**Working title**: High temperature structures *Drosophila* community composition in a tropical lowland rainforest

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**Abstract:**

Focus: Impact of warming on species distribution

Tropical insects are challenged by global warming, resulting in altered distribution and their related ecosystem functions. Multiple abiotic factors and biotic factors can influence species response to climate change. Therefore, to precisely estimate the real magnitude of the impact of warming on tropical species, the correlation between species’ fundamental thermal niche and realized distribution needs to be examined at the first place. We measured multiple thermal traits in both low and high temperature of *Drosophila* species and examine the relationship with their altitudinal distribution in Australian rainforest mountains. Our findings reveal that the tropical lowland community is structured by high temperature as the occupying *Drosophila* species are higher in the upper thermal limit of reproduction. Physiological tolerance to extremely high temperatures is also higher of lowland-biased species. In contrast, highland-biased species don’t show stronger cold tolerance. These results provide the foundation for elucidating the role of temperature in structuring the biological community on tropical mountains and imply the vulnerability of lowland tropical insects to future warming.

**Abstract:**

Focus: Comparison between different thermal traits

As climate change influences species distribution and abundance, thermal traits are often used in an attempt to predict organismal responses to global warming. Often the choice of performance traits is driven by expediency, rather than by validated links to species distribution. However, they reflect different aspects of fitness that are not necessarily correlated. Fecundity reflects the population-level performance of species at different temperatures. Physiological response to sublethal condition links more tightly with organisms’ capacity to survive during a short period of extreme weather. Here we examined the relationships between thermal traits and compared their predictability on species distribution on a temperature gradient on Australian rainforest mountains. Heat tolerances using reproductive measurement and physiological measurement were correlated with themselves and with altitudinal distribution of the *Drosophila* species. In contrast, different cold tolerances were not correlated and they all gave poor prediction on species distribution in high altitude. Heat tolerance was not found to have a trade-off relationship with cold tolerance. These results illustrated what traits may involve as the fitness components of insects in adapting to low and high temperature in tropical areas, providing information for future study to evaluate biodiversity response to climate change.

**Background**

Tropical species, which account for the greatest global biodiversity of the world (Gaston 2000), are threatened by climate change. Insects deliver crucial ecosystem functions and services (Greenwood 1987; Schowalter, Noriega, and Tscharntke 2018; Weisser and Siemann 2008; Yang and Gratton 2014). They are especially challenged by warming because their locomotion, growth, and reproduction are dictated by environmental temperature (Deutsch et al. 2008; Hoffmann 2010; Wilson and Maclean 2011). Although the last three decades witness an exponential increase in range shift research, there is a substantial lack of study evaluating the impact of climate change on species in tropical regions where historical records are relatively scarce (Cheng et al. 2019; Lenoir and Svenning 2015).

Nevertheless, tropical species are expected to be particularly vulnerable to warming. Tropical species have narrower thermal tolerance range and are more likely to experience environmental temperature exceeding their upper limit under predicted warming (Deutsch et al. 2008; Kellermann, Overgaard, et al. 2012; Khaliq et al. 2014). As species distribution shifts poleward or upslope following climate change (Freeman and Class Freeman 2014), no species source pools are available to replace climatically displaced or declining species in the tropical lowland ecosystem (Colwell et al. 2008; Lenoir and Svenning 2015). Tropical species with narrow ranges are especially worrisome as their dispersal is challenged by larger gaps between current and projected ranges (Colwell et al. 2008). Consequently, tropical regions may face a higher risk of biodiversity attrition (Colwell et al. 2008). What’s worse, the future disruption of ecological assemblage is recently predicted to be simultaneous and abrupt, hitting foremost tropical oceans and tropical forests (Trisos, Merow, and Pigot 2020).

However, internal and external factors complicate species’ response to climate change. Thermal niches deduced from realized distribution are possibly underestimated concerning their true tolerances, especially among tropical lowland species (Feeley and Silman 2010). If so, these species may persist despite warming in tropics, reducing rates of biotic attrition (Feeley and Silman 2010). Abundance and distribution of species are also linked with extreme weather, precipitation, ocean acidification, etc., which are additional consequences underlying climate change (Allen and Breshears 1998; Devoto et al. 2009; Dutkiewicz et al. 2015; Engelbrecht et al. 2007; Smith 2011; Wernberg et al. 2013; Zhu et al. 2014). Increasing evidence on behavioral thermoregulation (Bonebrake et al. 2014; Kearney, Shine, and Porter 2009) emphasized the influence of habitat heterogeneity on species distribution and resilience to climate change. Last but not the least, tropical forests are notable for their intense biotic interactions (Roslin et al. 2017), which may decisively refine realized distribution (DeRivera et al. 2005; Wisz et al. 2013) and alter their individualist species response to warming ((Davis et al. 1998)). Therefore, to precisely estimate the real magnitude of the impact of warming on tropical species, the correlation between species’ fundamental thermal niche and realized distribution needs to be examined. This serves as a foundation to investigate the causes and drives of distribution and abundance (Kearney and Porter 2004).

Studies compares field distribution and thermal traits show an indefinite and incomplete picture. Both upper thermal limit (Batista, Rocha, and Klaczko 2018; Kellermann, Overgaard, et al. 2012) and cold and/or desiccation resistance (Batista et al. 2018; Bishop et al. 2017; Kellermann et al. 2009; Kellermann, Loeschcke, et al. 2012; de La Vega and Schilman 2018; Overgaard, Kearney, and Hoffmann 2014) were shown significant correlation with species distribution and abundance. However, it has been suggested that upper thermal limit varies less than cold/desiccation resistance (Hoffmann 2010; Sunday, Bates, and Dulvy 2011), and it doesn’t constrain species occupation of the area toward lower latitude(de La Vega and Schilman 2018; Overgaard et al. 2014). In other studies, none of the thermal tolerance traits comply with realized distribution (Freeman 2016; Khaliq et al. 2014; Sánchez-Fernández et al. 2012), implying a more important role of other abiotic factors and biotic factors in those systems. Research effort on different taxa (e.g. endotherm vs ectotherm), regions (e.g. temperate vs tropic), and geographic scales (e.g. continental vs local) are disproportionate. Studies in tropical areas and local scales are needed to understand the generality of the relationship between species distribution and thermal traits.

Differences in criteria used to evaluate thermal tolerance adds furthermore complication. Compared with the life-history traits or population growth rate, physiological traits (e.g. development rate, metabolism, mobility) are more instantaneous measures of individual performance to thermal stress. They are often the only feasible traits to measure among species with long lifespans or that are difficult to establish in the laboratory population. Therefore, most existing studies have used short-term physiological measurements to understand distribution patterns and inform future changes. However, they reflect different aspects of fitness that are may not necessarily correlate with reproduction-related traits (Overgaard et al. 2014; Sinclair et al. 2016). Reproduction generally reflects the population-level performance of species at different temperatures. Physiological response to sublethal condition links more tightly with organisms’ capacity to survive during a short period of extreme climate (Overgaard et al. 2014). Additionally, thermal tolerance estimated using different traits or using different stress exposure regimes sometimes differ markedly ((Hoffmann 2010; Sinclair et al. 2016; Terblanche et al. 2007)). There have been very few comparative studies exploring their relationship between each other and their predictability over distribution.

In this study, we investigated the relationship between fundamental thermal niche and species turnover of tropical *Drosophila* on continuous temperature gradients on the Australian rainforest mountains. As the species involved in this study interact in connected communities, it tests the hypothesis that cold and/or warm temperatures drive an abundance pattern across the altitude gradient in a local scale. In addition, lab-reared populations control for the influence of maternal effect and acclimation on measurement, and enable our comparison between the physiological and reproductive tolerance. Our findings reveal that the tropical lowland community is structured by high temperature as the occupying *Drosophila* species are higher in the upper thermal limit of reproduction. Physiological tolerance to extremely high temperatures is also higher of lowland-biased species, correlating with the reproduction upper limit. In contrast, highland-biased species don’t show stronger cold tolerance. These results provide the foundation for elucidating the role of temperature filtering in structuring the biological community on tropical mountains and imply the vulnerability of lowland tropical insects to future warming.

**Methods**

1. Study system

The field survey was conducted and laboratory cultures were collected from Australian mountainous rainforest: Paluma Range (S18° 59.031' E146° 14.096') and Kirrama Range (S18° 12.134' E145° 53.102'). The two transects spanned from 59 – 916 meters above sea level and experienced an average temperature ranged from 21°C to 26°C. These sites represent the three “ecologically significant climatic zones” based on forest structure across the altitude range of each mountain.

*Drosophila* isofemale cultures were established from pupae collected from both high- and low-altitude sites. All cultures were maintained at 24°C and 12/12 L/D cycle at the Biology Centre, Czech Academy of Sciences since 2017 and 2018. They were transferred and maintained at 25°C and 12/12 L/D cycle at the Department of Zoology, the University of Oxford since December 2018. Theywere maintained on standard *Drosophila* medium (corn flour, yeast, sugar, agar, and methyl-4-hydroxybenzoate) for approximately 15 to 30 non-overlapping generations in Czech Republic and additional approximately 4-7 non-overlapping generations in Oxford before they were used to construct mass bred lines.

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 ??CITATION??. Relative abundance was surveyed for three sites representing the highest, lowest, and most central points of each of the two transects. 182 pupae were sampled at each site. 716 pupae were successfully identified to species by DNA metabarcoding (??CITATION??), with 86 – 134 pupae in each site. *D. serrata* (1 sample) and *D. immigrans* (4 samples) were excluded from the distribution analysis due to small occurrence.

1. Preparation of experimental animals

To revive genetic variation, we constructed mass bred lines (MBLs) by combining four isofemale lines of each *Drosophila* species except *D. pandora* (only three isofemale lines were available). The selected four lines included as much the geographic variation of the cultured isofemale lines as possible. Each population cage was initiated using two MBLs independently crossed by the chosen isofemale lines. Cages are maintained at 25°C and 12/12 L/D cycle for more than four generations’ time before the experiment. Therefore, the difference in thermal traits measured below was not influenced by maternal effect, acclimation, or isofemale line. MBLs of *D. rubida* and *D. pseudotakahashii* were not constructed because their isofemale lines were not in good condition. The other eight species and Dah strain of *D. melanogaster* were used for the following laboratory measurement. *D. melanogaster* was measured together with the focal species because it served as a reference for comparison if more species will be measured in the future.

Fly eggs collected from the population cage were reared under low-density (less than 100 eggs per vial) at 25°C and 12/12 L/D cycle. Within 12 hours of emergence, virgin females and males were separately kept at 25°C and 12/12 L/D cycle. We additionally mixed five females and males in each of two vials to monitor their reproductive activity every day. As different species have different development time and sexually maturation time, eggs of different species were collected from cages on different days so that their first day of egg-laying were synchronized. Two days after sexual maturation, half of the adults were subjected to fecundity measurement and the other half, which were siblings, were subjected to physiological measurement.

1. Fecundity measurement

Two virgin females were paired with two virgin males on a 4ml standard *Drosophila* medium. They were randomly subjected to one of the seven constant temperature (14°C, 17°C, 20°C, 23°C, 26°C, 29°C, 32°C) and 12/12 L/D cycle. Vials were submerged in the water baths set at the constant temperature. 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 humidity was similar to the 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.

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 fitness in the corresponding temperature. After eight-day temperature treatments, all flies were kept at 25°C for another four days to examine the recovery of fecundity. Surviving flies were recorded at the beginning and end of each period. Vials containing eggs produced during the testing periods were maintained at their corresponding temperature for development. Vials were examined daily for emergence. The first emergence dates were recorded for different temperatures and the numbers of the next-generation adults were counted 5-7 days later. For each species and each temperature treatment, eight replicates were evenly split between two blocks. Detailed schedule was shown in supplementary figure 2.

1. Physiological 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. 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 were kept in groups at 25°C and 12/12 L/D cycle for 9-10 days before randomly-selected individuals were allocated separately in empty flat-bottom 3ml insect tubes. All species were placed on a portable observation shelf which was moved into the incubator when heat and cold treatment started. Every tube was examined once every minute and the flies that lost or recovered their motor ability were recorded. Each sex was measured separately, with seven replicates per species for each block and repeated for three blocks.

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 mean relative altitude (hIndex) of distribution, the relative altitude of each sample was assigned 0, 0.5, and 1 if it was collected at low-, middle- and high-altitude sites. In addition, logistic regression was used to determine how detection probability changes along 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) provided by *lmer* package. The above two ways of describing distribution patterns were compared by the Spearman’s rank test.

*Thermal performance curve*. Multi-level non-linear piecewise model was fitted under the Bayesian framework using MCMC sampling provided by *rstan* package in R. Total offspring numbers were calculated from combining the offspring numbers of day 1-2 and day 7-8. The average daily fecundity per female was calculated, then transformed by square root rule. Square rooted daily fecundity was modeled by Briere2 function:

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

Y = 0 (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 by 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, compared 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. 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 didn’t 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.

The model parameters were also estimated by the maximum likelihood method using *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.

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

*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 of 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.

*Physiological tolerance*. The six measurements of physiological tolerance were modeled by hIndex, block, and tube position as fix effects, species as a random effect in the linear mix-effect model.

*Correlation analysis*. The pairwise correlation among thermal traits was evaluated by Spearman’s rank test in *Hmisc* package. Traits included RTmin, RTmax, RTopt, median knockdown time to hot of female (FKDHEAT), median knockdown time to hot of male (MKDHEAT), median knockdown time to cold of female (FKDCOLD), median knockdown time to cold of male (MKDCOLD), median recovery time from cold of female (FRCCOLD), median recovery time from cold of male (MKDCOLD).

**Results**

1. Field distribution

The nine *Drosophila* species shown in figure 1a accounted for 99% of samples. The proportion of occurrence among low-, medium- and high-altitude sites differed among species. The regression coefficient evaluated how detection probability change along with altitude (supplementary figure 4). *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 biases in altitude. *D. birchii* was most likely to bias towards highland. *D. palidifrons* and *D. pseudotakahashii* were significantly enriched in high altitudes. There were only six samples of BUN sampled in our survey, which might explain the peculiar value of its estimated coefficient (coefficient = -69, not shown in figure 1b) and its large standard error (se = 21603). Nevertheless, BUN has been categorized as lowland-biased species.

Values of the regression coefficients correlated tightly with the mean relative altitude (hIndex) of species distribution (rho = 0.98, p-value < 0.001, Spearman’s rank test). For simplification, the hIndex was used to represent the distribution pattern in the following analysis.

1. Reproductive thermal performance

The thermal performance curve, measured by combined daily fecundity, is shown in figure 2 (supplementary figure 5 shows data points and fitted curve for individual species). Table 1 shows estimates of the parameters of the Briere’s function for each species. Species whose distribution were biased towards lowland show higher RTmax (coefficient = -2.52, 95% ci = -3.68 - 1.36, p = 0.00125). RTmin had more variation among species than RTmax. Their values had no relationship with the species distribution patterns (coefficient = 0.024, 95% ci = -2.47 – 2.52). The temperature for optimal reproductive performance (RTopt) also didn’t correlate with their distribution patterns (coefficient = 0.068, 95% ci = -1.93 – 2.07).

As shown in figure 3, in 29°C treatment, daily fecundity decreased with hIndex (coefficient = -5.09, p < 0.0001). In contrast, highland-biased species didn’t show higher fecundity at 17°C (p = 0.788). 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. All species recovered their fecundity after eight-day exposure to 14C. This recovered fecundity showed a minor but not significant increase (coefficient = 0.35, p = 0.105) for the species whose distribution were biased towards the higher altitude.

1. Physiological tolerance

Heat tolerance, measured by knockdown time by 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). Except for *D. simulans* and *D. melanogaster*, the other *Drosophila* species show relatively weak resistance to low temperature with no difference (male: p = 0.18; female: p = 0.53). Species whose distribution were biased towards high altitude spent a longer time to recover from the chill coma (male: p = 0.054; female: p = 0.029), which is presumably disadvantaged in the low-temperature environment. The experimental block and the position on the observation rack sometime showed a significant effect, which might be explained by the temperature fluctuation of the incubator and the difference of microenvironment.

1. Correlation among thermal traits

Table 2 shows the results of the Spearman’s rank correlation test. Species which had higher RTmax stayed active for longer in extreme high temperature. In contrast, species that had lower RTmin didn’t show any advantage in cold resistance or cold recovery. RTopt was not correlated with any other traits. For each of the 3 physiological measurements, male performance correlated significantly with female performance. Heat tolerance was not found to have a trade-off relationship with cold tolerance: RTmin and RTmax were not significantly correlated (supplementary figure 6); Species that resisted heat for longer also showed higher tolerance to cold (longer resistance to chill coma and shorter recovery).

**Discussions**

1. Interpretation of the results: Our analysis, taken together, show that species that have a higher tolerance to high temperature bias towards the low altitude. However, the pattern doesn’t hold for the cold end.
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; 2) the tight correlation between thermal trait and distribution doesn’t mean there is no effect of biotic interaction ---- temperature can change the competitive hierarchy among species, the realized community composition might be a combined effect of temperature and competition.
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.**

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**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.**

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**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.**

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**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.**

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**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. 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 |

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

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Supplementary figure 2. Time table of fecundity measurement.

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

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

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

A bunch of different colors

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

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**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.