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

Running title: Biotic and abiotic effects on distributions

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

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There is no conflict of interest to declare.

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Abstract:

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

Keywords:

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

Introduction

Temperature has a fundamental impact on the reproduction, survival, growth, and behaviour of organisms (Huey and Kingsolver 1989; Huey and Stevenson 1979), strongly influencing species' ranges and abundances (Hoffmann and Blows 1994; Wilson et al. 2005). As a result, estimates of thermal tolerances based on laboratory assays or species' distributions have been used widely to evaluate species' sensitivity to climate change (Deutsch et al. 2008; Kearney and Porter 2009). Tropical ecosystems comprise many species that live close to their upper thermal limits (Deutsch et al. 2008; Diamond et al. 2012; Huey et al. 2009), and which may not tolerate or adapt to warmer temperatures (Bonebrake and Deutsch 2012; Kellermann et al. 2012). The narrow thermal ranges of tropical insects (Khaliq et al. 2014) also mean that they will need to undertake relatively large latitudinal or elevational range-shifts to track their climate envelopes as the climate warms, increasing the risk of extinction and community disassembly (Colwell et al. 2008; Sheldon, Yang, and Tewksbury 2011).

Despite high relevance for understanding species' responses to climate change in tropical ecosystems (Corlett 2012), it remains questioned whether temperature is the major proximate factor setting the position of the 'warm' (low-latitude or low-elevation) limits of species' distributions. Global analyses found smaller distribution shifts in response to warming at warm range limits than at cool range limits (Chen, Hill, Shiu, et al. 2011; Sunday, Bates, and Dulvy 2012), and some laboratory assays have found similar upper thermal limits for tropical and temperate species (MacLean et al. 2019; Nowrouzi et al. 2018; Overgaard, Kearney, and Hoffmann 2014). Instead, other abiotic factors (e.g. precipitation) or biotic

interactions may be critical in determining range limits in the tropics (Engelbrecht et al. 2007; Louthan, Doak, and Angert 2015).

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

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

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

Methods

1. Species distributions along elevational gradients

Field survey. Field data on *Drosophila* communities were collected from rainforest sites at Paluma Range (S18° 59.031' E146° 14.096') and Kirrama Range (S18° 12.134' E145° 53.102'), Queensland, Australia. These sites lie within the Wet Tropics bioregion which has high levels of endemism associated with cool and moist upland

138 refugia (Williams et al. 2003). Abundances of *Drosophila* at these sites peak during
139 the period March to June. *Drosophila* pupae were sampled using bottle traps baited
140 with fermented banana from 11th March – 12th April 2016 for three sites at elevation
141 of 70m, 350-390m, and 730-880m (subsequently referred to as low-, mid- and high-
142 elevation, respectively) on the two mountains. 182 pupae were sampled at each site
143 and 716 pupae in total were successfully identified to species by DNA
144 metabarcoding (Jeffs et al. 2021), with 86 – 134 pupae at each site. Two infrequent
145 species, *Drosophila serrata* (1 sample) and *D. immigrans* (4 samples), were
146 excluded from analyses.
147 *Distribution analysis*. Two methods were used to assess trends in relative
148 abundance with elevation for individual species. First, the probability of detecting a
149 particular *Drosophila* species among other co-occurring *Drosophila* species was
150 modeled as a function of *elevation*, *transect* and their interaction in a generalized
151 linear model (family = "binomial") using the *stats* package in R. For each species, the
152 response variable was 1 if the pupa was identified as the focal species and 0 if it was
153 identified as any other species. Second, since none of the species had a bimodal
154 distribution with elevation, abundance-weighted mean elevation (*hIndex*) was used
155 as a simple alternative method to quantify distributions. The location of each sample
156 was assigned a value of 0, 0.5, or 1 if it was collected at low-, mid- and high-
157 elevation sites, respectively. *hIndex* was calculated for each species by averaging
158 these values for samples from both transects. Correspondence between the two
159 measures was tested by Spearman's rank correlation test between the coefficient
160 value for *elevation* in the regression and *hIndex* across species.
161
162 2. Maintenance of laboratory culture

163 *Drosophila* isofemale cultures were established in 2017 and 2018 from adults
164 collected from high- and low-elevation sites. Cultures were maintained initially at
165 24°C at the Biology Centre, Czech Academy of Sciences, and then transferred to the
166 Department of Zoology, University of Oxford, UK, in December 2018, where they
167 were maintained at 25°C. Cultures and all experiments mentioned below were
168 maintained under a 12h/12h light/dark cycle. There were approximately 15 to 30
169 non-overlapping generations in the Czech Republic and four to seven non-
170 overlapping generations in Oxford before cultures were used to establish mass bred
171 lines (MBLs).
172 To revive genetic variation, we made MBLs of each *Drosophila* species by
173 combining four isofemale lines (except for *D. pandora*, where only three isofemale
174 lines were available). The four lines were selected from different mountains and
175 different elevations if possible (detailed information in Supplementary Table 1). Each
176 mass bred population was initiated using two independently-reared MBLs of the
177 same species. Large populations were maintained at 25°C for more than four
178 generations before measuring the thermal traits. The MBLs were maintained at 25°C
179 until 2020 and at 23°C thereafter. In this way, measurements should not have been
180 influenced by maternal effects, acclimation, or isofemale line effects.
181 Nine tropical *Drosophila* species (listed in Supplementary Table 1) were included
182 in laboratory thermal trait measurements. Of the available species, only *D. rubida*
183 was not included because it was difficult to raise in large numbers and to
184 synchronize with the other species. Isofemale lines of *D. pseudotakihashii* were
185 contaminated by another species before measuring the thermal traits. Therefore, its
186 thermal traits were not measured. A new MBL originated from the two available *D.*
187 *pseudotakihashii* isofemale lines was used in the competition experiment. *Drosophila*

188 *melanogaster* does not occur naturally at the study sites, but a laboratory strain (wild
189 type, *Dah* strain) was measured for thermal performance together with the focal
190 species, as a benchmark for future comparisons.
191
192 3. Reproductive thermal performance
193 *Experimental measurements*: We exposed flies to temperatures ranging from
194 14°C to 32°C and measured how their reproductive success changed with
195 temperature (procedures are detailed in Supplementary Figure 1A). To generate the
196 adults flies to initiate the experiment, fly eggs collected from the population cage
197 were reared at low density (<100 eggs per vial) at 25°C. Emerging adults were
198 separated by sex within 12 hours to guarantee that they were unmated. Rearing was
199 started on different days for different species to synchronize the first day of egg-
200 laying. Two additional vials with five pairs of flies were monitored daily for sexual
201 maturation, indicated by egg laying. Two days after the first observation of egg-laying
202 in both vials, two virgin females and two virgin males were paired in a new vial
203 containing 4ml *Drosophila* medium (weight/volume concentration: 8% corn flour, 4%
204 yeast, 5% sugar, 1% agar, and 1.67% methyl-4-hydroxybenzoate). Vials were
205 randomly assigned to water baths set at one of seven constant temperatures (14°C,
206 17°C, 20°C, 23°C, 26°C, 29°C, 32°C). For each species and each temperature
207 treatment, eight replicates were evenly split between two experimental blocks,
208 making up a total of 1512 vials. Vials were submerged with the water level kept
209 above the zone within which flies could freely move. The temperature and relative
210 humidity of vials in each water bath were monitored within two empty tubes placed at
211 the centre and the corner. Observed temperatures varied ±0.5°C around the set
212 temperature. Temperatures in the centre of the water bath were on average 0.5°C

higher than at the corners; the average of the former during the experimental period was used as the corrected temperature in analyses. Relative humidity levels were similar to field conditions, ranging between 80% - 95%.

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

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

$$P_i(T) = \begin{cases} a_i \times T \times (T - RTmin_i) \times \ (RTmax_i - T)^{1/b_i} \ \ (for\ RTmax_i > T > RTmin_i) \\ 0 \ \ (for\ T \leq RTmin_i\ or\ T \ \geq RTmax_i) \end{cases}$$

where $P_i(T)$ is the reproductive performance of species *i* in temperature *T*, *RTmin* and *RTmax* are the minimum and maximum temperatures for the species to reproduce, *a* is a scaling factor and *b* is a shape factor of the curve. Values of *RTmin* of the nine species were modelled by a Gaussian distribution whose mean, μ_{RTmin}, and variance, ${\sigma_{RTmin}}^2$, were the hyper parameters in the multi-level model. The same applied to $RTmax_i$, a_i, and b_i. *P(T)* was modelled assuming a Gaussian distribution of errors. Although a Gaussian distribution is not ideal to model the transformed count data, which are all positive, models using untransformed count

numbers with Poisson, zero-inflated Poisson, negative binomial, and lognormal distributions did not adequately converge, potentially due to the piecewise nature of the thermal performance functions. The standard deviations of the Gaussian distribution of *P(T)* were assumed different in different temperature treatments and followed a Gaussian distribution, $\sigma_{P(T)} \sim Gaussian(\mu_{\sigma_P},\ {\sigma_{\sigma_P}}^2)$. This generated better fits than assuming the same standard deviation of *P(T)* across temperature treatments, as judged by leave-one-out cross-validation. This was because when the temperature was equal or close to *RTmin* and *RTmax*, *P(T)* was close to zero and its standard deviation was also small.

The prior distributions of the hyper parameters μ_{RTmin}, μ_{RTmax}, μ_a, μ_b, and μ_{σ_P} are Gaussian distributions with reasonable means (15°C, 30°C, 0, 0, 0) and relatively large standard deviations (10, 10, 1, 10, 10). The prior distribution of the hyper parameters ${\sigma_{RTmin}}^2$, ${\sigma_{RTmax}}^2$, ${\sigma_a}^2$, ${\sigma_b}^2$, and ${\sigma_{\sigma_P}}^2$ is inverse-gamma (0.001, 0.001), which is a commonly used non-informative distribution for priors of variance. The values of a_i and b_i were bounded to be positive. The values of $RTmin_i$ were bounded between 0°C to 17°C and the values of $RTmax_i$ were bounded between 26°C to 35°C based on prior knowledge of the range of temperatures under which Australian *Drosophila* can reproduce. The multi-level model was fitted under a Bayesian framework using MCMC sampling within the *rstan* package (Stan Development Team, 2021) in R. Models converged and performance was acceptable in diagnostic plots (Supplementary Figure 2). Medians of the posterior distributions were used as the parameter values to construct the thermal performance curves. Optimal temperatures for peak reproduction were calculated directly from known parameters:

$$RTopt = \frac{2bRTmax+(b+1)RTmin+\sqrt{4b^2RTmax^2+(b+1)^2RTmin^2-4b^2RTminRTmax}}{4b+2}$$

263 *Regression analysis*: The medians of the posterior distributions of *RTmin*,
264 *RTmax*, and *RTopt* were modelled by *hIndex* in a linear model with phylogenetic
265 correction. Phylogenetic tree were modified from Finet et al. 2021 (Supplementary
266 Figure 3). Fecundity at 29°C and 17°C, and recovered fecundity after 29°C and
267 14°C, were used as direct measurements of performance in high or low
268 temperatures. These fecundities were modelled as a function of *hIndex* and
269 *experimental block* as fixed effects, and *species* as a random effect in a generalized
270 linear mixed-effect model (family = "negative binomial") with phylogenetic correction.
271 These regression analyses were conducted using the *brms* package (Bürkner 2021)
272 in R. *Drosophila melanogaster* and *D. simulans* were not included in the regression
273 because their distribution patterns were unavailable from the field survey.
274
275 4. Thermal knockdown
276 *Experimental measurements*: Resistance to extreme cold temperature was
277 measured as knockdown time for each individual at 5°C and the time for recovery of
278 mobility after a 30-minute exposure to 5°C. The constant temperature chosen for
279 cold stress studies is often around 0°C (Gibert et al. 2001). As tropical species often
280 have significantly weaker cold resistance (Gibert et al. 2001), 5°C was used instead
281 to increase the variation among the tested species after pilot trials. Heat stress was
282 chosen to be 40°C, which follows common practice for *Drosophila* species
283 (Hoffmann, Sørensen, and Loeschcke 2003) and is expected to capture the
284 between-species variance in heat tolerance over a time scale which is convenient to
285 measure (Jørgensen, Malte, and Overgaard 2019). After being knocked down by
286 heat (40°C), most flies did not survive. In this case, only knockdown time was used

287 to evaluate resistance to heat. The knockdown experiments were conducted from
288 May to Jun 2019.
289 Virgin adult flies (siblings of those used for reproduction measurements) were
290 kept in same-sex groups at 25°C for 9-10 days before knockdown assays. Assays
291 were conducted for male and female flies separately. An observation rack was
292 divided into nine (3x3) cells. Each cell was randomly assigned one of the nine
293 *Drosophila* species and held seven flat-bottomed 3ml glass vials, each with a
294 randomly-selected individual of the allocated species. One set of observations on
295 such a set-up represents a single block. We repeated measurements for three
296 experimental blocks, and the allocation of species to cells was redrawn for each
297 block. In total, we measured 21 individuals per species per sex . During
298 measurement, the observation rack was moved immediately into the incubator pre-
299 set at 5°C or 40°C. Every tube was examined once every minute and flies that lost
300 their ability to stand in that minute were recorded. After exposure to 5°C for 30
301 minutes, all flies were in a chill coma. The observation rack was moved to a 25°C
302 room. Flies were left undisturbed and the time taken until each fly regained its ability
303 to stand was recorded.
304 *Regression analysis*: The knockdown time by heat, knockdown time by cold and
305 recovery time from cold of both sexes were first compared among species using
306 ANOVA. When interspecific variation was observed, they were modelled by *hIndex*,
307 *block*, and *cell position* as fixed effects, and *species* as a random effect in linear
308 mixed-effect models with phylogenetic correction using the *brms* package in R.
309 *Species* was included as a random effect to account for repeated measures.
310
311 5. Short-term competition

312 *Experimental design*: Pairs of species were reared in the same vials for one
313 generation to evaluate how the densities of intraspecific and interspecific competitors
314 influenced reproductive success under temperature regimes typical of lowland and
315 upland sites. We used incubators set at alternating temperatures mimicking day-time
316 and night-time temperatures in February at high-elevation (23°C / 21°C) and low-
317 elevation sites (28.5°C / 24°C) (Supplementary Figure 4). Adults to establish the
318 competition experiment were reared at moderate density (300 – 500 per bottle) at
319 their testing temperature regimes. After eclosion, individuals that emerged within the
320 same 48 hours were kept together in mixed-sex containers. Two days after the first
321 observation of egg-laying, adults of different sexes were separated, and from the
322 following day were allowed to compete and lay eggs in the 5 ml experimental vials
323 for two days before being discarded.
324 Five species were chosen as representative species for upland-biased, elevation-
325 generalist and lowland-biased distribution types (see Results for definition of
326 distribution types). The two-species combinations were: lowland species vs. upland
327 species: *D. bipectinata* vs. *D. pallidifrons*, *D. bipectinata* vs. *D. pseudotakahashii*,
328 *D.pandora* vs. *D. pallidifrons*; lowland species vs. lowland species: *D. bipectinata* vs.
329 *D.pandora*; lowland species vs. elevation-generalist: *D. bipectinata* vs. *D.*
330 *sulfurigaster*; upland species vs. elevation-generalist: *D. pallidifrons* vs. *D.*
331 *sulfurigaster*. Each combination was tested at different founding densities in a
332 factorial design: (4 pairs of species A, 2 pairs of species B), (4A, 4B), (4A, 8B), (2A,
333 4B) and (8A, 4B). We also included monocultures of each species with 2, 4, and 8
334 pairs. Each density and species combination was replicated ten times across two or
335 three blocks staggered by two days (two blocks for the *D. pandora* vs. *D. pallidifrons*
336 combination; three blocks for the other five pairs). Offspring that successfully

337 developed to adulthood were identified to species and counted. Experiment of *D.*
338 *pandora* vs. *D. pallidifrons* combination was conducted from September to
339 December, 2020 and the other five pairs were experimented from January to March,
340 2021.

341 *Competition models*: We used the Beverton-Holt model to describe the
342 population growth of a single generation of flies on discrete and temporary
343 resources:

344 $$N_{focal,t+1} = \frac{R_0 N_{focal,t}}{1 + a(N_{focal,t} + \beta N_{competitor,t})}$$

345 where R_0 is the generational reproduction rate and a is a constant defining the
346 form of the density-dependence relationship. β represents the interspecific
347 competition coefficient of the competitor species to the focal species, which defines
348 the equivalence between the two competing species. Offspring numbers of the focal
349 species were modelled assuming a negative binomial error distribution, under a
350 Bayesian framework using MCMC sampling within the *rstan* (Stan Development
351 Team 2021) package in R, as described by Terry et al. (2021). Model diagnostics are
352 shown in Supplementary Figure 5A-B. The medians of the posterior distribution were
353 used as the parameter values to infer the equilibrium state of each pair following
354 Hassell and Comins (1976).

355

356 6. Long-term competition

357 *Experimental design*: To evaluate the long-term impact of temperature and
358 competition on population size, a lowland species, *D. pandora*, and an upland
359 species, *D. pallidifrons*, were reared in monoculture and mixed-culture environments
360 for multiple generations under low-elevation and high-elevation temperature regimes
361 (Supplementary Figure 4).

362 Four monocultures of each species and eight mixed-species cultures were
363 maintained at each temperature regime for 13 weeks, totalling 24 cultures.
364 Monocultures were started with ten pairs of individuals. Mixed-species cultures were
365 started with ten pairs of individuals of each species. The starting density was very
366 low compared to the equilibrium density. The cultures were evenly divided into two
367 blocks starting on different dates. Each was maintained in a series of five bottles
368 following Ayala, Gilpin, and Ehrenfeld (1973). At the start of each week, adults
369 surviving in the most recent bottle and adults which were freshly emerged in the
370 older four bottles were separately collected, photographed and transferred together
371 into a new bottle with fresh food. In this way, adult survival and reproduction were
372 recorded separately. The total population size of each species was counted at the
373 end of the experiment. To avoid pseudo-replication introduced by 'incubator' effects,
374 the two incubators were switched between temperature regimes every week, with
375 their contents moved accordingly. Trays were shuffled inside the incubator every two
376 days. Temperature and humidity were recorded and the temperature regimes were
377 confirmed during and at the end of experiments. The experiment was conducted
378 from September to December, 2020.
379 *Data analysis*: Population sizes were modelled as a function of *temperature*,
380 *species*, *presence/absence of competitors*, and their interactions, with *culture ID* as a
381 random effect with a generalized linear mixed-effect model (family = "zero-inflated
382 negative binomial") using the *brms* package in R. Model diagnostics are shown in
383 Supplementary Figure 5C. To visualize the three-way interactive effects, the
384 posterior estimates of the high-temperature effect were plot against zero for the two
385 species with or without the presence of competitors; the posterior estimates of the

386 effect of the competition were plotted against zero for the two species in each
387 temperature regime.
388
389 All statistics mentioned in the *Methods* section were performed with R statistical
390 software, version 4.0.3 (R Core Team, 2020). Original data are achieved on Zenedo
391 (10.5281/zenodo.6400611) and code of analysis are available on GitHub
392 (https://github.com/Jinlinc/DistributionRegulators.git).
393
394
395 **Results**
396 *1.Field distributions*
397 The numbers of samples found at low-, medium- or high-elevation sites for
398 each of the nine major *Drosophila* species (accounting for 99% of all samples) are
399 shown in Figure 1. Distributions quantified using regression and by weighted
400 elevation (*hIndex*) were consistent (Spearman's rank correlation rho = 0.93, p value
401 = 0.0007). *Drosophila bipectinate* and *D. pandora* had significantly decreased
402 detection probability with increasing elevation and were thus categorized as lowland-
403 biased species with high confidence. *Drosophila pseudoananassae* showed a
404 lowland bias on one of the two mountains, and a mid-elevation peak on the other.
405 *Drosophila rubida*, *D. sulfurigaster* and *D. birchii* showed no significant change with
406 elevation and were thus defined as elevation generalists. *Drosophila pallidifrons* and
407 *D. pseudotakahashii* were significantly more likely to be found at high elevations and
408 were thus defined as upland-biased species. Coefficients and p values of the
409 regressions and *hIndex* are shown in Supplementary Table 2. For *D. bunnanda,* the
410 six records were insufficient for model fitting but all occurred at lowland sites, and

411 data from a larger-scale study suggest it is a lowland-biased species (Schiffer and
412 McEvey 2006).
413
414 *2.Thermal performance curves*
415 Thermal performance curves of daily fecundity per female vary among
416 species in terms of the range, optimal temperature, peak fecundity, and shape
417 factors (Figure 2; Table 1; see Supplementary Figure 2C for original data and fitted
418 curves for each species). The temperature for optimal reproductive performance,
419 *RTopt*, did not correlate with distribution patterns (Coefficient = 0.09, 95% credible
420 interval = -2.83 – 3.01; Supplementary Figure 6A). There was no general trade-off
421 between cold tolerance (estimated *RTmin*) versus heat tolerance (estimated *RTmax*)
422 corresponding to species' distribution types (Spearman's rank correlation rho = -0.6,
423 p value = 0.10; Supplementary Figure 6B). For example, the lowland-biased species
424 *D. bunnanda* has higher heat tolerance and lower cold tolerance than its elevation-
425 generalist relative, *D. birchii*. In contrast, *D. sulfurigaster* outperforms its upland-
426 biased relative, *D. pallidifrons*, across the temperature range.
427
428 *3.Cold tolerance*
429 Values of *RTmin* were not correlated with species distribution patterns (Figure
430 3a. Coefficient = -0.41, 95% credible interval = -4.15 – 3.43). Similarly, upland-
431 biased species did not show higher fecundity at the low temperature, 17°C (Figure
432 3b. Coefficient = -0.27, 95% credible interval -3.80 – 3.05). When exposed to acute
433 sublethal low temperature (5°C), all seven tropical *Drosophila* species showed
434 similarly poor performance compared to *D. simulans* and *D. melanogaster*
435 (Supplementary Table 3 for Tukey multiple pairwise comparisons). All species

436 recovered their fecundity after eight days at 14°C. This recovered fecundity was
437 slightly but non-significantly higher among upland species (Figure 3c. Coefficient =
438 0.33, 95% credible interval = -0.55 – 1.14). It took longer for upland species to regain
439 mobility after chill coma (Figure 3d. Male: coefficient = 13.04 (-9.31 – 35.05); female:
440 coefficient = 8.49 (-2.18 – 20.02).)
441
442 *4.Heat tolerance*
443 Regardless of the small variation of *RTmax* (standard deviation = 1.01)
444 compared with *RTmin* (standard deviation = 2.82), *RTmax* was significantly higher
445 among species with more lowland-biased distributions (Figure 3e. Coefficient = -
446 3.06, 95% credible interval = -5.30 – -0.88). Reproductive performance at 29°C also
447 decreased with *hIndex* (Figure 3f. Coefficient = -5.80, 95% credible interval = -9.37 –
448 -2.50). After exposure to 29°C for eight days, neither upland species could
449 reproduce when transferred back to 25°C, while four out of the five elevation-
450 generalist and lowland-biased species resumed reproduction (Figure 3g).
451 Knockdown time at lethally high temperature (40°C) was shorter among upland
452 species (Figure 3h. Male: coefficient = -6.71 (-13.70 – 0.50); female: coefficient = -
453 1.99 (-9.48 – 5.71)), indicating these species lose their mobility faster at high
454 temperatures.
455
456 *5.Interspecific competition*
457 When raised in a laboratory condition mimicking the warmer, lowland sites,
458 reproductive success was highest for the two lowland-biased species, followed by
459 the elevation-generalist species, *D. sulfurigaster*. The two upland species could
460 barely reproduce regardless of the presence of competitors (proliferation rates (R0)

461 of *D. pallidifrons* and *D. pseudotakahashii* were lower than 1; Figure 4; Table 2).
462 Their competitive effect on lowland species was minimal, indicated by low β values
463 (Table 2). The two lowland species, *Drosophila pandora* and *D. bipectinata*, are
464 expected to coexist stably, based on their reproductive and competitive parameters.
465 When raised a under cooler, upland condition, all species could reproduce
466 and sustain their populations (all proliferation rates (R0) were higher than 1; Table
467 2). Lowland species were strongly affected by the density of *D. pallidifrons*, an
468 upland species, while upland species were significantly less affected by lowland
469 species. Competition with *D.pallidirons* under upland conditions was predicted to
470 drive *D. pandora* and *D. bipectinata* to exclusion (Table 2).
471 In the long-term competition experiment, high temperature drove the upland-
472 species *D. pallidifrons* to extinction regardless of the starting species composition
473 (monoculture versus mixed-species culture) (Figure 5a). In contrast, the monoculture
474 of the lowland-species *D. pandora* remained abundant at both temperatures (Figure
475 5b: 90% credible interval of temperature effect in monoculture overlaps with zero).
476 The interspecific competition effect was only significant at the upland regime (Figure
477 5c), which significantly reduced the population size of *D. pandora* in mixed-species
478 cultures compared to the monocultures (Figure 5a and Figure 5b).
479
480
481 **Discussion**
482 Counter to expectation, our results do not support the common assumption
483 that cool boundaries to species' ranges are constrained abiotically (reflecting thermal
484 niches), while biotic interactions (such as interspecific competition) define warm
485 boundaries. Instead, we found that maximum temperatures experienced on a daily

basis at lowland sites were sufficiently high to serve as an environmental filter. In cooler, high-elevation conditions, lowland species were outcompeted by certain upland species whose distribution were confined to high elevations as a result of their intolerance to heat.

1.Contributions of abiotic and biotic factors in determining distributions

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

511 interactions may be needed to understand how and why the governing rules might
512 change under different thermal and biotic regimes (Amundrud and Srivastava 2020;
513 Srinivasan et al. 2018).

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

526 Field experiments on *D. birchii* (O'Brien et al. 2017, 2020), found that its
527 reproduction when raised alone was highest at low elevations, where both
528 intraspecific and interspecific competition with its sister species *D. bunnanda* were
529 also high. In our experiments, *D. birchii* was one of the most heat-sensitive species,
530 and its populations were unable to persist at high temperatures typical of low
531 elevations (pilot experiment, unpublished data). This discrepancy can be explained
532 by different temperature regimes used in our study (corresponding to February
533 temperatures in the field, with an average of 26°C and average daily maximum of
534 30.6°C at lowland) and by O'Brien et al. (April temperatures, which are around 2°C
535 lower on average temperature and on average daily maximum). A further possibility

536 is that single-generation experiments initiated with mated adult flies did not capture
537 the impact of high temperatures prior to sexual maturation. These observations
538 further highlight the sensitivity of measures of thermal performance to experimental
539 conditions. They also suggest that the relative importance of abiotic and biotic
540 factors in determining species distributions may change seasonally.
541
542 *2.Low variation in upper thermal limits*
543 Upper thermal limits vary little among species (Hoffmann 2010) and have
544 limited adaptive potential (van Heerwaarden and Sgrò 2021; Kellermann et al. 2012).
545 Consistent with other studies (Goulet, Thompson, and Chapple 2017; Hangartner
546 and Hoffmann 2016), we found that heat tolerance is a systematic trait manifested in
547 critical temperatures, fecundities at sub-sterile temperatures, recovered fecundities,
548 and locomotive responses. Modest changes in critical temperature can therefore
549 lead to pronounced differences in overall performance under real and variable
550 temperature regimes.
551 Such small thermal safety margins suggest a severe threat of biotic attrition in
552 tropical lowlands (Colwell et al. 2008; Deutsch et al. 2008; Duarte et al. 2012; van
553 Heerwaarden and Sgrò 2021). Laboratory-measured critical temperatures can be
554 sensitive to experimental conditions, making it difficult to relate them to climatological
555 means or maxima, and hence the threat of rising temperatures (Sinclair et al. 2016).
556 In this context, our study benefits from a comparative approach, which reveals that
557 upland species are already constrained by high temperatures, and that a very small
558 difference (about 1°C) distinguishes the upper thermal limits (*RTmax*s) of lowland
559 and upland species. Given the low evolutionary potential of heat tolerance
560 (Hoffmann, Chown, and Clusella-Trullas 2013), both lowland species and upland

species are likely to be vulnerable to modest temperature increases across the elevation gradient. Thus, lowland biotic attrition and upland range contraction are likely to occur with future warming, leading to cascading effects in lowland communities and threatening endemic upland species on tropical mountains such as those in the Australian Wet Tropics (Freeman et al. 2018).

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

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

4. Context-dependence of thermal performance

Inevitably, thermal traits measured under laboratory conditions cannot account for the tendency of thermal tolerances to vary with a range of abiotic factors including, but not limited to precipitation (Bozinovic and Pörtner 2015; Kellermann et al. 2012), diet, and larval conditions (Bubli, Imasheva, and Loeschcke 1998). Such

variables are to a large extent standardized in our study, allowing dependence on abiotic conditions to be resolved. Our long-term experiment showed that the thermal response of population size to temperature depended on competitive conditions, highlighting the difference between thermal performance curves of organisms and their populations when biotic interactions are present (Davis et al. 1998). There is a growing realisation that sensitivity to temperature depends on the biotic environment (Gilman et al. 2010): it is not only the demographics of the interacting species that are affected by temperature (Huey and Kingsolver 1989); interactions themselves are altered (Burnside et al. 2014).

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

5. Conclusions

Tropical ecosystems host an exceptional diversity of endemic species (Laurance et al. 2011). Predicting their sensitivity to climate change is a high priority and requires an understanding of the proximate causes of current distributions as well as the interactions between thermal tolerance and other environmental factors, both biotic and abiotic. Our study contributes to the growing literature demonstrating

that species can be sensitive to warming at the warm boundaries of their distributions. In particular, we highlight the important role of daily maximum temperatures in structuring community at tropical lowlands, the essential role of interspecific competition at uplands, and the vulnerability of species there to increasing mean temperatures and temperature extremes.

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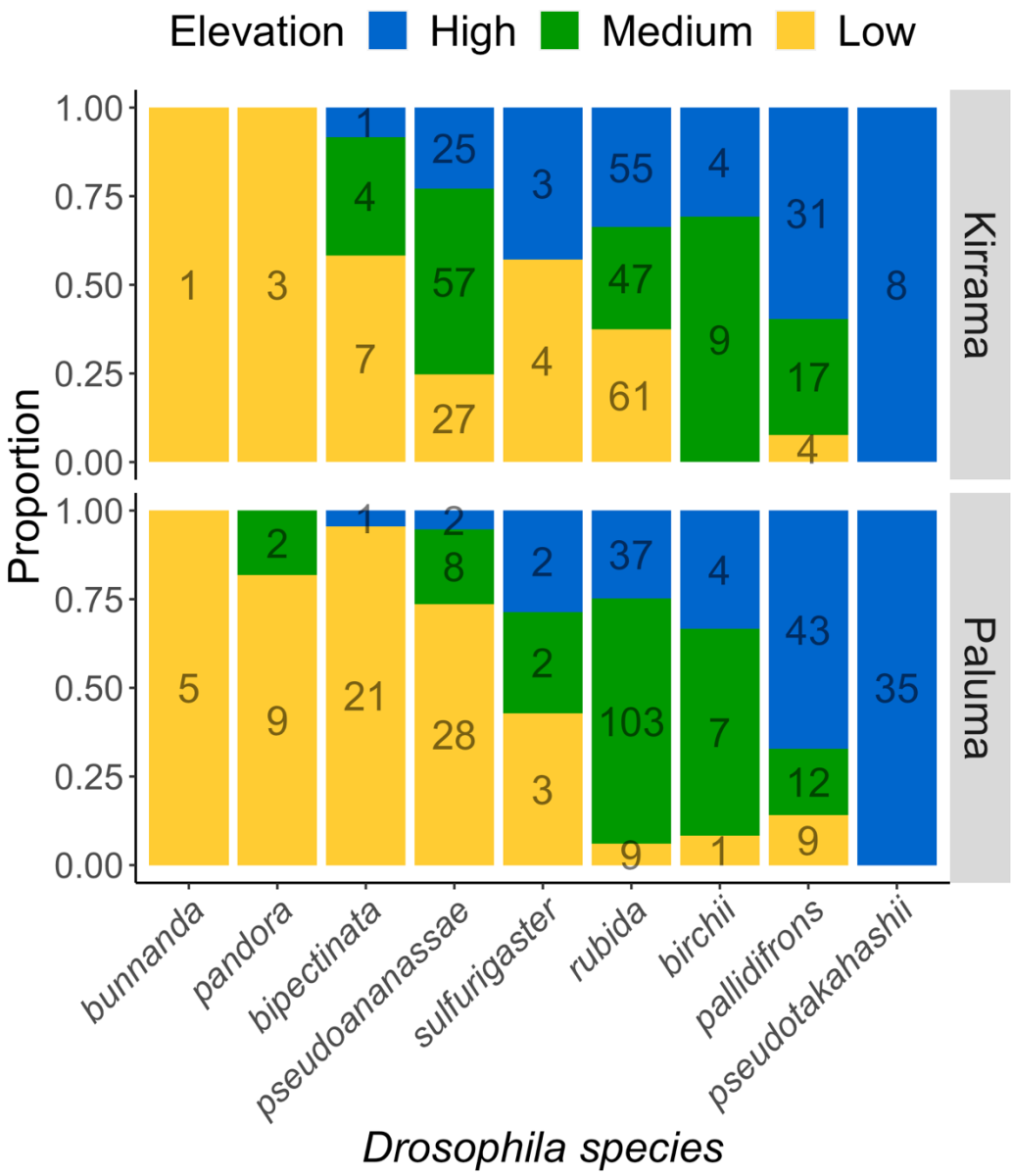
891 **Figure 1.** Distribution patterns of nine rainforest *Drosophila* species at two study
892 sites, Mount Kirrama and Mount Paluma. The proportion of samples found at sites at
893 low (yellow), medium (green) and high (blue) elevations is shown for each species.
894 Numbers on the bars show the counts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



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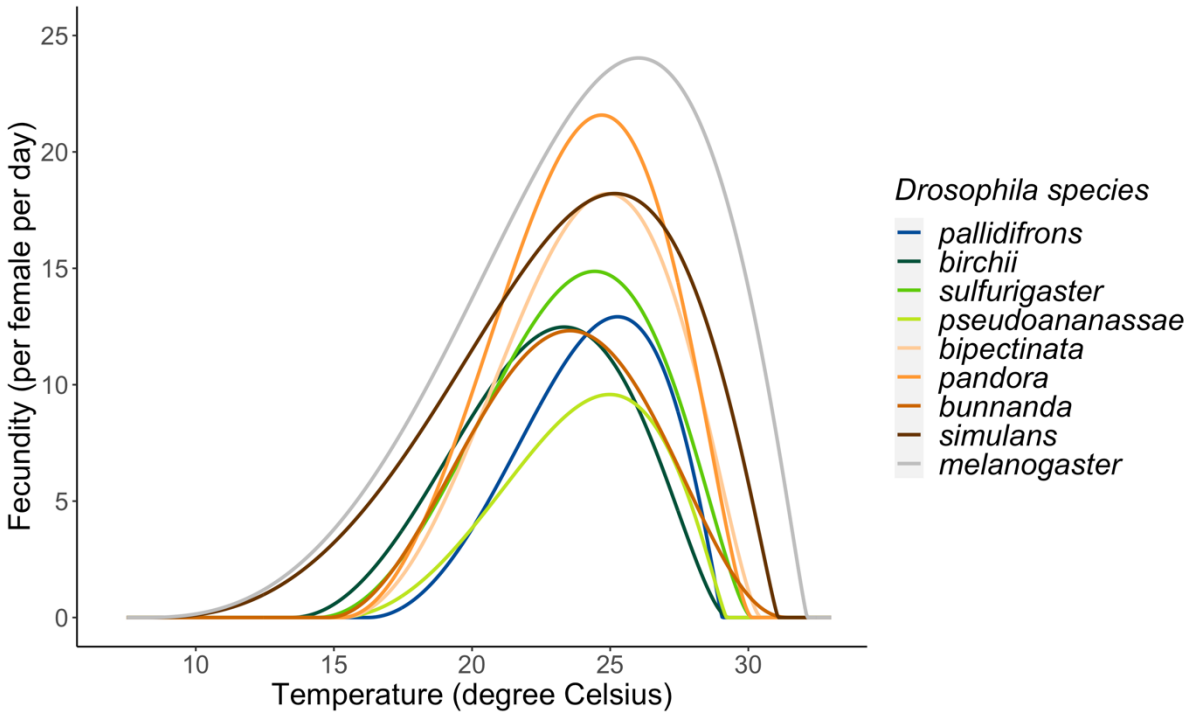
897

898 **Figure 2.** Thermal performance curves for reproduction of nine *Drosophila*
899 species. The fitted numbers of adult offspring produced per female parent per day
900 are plotted against temperature. Line colours indicate species' distribution types, with
901 upland-biased species represented by cold colours and lowland-biased species
902 represented by warm colours.



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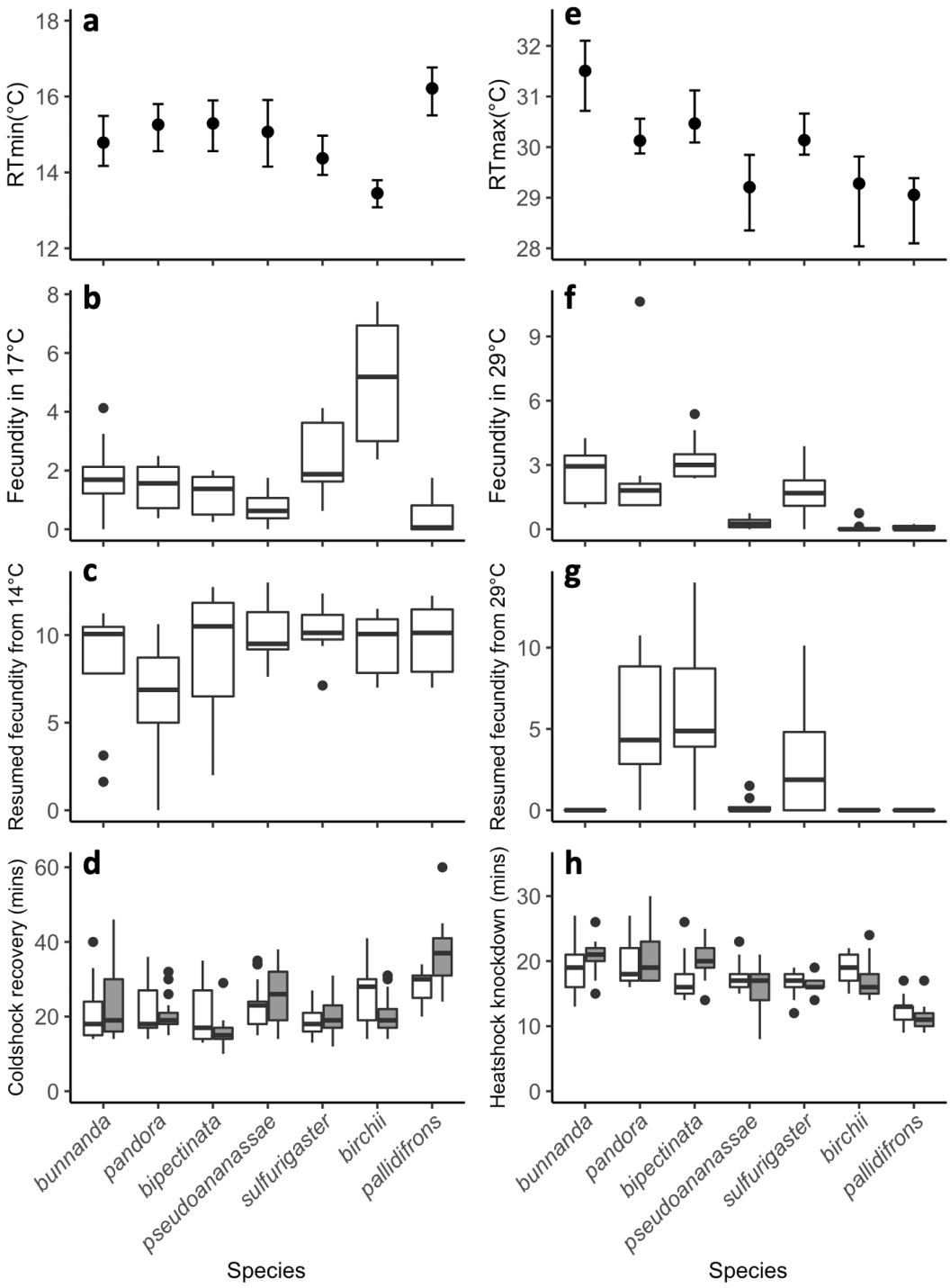
905 **Figure 3.** Reproductive and physiological thermal tolerance of seven tropical
906 *Drosophila* species. Species are ordered by their distribution patterns, from lowland-
907 biased species (left) to upland-biased species (right). Cold tolerance is represented
908 by *RTmin* (a), fecundity at 17°C (b), recovered fecundity after 14°C (c) and recovery
909 time after chill coma (d). Heat tolerance is represented by *RTmax* (e), fecundity at
910 29°C (f), recovered fecundity after 29°C (g) and knockdown time at high temperature
911 (h). Graph (a) and (e) show the medians and 90% credible intervals of the posterior
912 distribution of the estimated parameters. Boxplots in b-d and f-h show the minimum,
913 25th percentile, median, 75th percentile, maximum and potential outliers. In (d) and
914 (h), data for females (shaded) and males (unshaded) are plotted separately.


a
RTmin(°C)
18
16
14
12
e
RTmax(°C)
32
31
30
29
28
b
Fecundity in 17°C
8
6
4
2
0
f
Fecundity in 29°C
9
6
3
0
c
Resumed fecundity from 14°C
10
5
0
g
Resumed fecundity from 29°C
10
5
0
d
Coldshock recovery (mins)
60
40
20
0
h
Heatshock knockdown (mins)
30
20
10
0
bunnanda
pandora
bipectinata
pseudoananassae
sulfurigaster
birchii
pallidifrons
Species
Species

915

916

Figure 4. Interspecific competition for species pairs under upland (left) and lowland (right) temperature regimes. Each line shows the fecundity of the focal species when its founder number is kept at four pairs while changing the number of individuals of the competing species. The identity of the focal species in each pair is indicated by the colour of the line (see legend) and by the label (e.g. for BIP_PST, the first code BIP is the focal species and the competitor species is PST). The shaded area indicates the 90% credible interval of the fitted values under the Beverton-Holt model for pairwise species competition. PST = *D. pseudotakahashii*, PAL = *D. pallidifrons*, SUL = *D. sulfurigaster*, BIP = *D. bipectinata*, PAN = *D. pandora*.

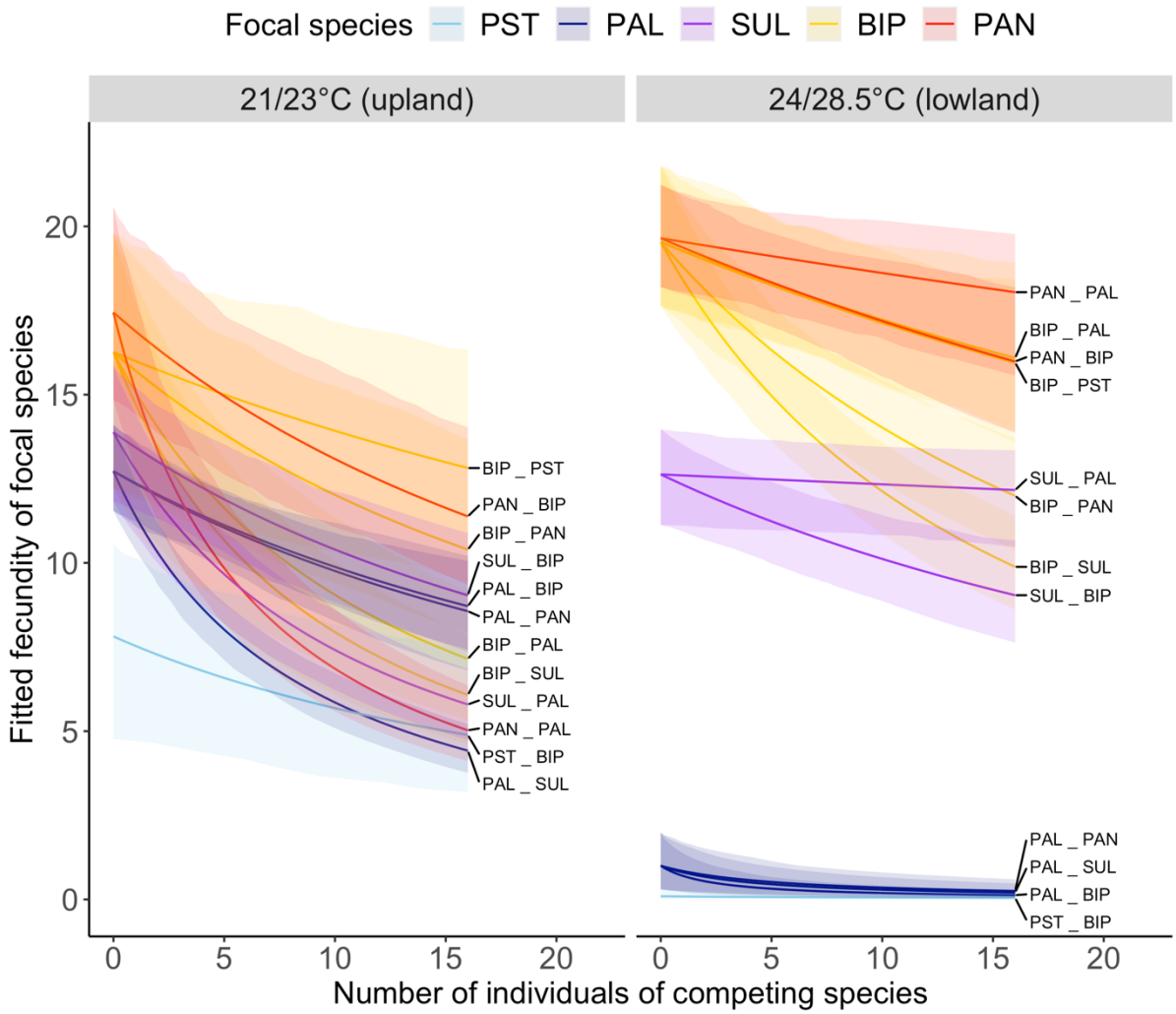
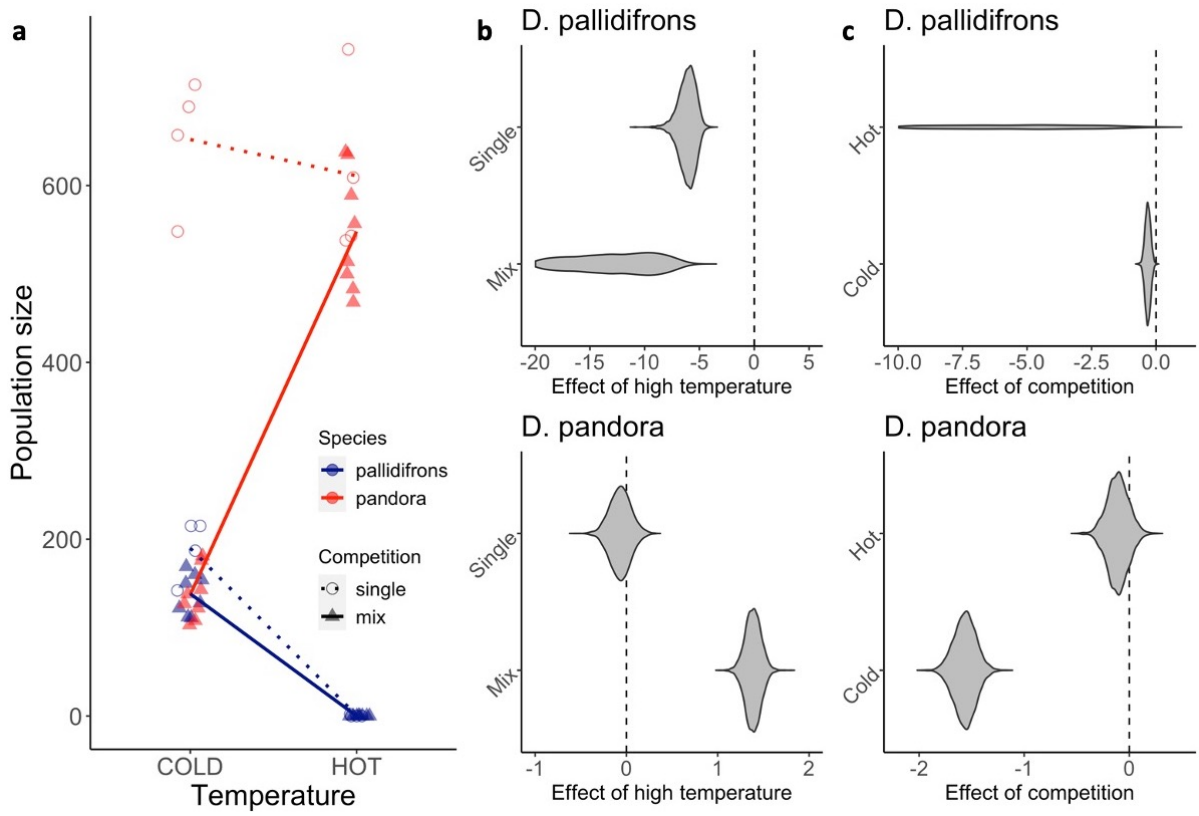


Figure 5. Effects of temperature and interspecific competition on population sizes of *D. pallidifrons* and *D. pandora*. a) The ending population sizes of *D. pallidifrons* and *D. pandora* which were initiated in monoculture (single) or mixed-species culture (mix) under upland (cold) and lowland (hot) temperature regimes. b) the posterior distributions of the effect of high temperature (coefficient of temperature) when the indicated species were maintained alone (single) or with the other species (mix). c) the posterior distributions of the effect of competition (coefficient of competition) when the indicated species were maintained at upland (cold) and lowland (hot) temperature regimes.



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

species	a	a.ci90	b	b.ci90	RTmin	RTmin.ci90	RTmax	RTmax.ci90
D. bipectinata	0.0046	0.0031 - 0.0060	1.27	1.02 - 1.55	15.28	14.54 - 15.87	30.44	30.08 - 31.05
D. birchii	0.0034	0.0022 - 0.0056	1.17	0.95 - 1.56	13.45	13.07 - 13.77	29.27	28.12 - 29.81
D. bunnanda	0.0016	0.0012 - 0.0025	0.88	0.81 - 1.06	14.57	14.10 - 15.18	31.21	30.60 - 31.78
D. melanogaster	0.0037	0.0032 - 0.0042	1.72	1.48 - 2.03	8.29	6.89 - 9.37	32.13	32.03 - 32.28
D. pallidifrons	0.0073	0.0056 - 0.0098	1.74	1.37 - 2.38	16.23	15.54 - 16.75	29.06	28.19 - 29.38
D. pandora	0.0052	0.0037 - 0.0065	1.26	1.03 - 1.51	15.25	14.58 - 15.80	30.13	29.87 - 30.58
D. pseudoananassae	0.0053	0.0035 - 0.0071	1.68	1.24 - 2.32	15.06	14.17 - 15.89	29.22	28.38 - 29.77
D. simulans	0.0035	0.0027 - 0.0046	1.69	1.36 - 2.21	8.51	6.94 - 9.64	31.09	30.39 - 31.78
D. sulfurigaster	0.0040	0.0028 - 0.0051	1.27	1.03 - 1.52	14.37	13.91 - 14.96	30.11	29.84 - 30.63

941

942 **Table 2.** Fitted values of the parameters of the competition model and predicted equilibrium states for pairwise interspecific
943 competition. R_0 is the reproductive rate, α is a constant defining the form of the density-dependence relationship, β is the
944 interspecific competition coefficient, and ci90 represents the 90% credible intervals of each parameter. The equilibrium states of the
945 focal species are inferred based on Hassell and Comins (1976).

Temperature	Focal species	R_0	R_0.ci90	α	α.ci90	Competitor	β	β.ci90	Equilibrium state of the focal species
Cool, upland	BIP	11.36	8.06-15.21	0.05	0.02-0.09	PAL	2.26	1.4-4.08	excluded
						PAN	0.99	0.47-1.95	excluded
						PST	0.47	0.12-1.09	stable coexistence
						SUL	2.95	1.91-5.25	excluded
	PAL	27.94	19.52-38.66	0.42	0.27-0.64	BIP	0.3	0.15-0.49	dominant
						PAN	0.32	0.14-0.52	dominant
						SUL	1.22	0.9-1.62	unstable coexistence
	PAN	13.68	10.4-17.85	0.07	0.04-0.12	BIP	0.74	0.33-1.36	dominant
						PAL	3.41	2.26-5.59	excluded

	PST	6.27	3.4-10.66	0.08	0.03-0.19	BIP	0.79	0.35-1.76	stable coexistence
	SUL	20.96	14.27-31.13	0.25	0.14-0.44	BIP	0.41	0.19-0.67	dominant
						PAL	1.05	0.71-1.53	unstable coexistence
Warm, lowland	BIP	15.35	12.51-19.05	0.07	0.05-0.11	PAL	0.29	0.07-0.63	dominant
						PAN	0.87	0.54-1.35	stable coexistence
						PST	0.31	0.07-0.63	dominant
						SUL	1.35	0.93-2	excluded
	PAL	0.99	0.19-2.37	0.12	0.02-0.46	BIP	6.81	2.98-22.27	unable to establish
						PAN	2.99	1.52-8.93	unable to establish
						SUL	3.98	1.77-12.83	unable to establish
	PAN	17.18	14.2-21.24	0.09	0.06-0.14	BIP	0.27	0.08-0.51	stable coexistence
						PAL	0.11	0.01-0.29	dominant
	PST	0	\	\	\	BIP	\	\	unable to establish
	SUL	13.65	10.26-19.03	0.15	0.09-0.24	BIP	0.37	0.18-0.62	dominant
						PAL	0.04	0-0.14	dominant