, 32°C). For each species and each temperature treatment, eight replicates were evenly split between two experimental blocks, making up a total of 1512 vials. Vials were submerged with the water level kept above the zone within which flies could freely move. The temperature and relative humidity of vials in each water bath were monitored within two empty tubes placed at the centre and the corner. Observed temperatures varied ±0.5°C around the set temperature. Temperatures in the centre of the water bath were on average 0.5°C higher than at the corners; the average of the former during the experimental period was used as the corrected temperature in analyses. Relative humidity levels were similar to field conditions, ranging between 80% - 95%.

As temperature influenced how fecundity changed through time (Supplementary Figure 1B), offspring numbers were measured for the 1st – 2nd day and the 7th – 8th day and then averaged to reflect relative fecundity in early adult life. After eight days of exposure to temperature treatments, all flies were returned to 25°C for another four days to examine their recovery of reproduction. Surviving flies were recorded at the beginning and end of each period. Eggs produced during the test periods developed at the same temperature as their parents. Vials were left for 5 – 7 days after the first emergence was observed and then the offspring was counted. The experiment was conducted from May to August 2019.

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

where *Pi(T)* is the reproductive performance of species *i* in temperature *T*, *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 *RTmin* of the nine species were modelled 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 modelled assuming a Gaussian distribution of errors. Although a Gaussian distribution is not ideal to model the transformed count data, which are all positive, models using untransformed count numbers with Poisson, zero-inflated Poisson, negative binomial, and lognormal distributions 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, . This generated better fits 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.

The prior distributions of the hyper parameters , , , , and are Gaussian distributions with reasonable means (15**°**C, 30**°**C, 0, 0, 0) and relatively large standard deviations (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 under a Bayesian framework using MCMC sampling within the *rstan* package (Stan Development Team, 2021) in R. Models converged and performance was acceptable in diagnostic plots (Supplementary Figure 2). Medians of the posterior distributions were used as the parameter values to construct the thermal performance curves. Optimal temperatures for peak reproduction were calculated directly from known parameters:

*Regression analysis*: The medians of the posterior distributions of *RTmin*, *RTmax*, and *RTopt* were modelled by *hIndex* in a linear model with phylogenetic correction. Phylogenetic tree were modified from Finet et al. 2021 (Supplementary Figure 3). Fecundity at 29°C and 17°C, and recovered fecundity after 29°C and 14°C, were used as direct measurements of performance in high or low temperatures. These fecundities were modelled as a function of *hIndex* and *experimental block* as fixed effects, and *species* as a random effect in a generalized linear mixed-effect model (family = “negative binomial”) with phylogenetic correction. These regression analyses were conducted using the *brms* package (Bürkner 2021) in R. *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, Sørensen, and Loeschcke 2003) and is expected to capture the between-species variance in heat tolerance 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 heat. The knockdown experiments were conducted from May to Jun 2019.

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 . During measurement, the observation rack was moved immediately into the incubator pre-set at 5°C or 40°C. Every tube was examined once every minute and flies that lost their ability to stand in that minute were recorded. After exposure 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 time taken until each fly regained its ability to stand 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 using ANOVA. When interspecific variation was observed, they were modelled by *hIndex*, *block*, and *cell position* as fixed effects, and *species* as a random effect in linear mixed-effect models with phylogenetic correction using the *brms* package in R. *Species* was included as a random effect to account for repeated measures.

1. Short-term competition

*Experimental design*: Pairs of species were reared in the same vials for one generation to evaluate how the densities of intraspecific and interspecific competitors influenced reproductive success under temperature regimes typical of lowland and upland sites. We used incubators set at alternating temperatures mimicking day-time and night-time temperatures in February at high-elevation (23°C / 21°C) and low-elevation sites (28.5°C / 24°C) (Supplementary Figure 4). Adults to establish the competition experiment were reared at 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 from the following day were allowed to compete and lay eggs in the 5 ml experimental vials for two days before being discarded.

Five species were chosen as representative species for upland-biased, elevation-generalist and lowland-biased distribution types (see Results for definition of distribution types). The two-species combinations were: lowland species vs. upland species: *D. bipectinata* vs. *D. pallidifrons*, *D. bipectinata* vs. *D. pseudotakahashii*, *D.pandora* vs. *D. pallidifrons*; lowland species vs. lowland species: *D. bipectinata* vs. *D.pandora*; lowland species vs. elevation-generalist: *D. bipectinata* vs. *D. sulfurigaster*; upland species vs. elevation-generalist: *D. pallidifrons* vs. *D. sulfurigaster*. Each combination was tested 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 with 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* vs. *D. pallidifrons* combination; three blocks for the other five pairs). Offspring that successfully developed to adulthood were identified to species and counted. Experiment of *D. pandora* vs. *D. pallidifrons* combination was conducted from September to December, 2020 and the other five pairs were experimented from January to March, 2021.

*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 defines the equivalence between the two competing species. Offspring numbers of the focal species were modelled assuming a negative binomial error distribution, under a Bayesian framework using MCMC sampling within the *rstan* (Stan Development Team 2021) package in R, as described by Terry et al. (2021). Model diagnostics are shown in Supplementary Figure 5A-B. 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*: To evaluate the long-term impact of temperature and competition on population size, a lowland species, *D. pandora*, and an upland species, *D. pallidifrons*, were reared in monoculture and mixed-culture environments for multiple generations under low-elevation and high-elevation temperature regimes (Supplementary Figure 4).

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 cultures were evenly divided into two blocks starting on different dates. Each was maintained in a series of five bottles following Ayala, Gilpin, and Ehrenfeld (1973). At the start of each week, adults surviving in the most recent bottle and adults which were freshly emerged in the older four bottles were separately collected, photographed and transferred together 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 regimes 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. The experiment was conducted from September to December, 2020.

*Data analysis*: Population sizes were modelled as a function of *temperature*, *species*, *presence/absence of competitors*, and their interactions, with *culture ID* as a random effect with a generalized linear mixed-effect model (family = “zero-inflated negative binomial”) using the *brms* package in R. Model diagnostics are shown in Supplementary Figure 5C. 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 each temperature regime.

All statistics mentioned in the *Methods* section were performed with R statistical software, version 4.0.3 (R Core Team, 2020). Original data are achieved on Zenedo (10.5281/zenodo.6400611) and code of analysis are available on GitHub (https://github.com/Jinlinc/DistributionRegulators.git).

**Results**

*1.Field distributions*

The numbers of samples found at low-, medium- or high-elevation sites for each of the nine major *Drosophila* species (accounting for 99% of all samples) are shown in Figure 1. Distributions quantified using regression and by weighted elevation (*hIndex*) were consistent (Spearman’s rank correlation rho = 0.93, p value = 0.0007). *Drosophila bipectinate* and *D. pandora* had significantly decreased detection probability with increasing elevation and were thus categorized as lowland-biased species with high confidence. *Drosophila pseudoananassae* showed a lowland bias on one of the two mountains, and a mid-elevation peak on the other. *Drosophila rubida*, *D. sulfurigaster* and *D. birchii* showed no significant change with elevation and were thus defined as elevation generalists. *Drosophila pallidifrons* and *D. pseudotakahashii* were significantly more likely to be found at high elevations and were thus defined as upland-biased species. Coefficients and p values of the regressions and *hIndex* are shown in Supplementary Table 2. For *D. bunnanda,* the six records were insufficient for model fitting but all occurred at lowland sites, and data from a larger-scale study suggest it is a lowland-biased species (Schiffer and McEvey 2006).

*2.Thermal performance curves*

Thermal performance curves of daily fecundity per female vary among species in terms of the range, optimal temperature, peak fecundity, and shape factors (Figure 2; Table 1; see Supplementary Figure 2C for original data and fitted curves for each species).The temperature for optimal reproductive performance, *RTopt*, did not correlate with distribution patterns (Coefficient = 0.09, 95% credible interval = -2.83 – 3.01; Supplementary Figure 6A). There was no general trade-off between cold tolerance (estimated *RTmin*) versus heat tolerance (estimated *RTmax*) corresponding to species’ distribution types (Spearman’s rank correlation rho = -0.6, p value = 0.10; Supplementary Figure 6B). For example, the lowland-biased species *D. bunnanda* has higher heat tolerance and lower cold tolerance than its elevation-generalist 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 species distribution patterns (Figure 3a. Coefficient = -0.41, 95% credible interval = -4.15 – 3.43). Similarly, upland-biased species did not show higher fecundity at the low temperature, 17°C (Figure 3b. Coefficient = -0.27, 95% credible interval -3.80 – 3.05). When exposed to acute sublethal low temperature (5°C), all seven tropical *Drosophila* species showed similarly poor performance compared to *D. simulans* and *D. melanogaster* (Supplementary Table 3 for Tukey multiple pairwise comparisons). All species recovered their fecundity after eight days at 14°C. This recovered fecundity was slightly but non-significantly higher among upland species (Figure 3c. Coefficient = 0.33, 95% credible interval = -0.55 – 1.14). It took longer for upland species to regain mobility after chill coma (Figure 3d. Male: coefficient = 13.04 (-9.31 – 35.05); female: coefficient = 8.49 (-2.18 – 20.02).)

*4.Heat tolerance*

Regardless of the small variation of *RTmax* (standard deviation = 1.01) compared with *RTmin* (standard deviation = 2.82), *RTmax* was significantly higher among species with more lowland-biased distributions (Figure 3e. Coefficient = -3.06, 95% credible interval = -5.30 – -0.88). Reproductive performance at 29°C also decreased with *hIndex* (Figure 3f. Coefficient = -5.80, 95% credible interval = -9.37 – -2.50). After exposure to 29°C for eight days, neither upland species could reproduce when transferred back to 25°C, while four out of the five elevation-generalist and lowland-biased species resumed reproduction (Figure 3g). Knockdown time at lethally high temperature (40°C) was shorter among upland species (Figure 3h. Male: coefficient = -6.71 (-13.70 – 0.50); female: coefficient = -1.99 (-9.48 – 5.71)), indicating these species lose their mobility faster at high temperatures.

*5.Interspecific competition*

When raised in a laboratory condition mimicking the warmer, lowland sites, reproductive success was highest for the two lowland-biased species, followed by the elevation-generalist species, *D. sulfurigaster*. The two upland species could barely reproduce regardless of the presence of competitors (proliferation rates (R0) of *D. pallidifrons* and *D. pseudotakahashii* were lower than 1; Figure 4; Table 2). Their competitive effect on lowland species was minimal, indicated by low values (Table 2). The two lowland species, *Drosophila pandora* and *D. bipectinata*, are expected to coexist stably, based on their reproductive and competitive parameters.

When raised a under cooler, upland condition, all species could reproduce and sustain their populations (all proliferation rates (R0) were higher than 1; Table 2). Lowland species were strongly affected by the density of *D. pallidifrons*, an upland species, while upland species were significantly less affected by lowland species. Competition with *D.pallidirons* under upland conditions was predicted to drive *D. pandora* and *D. bipectinata* to exclusion (Table 2).

In the long-term competition experiment, high temperature drove the upland-species *D. pallidifrons* to extinction regardless of the starting species composition (monoculture versus mixed species culture) (Figure 5a). In contrast, the monoculture of the lowland-species *D. pandora* remained abundant at both temperatures (Figure 5b: 90% credible interval of temperature effect in monoculture overlaps with zero). The interspecific competition effect was only significant at the upland regime (Figure 5c), which significantly reduced the population size of *D. pandora* in mixed-species cultures compared to the monocultures (Figure 5a and Figure 5b).

**Discussion**

Counter to expectation, our results do not support the common assumption that cool boundaries to species’ ranges are constrained abiotically (reflecting thermal niches), while biotic interactions (such as interspecific competition) define warm boundaries. Instead, we found that maximum temperatures experienced on a daily basis at lowland sites were sufficiently high to serve as an environmental filter. In cooler, high-elevation conditions, lowland species were outcompeted by certain upland species whose distribution were confined to high elevations as a result of their intolerance to heat.

*1.Contributions of abiotic and biotic factors in determining distributions*

The idea that the contributions of abiotic versus biotic factors might differ for species’ warm and cool range boundaries can be traced back to Charles Darwin, and remains an area of active debate (Cahill et al. 2014; Hargreaves, Samis, and Eckert 2014; Schemske et al. 2009). There is especially mixed evidence on the importance of upper thermal limits in deciding warm boundaries (positive relationships: Batista et al. 2018; Duarte et al. 2012; García-Robledo et al. 2016; Kellermann et al. 2012; Merrill et al. 2008; null relationships: Gaston and Chown 1999; Huang and Tu 2008; Kimura 2004; Nowrouzi et al. 2018). This inconsistency could arise for at least three reasons. First, data available for synthetic studies are over-represented by systems from temperate latitudes in the northern hemisphere (Feeley et al. 2017) and relate largely to cool limits (Cahill et al. 2014); a recent synthesis finds that the difference in the relative importance of biotic factors to warm versus cool boundaries tends to disappear toward the equator (Paquette and Hargreaves 2021). Second, mechanisms governing latitudinal or elevational distribution patterns could differ (Chen, Hill, Ohlemüller, et al. 2011), and may only become apparent as more datasets accumulate from less-studied locations (Duarte et al. 2012; Freeman et al. 2021). Third, some of the uncertainty might reflect methodological differences when choosing, defining and measuring thermal traits. Studies like the current one that examine a range of thermal traits and biotic interactions may be needed to understand how and why the governing rules might change under different thermal and biotic regimes (Amundrud and Srivastava 2020; Srinivasan et al. 2018).

Indeed, different choices of indicators of thermal performance, and different experimental methods may explain why some of our results contrast with some other studies of Australian rainforest *Drosophila*. Overgaard et al. (2014) found no evidence that thermal traits of tropical rainforest *Drosophila* differed among species. Unlike Overgaard et al. (2014), we measured parental fecundity by allowing their eggs to develop to adults under the relevant temperature; we observed that eggs laid at high temperatures never hatched, perhaps as a result of sperm sterilization (Parratt et al. 2021). We also observed different speeds of senescence after exposure to different temperatures (Supplementary Figure 1B), which the three-day period of fecundity measurement in Overgaard’s study would not have captured. Nevertheless, both studies highlight the conserved nature of heat tolerance and suggest small thermal safety margins to future warming.

Field experiments on *D. birchii* (O’Brien et al. 2017, 2020), found that its reproduction when raised alone was highest at low elevations, where both intra-specific and inter-specific competition with its sister species *D. bunnanda* were also high. In our experiments, *D. birchii* was one of the most heat-sensitive species, and its populations were unable to persist at high temperatures typical of low elevations (pilot experiment, unpublished data). This discrepancy can be explained by different temperature regimes used in our study (corresponding to February temperatures in the field, with an average of 26°C and average daily maximum of 30.6°C at lowland) and by O’Brien et al. (April temperatures, which are around 2°C lower on average temperature and on average daily maximum). A further possibility is that single-generation experiments initiated with mated adult flies do not capture the impact of high temperatures prior to sexual maturation. These observations further highlight the sensitivity of measures of thermal performance to experimental conditions. They also suggest that the relative importance of abiotic and biotic factors in determining species distributions may change seasonally.

*2.Low variation in upper thermal limits*

Upper thermal limits vary little among species (Hoffmann 2010) and have limited adaptive potential (van Heerwaarden and Sgrò 2021; Kellermann et al. 2012). Consistent with other studies (Goulet, Thompson, and Chapple 2017; Hangartner and Hoffmann 2016), we found that heat tolerance is a systematic trait manifested in critical temperatures, fecundities at sub-sterile temperatures, recovered fecundities, and locomotive responses. Modest changes in critical temperature can therefore lead to pronounced differences in overall performance under real and variable temperature regimes.

Such 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; van Heerwaarden and Sgrò 2021). Laboratory-measured critical temperatures can be sensitive to experimental conditions, making it difficult to relate them to climatological means or maxima, and hence the threat of rising temperatures (Sinclair et al. 2016). In this context, our study benefits from a comparative approach, which reveals that upland species are already constrained by high temperatures, and that a very small difference (about 1°C) distinguishes the upper thermal limits (*RTmax*s) of lowland and upland species. Given the low evolutionary potential of heat tolerance (Hoffmann, Chown, and Clusella-Trullas 2013), both lowland species and upland species are likely to be vulnerable to modest temperature increases across the elevation gradient. Thus, lowland biotic attrition and upland range contraction are likely to occur with future warming, leading to cascading effects in lowland communities and threatening endemic upland species on tropical mountains such as those in the Australian Wet Tropics (Freeman et al. 2018).

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

Daily maxima can be more important than mean temperatures in structuring distributions (Lynch et al. 2014). In our study system, the mean temperature during the study season at our lowland sites is around 26°C. At this temperature, all study species are close to their peak reproductive performance. However, upland and lowland sites differ greatly in the average daily maximum temperature and the daily duration that the temperature reaches or exceeds stressful levels for *Drosophila* reproduction (Supplementary Figure 7). Brief exposure to stressful thermal environments can have similar fitness costs to continuously stressful conditions (Saxon, O’Brien, and Bridle 2018). This highlights the importance of considering daily temperature variations and extreme temperature events when studying species distributions and projecting responses to climate change (Kingsolver, Diamond, and Buckley 2013; Ma, Hoffmann, and Ma 2015).

*4. Context-dependence of thermal performance*

Inevitably, thermal traits measured under laboratory conditions cannot account for the tendency of thermal tolerances to vary with a range of abiotic factors including, but not limited to precipitation (Bozinovic and Pörtner 2015; Kellermann et al. 2012), diet, and larval conditions (Bubli, Imasheva, and Loeschcke 1998). Such variables are to a large extent standardized in our study, allowing dependence on abiotic conditions to be resolved. Our long-term experiment showed that the thermal response of population size to temperature depended on competitive conditions, highlighting the difference between thermal performance curves of organisms and their populations when biotic interactions are present (Davis et al. 1998). There is a growing realisation that 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 are altered (Burnside et al. 2014).

Finally, between-population variation and local adaptation (Hoffmann, Anderson, and Hallas 2002), especially adaptation to abiotic environments at distribution boundaries (Peterson, Doak, and Morris 2019), should not be neglected if studying distributions at a large spatial scales (e.g., across latitudes). The relatively restricted spatial scale of our study (within a mountain range), the use of mass bred lines sourced from different elevations, and the limited plasticity and local adaptation documented for our study species (MacLean et al. 2019; O’Brien et al. 2017) mean that plastic and evolutionary response to thermal conditions are unlikely to complicate interpretation of our results.

*5.Conclusions*

Tropical ecosystems host an exceptional diversity of endemic species (Laurance et al. 2011). Predicting their sensitivity to climate change is a high priority and requires an understanding of the proximate causes of current distributions as well as the interactions between thermal tolerance and other environmental factors, both biotic and abiotic. Our study contributes to the growing literature demonstrating that species can be sensitive to warming at the warm boundaries of their distributions. In particular, we highlight the important role of daily maximum temperatures in structuring community at tropical lowlands, the essential role of interspecific competition at uplands, and the vulnerability of species there to increasing mean temperatures and temperature extremes.

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**Figure 1.** Distribution patterns of nine rainforest *Drosophila* species at two study sites, Mount Kirrama and Mount Paluma. The proportion of samples found at sites at low (yellow), medium (green) and high (blue) elevations is shown for each species. Numbers on the bars show the counts of samples for each combination of species and site.

Chart, bar chart

Description automatically generated

**Figure 2.** Thermal performance curves for reproduction of nine *Drosophila* species. The fitted numbers of adult offspring produced per female parent per day are plotted against temperature. Line colours indicate species’ distribution types, 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 seven tropical *Drosophila* species. Species are ordered by their distribution patterns, from lowland-biased species (left) to upland-biased species (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). Heat tolerance is represented by *RTmax* (e), fecundity at 29°C (f), recovered fecundity after 29°C (g) and knockdown time at high temperature (h). Graph (a) and (e) show the medians and 90% credible intervals of the posterior distribution of the estimated parameters. Boxplots in b-d and f-h show the minimum, 25th percentile, median, 75th percentile, maximum and potential outliers. In (d) and (h), data for females (shaded) and males (unshaded) are plotted separately.

Diagram, engineering drawing

Description automatically generated

**Figure 4.** Interspecific competition for species pairs under upland (left) and lowland (right) 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 individuals of the competing species. The identity of the focal species in each pair is indicated by the colour of the line (see legend) and by the label (e.g. for BIP\_PST, the first code BIP is the focal species and the competitor species is PST). The shaded area indicates the 90% credible interval of the fitted values under the Beverton-Holt model for pairwise species competition. PST = *D. pseudotakahashii*, PAL = *D. pallidifrons*, SUL = *D. sulfurigaster*, BIP = *D. bipectinata*, PAN = *D. pandora*.

Chart

Description automatically generated

**Figure 5.** Effects of temperature and interspecific competition on 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 under upland (cold) and lowland (hot) temperature regimes. b) the posterior distributions 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 distributions of the effect of competition (coefficient of competition) when the indicated species were maintained at upland (cold) and lowland (hot) temperature regimes.

Diagram

Description automatically generated

**Table 1.** Estimated parameters of thermal performance functions and their 90% credible intervals (ci90) of the nine species. *RTmin*and *RTmax* are the minimum and maximum temperatures for the species to reproduce. *a* is the scaling factor and *b* is the shape factor of the Briere2 function.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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 model and predicted equilibrium states for pairwise interspecific competition. *R0* is the reproductive rate, is a constant defining the form of the density-dependence relationship, is the interspecific competition coefficient, and ci90 represents the 90% credible intervals of each parameter. The equilibrium states of the focal species are inferred based on Hassell and Comins (1976).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Temperature** | **Focal species** | **R0** | **R0.ci90** |  | **.ci90** | **Competitor** |  | **.ci90** | **Equilibrium state of the focal species** |
| Cool, 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 |
| Warm, 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 |