**Small difference in upper thermal limits and competition structures *Drosophila* distribution along a tropical altitude gradient**

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

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**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.

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**Abstract:**

Tropical insects are challenged by global warming. The role of high temperature in influencing species’ distribution at the warmer boundaries is still controversial. To precisely estimate the real magnitude of the impact of warming on tropical species, the mechanisms governing species distribution needs to be examined in the first place. Drosophila species in the Australian rainforest mountains were shown to exhibit contrasting distribution patterns along the elevation gradients. We used this trackable system as a case study to understand the contribution of temperature and interspecific competition in structuring community composition along elevational gradient. We compared several lab-measured thermal traits among those species, and examined how their difference translates into their competitive performance at simulated lowland and highland temperature. Combined, we studied the relative importance of temperature and inter-specific competition in determining species distribution patterns. Our findings reveal that upper thermal limits show smaller variation compared to lower limits, nevertheless, this small difference could lead to different outcome of environmental sorting by high temperature at tropical lowland. In contrast, upland community composition is mainly driven by inter-specific competition rather than cold limits. These results provide the foundation for elucidating the role of temperature in structuring the biological community on tropical mountains and imply the vulnerability of tropical insect communities along the whole elevation gradient to the future warming.

There are a lot of studies examine the correlations between thermal performance and distribution (i.e. central point). A positive relationship doesn’t really tell you the limiting factor of distribution. Even a positive relationship is shown, a species can still sustain its population in high temperature when with its own, but fail so in the presence of other competitors.

How proximity of current temperature to critical temperature translates into competitive outcomes and eventually distribution?

The relationships between heat stress and competition in low latitude/altitude boundaries?

**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 behavior 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. Documented changes of insects’ distribution, composition and phenology have imposed direct environmental and economic threats (Deutsch et al. 2018; Logan, Régnière, and Powell 2003; Pecl et al. 2017).

Thermal tolerance, which is derived 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 to tropical species is still much debated, from least to most concern among major biotas (Corlett 2011). Tropic species on average have narrower range than temperate species (Khaliq et al. 2014) and have been living near their upper thermal limits suggested by laboratory measurements (Deutsch et al. 2008; Diamond et al. 2012; Huey et al. 2009). Tropical species are thus predicted to face particular difficulty to tolerate or adapt to the projected warming in their current location (Bonebrake and Deutsch 2012; Deutsch et al. 2008; Kellermann et al. 2012), and to track the relatively large shift of their “climate envelope”, the projected suitable climate zone that moves toward higher latitude or altitude (Colwell et al. 2008; Sheldon, Yang, and Tewksbury 2011).

However, it is uncertain whether thermal tolerance is the key predictor of distribution in the warmer margins. The observation that species occupying the cooler environment have similar upper thermal limits with tropical species poses doubt on the importance 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) and biotic interactions may become the determining factors in the tropics (Engelbrecht et al. 2007; Jankowski et al. 2013; Louthan, Doak, and Angert 2015). A commonly-hold 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 observed smaller change at the species’ warm boundaries than cold boundaries in response to warming (Chen et al. 2011; Sunday, Bates, and Dulvy 2012), undermining the gloom prediction for the tropical species in response to warming.

Despite the controversy and urgent nature of this research topic, limited amount of empirical evidence come from species-rich tropical systems (XXX), and most of them only examine the correlational relationship between thermal tolerance and distribution. It remains a crucial link how laboratory-measured thermal traits, e.g. critical temperature and optimal temperature, are ecologically relevant in realistic climate conditions. Additionally, biotic interactions themselves are regulated by temperature. Sensitivity of a particular species may depend on its interacting species’ response to temperature. Therefore, a significant correlation between tolerance and distribution does not rule out the role of biotic interaction, and the species of interest can still be sensitive to temperature change even when biotic interaction is 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 interaction. Such empirical studies will contribute to the theory unifying the long-separate concepts of environmental filter and biotic filter, and provide practical information for wildlife management and conservation in face with climate change.

Tropical mountains provide the natural gradient to test the sensitivity of tropical species to temperature (Corlett 2011). Rainforest in the Wet Tropics bioregion in north-eastern Australia has high biodiversity values especially for its high degree of endemism in its cool, moist upland refugia (Williams, Bolitho, and Fox 2003). Species composition significantly changes along the altitude gradient on those tropical mountains (Williams et al. 2003). Frugivore flies are sensitive to temperature on the organismal level (Batista, Rocha, and Klaczko 2018), while their population size is additionally regulated by humidity, food availability, competition, and natural enemies (e.g. parasitoid wasps) (Fletcher 1973; Krebs and Barker 1991; Mitsui et al. 2007). Methods have been established to quantify their field distribution (Jeffs et al. 2020), laboratory thermal performance (Hoffmann, Sørensen, and Loeschcke 2003) and competitive ability (CHRIS, CHEN AND LWEIS 2021). Thus, this system offers a unique opportunity to investigate the relationships between heat stress and biotic competition in determining warm and cold boundaries in tropics.

We hypothesize that species turnover observed along the tropical altitudinal gradient is the result of thermal constrains at highland and competitive exclusion at lowland. Thus we predict that cold tolerances correlate with species distribution types. In contrast, species which have reduced abundance towards lowland (compared with themselves) do not necessarily have the weaker heat tolerance, but their population dynamics are negatively influenced by the presence of lowland-biased species as the competitors. To test the hypotheses, we first demonstrated the species turnover patterns along the altitude gradients. We then examined the correlations between species distribution patterns and multiple thermal tolerance traits. Thirdly, pairs of species with similar or different distribution patterns were placed in simulated highland and lowland temperature to examine their competitive outcomes in both of short and long terms. Our results contrast with our original hypotheses, suggesting that inter-specific competition significantly reduces lowland-biased species at highland, whereas high temperature, regardless of biotic competition, already constrains the upland *Drosophila* species at Australian tropical lowland. Additionally, the 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 both high- and low-altitude 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, the University of Oxford, UK, since December 2018. Theywere maintained for approximately 15 to 30 non-overlapping generations in Czech Republic and additional approximately four to seven non-overlapping generations in Oxford before they were used to construct mass bred lines.

To revive genetic variation, we constructed mass bred lines (MBLs) by combining four isofemale lines of each *Drosophila* species (except for *D. pandora*, where only three isofemale lines were available). The four lines were selected from different mountains and different altitude if possible (Detailed arrangement was shown in supplementary table 1). Each population cage was initiated using two independently-reared MBLs of the same species. Large population size within cage was maintained at 25°C and 12h/12h L/D cycle for more than four generations before the thermal traits measurement. Therefore, traits should not have been influenced by maternal effect, acclimation, or isofemale line effect. The MBLs are maintained in 23°C and 12h/12h L/D since 2020.

Nine tropical *Drosophila* species were included in laboratory measurements. *D. rubida* was not included in laboratory measurement because it has low reproduction rate and much longer development and sexually maturation time than other species, making it practically difficult to raise to large number and also synchronized with other species. Isofemale lines of *D. pseudotakihashii* was contaminated by other species before the thermal traits were measured. 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 measurement. *D. melanogaster* does not occur naturally at the study sites. One 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. Details were described in 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. 182 pupae were sampled at each site. 716 pupae were successfully identified to species by DNA metabarcoding (Jeffs et al. 2020), with 86 – 134 pupae at each site. *D. serrata* (1 individual) and *D. immigrans* (4 individuals) were excluded from the distribution analysis due to infrequent occurrence.

1. Preparation of experimental animals

Fly eggs collected from the population cage were reared under low-density (less than 100 eggs per vial) at 25°C and 12h/12h L/D cycle. To collect virgin flies for measuring thermal trait, adults emerged within 12 hours were separated. For competition analysis, flies emerged within the same 48 hours were kept together in mixed-gender containers. We additionally mixed five females and five males in each of two vials to monitor their reproductive activity every day. As different species have different development times and sexual maturation times, different species started on different days so that their first day of egg-laying was synchronized.

1. Fecundity measurement

Two days after first egg-laying was observed, 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 the seven constant temperature (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 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, which was used as the corrected temperature in 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), the 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 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

Relative tolerance 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 are often chosen around 0°C (Gibert et al. 2001). As tropical species often have significantly lower cold resistance (Gibert et al. 2001), 5°C was used instead to increase the variation among the tested species after piloting trials. Heat stress is chosen to be 40°C, which follows common practice (Hoffmann et al. 2003) and is expected to capture the between-species variance and produce the time scale which is convenient to measure (Jørgensen et al. 2019). After being knocked down by heat (40°C), most flies did not survive. In this case, only knockdown time was used to evaluate tolerance 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 randomly-selected individuals were allocated separately in empty flat-bottom 3ml insect tubes. An observation rack was divided into 3X3 grids and each grid held seven tubes containing the same species. Nine species were assigned in random order to one 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 the flies that lost or recovered their motor ability in that minute were recorded. For each sex, the above procedures were repeated three times

1. Competition measurements

Short-term competition: two days after first egg-laying was observed, adults of different sex were separated and then they were used as the founders in 5ml-food vials at the next day. Each two-species combination (6 combinations in total) 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 of 2, 4, 8 pairs. Each density replicated ten times divided in 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 they were dispose of. Offspring of the founders developed in the vials and experienced intra- and inter-specific competition over food and space. Offspring that successfully developed to adulthood were counted. Such competition set-up was conducted in incubators setting at alternating temperature regimes mimicking day/night temperature 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) of our study site (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 populations starting with the mixture of the above two species were maintained in the simulated highland and lowland temperature for 13 weeks. Monoculture populations were started by 10 pairs of individuals, and mixed-species populations were started by 10 pairs of each species. The populations were evenly divided into two blocks starting at different dates. Each population was maintained in a series of five bottles following Ayala’s type 1 system (Ayala 1973??). On every same day of the week, individuals surviving in the latest bottles and individuals newly 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 new addition of adults were separately recorded. Total population size of each species was counted at the end of the experiment.

To avoid pseudo-replication, the two incubator switched to the other’s 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 altitude (hIndex) of distribution, the relative location of each sample was assigned 0, 0.5, and 1 if it was collected at low-, middle- and high-altitude sites. In addition, intra-specific abundance patterns were assessed by logistic regression of detection probability with altitude. 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 the altitude as the fixed effect and the transect as the random effect in 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 here is to compare the abundance of a species with itself along the altitude, rather than to compare the abundance of multiple species in a given location. By this definition, an upland-biased species may have 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* is the minimum and maximum temperature for the species to reproduce, *a* is a scaling factor and *b* is a shape factor of the curve. *a*, *b*, *RTmin*, and *RTmax* of the nine species were assumed to share normal distribution respectively. 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. It 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 converged results, potentially due to the piecewise nature of the thermal performance function. Diagnostics were performed and the model performance is acceptable (supplementary figure 3). Non-informative priors were chosen for all parameters. 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.

Median of the posterior distribution of *a*, *b*, *RTmin*, and *RTmax* were used as the model parameters to construct the thermal performance curve.

The model parameters were also estimated by the maximum likelihood method using the *bbmle* package. Total offspring numbers were modeled by 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 badly 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 conducted. 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 by hIndex, block, and tube position as fixed effects, species as a random effect in the linear mix-effect model (family = “gaussian”).

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

R0 is the generational reproduction rates, α represents intra-specific competition coefficients. β represents the inter-specific competition coefficients, which defines the equivalence between the two particular species. Their values and 90% credible intervals were estimated using the same Bayesian statistical method detailed in Terry, Chen and Lewis 2021. Equilibrium state of each pair tested was predicted as described in XXXXX.

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

**Results**

Figure 1a shows the absolute numbers of identified samples found on low-, medium- or high-altitude sites of each of the nine major *Drosophila* species (accounting for 99% of total samples). Distribution evaluated by regression of detection probability along altitude and by weighted central altitude (hIndex) showed consistent patterns (figure 1b), which show different altitudinal biases among species. *D. bipectinate* and *D. pandora* were categorized as lowland-biased species with high confidence. *D. pseudoananassae* was most likely to bias towards lowland. *D. rubida* and *D. sulfurigaster* showed no significant change with altitude. *D. birchii* was most likely to bias towards highland. *D. palidifrons* and *D. pseudotakahashii* were significantly more abundant in high altitudes. The only six samples of *D. bunnanda* were all found at low altitude, 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 that it was found predominantly at lowland (Schiffer and McEvey 2006). Therefore, *D. bunnanda* was categorized as lowland-biased species. 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). There was no general trade-off between performances in cold versus warm environment that correspond to their distribution types (supplementary figure 6, p = yyy). For example, the lowland-biased species *D. bunnanda* have higher minimal temperature, optimal temperature and maximal temperature than it upland-biased relative, *D. birchii*. In contrast, *D. sulfurigaster* always outperforms its upland-biased relative, *D. palidifrons.* “hotter better” and “jack for all, master for none” hypotheses are not supported.

Values of RTmin had no relationship with the species distribution patterns (coefficient = 0.024, 95% ci = -2.47 – 2.52). Similarly, upland-biased species did not show higher fecundity at the stressfully-low temperature, 17°C (p = 0.788). When exposed to acute sublethal low temperature (5°C), except for *D. simulans* and *D. melanogaster*, the other *Drosophila* species all show similarly weak resistance (male: p = 0.18; female: p = 0.53). All species recovered their fecundity after eight-day exposure to 14°C. This recovered fecundity showed a minor but not significant increase (coefficient = 0.35, p = 0.105) for the upland-biased species. They also spent a longer time to regain mobility after the chill coma (male: p = 0.054; female: p = 0.029), which is also presumably disadvantaged in the upland environment.

Regardless of the small variation of RTmaxs compared with RTmins, species whose distribution were biased towards lowland consistently had higher RTmax (coefficient = -2.52, 95% ci = -3.68 - 1.36, p = 0.00125). Reproductive performance at 29°C also decrease with hIndex (coefficient = -5.09, p < 0.0001). 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. Heat tolerance, measured by knockdown time at high temperature, was lower among species whose distribution were biased towards high latitude (male: coefficient = -9.1, p = 0.0013; female: coefficient = -5.4, p = 0.056).

In 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 3a). In simulated upland environment, all species can reproduced and sustained their populations. Lowland species were strongly influenced by the density of *D. palidifrons*, an upland species. While upland species were significantly less affected by upland species, shown by lower inter-specific competition coefficients (figure 3a, table 2). The competitive effect at cold upland environment 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, population size of *D. pandora* kept high in both temperature when they were raised alone. But their populations were significantly reduced by low temperature only when in the presence of inter-specific competitors (Figure 4).

[Should I do a multi-variate analysis of thermal traits – distribution index? I personally think NO, as I don’t want to combine heat tolerance trait and cold tolerance trait into PCA. I am testing two hypotheses separately. ]

Thermal breadth:

Tbr was calculated as the temperature range where performance was above an 80% threshold (Huey et al., 2012; Huey and Stevenson, 1979) and as such Tbr can be used as a measure of the flatness of the TPC. (Kellermann, 2019)

[GUT FEELING: By this method, then of course “jack for all” hypothesis holds. Because this is using percentage]

Choice of TPC curve:

>Try out the new TPC fitting package in R [I have tried “rTPC”. There is no way to build hierarchy in the modeling strategy as I did with my customerized rstan. I could learn from the functions in their library (but I can’t find the original paper of some)]

>Check what functions did others used:

Three studies examined TPCs in fecundity (Condon et al., 2014; Cooper et al., 2010; Klepsatel et al., 2013)

TPCs in egg-to-adult viability (Cohet et al., 1980; Petavy et al., 2001; Schou et al., 2017),

two studies examined TPCs in both fecundity and egg-to-adult viability (Clemson et al., 2016; Overgaard et al., 2014).

2019 kellermann: The full model for activity included developmental temperature (scaled) as a quadratic fixed effect, test temperature (scaled) as a linear fixed effect, as well as the interactions between the two fixed effects. [This is not the performance at the vairous temperature. This is the performance of adults which used to develop at the various temperature] [Quadratic function – not good! Negative values, symmetric]

2013 Kingslover: RG model vs. Deustch’s model (To evaluate whether the simulation results are sensitive to the specific model used for thermal performance)

urn:x-wiley:02698463:media:fec12145:fec12145-math-0001(eqn 1)

For each species, we used a Gaussian function to model *R*0 as a function of temperature (*T*). [So this is also a symmetric function]

urn:x-wiley:02698463:media:fec12145:fec12145-math-0002(eqn 2)

Similarly, we used an asymptotic function to model *G*−1 as a function of temperature:

urn:x-wiley:02698463:media:fec12145:fec12145-math-0003(eqn 3)

>Phylogenetic correction: [EMAILED KELLERMANN, NO ANSWER]

Literature:

In the present study, we employed a suite of

analytical approaches to analyze phylogenetic signal. Although

these analytical methods are related in their aims, their different

assumptions may potentially lead to different conclusions and

we therefore based our conclusions on the consensus findings

(Cooper et al. 2010).

To examine the proportion of trait variation that could be entirely

attributed to climatic variables, we controlled for phylogenetic

effects using two independent methods of analysis. This was done

by computing phylogenetic independent contrasts (PIC) and the

SLOUCH model (SLOUCH, described below). These approaches

were calculated in ape and SLOUCH (Hansen et al. 2008),

respectively.

**Discussions**

Key sentences of each paragraph of discussion:

Small difference in critical temperature could mean big difference in performance in realistic situation

Thermal safety margins for all species are very small in tropics

Mixed messages of the role of temperature in determining warm limits – we need more evidence from tropical area; we have to recognize the regional difference; we need improved, widely recognized/standardized methods

Daily peak temperature is an important climate elements. With numerous effort to use biophysical model to predict species range and response to climate change (Kearney and Porter 2009), it is fundamental to understand which aspects of climate elements constrain distribution.

Our quantifications of thermal traits and competition have limitation. Alternative hypotheses (?).

[\*interpretation] adaptation or filtering? (Kellermann 2012)Using a suite of analyses we showed that phylogenetic signal in heat resistance reflects phylogenetic inertia rather than common selection pressures. Current species distributions are therefore more likely to reflect environmental sorting of lineages rather than local adaptation.

The generality of this relationship is essential to realistically estimate the magnitude of the impact of warming on tropical species.

[discussion] adaptation?

[Implication] Combined with the temperature data, the thermal performance results implied the importance of extreme and/or stressful temperature rather than mean temperature in structuring distribution. The mean temperature during the survey season in the lowland is around 26°C, which all the species are around its peak reproductive performance. In addition to difference in mean temperature, highland and lowland sites significantly differ in the number of days and the daily duration that the temperature reached above 28°C (supplementary figure xxx). The lowland-biased and upland-biased species show significant distinction in their reproduction and recovery from our stressful testing temperature, 29°C. Saxon et al. showed that brief exposure to stressful thermal environment has similar fitness costs to continuously stressful conditions (Saxon, O’Brien, and Bridle 2018). These results stress the necessity to consider daily temperature variation and extreme temperature event in research studying the relationship between the environmental factors and distribution and future projection (Kingsolver, Diamond, and Buckley 2013).

Buckley, Lauren B., and Raymond B. Huey. "How extreme temperatures impact organisms and the evolution of their thermal tolerance." *Integrative and comparative biology* 56.1 (2016): 98-109.

Thermal extremes can drive organisms in temperate and tropical sites to have similar thermal tolerances despite major differences in mean temperatures

[implication] Laurance, William F., et al. "Global warming, elevational ranges and the vulnerability of tropical biota." *Biological Conservation* 144.1 (2011): 548-557.: a relatively high proportion of plants and ectothermic vertebrates (amphibians and reptiles) are upper-zone specialists.

[Bigger context] On the contrary to our results, high temperature is not usually regarded as the limiting factor to local distribution patterns. Climate factors are generally viewed as drivers operating at the continental or regional scale, while at the local scales, biological variables are thought to become increasingly important (Hortal et al. 2010). Heat tolerance traits has less variation (Hoffmann 2010), especially in studies of local scales rather than continental scales (Nowrouzi et al. 2018; Overgaard et al. 2014).

[Bigger context] Tolerance-competition has been widely used to explain species turnover along environmental gradients [CITATION]. However, compared with cold tolerance [CITATION], the heat tolerance – competition trade-off and its influence on distribution is not widely recognized [CITATION]. The ones that occupy the warm area is often regarded as better competitor… The lack of study may be partly explained by the bias in geographic coverage. ANY POSITIVE STUDY? [1. PNG birds’ cold tolerance can’t explain upper distribution (Freeman 2016)]

[] Recent studies focusing on the tropic area have SOME POSITIVE RESULTS… Our study included most of the Drosophila species in the community, offering a systematic examination on the relationship between heat tolerance and distribution in the local scale. Our study’s meaning to the field: driving factors of distribution are not the same across geographic areas.

1. thermal performance of sperm activity is closely associated with the long-term abundance patterns in variable thermal environment among two sister Drosophila species occurring at the tropics.
2. Species’ northern and southern range limits are related to their tolerance of low and high temperatures respectively among European diving beetles.
3. Butterfly’s altitude distribution: host vs. temperature

[Implication] Reproductive success, recovered reproduction, knockdown time reflect different aspects of fitness. The correlations between thermal performance traits were challenged (Hoffmann et al. 1997; Overgaard et al. 2014; Sinclair et al. 2016). [Compare with Overgaard’s study (because it is so similar!)]. Our results show that rank of knockdown time to heat could be used as an approximate of the rank of reproduction upper limit. OTHER POSITIVE RESULTS??. These correlation may reflect a common biochemical mechanism (for example, heat-shock protein??) mediating thermal tolerance of organism’s metabolism, reproduction, development and behavior. With that said, tolerance using multiple traits are still complementary to each others to better understand fitness and thermal adaptation across environment (Kellermann et al. 2019).

[performance is dependent on the thermal history, rate of temperature change and exposure time. Ma et al 2020]

[No trade-off between heat tolerance and cold tolerance, indicated by the artificial selection exp. After 12 generations of artificial selection, lines diverged significantly for high KRHT only. Sambucetti, 2010]

[Limitation] These lab-measured thermal tolerance may not be representative among species with wide geographic range [POPULATION VARIATION CITATION], and cannot reflect the plasticity [CITATION] and evolutionary response [CITATION] to climate change. Thermal tolerance may be influenced by precipitation (Bozinovic and Pörtner 2015; Kellermann et al. 2012), diet, larval conditions (Bubli, Imasheva, and Loeschcke 1998), etc. [CITATION]. It is unsure whether the impact to different species is of similar degree. Thus, ignoring XXX may lead to overestimation of the severity of the impact of climate change [CITATION]. Nevertheless, it has been suggested that tropical species has low plasticity and evolutionary potential in adapting to warming. Geographic variation (and gene flow) and laboratory evolution can help with the first issue [CITATION]. Long-term monitoring data of population trend and climate [synchronization is also a sign!] can help to answer the second issue [CITATION] (Gade et al. 2020).

The lowland species’ safety margin is unknow from the spatial analysis. Temporal analysis with long-term monitoring data is needed. An example (Gade et al. 2020)

[Ending conclusion] Tropical lowland has high biodiversity, therefore very important (Gaston, 2000). Tropical insects have high diversity and they are important (Greenwood 1987). This study contribute to the growing literatures which show the warmer margins are also sensitive to warming (Wilson et al. 2005). We show the species turnover along altitude gradient is highly likely to result from physiological and reproductive constrains by high temperature. Upland tropical species will especially face range contraction by future warming. With numerous effort to use biophysical model to predict species range and response to climate change (Kearney and Porter 2009), it is fundamental to understand which aspects of climate elements constrain distribution.

Here, we provide evidence that tropical insect species have already undertaken altitude increases, confirming the global reach of climate change impacts on biodiversity. (Chen et al. 2009) High-altitude refuge…

[TPC implication] 1) thermal characteristics are diverse among community components. Upper thermal limits differ among and within component species in a tritrophic hostparasitoid- hyperparasitoid system (Agosta et al. 2018); 2) Thermodynamic effects on organismal performance: Is hotter better? In general, yes. (Angilletta et al. 2010)

[implication] systematic bias in climate change response study: in particular, tropical and marine systems are grossly underrepresented, as are plants and endothermic animals.

[implication] extreme tolerance is more related to range size. (Pither 2001)

[implication] We found that thermal niche estimates derived from both approaches lack general congruence (Sánchez-Fernández et al. 2012)

[implication] Even though there might be truncated thermal limit estimate (Feeley), as shown by our results, upland species will face contraction because combined effect of temperature and competition. For lowland species, it’s important to measure its real tolerance to use for predictive model (Martínez et al 2015), and to find a reliable link lab-measured tolerance with reality projection. Long-term monitoring data could be one way to understand the temperature limit for field populations.

(Martínez et al 2015) The thermal thresholds obtained in growth and survival experiments were used as proxies of the fundamental niches of two foundational marine macrophytes. The geographic projections of these species' distributions obtained using these thresholds and existing SDMs were similar in areas where the species are either absent-rare or frequent and where their potential and realized niches match, reaching consensus predictions.

[method limitation]

Measures of CTmin, LTe50 and LTi50 proved to be the best predictors to describe the variation in realized latitudinal distributions. there was only a weak correlation between the entrance into coma (CTmin) and the recovery from chill coma (CCRT) (Anderson, 2015)

Critical temperatures (CTmin and CTmax), half lethal temperature (LT50cold and LT50heat), heat stress survival, knockdown (heat coma and chill coma) time, recovery time, etc. have been common practice to compare relative resistance to heat stress and cold stress (Gibert et al. 2001; Hoffmann et al. 2003). Critical temperature becomes more popular for its direct association of climate data. However, the absolute value of CTmin and CTmax is significantly influenced by the rate of temperature change (Terblanche et al. 2007), making it difficult to compare between studies. Knockdown time and recovery time are simpler measure which also serve the purpose of comparing thermal tolerance. Variation has been observed among species and geographic ranges (Gibert et al. 2001; Hoffmann, Anderson, and Hallas 2002), and knockdown/recovery time were correlated with other measurement, such as critical temperature (Andersen et al. 2015).

[Impact]

We show that lower elevational limits for 16 butterfly species in central Spain have risen on average by 212 m (± SE 60) in 30 years, accompanying a 1.3°C rise (equivalent to c. 225 m) in mean annual temperature. (Wilson et al., 2005)

We show that recent warming constitutes an “escalator to extinction” for birds on a remote Peruvian mountain—high-elevation species have declined in both range size and abundance, and several previously common mountaintop residents have disappeared from the local community. (Freeman et al. 2018)

Rapid upslope shifts in New Guinean birds illustrate strong distributional responses of tropical montane species to global warming (Freeman, 2014)

[research effort] publications treating latitudinal range shifts have focused primarily on changes at the distributional margins (shifts or abundance changes at the leading and trailing edges) rather than on changes within the distribution (shifts or abundance changes at the optimum position. Additionally, we found that reports studying elevational range shifts have mostly focused on changes within the core of the distribution, rather than on changes at the distributional margins. (Lenoir and Sevenning 2015)

[reflection] there are various physical constrain and biotic surrounding that could change along the altitude gradient (Jankowski et al., 2013). The correlation we found is not direct evidence of the causal effect. It’s an indication of xxx.

[tolerance and distribution study]

Negative:

1. geographic variation in thermal tolerance within species was low or negligible. Cold tolerance – northern and southern margins. no relation was observed between heat tolerance and latitudinal distribution. Heat tolerance was higher in species inhabiting openlands or the forest canopy than in those inhabiting the forest understorey. (Kimura 2004)
2. We conclude that the heat tolerance of T. hsuehshanensis (lizard) is not a crucial factor limiting its current altitudinal distribution. ShuPing & MingChun, 2008
3. Is thermal limitation the primary driver of elevational distributions? Not for montane rainforest ants in the Australian Wet Tropics (Nowrouzi et al 2018)
4. Elevation and Climatic Tolerance: A Test Using Dung Beetles (1999)

Positive:

1. Despite their smaller size, high altitude bumble bees tolerated colder air temperatures: they had ~1 °C lower CTmin and recovered from cold exposure at ~3-4 °C lower air temperatures. Conversely, low altitude bees tolerated ~5 °C hotter air temperatures. These altitudinal differences in thermal tolerance parallel differences in average daily minimum (1.2 °C) and maximum (7.5 °C) temperatures between these sites. (Oyen, 2016)
2. Climatic limitation is the most likely explanation for the low elevation range margin of A. crataegi, whereas the absence of host plants from high elevations sets the upper limit. (Merrill et al. 2008)
3. In general, species found at middle elevations and on mountaintops are less tolerant to high temperatures than species restricted to lowland habitats. [genotypic adaptation of local population] High-elevation beetle haplotypes are characterized by low thermal limits; this pattern supports the hypothesis that populations along elevational gradients are locally adapted genotypes. (García-Robledo et al. 2016)
4. Opposing clines for high and low temperature resistance in Drosophila melanogaster (Hoffmann, 2002)
5. species' northern and southern range limits are related to their tolerance of low and high temperatures respectively. (Calosi, 2010)
6. Upper thermal tolerances of tadpoles were positively correlated (controlling for phylogeny) with maximum pond temperatures, although the slope was steeper in subtropical than in temperate species. (Duarte et al 2012)

[IS THERE ANY REVIEW OF THE RELATIONSHIP BETWEEN COLD TOLERANCE AND HOT TOLERANCE WITH TEMPERATURE?]

**address the wider literature and how it relates to your results (consistent / inconsistent, & why, etc.**

1. Constrained upper thermal limits in the larger scale – but difference and environmental filter still act in the local scale like my study. (Hoffman 2013)
2. Cold tolerance vs. warm tolerance (driving factors of distribution are not the same across geographic areas)

Species from hot and relatively dry regions had higher resistance, whereas resistance was uncorrelated with temperature in wetter regions. Kellermann 2012: Current species distributions are therefore more likely to reflect environmental sorting of lineages rather than local adaptation.

1. Cold tolerance – competition trade-off

Combined effects of climate and biotic interactions on the elevational range of a phytophagous insect ----- high temperature constrain lowland distribution!

1. Abundance is sensitive to temperature gradient. Most of the literature I have seen is about range size!!!
2. Extreme vs. constant

The important influence to tropical species of daily temperature variation and extreme temperature event

1. Comparison of performance measurement

Variation in the TPC across traits and time scales suggests that TPCs using single traits may not be an accurate predictor of fitness and thermal adaptation across environments.

1. Interpretation of the results
2. Cold vs warm limit of distribution: The relationship between this finding and the commonly-accepted understanding of latitudinal distribution pattern.
3. Relationship with biotic interaction: 1) environmental filtering and biotic interaction have different relative importance in different areas; [It might not be good to compare the relative role of environmental filtering and biotic interaction. First, definition problem of environmental filtering; second, if they work together to generate the end result – extirpation, then how to rigorously compare their ‘importance’?]
4. Limitation: Thermal performance curves are not fixed (acclimation, trans-generational effect, diet…). Behavior tolerance, plasticity, inter-population variability, and evolution were not addressed in this study. What do we already know about them in terms of tropical species?
5. Implication: 1) driving factors of distribution are not the same across geographic areas; 2) The important influence to tropical species of daily temperature variation and extreme temperature event; 3) Tropical species are already living close to their upper thermal limits; 4) Thermal performance curve could be used to estimated individual species effect to warming (but competition is not considered. 5) Rank of knockdown time to heat could be used as an approximate of the rank of reproduction upper limit. They may all involve in the fitness of insects in adapting to high temperatures.

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**Figure 1. a) Proportion of samples found in the low-, middle- and high-altitude site for the nine Drosophila species. b) Regression coefficients and hIndex unanimously describe altitudinal distribution patterns. *D. bunnanda* is not included in the graph 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).

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 4. 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

**Figure 3. Reproduction in stressful temperature treatment and after the temperature treatment. The species on the horizontal axis are ordered descendingly by the mean relative altitude of their distribution (hIndex). hIndex of *D. simulans* and *D. melanogaster* is unavailable.**

A close up of text on a white surface

Description automatically generated

**Figure 4. Physiological responses to lethal heat stress and cold stress. The species on the horizontal axis are ordered descendingly by the mean relative altitude of their distribution (hIndex). hIndex of *D. simulans* and *D. melanogaster* is unavailable. Measurements of females were labeled by red, males were labeled by blue.**

**A screenshot of a cell phone

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 altitude. “High” means sites from high altitude.
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.

**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 |