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

**Small differences in upper thermal limit and competition structure lowland and upland *Drosophila* community composition respectively in tropical rainforest**

**Running title:** abiotic and biotic regulators of distribution

**Authors**:

Jinlin Chen1 0000-0002-0626-9938

Owen T. Lewis1 0000-0001-7935-6111

1. Department of Zoology, University of Oxford

**Author Contributions**:

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**:

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

**Acknowledgements**:

We thank Hrček lab (Czech Academy of Sciences) and XXX (Australian collaborators) for providing Drosophila laboratory cultures. We thank Jan Hrček, Chia-Hua Lue, Nick Pardikes and Mélanie Thierry and the Oxford Fly group for their advice and share of facilities. We thank Chris Terry for advice on analysis and manuscript.

**Abstract:**

Species turnover along temperature gradients (e.g. latitude, elevation) is a widespread phenomenon and provides valuable information on how ecological communities might reorganize as the climate warms. However, the precise response of species distributions to climate change will depend on the mechanistic causes of range limits. Wein nine species of *Drosophila* al Characterised by species with small thermal safety margins and increasing climate anomaly, the tropics face multiple threats to biodiversity such as lowland biotic attrition and upland community contraction. Evidence for the contribution of temperature to species distributions on tropical mountains is limited and the proximate causes (i.e. thermal tolerance, biotic competition) are rarely examined. Upper thermal limits varied less among species than lower limits; nevertheless, these small differences could lead to a different outcome of environmental sorting by the high temperature in tropical lowlands. In contrast, our results indicate that upland community composition is driven by interspecific competition rather than cold tolerance. These results contribute to the discussion about the role of abiotic and biotic factors in structuring tropical communities and indicate that there tropical insects may be vulnerable to future warming wherever their distributions lie along elevational and other temperature gradients.

**Introduction**

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

Thermal tolerance estimated from either laboratory measures or observed distribution has been used in species distribution models to evaluate species’ sensitivity to climate change (Kearney and Porter 2009). The impact of warming on tropical species is still much debated, from least to most concern among major biotas (Corlett 2011). Tropical species generally occur within a narrower thermal range than temperate species (Khaliq et al. 2014) and live near to their upper thermal limits (Deutsch et al. 2008; Diamond et al. 2012; Huey et al. 2009). Tropical species are therefore predicted to face particular difficulty to tolerate or adapt to the projected warming (Bonebrake and Deutsch 2012; Deutsch et al. 2008; Kellermann et al. 2012), or to track the relatively large shift of their “climate envelope”, the projected suitable climate zone that moves toward higher latitude or elevation (Colwell et al. 2008; Sheldon, Yang, and Tewksbury 2011).

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

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

To investigate the roles of thermal tolerances and biotic interactions in determining species’ range margins and structuring communities, we investigated the community of *Drosophila* flies occupying rainforest habitats in the 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 form many taxa changes significantly along the elevation gradient on these tropical mountains (Williams et al. 2003). *Drosophila* flies are sensitive to temperature at the organismal level (Batista, Rocha, and Klaczko 2018), while their population sizes are additionally regulated by humidity, food availability, competition, and natural enemies (e.g. parasitoid wasps) (Fletcher 1973; Krebs and Barker 1991; Mitsui et al. 2007). At out study sites, methods have been established to quantify their field distribution (Jeffs et al. 2020), laboratory thermal performance (Hoffmann, Sørensen, and Loeschcke 2003) and competitive ability (Terry, Chen, and Lewis 2021). Thus, this system offers an ideal opportunity to investigate the roles of temperature and competition in determining warm and cold boundaries in the tropics.

We hypothesize that species turnover observed on the altitudinal 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 species’ distributions. In contrast, species that have reduced abundance at low elevations, relative to high elevations will not necessarily have lower heat tolerance; rather their abundances are predicted to be reduced by competition with lowland-biased species. To test these hypotheses, we first demonstrated the species turnover patterns along the elevation gradients. We then examined correlations between species distribution patterns and multiple thermal tolerance traits. Thirdly, pairs of species with similar or different distribution types were placed in simulated highland and lowland temperatures to examine their competitive outcomes in both the short and long term. Counter to our original predictions, our results suggest that interspecific competition significantly reduces lowland-biased species at high elevations, whereas high temperature, regardless of competition, constrains upland *Drosophila* species at low elevations. Small difference in their upper thermal limits implies the vulnerability of all species to future warming.

**Methods**

1. Study system

Field data were collected, and laboratory cultures were initiated, from rainforest sites spanning elevations of from 59 – 916 m at Paluma Range (S18° 59.031' E146° 14.096') and Kirrama Range (S18° 12.134' E145° 53.102'), Queensland, Australia. Mean temperatures at study sites ranged from 21°C to 26°C.

*Drosophila* isofemale cultures were established in 2017 and 2018 from pupae collected from high- and low-elevation sites. Cultures had been maintained at 24°C and 12h/12h L/D cycle at the Biology Centre, Czech Academy of Sciences since collection and transferred and maintained at 25°C and 12h/12h L/D cycle at the Department of Zoology, University of Oxford, UK, since December 2018. 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 and 12h/12h L/D cycle 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 in 23°C and 12h/12h L/D since 2020.

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

1. Field distribution survey

*Drosophila* pupae were sampled using bottle traps baited with fermented banana from 11th March – 12th April 2016 (Jeffs et al. 2020). Relative abundance was surveyed for three sites representing the highest, lowest, and most central points of each of the two mountains. In total, 182 pupae were sampled at each site and 716 pupae were successfully identified to species by DNA metabarcoding (Jeffs et al. 2020), with 86 – 134 pupae at each site. *Drosophila serrata* (1 individual) and *D. immigrans* (4 individuals) were excluded from the distribution analysis due to infrequent occurrence.

1. Preparation of experimental animals

For thermal trait measurement, fly eggs collected from the population cage were reared at low density (less than 100 eggs per vial) at 25°C and 12h/12h L/D cycle. Adults that emerged within 12 hours were separated to guarantee that they were unmated. For the competition experiment, fly eggs collected from the population cage were reared under moderate density (300 – 500 per bottle) at 21°C/23°C (upland) or 24°C/28.5°C (lowland) and 12h/12h L/D cycle. Flies that emerged within the same 48 hours were kept together in mixed-sex containers. These individuals were the founders of the competition. The temperature of competition was the same as the founders in the larval stage. For both experiments, we additionally mixed five females and five males in each of two vials to monitor their reproductive activity every day. Some species started on different days to synchronize the first day of egg-laying of all species. Two days after the first observation of egg-laying, measurements of thermal traits or competition started.

1. Fecundity measurement

Two virgin females were paired with two virgin males on a 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) and 12h/12h L/D cycle. 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. The level of humidity was similar to field condition, ranging between 80% - 95%. The observed temperature showed ±0.5°C fluctuation around the mean temperature, the average of which was used as the corrected temperature in the analysis (Supplementary table 2 [both centre and corner logs]).

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

1. Thermal knockdown measurement

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

Virgin adult flies, which were siblings of those in fecundity measurement, were kept in groups at 25°C and 12h/12h L/D cycle for 9-10 days before allocation. Randomly-selected individuals were allocated separately in empty flat-bottom 3ml vials. An observation rack was divided into a 3x3 grid with each cell in the grid holding seven tubes containing the same species. All nine species were assigned in random order, with one species in each cell of the grid. The observation rack was moved immediately into the incubator, representing the start of the heat or cold treatment. Every tube was examined once every minute and flies that lost or recovered their motor ability in that minute were recorded. For each sex, the above procedure was repeated three times.

1. Competition measurements

Short-term competition: adults of different sexes were separated and then they were used as the founders in 5ml-food vials the next day. Each two-species combination (6 combinations in total) was measured at different founding densities in a factorial design: (4 pairs of species A, 2 pairs of species B), (4A, 4B), (4A, 8B), (2A, 4B) and (8A, 4B). We also included monocultures of each species of 2, 4, 8 pairs. Each density and species combination was replicated ten times across two or three blocks staggered by two days (two blocks for PAN-PAL combination, which was conducted before the other five pairs; three blocks for the other five pairs.). Founders laid eggs in vials for two days before being discarded. Offspring of the founders experienced intra- and inter-specific competition over food and space in the vials. Offspring that successfully developed to adulthood were identified to species and counted. The competition experiments were conducted in incubators set at alternating temperature regimes mimicking day/night temperatures during the first month of breeding season at highland (23°C /21°C, 12h/12h L/D) and lowland (28.5°C /24°C, 12h/12h L/D) (supplementary figure XXX).

Long-term competition: four populations of *D. pandora* (lowland-biased species) monoculture, four populations of *D. palidifrons* (highland-biased species) monoculture and eight mixed-species populations were maintained in the simulated highland and lowland temperature for 13 weeks. Monoculture populations were started with 10 pairs of individuals. Mixed-species populations were started with 10 pairs of each species. 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 1 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.

1. Data analysis

All statistics were performed with R statistical software (version 3.6.0 ). All analysis code is available in ??GITHUB or SUPP??. *D. melanogaster* and *D. simulans* were not included in analyses involving field distribution, because their distribution patterns were unavailable.

*Distribution*. To calculate the abundance-weighted central elevation (hIndex) of distribution, the location of each sample was assigned values of 0, 0.5, and 1 if it was collected at low-, middle- and high-elevation sites, respectively. In addition, intra-specific abundance patterns were assessed by logistic regression of detection probability with elevation. For each species, pupal identity was labeled as 1 if the pupa was identified as the focal species and 0 if it was identified as any other species. Their identity was fitted against elevation as a fixed effect and the transect as a random effect in a generalized linear mix-effect model (varying intercept, varying slope) using the *lmer* package. These two ways of describing distribution patterns were compared using a Spearman’s rank test. For simplification, the hIndex was used to represent the distribution pattern in the following analysis.

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

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

Y = a \* T \* (T - RTmin) \* (RTmax - T)^(1/b) (if RTmin < T < RTmax),

Y = 0 (if T <= RTmin or R >= RTmax),

where T is the temperature, *RTmin* and *RTmax* are the minimum and maximum temperatures for the species to reproduce, *a* is a scaling factor and *b* is a shape factor of the curve. Across the nine species, each of *a*, *b*, *RTmin*, and *RTmax* were assumed to share a normal distribution. Square rooted daily fecundity was modeled using a normal distribution with temperature-dependent standard deviation. Assuming temperature dependency of standard deviation generated better fitting than assuming the same standard deviation across temperature treatments, as judged by leave-one-out cross-validation. This was because when the temperature was equal or close to *RTmin* and *RTmax*, the standard deviation should be zero or close to zero. A normal distribution is not ideal to model the transformed count data, which are all positive. However, modeling offspring counts with Poisson, zero-inflated Poisson, negative binomial, lognormal distribution did not produce model convergence results, potentially due to the piecewise nature of the thermal performance function. Diagnostics were performed and model performance was acceptable (supplementary figure 3). Priors of parameters were sampled from non-informative distribution. The values of *a* were bounded to be positive. The values of *b* were bounded to be larger than 0.8 to ensure that the thermal performance curve has a steeper slope on the right side. The values of *RTmin* were bounded to be lower than 17°C and the values of *RTmax* were bounded between 26°C - 35°C according to experience. Medians of the posterior distributions were used as the parameter values to construct the thermal performance curve.

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

*Reproduction-related traits*: The posterior distributions of *RTmin*, *RTmax*, and *RTopt* (6000 samples of each parameter of each species) were modeled by hIndex as the fixed effect and species identity as the random effect in the linear mix-effect model. Fecundity at 29°C and 17°C, recovered fecundity after 29°C and 14°C were used as direct measurements of their performance in the high and low temperatures. The offspring numbers were modeled by hIndex and experimental block as fix effects and species as a random effect in the generalized linear mix-effect model (family = “negative binomial”). Diagnostics of the models were examined. Data points with extreme leverage value were excluded and the model was fitted again to test if the statistical significance still holds.

*Knockdown tolerance*. The six measurements of physiological tolerance were modeled as a function of hIndex, block, and tube position as fixed effects, and species as a random effect in linear mix-effect models (family = “gaussian”).

*Short-term competition.* We used the Beverton-Holt model to describe the population growth of a single generation of flies on discrete and temporary resources:

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

*Long-term competition*. The population sizes were modelled by a three-way interaction of temperature treatment, species identity and the presence/absence of competitors, with the population identity as the random effect in the generalized linear mix-effect model (family = “zero-inflated negative binomial”) using the brms package. 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.

**Results**

Figure 1a shows the absolute numbers of identified samples found at low-, medium- or high-elevation sites for each of the nine major *Drosophila* species (accounting for 99% of total samples). Distribution quantified using regression of detection probability against elevation and by weighted central elevation (hIndex) showed consistent patterns (figure 1b) regarding the altitudinal biases of species. *Drosophila bipectinate* and *D. pandora* were categorized as lowland-biased species with high confidence. *D. pseudoananassae* was most 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 highland. *D. palidifrons* and *D. pseudotakahashii* were significantly more abundant at high elevations. The only six samples of *D. bunnanda* were all found at low elevation, which might explain the peculiar value of its estimated 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). No significant phylogenetic signal of distribution pattern was detected (XXXX = yyy).

Thermal performance curves of daily fecundity per female vary in the range, optimal temperature, peak fecundity, and shape factors among species (figure 2, see table 1 for the value of the curve parameters).The temperature for optimal reproductive performance (RTopt) did not correlate with their distribution patterns (coefficient = 0.068, 95% ci = -1.93 – 2.07, df = xxx). There was no general trade-off between performances in the cold versus warm environments that correspond to their distribution types (supplementary figure 6, p = yyy). For example, the lowland-biased species *D. bunnanda* have a higher minimum temperature, optimal temperature and maximum temperature than its upland-biased relative, *D. birchii*. In contrast, *D. sulfurigaster* always outperforms its upland-biased relative, *D. palidifrons.* The “jack-of-all-temperature, master of none” hypothesis is not supported.

Values of RTmin were not correlated with the species distribution patterns (figure 3. coefficient = 0.024, 95% ci = -2.47 – 2.52, df = xxx). Similarly, upland-biased species did not show higher fecundity at the low temperature, 17°C (figure 3. p = 0.788, df = xxx). When exposed to acute sublethal low temperature (5°C), except for *D. simulans* and *D. melanogaster*, the other *Drosophila* species show similarly weak resistance (figure 3. male: p = 0.18, df = xxx; female: p = 0.53, df = xxx). All species recovered their fecundity after eight-day exposure to 14°C. This recovered fecundity showed a minor but non-significant increase (figure 3. coefficient = 0.35, p = 0.105, df = xxx) among the upland-biased species. It took longer for upland-biased species to regain mobility after the chill coma (figure 3. male: p = 0.054, df = xxx; female: p = 0.029, df = xxx), presumably disadvantaged in the upland environment.

Regardless of the small variation of RTmax compared with RTmin, species whose distribution were biased towards lowland consistently had higher RTmax (figure 3. coefficient = -2.52, 95% ci = -3.68 - 1.36, p = 0.00125, df = xxx). Reproductive performance at 29°C also decrease with centred elevation (figure 3. coefficient = -5.09, p < 0.0001, df = xxx). After exposure to 29°C for eight days, neither of the two highland-biased species could reproduce when transferred back to mild temperature. Five out of the six non-biased and lowland-biased species resumed reproduction (figure 3). Heat tolerance, measured by knockdown time at high temperature, was lower among species whose distribution were biased towards high latitude (figure 3. male: coefficient = -9.1, p = 0.0013, df = xxx; female: coefficient = -5.4, p = 0.056, df = xxx).

In the simulated lowland environment, the reproductive success of lowland-biased species remained the highest, followed by the widespread species, *D. sulfurigaster*. The upland-biased species could barely reproduce regardless of the presence of competitors (Figure 4a). In the simulated upland environment, all species can reproduce and sustain their populations. Lowland species were strongly affected by the density of *D. palidifrons*, an upland species. While upland species were significantly less affected by upland species, shown by lower interspecific competition coefficients (figure 4a, table 2). Competition under upland conditions was predicted to drive lowland species to exclusion at equilibrium (Table 2, SI 3abf). For long-term monoculture populations and mixed-species communities, high temperature drove *D. palidifrons* to extinction regardless of the starting species composition. In contrast, the monoculture of *D. pandora* remained high in size in both temperatures when they were raised alone. But their populations were significantly reduced at low temperature only when in the presence of interspecific competitors (Figure 5).

**Discussion**

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

*1.Low variation of upper thermal limit*

Upper thermal limits show low levels of variation among species (Hoffmann 2010). As the exact values of laboratory measurements depend on the precise experimental conditions, e.g. the rising speed of temperature for CTmax (Terblanche et al. 2007), it is difficult to relate a critical temperature or the fecundity at a constant temperature to performance in the field (Sinclair et al. 2016). Consistent with others (Goulet, Thompson, and Chapple 2017; Hangartner and Hoffmann 2016), our study showed that heat tolerance is a systematic trait manifested in the critical temperature, the fecundity at a sub-sterile temperature, the recovered fecundity and the locomotive response. Therefore, a modest difference in critical temperature may represent a distinct difference in overall performance when organisms are operating at real, variable temperature regimes.

Small thermal safety margins suggest a severe threat of biotic attrition in tropical lowlands (Colwell et al. 2008; Deutsch et al. 2008; Duarte et al. 2012). Discussion about biotic attrition faces great uncertainty. The thermal niches of tropical lowland species estimated by their extant distribution may be truncated because hotter areas are unavailable for species to occupy (Feeley and Silman 2010). Laboratory-measured critical temperatures are sensitive to experimental conditions, making it difficult to relate these temperatures to climatological means or maxima, and thus the threat of rising temperatures (Sinclair et al. 2016). This study benefits from a comparative approach with detailed quantification of species with different thermal traits and distribution. We first showed that upland species is already constrained by lowland temperature, and also showed the marginal difference in upper thermal limits between lowland species and upland species. Given the low evolutionary potential of the heat tolerance (Hoffmann, Chown, and Clusella-Trullas 2013), the lowland species are likely vulnerable to even 1 degree 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 at lowland and threatening endemic upland species (Freeman et al. 2018).

*2.Controversy over the contribution of abiotic and biotic factor in deciding distribution*

The idea of comparing the contribution of abiotic factor versus biotic factor in warm versus cold boundaries has a deep root since Charles Darwin but remain 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 correlation: (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)). The discrepancy could arise from at least three reasons: geographic bias of research effort, the distinctive nature of mechanisms governing latitudinal versus altitudinal distribution and methodological difference 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 preprint). Limited and mixed results from tropical systems cast great uncertainty on the importance of biotic and abiotic contribution in tropics (Cahill et al. 2014). Second, mechanisms governing latitudinal or altitudinal distribution patterns could be very different, indicated by that their recorded responses to past warming are very different (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 sensitivity of demographic traits to temperature are indistinguishable between widespread and tropical species and are thus a poor predictor of distribution. While in this study, we showed that the interspecific difference in the demographic response to heat correlates well with the altitudinal distribution types. Our main differences in measuring demographic traits are that 1) we used eggs produced in the tested temperature to measure their egg-adult survival, 2) adults are virgins before testing fecundity, 3) we combined two time periods after exposing to the tested temperature. In our experiment, individuals laid eggs at high temperatures while those eggs did not eventually hatch, presumably due to the sterilization of sperm at high temperature (Parratt et al. 2021). We also observed different speeds of senescence after exposure to different temperatures (supplementary figure xxx), which the three-day period of fecundity measurement in Overgaard’s study would not capture. Nevertheless, both studies 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 increase with temperature (in low elevation). My result expect birchii to be already constrained by heat at lowland.

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

Daily high temperature rather than mean contributes more to structuring distribution (Lynch et al. 2014). The mean temperature during the survey season at lowland is around 26°C, of which all the species are around their peak reproductive performance. In addition to the mean, highland 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, e.g. 28°C (supplementary figure xxx). A preliminary experiment raising long-term populations in constant 20°C and 26°C showed that *D. palidifrons* always out-numbered *D. pandora* in mixed-species culture, contrasting the poor performance of *D. palidifrons* when raised in 24°C /28.5°C scenario. Brief exposure to stressful thermal environments has 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 both population size and climate is especially valuable to understand abiotic limits for field populations (Corlett 2011; Gade et al. 2020).

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

*5.Conclusions*

This study contributes to the growing literature that shows the warmer margins of distribution are also sensitive to warming (Wilson et al. 2005). Benefiting from the Drosophila system, we could examine the roles of both abiotic (temperature) and biotic factors as direct determinants of species abundance along environmental gradients. The generality of their 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 to increasing temperature 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 need to update 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, which requires identification of proximate causes of current distribution and understanding about the interactions between thermal tolerance and other environmental (abiotic and biotic) factors.

**References:**

Amundrud, Sarah L. and Diane S. Srivastava. 2020. “Thermal Tolerances and Species Interactions Determine the Elevational Distributions of Insects.” *Global Ecology and Biogeography* 29(8):1315–27.

Ayala, Francisco J., Michael E. Gilpin, and Joan G. Ehrenfeld. 1973. “Competition between Species: Theoretical Models and Experimental Tests.” *Theoretical Population Biology* 4(3):331–56.

Batista, Marcos Roberto Dias, Felipe Bastos Rocha, and Louis Bernard Klaczko. 2018. “Altitudinal Distribution of Two Sibling Species of the Drosophila Tripunctata Group in a Preserved Tropical Forest and Their Male Sterility Thermal Thresholds.” *Journal of Thermal Biology* 71:69–73.

Beauchesne, David, Kevin Cazelles, Philippe Archambault, Laura E. Dee, and Dominique Gravel. 2021. “On the Sensitivity of Food Webs to Multiple Stressors” edited by T. Wootton. *Ecology Letters* ele.13841.

Bonebrake, Timothy C. and Curtis A. Deutsch. 2012. “Climate Heterogeneity Modulates Impact of Warming on Tropical Insects.” *Ecology* 93(3):449–55.

Bozinovic, Francisco and Hans Otto Pörtner. 2015. “Physiological Ecology Meets Climate Change.” *Ecology and Evolution* 5(5):1025–30.

Briere, Jean Francois, Pascale Pracros, Alain Yves Le Roux, and Jean Sebastien Pierre. 1999. “A Novel Rate Model of Temperature-Dependent Development for Arthropods.” *Environmental Entomology* 28(1):22–29.

Bubli, Oleg A., Alexandra G. Imasheva, and Volker Loeschcke. 1998. “Selection for Knockdown Resistance to Heat in Drosophila Melanogaster at High and Low Larval Densities.” *Evolution* 52(2):619–25.

Burnside, William R., Erik B. Erhardt, Sean T. Hammond, and James H. Brown. 2014. “Rates of Biotic Interactions Scale Predictably with Temperature despite Variation.” *Oikos* 123(12):1449–56.

Cahill, Abigail E., Matthew E. Aiello-Lammens, M. Caitlin Fisher-Reid, Xia Hua, Caitlin J. Karanewsky, Hae Yeong Ryu, Gena C. Sbeglia, Fabrizio Spagnolo, John B. Waldron, and John J. Wiens. 2014. “Causes of Warm-Edge Range Limits: Systematic Review, Proximate Factors and Implications for Climate Change” edited by W. Daniel Kissling. *Journal of Biogeography* 41(3):429–42.

Chen, I. Ching, Jane K. Hill, Ralf Ohlemüller, David B. Roy, and Chris D. Thomas. 2011. “Rapid Range Shifts of Species Associated with High Levels of Climate Warming.” *Science* 333(6045):1024–26.

Chen, I. Ching, Jane K. Hill, Hau Jie Shiu, Jeremy D. Holloway, Suzan Benedick, Vun Khen Chey, Henry S. Barlow, and Chris D. Thomas. 2011. “Asymmetric Boundary Shifts of Tropical Montane Lepidoptera over Four Decades of Climate Warming.” *Global Ecology and Biogeography* 20(1):34–45.

Colwell, Robert K., Gunnar Brehm, Catherine L. Cardelús, Alex C. Gilman, and John T. Longino. 2008. “Global Warming, Elevational Range Shifts, and Lowland Biotic Attrition in the Wet Tropics.” *Science* 322(5899):258–61.

Corlett, Richard T. 2011. “Impacts of Warming on Tropical Lowland Rainforests.” *Trends in Ecology and Evolution* 26(11):606–13.

Corlett, Richard T. 2012. “Climate Change in the Tropics: The End of the World as We Know It?” *Biological Conservation* 151(1):22–25.

Crespo-Pérez, Verónica, Elena Kazakou, David W. Roubik, and Rafael E. Cárdenas. 2020. “The Importance of Insects on Land and in Water: A Tropical View.” *Current Opinion in Insect Science* 40:31–38.

Davis, A. J., L. S. Jenkinson, J. H. Lawton, B. Shorrocks, and S. Wood. 1998. “Making Mistakes When Predicting Shifts in Species Range in Response to Global Warming.” *Nature* 391(6669):783–86.

Deutsch, Curtis A., Joshua J. Tewksbury, Raymond B. Huey, Kimberly S. Sheldon, Cameron K. Ghalambor, David C. Haak, and Paul R. Martin. 2008. “Impacts of Climate Warming on Terrestrial Ectotherms across Latitude.” *Proceedings of the National Academy of Sciences of the United States of America* 105(18):6668–72.

Deutsch, Curtis A., Joshua J. Tewksbury, Michelle Tigchelaar, David S. Battisti, Scott C. Merrill, Raymond B. Huey, and Rosamond L. Naylor. 2018. “Increase in Crop Losses to Insect Pests in a Warming Climate.” *Science* 361(6405):916–19.

Diamond, Sarah E., D. Magdalena Sorger, Jiri Hulcr, Shannon L. Pelini, Israel Del Toro, Christopher Hirsch, Erik Oberg, and Robert R. Dunn. 2012. “Who Likes It Hot? A Global Analysis of the Climatic, Ecological, and Evolutionary Determinants of Warming Tolerance in Ants.” *Global Change Biology* 18(2):448–56.

Duarte, Helder, Miguel Tejedo, Marco Katzenberger, Federico Marangoni, Diego Baldo, Juan Francisco Beltrán, Dardo Andrea Martí, Alex Richter-Boix, and Alejandro Gonzalez-Voyer. 2012. “Can Amphibians Take the Heat? Vulnerability to Climate Warming in Subtropical and Temperate Larval Amphibian Communities.” *Global Change Biology* 18(2):412–21.

Engelbrecht, Bettina M. J., Liza S. Comita, Richard Condit, Thomas A. Kursar, Melvin T. Tyree, Benjamin L. Turner, and Stephen P. Hubbell. 2007. “Drought Sensitivity Shapes Species Distribution Patterns in Tropical Forests.” *Nature* 447(7140):80–82.

Feeley, Kenneth J. and Miles R. Silman. 2010. “Biotic Attrition from Tropical Forests Correcting for Truncated Temperature Niches.” *Global Change Biology* 16(6):1830–36.

Feeley, Kenneth J., James T. Stroud, and Timothy M. Perez. 2017. “Most ‘Global’ Reviews of Species’ Responses to Climate Change Are Not Truly Global” edited by I. Kühn. *Diversity and Distributions* 23(3):231–34.

Fletcher, B. S. 1973. “The Ecology of a Natural Population of the Queensland.” *Australian Journal of Zoology* 21(4):437–75.

Freeman, Benjamin G., Micah N. Scholer, Viviana Ruiz-Gutierrez, and John W. Fitzpatrick. 2018. “Climate Change Causes Upslope Shifts and Mountaintop Extirpations in a Tropical Bird Community.” *Proceedings of the National Academy of Sciences of the United States of America* 115(47):11982–87.

Freeman, Benjamin G., Yiluan Song, Kenneth J. Feeley, and Kai Zhu. 2021. “Montane Species Track Rising Temperatures Better in the Tropics than in the Temperate Zone.” *Ecology Letters* ele.13762.

Gade, Meaghan R., Grant M. Connette, John A. Crawford, Daniel J. Hocking, John C. Maerz, Joseph R. Milanovich, and William E. Peterman. 2020. “Predicted Alteration of Surface Activity as a Consequence of Climate Change.” *Ecology*.

García-Robledo, Carlos, Erin K. Kuprewicz, Charles L. Staines, Terry L. Erwin, and W. John Kress. 2016. “Limited Tolerance by Insects to High Temperatures across Tropical Elevational Gradients and the Implications of Global Warming for Extinction.” *Proceedings of the National Academy of Sciences of the United States of America* 113(3):680–85.

Gaston, Kevin J. and Steven L. Chown. 1999. “Elevation and Climatic Tolerance: A Test Using Dung Beetles.” *Oikos* 86(3):584.

Gibert, Patricia, Brigitte Moreteau, Georges Pétavy, Dev Karan, and Jean R. David. 2001. “Chill-Coma Tolerance, a Major Climatic Adaptation among Drosophila Species.” *Evolution* 55(5):1063–68.

Gilman, Sarah E., Mark C. Urban, Joshua Tewksbury, George W. Gilchrist, and Robert D. Holt. 2010. “A Framework for Community Interactions under Climate Change.” *Trends in Ecology and Evolution* 25(6):325–31.

Goulet, Celine T., Michael B. Thompson, and David G. Chapple. 2017. “Repeatability and Correlation of Physiological Traits: Do Ectotherms Have a ‘Thermal Type’?” *Ecology and Evolution* 7(2):710–19.

Hangartner, Sandra and Ary A. Hoffmann. 2016. “Evolutionary Potential of Multiple Measures of Upper Thermal Tolerance in Drosophila Melanogaster.” *Functional Ecology* 30(3):442–52.

Hargreaves, Anna L., Karen E. Samis, and Christopher G. Eckert. 2014. “Are Species’ Range Limits Simply Niche Limits Writ Large? A Review of Transplant Experiments beyond the Range.” *American Naturalist* 183(2):157–73.

Hassell, M. P. and H. N. Comins. 1976. “Discrete Time Models for Two-Species Competition.” *Theoretical Population Biology* 9(2):202–21.

HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. “Rethinking Community Assembly through the Lens of Coexistence Theory.” *Annual Review of Ecology, Evolution, and Systematics* 43(1):227–48.

Hoffmann, A. A. 2010. “Physiological Climatic Limits in Drosophila: Patterns and Implications.” *Journal of Experimental Biology* 213(6):870–80.

Hoffmann, Ary A., Alisha Anderson, and Rebecca Hallas. 2002. “Opposing Clines for High and Low Temperature Resistance in Drosophila Melanogaster.” *Ecology Letters* 5(5):614–18.

Hoffmann, Ary A. and Mark W. Blows. 1994. “Species Borders: Ecological and Evolutionary Perspectives.” *Trends in Ecology and Evolution* 9(6):223–27.

Hoffmann, Ary A., Steven L. Chown, and Susana Clusella-Trullas. 2013. “Upper Thermal Limits in Terrestrial Ectotherms: How Constrained Are They?” edited by C. Fox. *Functional Ecology* 27(4):934–49.

Hoffmann, Ary A., Jesper G. Sørensen, and Volker Loeschcke. 2003. “Adaptation of Drosophila to Temperature Extremes: Bringing Together Quantitative and Molecular Approaches.” *Journal of Thermal Biology* 28(3):175–216.

Huang, Shu Ping and Ming Chung Tu. 2008. “Heat Tolerance and Altitudinal Distribution of a Mountainous Lizard, Takydromus Hsuehshanensis, in Taiwan.” *Journal of Thermal Biology* 33(1):48–56.

Huey, Raymond B., Curtis A. Deutsch, Joshua J. Tewksbury, Laurie J. Vitt, Paul E. Hertz, Héctor J. Álvare. Pérez, and Theodore Garland. 2009. “Why Tropical Forest Lizards Are Vulnerable to Climate Warming.” *Proceedings of the Royal Society B: Biological Sciences* 276(1664):1939–48.

Huey, Raymond B. and Joel G. Kingsolver. 1989. “Evolution of Thermal Sensitivity of Ectotherm Performance.” *Trends in Ecology and Evolution* 4(5):131–35.

Huey, Raymond B. and R. D. Stevenson. 1979. “Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches.” *Integrative and Comparative Biology* 19(1):357–66.

Jankowski, Jill E., Gustavo A. Londoño, Scott K. Robinson, and Mark A. Chappell. 2013a. “Exploring the Role of Physiology and Biotic Interactions in Determining Elevational Ranges of Tropical Animals.” *Ecography* 36(1):1–12.

Jankowski, Jill E., Gustavo A. Londoño, Scott K. Robinson, and Mark A. Chappell. 2013b. “Exploring the Role of Physiology and Biotic Interactions in Determining Elevational Ranges of Tropical Animals.” *Ecography* 36(1):1–12.

Jeffs, Christopher T., J. Christopher D. Terry, Megan Higgie, Anna Jandová, Hana Konvičková, Joel J. Brown, Chia-Hua Lue, Michele Schiffer, Eleanor K. O’Brien, Jon Bridle, Jan Hrček, and Owen T. Lewis. 2020. “Molecular Analyses Reveal Consistent Food Web Structure with Elevation in Rainforest Drosophila - Parasitoid Communities.” *BioRxiv* 1-16 https://doi.org/10.1101/2020.07.21.213678.

Jørgensen, Lisa Bjerregaard, Hans Malte, and Johannes Overgaard. 2019. “How to Assess Drosophila Heat Tolerance: Unifying Static and Dynamic Tolerance Assays to Predict Heat Distribution Limits” edited by C. White. *Functional Ecology* 33(4):629–42.

Kearney, Michael and Warren Porter. 2009. “Mechanistic Niche Modelling: Combining Physiological and Spatial Data to Predict Species’ Ranges.” *Ecology Letters* 12(4):334–50.

Kellermann, Vanessa, Johannes Overgaard, Ary A. Hoffmann, Camilla Fljøgaard, Jens Christian Svenning, and Volker Loeschcke. 2012. “Upper Thermal Limits of Drosophila Are Linked to Species Distributions and Strongly Constrained Phylogenetically.” *Proceedings of the National Academy of Sciences of the United States of America* 109(40):16228–33.

Khaliq, Imran, Christian Hof, Roland Prinzinger, Katrin Böhning-Gaese, and Markus Pfenninger. 2014. “Global Variation in Thermal Tolerances and Vulnerability of Endotherms to Climate Change.” *Proceedings of the Royal Society B: Biological Sciences* 281(1789).

Kimura, Masahito T. 2004. “Cold and Heat Tolerance of Drosophilid Flies with Reference to Their Latitudinal Distributions.” *Oecologia* 140(3):442–49.

Kingsolver, Joel G., Sarah E. Diamond, and Lauren B. Buckley. 2013. “Heat Stress and the Fitness Consequences of Climate Change for Terrestrial Ectotherms” edited by J. Grindstaff. *Functional Ecology* 27(6):1415–23.

Krebs, Robert A. and J. S. F. Barker. 1991. “Coexistence of Ecologically Similar Colonising Species: Intra- and Interspecific Competition in Drosophila Aldrichi and D. Buzzatii.” *Australian Journal of Zoology* 39(5):499–508.

Laurance, William F., D. Carolina Useche, Luke P. Shoo, Sebastian K. Herzog, Michael Kessler, Federico Escobar, Gunnar Brehm, Jan C. Axmacher, I. Ching Chen, Lucrecia Arellano Gámez, Peter Hietz, Konrad Fiedler, Tomasz Pyrcz, Jan Wolf, Christopher L. Merkord, Catherine Cardelus, Andrew R. Marshall, Claudine Ah-Peng, Gregory H. Aplet, M. del Coro Arizmendi, William J. Baker, John Barone, Carsten A. Brühl, Rainer W. Bussmann, Daniele Cicuzza, Gerald Eilu, Mario E. Favila, Andreas Hemp, Claudia Hemp, Jürgen Homeier, Johanna Hurtado, Jill Jankowski, Gustavo Kattán, Jürgen Kluge, Thorsten Krömer, David C. Lees, Marcus Lehnert, John T. Longino, Jon Lovett, Patrick H. Martin, Bruce D. Patterson, Richard G. Pearson, Kelvin S. H. Peh, Barbara Richardson, Michael Richardson, Michael J. Samways, Feyera Senbeta, Thomas B. Smith, Timothy M. A. Utteridge, James E. Watkins, Rohan Wilson, Stephen E. Williams, and Chris D. Thomas. 2011. “Global Warming, Elevational Ranges and the Vulnerability of Tropical Biota.” *Biological Conservation* 144(1):548–57.

Logan, Jesse A., Jacques Régnière, and James A. Powell. 2003. “Assessing the Impacts of Global Warming on Forest Pest Dynamics.” Pp. 130–37 in *Frontiers in Ecology and the Environment*. Vol. 1. Ecological Society of America.

Louthan, Allison M., Daniel F. Doak, and Amy L. Angert. 2015. “Where and When Do Species Interactions Set Range Limits?” *Trends in Ecology and Evolution* 30(12):780–92.

Lynch, Heather J., Marc Rhainds, Justin M. Calabrese, Stephen Cantrell, Chris Cosner, and William F. Fagan. 2014. “How Climate Extremes—Not Means—Define a Species’ Geographic Range Boundary via a Demographic Tipping Point.” *Ecological Monographs* 84(1):131–49.

Ma, Gang, Ary A. Hoffmann, and Chun Sen Ma. 2015. “Daily Temperature Extremes Play an Important Role in Predicting Thermal Effects.” *Journal of Experimental Biology* 218(14):2289–96.

MacLean, Heidi J., Jesper G. Sørensen, Torsten N. Kristensen, Volker Loeschcke, Kristian Beedholm, Vanessa Kellermann, and Johannes Overgaard. 2019. “Evolution and Plasticity of Thermal Performance: An Analysis of Variation in Thermal Tolerance and Fitness in 22 *Drosophila* Species.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 374(1778):20180548.

Merrill, Richard M., David Gutiérrez, Owen T. Lewis, Javier Gutiérrez, Sonia B. Díez, and Robert J. Wilson. 2008. “Combined Effects of Climate and Biotic Interactions on the Elevational Range of a Phytophagous Insect.” *Journal of Animal Ecology* 77(1):145–55.

Mitsui, Hideyuki, Kees Van Achterberg, Göran Nordlander, and Masahito T. Kimura. 2007. “Geographical Distributions and Host Associations of Larval Parasitoids of Frugivorous Drosophilidae in Japan.” *Journal of Natural History* 41(25–28):1731–38.

Nowrouzi, Somayeh, Alan N. Andersen, Tom R. Bishop, and Simon K. A. Robson. 2018. “Is Thermal Limitation the Primary Driver of Elevational Distributions? Not for Montane Rainforest Ants in the Australian Wet Tropics.” *Oecologia* 188(2):333–42.

O’Brien, Eleanor K., Megan Higgie, Alan Reynolds, Ary A. Hoffmann, and Jon R. Bridle. 2017. “Testing for Local Adaptation and Evolutionary Potential along Altitudinal Gradients in Rainforest Drosophila: Beyond Laboratory Estimates.” *Global Change Biology* 23(5):1847–60.

Overgaard, Johannes, Michael R. Kearney, and Ary A. Hoffmann. 2014. “Sensitivity to Thermal Extremes in Australian Drosophila Implies Similar Impacts of Climate Change on the Distribution of Widespread and Tropical Species.” *Global Change Biology* 20(6):1738–50.

Parratt, Steven R., Benjamin S. Walsh, Soeren Metelmann, Nicola White, Andri Manser, Amanda J. Bretman, Ary A. Hoffmann, Rhonda R. Snook, and Tom A. R. Price. 2021. “Temperatures That Sterilize Males Better Match Global Species Distributions than Lethal Temperatures.” *Nature Climate Change* 11(6):481–84.

Pecl, Gretta T., Miguel B. Araújo, Johann D. Bell, Julia Blanchard, Timothy C. Bonebrake, I. Ching Chen, Timothy D. Clark, Robert K. Colwell, Finn Danielsen, Birgitta Evengård, Lorena Falconi, Simon Ferrier, Stewart Frusher, Raquel A. Garcia, Roger B. Griffis, Alistair J. Hobday, Charlene Janion-Scheepers, Marta A. Jarzyna, Sarah Jennings, Jonathan Lenoir, Hlif I. Linnetved, Victoria Y. Martin, Phillipa C. McCormack, Jan McDonald, Nicola J. Mitchell, Tero Mustonen, John M. Pandolfi, Nathalie Pettorelli, Ekaterina Popova, Sharon A. Robinson, Brett R. Scheffers, Justine D. Shaw, Cascade J. B. Sorte, Jan M. Strugnell, Jennifer M. Sunday, Mao Ning Tuanmu, Adriana Vergés, Cecilia Villanueva, Thomas Wernberg, Erik Wapstra, and Stephen E. Williams. 2017. “Biodiversity Redistribution under Climate Change: Impacts on Ecosystems and Human Well-Being.” *Science* 355(6332):1–9.

Peterson, Megan L., Daniel F. Doak, and William F. Morris. 2019. “Incorporating Local Adaptation into Forecasts of Species’ Distribution and Abundance under Climate Change.” *Global Change Biology* 25(3):775–93.

Saxon, A. D., E. K. O’Brien, and J. R. Bridle. 2018. “Temperature Fluctuations during Development Reduce Male Fitness and May Limit Adaptive Potential in Tropical Rainforest Drosophila.” *Journal of Evolutionary Biology* 31(3):405–15.

Schemske, Douglas W., Gary G. Mittelbach, Howard V. Cornell, James M. Sobel, and Kaustuv Roy. 2009. “Is There a Latitudinal Gradient in the Importance of Biotic Interactions?” *Annual Review of Ecology, Evolution, and Systematics* 40(1):245–69.

Schiffer, Michele and Shane F. McEvey. 2006. “Drosophila Bunnanda - A New Species from Northern Australia with Notes on Other Australian Members of the Montium Subgroup (Diptera: Drosophilidae).” *Zootaxa* (1333):1–23.

Sheldon, Kimberly S., Sylvia Yang, and Joshua J. Tewksbury. 2011. “Climate Change and Community Disassembly: Impacts of Warming on Tropical and Temperate Montane Community Structure.” *Ecology Letters* 14(12):1191–1200.

Sinclair, Brent J., Katie E. Marshall, Mary A. Sewell, Danielle L. Levesque, Christopher S. Willett, Stine Slotsbo, Yunwei Dong, Christopher D. G. Harley, David J. Marshall, Brian S. Helmuth, and Raymond B. Huey. 2016. “Can We Predict Ectotherm Responses to Climate Change Using Thermal Performance Curves and Body Temperatures?” edited by D. Vasseur. *Ecology Letters* 19(11):1372–85.

Srinivasan, Umesh, Paul R. Elsen, Morgan W. Tingley, and David S. Wilcove. 2018. “Temperature and Competition Interact to Structure Himalayan Bird Communities.” *Proceedings of the Royal Society B: Biological Sciences* 285(1874):20172593.

Sunday, Jennifer M., Amanda E. Bates, and Nicholas K. Dulvy. 2012. “Thermal Tolerance and the Global Redistribution of Animals.” *Nature Climate Change* 2(9):686–90.

Terblanche, John S., Jacques A. Deere, Susana Clusella-Trullas, Charlene Janion, and Steven L. Chown. 2007. “Critical Thermal Limits Depend on Methodological Context.” *Proceedings of the Royal Society B: Biological Sciences* 274(1628):2935–42.

Terry, J. Christopher D., Jinlin Chen, and Owen T. Lewis. 2021. “Natural Enemies Have Inconsistent Impacts on the Coexistence of Competing Species.” *Journal of Animal Ecology* 1365-2656.13534.

Williams, Stephen E., Elizabeth E. Bolitho, and Samantha Fox. 2003. “Climate Change in Australian Tropical Rainforests: An Impending Environmental Catastrophe.” *Proceedings of the Royal Society B: Biological Sciences* 270(1527):1887–92.

Wilson, Robert J., David Gutiérrez, Javier Gutiérrez, David Martínez, Rosa Agudo, and Victor J. Monserrat. 2005. “Changes to the Elevational Limits and Extent of Species Ranges Associated with Climate Change.” *Ecology Letters* 8(11):1138–46.

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

A close up of a logo

Description automatically generated

A screenshot of a cell phone

Description automatically generated

Figure 2. The thermal performance curve of reproduction. Color is ordered by their distribution pattern, with highland-biased species labeled by cold color and lowland-biased species labeled by warm color.

A close up of a device

Description automatically generated

Figure 3. Reproductive and physiological thermal tolerance of species with different altitudinal distribution patterns. Cold tolerance is represented by RTmin (a), fecundity at 17C (b), recovered fecundity after 14C (c) and recovery time after chill coma (d). Hot tolerance is represented by RTmax (e), fecundity at 29C (f), recovered fecundity after 29C (g) and knockdown time by high temperature (h).

Diagram, engineering drawing

Description automatically generated

Figure 4. The inter-specific competitive effect of the competing species on the focal species in upland and lowland temperature regimes. Each Solid line shows the fecundity of the focal species when its founder number is kept at 4 pairs, while changing the number of competing species. Colors 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 in behind. Shaded area indicates the 90% credible interval of the predicted fecundity by Beverton-Holt model.

Chart

Description automatically generated

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

Diagram

Description automatically generated with medium confidence

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

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **species** | **a** | **CI\_a** | **b** | **CI\_b** | **RTmin** | **CI\_RTmin** | **RTmax** | **CI\_RTmax** |
| *D. bipectinata* | 0.0046 | 0.0030 - 0.0059 | 1.26 | 1.01 - 1.55 | 15.28 | 14.56 - 15.88 | 30.45 | 30.08 - 31.05 |
| *D. birchii* | 0.0034 | 0.0022 - 0.0056 | 1.17 | 0.95 - 1.57 | 13.45 | 13.08 - 13.79 | 29.25 | 28.11 - 29.80 |
| *D. bunnanda* | 0.0017 | 0.0012 - 0.0026 | 0.88 | 0.81 - 1.07 | 14.58 | 14.09 - 15.20 | 31.19 | 30.61 - 31.77 |
| *D. melanogaster* | 0.0037 | 0.0032 - 0.0042 | 1.72 | 1.48 - 2.02 | 8.32 | 6.93 - 9.38 | 32.13 | 32.03 - 32.28 |
| *D. palidifrons* | 0.0073 | 0.0055 - 0.0099 | 1.74 | 1.36 - 2.39 | 16.23 | 15.51 - 16.77 | 29.07 | 28.14 - 29.39 |
| *D. pandora* | 0.0052 | 0.0037 - 0.0065 | 1.25 | 1.03 - 1.51 | 15.26 | 14.56 - 15.79 | 30.13 | 29.88 - 30.57 |
| *D. pseudoananassae* | 0.0053 | 0.0035 - 0.0071 | 1.67 | 1.22 - 2.33 | 15.07 | 14.15 - 15.91 | 29.22 | 28.42 - 29.80 |
| *D. simulans* | 0.0035 | 0.0027 - 0.0047 | 1.68 | 1.36 - 2.22 | 8.51 | 6.94 - 9.66 | 31.08 | 30.38 - 31.78 |
| *D. sulfurigaster* | 0.0040 | 0.0027 - 0.0051 | 1.26 | 1.03 - 1.53 | 14.37 | 13.92 - 14.94 | 30.12 | 29.84 - 30.63 |

**Table 2. Fitted values of the parameters of the competition and their predicted equilibrium states.** R0 is the reproductive rate. is intra-specific competition coefficient. is the inter-specific competition coefficients. ci90 represents the 90% credible intervals of each parameter. The equilibrium states of the focal species are inferred from Hassell and Comins 1974.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Temperature** | **Focal species** | **R0** | **R0.ci90** |  | **.ci90** | **Competitor** |  | **.ci90** | **Equilibrium state of the focal species** |
| cold | 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 |
| hot | BIP | 15.35 | 12.51-19.05 | 0.07 | 0.05-0.11 | PAL | 0.29 | 0.07-0.63 | dominant |
| PAN | 0.87 | 0.54-1.35 | stable coexistence |
| PST | 0.31 | 0.07-0.63 | dominant |
| SUL | 1.35 | 0.93-2 | excluded |
| PAL | 0.99 | 0.19-2.37 | 0.12 | 0.02-0.46 | BIP | 6.81 | 2.98-22.27 | die out |
| PAN | 2.99 | 1.52-8.93 | die out |
| SUL | 3.98 | 1.77-12.83 | die out |
| PAN | 17.18 | 14.2-21.24 | 0.09 | 0.06-0.14 | BIP | 0.27 | 0.08-0.51 | stable coexistence |
| PAL | 0.11 | 0.01-0.29 | dominant |
| PST | 0 | \ | \ | \ | BIP | \ | \ | die out |
| SUL | 13.65 | 10.26-19.03 | 0.15 | 0.09-0.24 | BIP | 0.37 | 0.18-0.62 | dominant |
| PAL | 0.04 | 0-0.14 | dominant |

Supplementary figure 1. The change of fecundity during 1st – 2nd day to 7th – 8th day in different temperature.

A close up of a whiteboard

Description automatically generated

Supplementary figure 2. Time table of fecundity measurement.

A screenshot of a cell phone

Description automatically generated

Supplementary figures 3. Diagnostics of model fitting of thermal performance curve.

A screenshot of a cell phone

Description automatically generatedA close up of a white wall

Description automatically generatedA close up of a map

Description automatically generated

Supplementary figure 4. Examples of logistic regression on occurrence data. *D. bipectinata* is lowland-biased species. *D. rubida* shows no bias. *D. palidifrons* is highland-biased species.

A screenshot of a video game

Description automatically generated

Supplementary figure 5. Daily fecundity and fitted thermal performance curve of each of the nine species.

A bunch of different colors

Description automatically generated

Supplementary figure 6. Scatter plot of posterior samples of RTmin and RTmax parameters.

A screenshot of a cell phone

Description automatically generated

Supplementary figure 6. Daily temperature of Feb. against the lowest CTmax and highest CTmin.

Chart, application

Description automatically generated

**Supplementary table 1. Isofemale line used to construct MBLs.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | Origin of cultured lines (yes/no) | | | | Lines for MBLs |
| Kirrama low1 | Kirrama high1 | Paluma low1 | Paluma high1 |
| *D. bunnanda* | yes | no | yes | no | KL87, KL134, KL127, PL114 |
| *D. pandora* | no | no | yes | no | PL17, PL21, PL012 |
| *D. bipectinata* | yes | no | yes | no | KL84, KL43, PL85, PL20 |
| *D. pseudoananassae* | yes | yes | yes | no | KL19, KH25, PL30, KH42 |
| *D. sulfurigaster* | yes | yes | yes | yes | KL08, KH10, PL51, PH18 |
| *D. rubida* | yes | yes | yes | yes | Construction unfinished3. |
| *D. birchii* | yes | yes | yes | yes | KL22, KH26, PL122, PH169 |
| *D. palidifrons* | no | yes | no | yes | KH20, KH69, PH183, PH184 |
| *D. simulans* | no | no | yes | no | PL45, PL34, PL42, PL43 |
| *D. pseudotakahashii* | no | yes | no | yes | Did not construct MBLs4 |

Note:

1. “Low” means sites from low elevation. “High” means sites from high elevation.
2. The three isofemale lines were the only lines cultured at the lab.
3. Construction is not finished by the start of the experiment in May. *D. rubida* grew poorly on the purchased fly medium before changing food recipe and made at the lab. Therefore, its crossing starts later than other species (April 5th). Additionally, *D. rubida* has significantly longer generation time than other species.
4. Only two isofemale lines were cultured at the lab.

**SI Table 2. Correlation matrix among thermal traits (RTmin, RTmax, RTopt, female knockdown time to heat, male knockdown time to heat, female knockdown time to cold, male knockdown time to cold, female recovery time from cold, male recovery time from cold). Spearman’s rank correlation rho is shown in the table. Significant correlation (p < 0.05) is labeled as bold.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | RTmin | RTmax | RTopt | FKDHEAT | MKDHEAT | FKDCOLD | MKDCOLD | FRCCOLD | MRCCOLD |
| RTmin | 1.00 | -0.55 | 0.02 | **-0.89** | -0.53 | -0.31 | -0.56 | 0.53 | 0.65 |
| RTmax |  | 1.00 | 0.17 | **0.71** | **0.93** | 0.64 | 0.61 | **-0.88** | **-0.85** |
| RTopt |  |  | 1.00 | -0.01 | 0.33 | 0.63 | 0.54 | -0.41 | -0.30 |
| FKDHEAT |  |  |  | 1.00 | **0.72** | 0.53 | **0.71** | -0.57 | **-0.68** |
| MKDHEAT |  |  |  |  | 1.00 | **0.79** | **0.71** | **-0.89** | **-0.83** |
| FKDCOLD |  |  |  |  |  | 1.00 | **0.85** | **-0.74** | **-0.75** |
| MKDCOLD |  |  |  |  |  |  | 1.00 | **-0.72** | **-0.70** |
| FRCCOLD |  |  |  |  |  |  |  | 1.00 | **0.93** |
| MRCCOLD |  |  |  |  |  |  |  |  | 1.00 |