Fitness effects of competition within and between species change across species' ranges, and reveal limited local adaptation in rainforest *Drosophila*

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Abstract

Competition within and between species can have large effects on fitness and may therefore drive local adaptation. However, these effects are rarely tested systematically, or considered when predicting species' responses to environmental change. We used a field transplant experiment to test the effects of intra and interspecific competition on fitness across the ecological niches of two rainforest *Drosophila* species that replace each other along an elevation gradient. For the species with the broader elevational range, we also tested for adaptation to the local abiotic and biotic environment. In both species, intraspecific competition reduced productivity more than interspecific competition at the centre of its elevational range, while interspecific competition had a stronger effect at the range edge, where the competing species is more abundant. Local adaptation was detected in the centre of the range of the more widespread species, but only in the presence of intraspecific competition. This study is the first to demonstrate that fitness effects of inter-specific competition increase at ecological margins, while intra-specific competition has more pervasive effects at range centres. This is a key assumption of "tangled bank" models of community evolution and has

34 important implications for predicting the resilience of ecological networks to
35 global change.

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39 **Introduction**

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42 Darwin (1859) used the metaphor of the “entangled bank” to describe the way
43 that interactions within and among species structure an ecological community,
44 due to the narrow range of conditions within which a given species can
45 successfully compete with a neighbouring species. Competitive interactions
46 can have large effects on fitness and are therefore likely to drive adaptive
47 divergence within species (e.g. Stuart et al. 2014, Hargreaves et al. 2019). If
48 we assume a trade-off between resistance to antagonistic interactions such
49 as competition, parasitism or predation, and tolerance of abiotic conditions
50 such as temperature or humidity, then antagonistic biotic interactions should
51 narrow a species’ environmental niche by reducing the range of conditions
52 within which it can persist, causing the evolution of ecological specialisation in
53 communities (Kneitel & Chase 2003; Poisot et al. 2011). Such theory predicts
54 that competition within a species (intraspecific competition) should have the
55 strongest effect on fitness at the centre of the species’ distribution but will
56 become less important towards the range edge. By contrast, at species’
57 margins, a given level of interspecific competition should have a bigger effect
58 on fitness, leading to species’ turnover along ecological gradients.
59 Interspecific competition should therefore increase in its effects on fitness at

60 or beyond the margins of a species' range, especially where other closely
61 related species (and likely competitors) increase in frequency.
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63 Spatial variation in the effects of competition on fitness should cause local
64 adaptation if these effects are consistent over time, especially where there is
65 a trade-off between competitive success and resistance to abiotic stress.
66 Studies in angiosperms have found that competition can either increase (e.g.
67 Bischoff et al. 2006; Rice & Knapp 2008) or decrease (e.g. Bischoff et al.
68 2006) the magnitude of local adaptation. In a meta-analysis of field studies,
69 mostly in plants, Hargreaves et al. (2019) found that local adaptation was
70 neither more prevalent nor stronger in the presence of biotic interactions
71 (including competition), compared with cases where biotic interactions were
72 excluded, despite strong effects of biotic interactions on fitness. However,
73 this effect varied with latitude: in tropical environments, local adaptation was
74 more prevalent when biotic interactions were left intact, however this was not
75 the case in temperate environments.
76
77 Strong and pervasive effects of competition on fitness may be more likely in
78 low latitude (tropical) ecosystems, where productivity and biodiversity are
79 generally much higher than in temperate ecosystems, where stronger
80 seasonal fluctuations continually reduce or reset biotic interactions (e.g. Coley
81 & Barone 1996; Schemske et al 2009). At a given latitude, the effects of biotic
82 interactions on fitness should also vary along elevational gradients, given their
83 pervasive effect on community structure, species turnover, and species'
84 ecology, as well as climatic factors (e.g. Rahbek 1995; Körner 2007; Morris et

85 al. 2015). Consistent with expectations along latitudinal gradients, biotic
 86 interactions should be a more important determinant of species' ecological
 87 limits at lower elevation ("warm") margins, compared with high elevation
 88 ("cold") margins where effects of abiotic factors (e.g. temperature,
 89 precipitation) are relatively stronger (e.g. Davis et al. 1998; Pearson &
 90 Dawson 2003).

91

92 Ecosystems where closely related and ecologically similar species replace
 93 each other across predictable environmental gradients provide excellent
 94 opportunities to test how competition within and among species affects fitness
 95 and drives local adaptation, and how these effects change as species
 96 approach their ecological limits. In practice however, it is difficult to
 97 disentangle the effects of competition from those of abiotic factors (e.g.
 98 temperature, precipitation) on fitness or as drivers of adaptive divergence,
 99 because different sources of environmental variation are typically correlated
 100 (Godsoe *et al.* 2017). Experiments that manipulate competition and abiotic
 101 environmental variation independently are therefore essential for
 102 understanding how environmental change mediates the evolution of species
 103 interactions.

104

105 In the Australian tropical rainforest fruit fly *Drosophila birchii*, transplant
 106 experiments have demonstrated that abiotic factors alone cannot explain the
 107 species' field abundance across its climatic range, suggesting an important
 108 role for biotic interactions. O'Brien et al. (2017) tested how the fitness of
 109 families of *D. birchii* varied when virgin flies were transplanted at a fixed

110 density into field cages at 10 sites along each of two elevation gradients and
 111 allowed to mate and produce offspring. They found that fitness (estimated by
 112 number of offspring produced) in field cages was highest at the warmest, low
 113 elevation sites and declined with increasing elevation. This pattern contrasted
 114 with patterns of field abundance, where *D. birchii* was rare at low elevation
 115 sites but increased in abundance with elevation up to ~900m asl. However,
 116 given these field cages contained only a single species at low density, the
 117 effects of competition and other biotic interactions on fitness were not
 118 included. The results from these transplant experiments therefore suggested
 119 that although abiotic factors (probably cold limits) can explain the upper limit
 120 to *D. birchii*'s elevational range, biotic interactions limit population growth at
 121 the warm edge of *D. birchii*'s range, leading to a mismatch between cage
 122 productivity and field abundance at low elevations. Despite observing
 123 substantial genetic variation in fitness of *D. birchii* in field cages (and clinal
 124 divergence in productivity under laboratory conditions), O'Brien et al (2017)
 125 did not detect any evidence for local adaptation in these field transplants: *D.*
 126 *birchii* families transplanted to their home site did not have higher fitness
 127 when compared with those transplanted from the opposite extreme of the
 128 species' elevational range. However, if competitive interactions are a major
 129 determinant of fitness across the elevational range of *D. birchii*, local
 130 adaptation may only be revealed in an environment that includes these
 131 interactions.

132

133 In this study, we use a large-scale field transplant experiment to quantify the
 134 effects of ecologically-realistic variation in the intensity of intraspecific and

135 interspecific competition on variation in fitness and life history traits of *D.*
 136 *birchii* and its phylogenetically and ecologically-close relative, *D. bunnanda*. In
 137 a novel advance on most previous studies, we conduct these assays of
 138 density dependent variation in fitness at sites along one of the elevational
 139 gradients used in O'Brien *et al.* (2017), which we already know includes the
 140 cold and warm extremes of these species' distributions. In addition, for *D.*
 141 *birchii*, which is found across a broader range of elevations than *D. bunnanda*,
 142 we tested for local adaptation by transplanting flies from different populations
 143 across their elevational range. This comparison tested whether local
 144 adaptation was revealed by comparing responses of flies from different
 145 populations to increasing inter and intraspecific competition at different
 146 elevations along the gradient.

147

148 We tested the following hypotheses:

149 (1) *The fitness effects of competition is strongest at the warm edge of the*
 150 *species' ranges:* Competition within and between *D. birchii* and *D.*
 151 *bunnanda* will reduce fitness, but the strength of this effect will vary
 152 across the elevational gradient. We predict a stronger effect of
 153 competition on fitness at lower elevations, consistent with the
 154 expectation that antagonistic biotic interactions are more ecologically
 155 important at the warm edges of species' ranges.

156

157 (2) *Competition within and between species causes species to replace*
 158 *each other along environmental gradients:* Increasing intraspecific
 159 competition will have a greater negative effect on fitness than

160 interspecific competition for a given species within cages transplanted
 161 into the elevational range where that species has the highest relative
 162 abundance (high elevation for *D. birchii*; low elevation for *D.*
 163 *bunnanda*). By contrast, increasing interspecific competition will have
 164 a greater effect than intraspecific competition within cages transplanted
 165 to elevations where the competitor species has higher relative
 166 abundance.

167

168 (3) *Selection on competitive ability at different elevations causes local*
 169 *adaptation within species: Drosophila birchii* reared from populations
 170 close to the transplant location will suffer lower reductions in fitness in
 171 response to increasing levels of competition than those reared from
 172 populations further away on the elevational gradient. At high elevation
 173 (where *D. birchii* is most abundant), intraspecific competition will be a
 174 stronger driver of fitness and therefore local adaptation. By contrast,
 175 local adaptation will be more strongly associated with interspecific
 176 competition at lower elevations, where *D. birchii* is outnumbered by the
 177 competitor species (*D. bunnanda*), and intraspecific interactions are
 178 relatively rare.

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182 **Methods**

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184 ***Study species***

185

186 *Drosophila birchii* and *D. bunnanda* (Diptera: Drosophilidae) are both confined
 187 to tropical rainforest habitat, with distributions that overlap at lower latitudes

188 and elevations. They are closely related, similar in size and likely to target
 189 similar food and oviposition resources at sites where they co-occur. They do
 190 not hybridise in the laboratory (personal observation), and there is no
 191 evidence for hybridisation in the wild. *Drosophila birchii* has a broader
 192 latitudinal range than *D. bunnanda* (van Heerwaarden et al. 2009), and a
 193 broader elevational range within latitudes, with *D. bunnanda* confined to
 194 warmer sites at low latitudes and elevations. At the sites sampled for this
 195 study, *D. bunnanda* typically outnumbers *D. birchii* ~3:1 in field traps below
 196 100m elevation, then it declines in abundance at higher elevations. The two
 197 species have roughly equal abundance ~400m asl, and *D. bunnanda* is
 198 virtually absent above 500m asl, where abundance of *D. birchii* increases,
 199 reaching its maximum around 900m asl (Figure 1; Bridle et al. 2009; O'Brien
 200 et al. 2017).

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Source of flies used in field transplant experiment

205 In April 2016, we established 80 *D. birchii* isofemale lines from field-mated
 206 females collected at two high elevation (~900m above sea level (a.s.l.)) and
 207 two low elevation (<100m a.s.l.) sites at each of two gradients separated by
 208 ~300 km of latitude: Paluma (19°00' S, 146°14' E) and Mt Lewis (16°35' S,
 209 145°19' E)(Eight sites in total). Each isofemale line was founded by a single,
 210 field-mated female. These ten *D. birchii* isofemale lines per site were
 211 maintained in the laboratory for 10 months (~20 generations). Two
 212 generations before establishment of the cage transplant experiment, we
 213 mixed lines from the same site together to create eight mass-bred *D. birchii*
 214 populations (Supplementary Note 1).

215

216 We established isofemale lines of the competitor species, *D. bunnanda*, using
 217 the same method as for *D. birchii*. However, because *D. bunnanda* is absent
 218 above 500m, these lines all came from low elevation sites at each of the two
 219 gradients where *D. birchii* was collected. We maintained five *D. bunnanda*
 220 lines from each of Paluma and Mt Lewis (10 *D. bunnanda* lines in total) in the
 221 laboratory over the same period and under the same conditions as for the *D.*
 222 *birchii* lines. We then combined them all to establish a single mass bred
 223 population of *D. bunnanda* (Supplementary Note 1).

224

225 All isofemale lines and mass-bred populations were maintained at 23 °C on a
 226 12:12hr light:dark cycle prior to establishment of the field experiment.

227

228

229 Establishment of field transplant experiment

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231 Two generations after mixing, we separated emergees from mass-bred
 232 populations by sex under light CO₂ anaesthesia within 24 hours of emergence
 233 every day over seven days, and held them in single-sex vials (maximum
 234 density 10 flies). This ensured they were unmated at the start of the
 235 experiment, and that all courtship and mating occurred within field cages. We
 236 kept them in single-sex vials for a minimum of 72 hours to recover from the
 237 effects of CO₂ before transplant into field cages. The long collection period
 238 was necessary to obtain sufficient numbers of flies but meant that
 239 experimental flies varied in age from 3 – 10 days when they were put in field
 240 vials. To avoid confounding effects of such variation in age, we mixed

241 emergees from different emergence days within each population prior to
 242 establishment of field vials.

243
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 245 We transplanted all populations of *D. birchii* and *D. bunnanda* in vials along
 246 one of the field elevation gradients where flies were sourced (Paluma). The
 247 two gradients from which the original lines were collected have very similar
 248 ranges of abiotic conditions (temperature and humidity), which change in the
 249 same way with increasing elevation (O'Brien *et al.* 2017). We established
 250 transplant cages at three elevations ('sites'): Low (80m above sea level
 251 (a.s.l.)), Mid (450m a.s.l.) and High (900m a.s.l.). The low and high transplant
 252 sites included the sites from which the Paluma isofemale lines were sourced.
 253 To account for localised environmental heterogeneity within each elevation,
 254 we divided each site into five sub-sites ('blocks') of roughly equal size, giving
 255 15 blocks in total. Details on variation in the abiotic environment along the
 256 gradient are provided in Supplementary Note 2 and Figure S1.

257
 258
 259 We transplanted flies in 30 ml plastic vials containing 5 ml of standard
 260 *Drosophila* media. Vials were closed with a square of muslin secured with a
 261 rubber band, which prevented flies from getting in or out, but allowed free air
 262 exchange with the outside, meaning conditions inside the vials tracked
 263 external temperature. We placed vials in holders constructed from 600 ml
 264 plastic bottles with two 135 x 95 mm windows cut out of the sides, ensuring
 265 maximal flow of air around the vial openings. We placed between two and
 266 four food vials in each bottle and hung bottles from tree branches at a height
 267 1.3 – 1.8 m above the ground. We suspended a 26cm plastic plate upside

268 down on the twine above each bottle to protect vials from rain and encased
 269 each bottle in strong wire mesh (20mm square holes) to prevent damage by
 270 vertebrates (particularly birds and small mammals).
 271
 272 We transplanted 19 656 virgin flies (11 808 *D. birchii* and 7 848 *D. bunnanda*)
 273 in 972 vials at a range of intraspecific and interspecific densities (see
 274 'competition treatments' below) into the three transplant elevations in May
 275 2017. We placed flies in vials less than 24 hours before they were installed in
 276 the field and left them in vials at their respective transplant sites for 10 days.
 277 Therefore, virtually all courtship, mating and egg-laying happened under field
 278 conditions. After 10 days, we removed and discarded any surviving flies, and
 279 left vials in the field until emergence began. This was 14 days after the
 280 establishment of vials at low elevation, 17 days at mid elevation, and 21 days
 281 at high elevation. On the day that the first emergence was observed, all vials
 282 at that transplant elevation were removed and taken to the laboratory to
 283 enable daily emergence to be recorded accurately. Vials were held in a
 284 constant temperature room set to the same mean temperature as the
 285 elevation at which they had been transplanted, determined using data from
 286 the dataloggers inside cages at that site (Supplementary Note 2; Figure S1),
 287 on a 12:12 hr light:dark cycle at 60% relative humidity (RH). At all transplant
 288 blocks, virtually all larvae had pupated by the time vials were brought in from
 289 the field.

291 **Competition treatments**

292 We used a response surface design (Inouye 2001), which independently
 293 varied the numbers of each species and enabled us to estimate the effects of
 294 intraspecific vs interspecific competition. We used 10 treatments, each
 295 defined by the number of *D. birchii* and *D. bunnanda* in a vial, with a total
 296 density of 6, 12, 24 or 48 flies (Figure 2). In a pilot study, we found that the
 297 average productivity of *D. birchii* declined with increasing intraspecific density
 298 across this range of densities (Supplementary Note 3; Figure S2),
 299 demonstrating that it was an appropriate range for detecting competition
 300 effects. We also verified that the size of flies emerging from each treatment
 301 lay within the size range of field-caught flies at the same elevation
 302 (Supplementary Note 4, Figure 3), implying that our competition treatments
 303 are ecologically realistic. We introduced equal numbers of unmated males
 304 and females (of each species, for mixed species treatments) into each vial,
 305 and transplanted five replicate vials of each Population x Treatment
 306 combination to each site (one replicate per block).

307

308 ***Measuring traits of flies emerging from field vials***

309 We removed and counted the number of emergees of each species and sex
 310 from each vial on the day emergence began at the transplant site, then daily
 311 for the next 10 days and then every three days for an additional nine days to
 312 capture any late emergence (20 days total from start of emergence). We
 313 undertook species identification and trait measurements blind with respect to
 314 treatment or transplant elevation. Male *D. birchii* and *D. bunnanda* were
 315 distinguished by their genital bristles (Schiffer & McEvey 2006). Females
 316 were identified based on differences in their pigmentation: the dark bands on

the dorsal abdomen are straight with sharp edges in *D. bunnanda*, whereas in *D. birchii* they rise in the centre and are more diffuse (M. Schiffer personal communication, and personal observation). For each species emerging from each vial, we recorded the number of male and female offspring emerging each day, to obtain values for the following: (1) productivity (total number of offspring per laying female), (2) offspring sex ratio (number of male offspring as a proportion of the total) for each vial, and (3) emergence time of each fly. We then mounted, photographed and landmarked the right wing of each fly to obtain a measure of (4) wing size, as a proxy for body size. Following the protocol described in Griffiths et al. (2005), we calculated wing centroid size by taking the square root of the sum of the squared distances between each of 10 wing landmarks and the wing centroid.

Data analysis

Testing effects of competition and elevation on traits

We fitted (generalized) linear mixed models to test for the effects of competition ('Intraspecific/Interspecific density': see Figure 2 for treatment combinations) and abiotic ('Elevation': low, mid or high) environmental variation on each trait. Separate models were fitted for each trait in each species. We applied a Bonferroni correction to adjust the significance level to account for multiple tests. In *D. birchii*, we additionally tested for an effect of the elevation from which the isofemale lines were originally sourced ('Source elevation': high or low), and its interactions with competition and transplant

344 elevation. All models were fitted using *lme4* (Bates *et al.* 2015), implemented
345 in *R* v 3.4.2.

346

347 For productivity and sex ratio, where vial was the unit of analysis, we fitted
348 models that included fixed effects of intraspecific density, interspecific density,
349 transplant elevation, source elevation and all two-way interactions. We
350 included source population as a random effect. For productivity, we square
351 root transformed data to conform to assumptions of normality, and fitted linear
352 mixed models with the factors described above. For offspring sex ratio, we
353 fitted generalized linear models with a binomial distribution and a logit link
354 function. For emergence time (day) and wing size (mm), which were
355 measured on individual flies, we fitted models with the same fixed and random
356 factors, but additionally included vial as a random effect. Both body size and
357 development time typically differ between males and females in *Drosophila*
358 species (e.g. Santos *et al.* 1994; Arthur *et al.* 2008), therefore separate
359 models were fitted for each sex. Emergence time and wing size data were
360 normally distributed and were left untransformed.

361

362 The significance of fixed and random effect terms in each model was
363 assessed by comparing the log likelihood of a model with or without the
364 relevant term using a chi-squared test. For several traits in both species, the
365 effects of intraspecific and interspecific density varied with elevation (indicated
366 by significant density x elevation terms; Table 1). To further explore the
367 relative effect of intra- and interspecific density on traits within each elevation,
368 we fitted separate linear mixed models for each transplant elevation (i.e. low,

mid and high), keeping the remaining fixed and random effects the same as in the full model. For each trait at each elevation, we assessed the relative importance of intraspecific vs interspecific competition in each species using the ratio of their partial regression coefficients, $\beta_{\text{intraspecific}} : \beta_{\text{interspecific}}$ (Anderson & Whiteman 2015).

Testing the power of competition to explain species' distributions

We tested whether the observed effects of intra and interspecific competition on productivity can explain the relative distributions of *D. birchii* and *D. bunnanda* across the elevation gradient, by comparing predicted productivities with and without competition between the species. We considered three different scenarios: (1) no interspecific competition and constant intraspecific competition across the gradient, (2) no interspecific competition and intraspecific interactions at a frequency proportional to field observations of the abundance of each species, and (3) intraspecific and interspecific interactions at frequencies proportional to the field abundance of each species. For (1), we used the observed productivity of flies in single-species field vials at intermediate density (density = 12). For (2) and (3), we used our field abundance counts (Figure 1) as the starting values for each species' density (and hence estimates of the frequencies of intraspecific (2 and 3) and interspecific (3) interactions) at each elevation, and used the equations from our elevation-specific models to calculate the expected productivity of each species across the gradient at these densities. Note that because *D. bunnanda* was not found at the high elevation site (density = 0), using this as the starting value meant that the predicted abundance of this species

394 remained zero under each of these scenarios. To enable comparison of
 395 observed and predicted abundances under each scenario, we calculated
 396 abundances (observed or predicted) of each species at each site relative to
 397 the abundance of *D. birchii* at the low elevation site. That is, we set the
 398 abundance of *D. birchii* at the low elevation site to 1 and calculated the
 399 relative abundance of *D. birchii* at the other sites and *D. bunnanda* at all sites
 400 by dividing their observed or predicted abundances by that of *D. birchii* at the
 401 low elevation site.

402

403 *Testing for local adaptation*

404 For *D. birchii*, where we transplanted populations from extreme ends of the
 405 species' elevational range, we were able to test for adaptation to the local
 406 abiotic or competitive environment. Local adaptation would be revealed by
 407 higher fitness of populations in their 'local' environment compared with their
 408 fitness in other environments ('home' vs 'away'), and/or by higher fitness of
 409 local populations than those transplanted from elsewhere in the species'
 410 range ('local' vs 'non-local')(Kawecki & Ebert 2004). Either of these should
 411 result in a source population x transplant environment interaction for fitness.
 412 We therefore examined the following interactions in the full models for each
 413 trait to test for local adaptation: (i) the 'Elevation x Source elevation'
 414 interaction to test for local adaptation to the abiotic environment (averaged
 415 across all competition treatments); (ii) the 'Intraspecific density x Source
 416 elevation' and 'Interspecific density x Source elevation' interactions to test for
 417 adaptation to the local intraspecific and interspecific competitive environments
 418 respectively (averaged across transplant elevations and the other form of

419 competition). Furthermore, because abiotic and biotic environmental factors
 420 may combine to drive adaptive divergence (if, for example, divergence in
 421 competitive ability depends upon the abiotic environment in which it is
 422 measured), we examined how Intraspecific/Interspecific density x Source
 423 elevation interactions varied across the three transplant elevations. We did
 424 not have enough statistical power to detect significant three-way interactions
 425 in our full model (i.e. Intraspecific/Interspecific density x Elevation x Source
 426 elevation). We therefore used the elevation-specific models and examined:
 427 (iii) Intraspecific density x Source elevation and Interspecific density x Source
 428 elevation interactions to test for divergence among source populations in their
 429 responses to intraspecific and interspecific competition respectively within
 430 each transplant elevation. Wherever one of the interactions described above
 431 was significant (after Bonferroni correction for multiple comparisons), we
 432 examined the pattern of fitness variation to determine whether it was
 433 consistent with local adaptation (i.e. superior performance of the 'home' or
 434 'local' population).

435

436 The traits we considered when testing for local adaptation were (i) productivity
 437 (since this may be considered a direct measure of fitness) and (ii) wing size,
 438 as a proxy for body size, which in *Drosophila* is positively correlated with a
 439 range of fitness measures including longevity, female fecundity and male
 440 mating success (e.g. Partridge & Farquhar 1983; Santos et al. 1992; McCabe
 441 & Partridge 1997).

442

443

444

Results

(1) Fitness effects of competition are strongest at the warm edge of the species' ranges

There was a strong effect of elevation on the overall productivity of both species (Table 1), consistent in direction and magnitude with that observed in *D. birchii* in O'Brien et al. (2017). Mean productivity (averaged across all density treatments) was highest in field cages at the warmest, low elevation site (*D. birchii* offspring per female mean $\pm$ SD = 5.52 ± 5.25 ; *D. bunnanda* mean $\pm$ SD = 3.44 ± 2.69 ; Table S2) and declined with elevation, so that at the high elevation site mean productivity was 24.5% and 27% of that at the low elevation site in *D. birchii* and *D. bunnanda* respectively (*D. birchii* offspring per female mean $\pm$ SD = 1.35 ± 2.02 ; *D. bunnanda* mean $\pm$ SD = 0.93 ± 1.05 ; Table S2).

Emergence time and wing size also varied with transplant elevation (Table 1), with flies of both sexes and species emerging later and at a larger size at higher elevations (Figure 3; Table S2). In *D. birchii*, emergence from cages at the high elevation site was, on average, 9.6 days (males) and 10.4 days (females) later than at the low elevation site (increases of 37.2% and 41.6% respectively; Table S2). In *D. bunnanda*, mean emergence times for males and females respectively increased by 11.7 days (46.4%) and 10.8 days (42.5%) from the low to the high elevation site (Table S2). The mean wing size of *D. birchii* emerging at the high elevation site was 11.0% (males) and 9.7% (females) greater than those emerging at the low elevation site (Table

474 S2). In *D. bunnanda*, these increases were 9.9% and 8.5% for males and
 475 females respectively (Table S2). We did not detect an effect of elevation on
 476 the sex ratio of offspring of either species (Table 1).
 477
 478 Both intra and interspecific competition greatly reduced the productivity of
 479 both species (Table 1; Figure 3) at all transplant elevations (Table 2). The
 480 strength of this effect varied with elevation (note the significant interactions of
 481 intra and interspecific density x elevation on productivity of *D. birchii*, and
 482 intraspecific density x elevation in *D. bunnanda*; Table 1). In both species, the
 483 reduction in productivity in response to intraspecific and interspecific
 484 competition was greatest at the low elevation transplant site, with weaker
 485 effects of both forms of competition at the mid and high elevation sites. In *D.*
 486 *birchii*, the reduction in productivity with each additional competitor of the
 487 same species (intraspecific competition; $\beta_{\text{Intraspecific}}$) or of the other species
 488 (interspecific competition; $\beta_{\text{Interspecific}}$) was, respectively, 1.6 and 1.8 times
 489 greater at the low elevation site than at the high elevation site. In *D.*
 490 *bunnanda*, $\beta_{\text{Intraspecific}}$ and $\beta_{\text{Interspecific}}$ were, respectively, 1.8 and 1.2 times
 491 greater at the low than the high elevation site (Table 2; Figure 3).
 492
 493 There were also strong effects of competition on emergence time and wing
 494 size of both species (Table 1). Increasing intensities of intra and interspecific
 495 competition resulted in smaller flies that emerged earlier (Figure 3). In
 496 contrast to productivity however, the size of the effect of intra and interspecific
 497 competition on these traits did not vary with elevation (note lack of significant
 498 interactions of intra and interspecific competition with elevation; Table 1).

499

500 Effects of competition on offspring sex ratio varied between the species and
 501 according to the type of competition and were evident only at the low
 502 elevation site (Table 2). In *D. birchii*, there was a small but significant
 503 increase in the proportion of male offspring emerging as a function of the
 504 intensity of interspecific competition at the low elevation site. The mean sex
 505 ratio ranged from slightly female-biased (mean prop males $\pm$ SD = $0.46 \pm$
 506 0.02) in field cages where *D. bunnanda* was absent (i.e. no interspecific
 507 competition) to slightly male-biased (mean $\pm$ SD = 0.54 ± 0.02) in mixed-
 508 species cages (Table S2). However, there was no effect of intraspecific
 509 competition on sex ratio (Table 1). By contrast, in *D. bunnanda*, we found the
 510 opposite: intraspecific competition, but not interspecific competition, increased
 511 the proportion of male offspring at the low elevation site (Table 2; Table S2).
 512 While the sex ratio of *D. bunnanda* offspring was female-biased in nearly all
 513 density treatments at this site (mean prop males $\pm$ SD = 0.4 ± 0.18), it ranged
 514 from mean $\pm$ SD = 0.35 ± 0.07 at the lowest intraspecific density to $0.46 \pm$
 515 0.02 at the highest intraspecific density (Table S2).

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518

519 **(2) Competition within and between species causes species to replace**
 520 **each other along environmental gradients**

521

522 *Effects of intra and interspecific competition on fitness:* In *D. birchii*,
 523 interspecific competition had a stronger effect in reducing productivity than
 524 intraspecific competition at the low and mid elevation sites (ratios of
 525 intraspecific to interspecific density coefficients ($\beta_{\text{Intraspecific}}/\beta_{\text{Interspecific}}$) are less
 526 than one; Table 2), where *D. bunnanda* is most abundant (Figure 1). By

527 contrast, at the high elevation site (where *D. birchii* is most abundant and *D.*
 528 *bunnanda* is absent), the negative effects of both forms of competition on
 529 productivity were equal ($\beta_{\text{Intraspecific}}/\beta_{\text{Interspecific}} = 1$; Table 2).
 530
 531 Similarly, in *D. bunnanda*, intraspecific competition reduced productivity more
 532 strongly than interspecific competition at the low and mid elevation sites
 533 ($\beta_{\text{Intraspecific}}/\beta_{\text{Interspecific}} > 1$; Table 2) where intraspecific interactions are most
 534 frequent in this species, whereas interspecific competition had a stronger
 535 effect in cages at the high elevation transplant site ($\beta_{\text{Intraspecific}}/\beta_{\text{Interspecific}} < 1$;
 536 Table 2) where this species is not normally found.
 537
 538 By contrast, the relative effects of intraspecific vs interspecific competition on
 539 other traits of the offspring in the cages (sex ratio, emergence time, wing size)
 540 did not show consistent contrasts in effect between the centre and edge of
 541 each species' elevational limits (Table 2).

542

543

544 *Testing the power of competition to explain species' distributions*

545 Using the likely frequencies of intraspecific and interspecific interactions at
 546 field sites, and the fitness effects of such intensities of interaction within cages
 547 to predict species' relative abundances at each site resulted in predictions that
 548 were closer to observed field abundances than predictions made assuming no
 549 interspecific competition (Figure S3). Using the productivity of each species
 550 in single-species vials to predict field relative abundance gave very misleading
 551 results: in particular, it predicted a higher abundance of *D. birchii* (compared
 552 to *D. bunnanda*) at the low elevation site and a higher abundance of *D.*
 553 *bunnanda* at the high elevation site (Figure S3B), which is the reverse of what

is observed in these ecological communities (Figure 1, Figure S3A). Predictions were significantly improved by assuming intraspecific interactions at a frequency proportional to observed field abundances (Figure S3C), and further by also assuming interspecific interactions occurred at field frequencies (Figure S3D). However, both of these approaches still predicted similar abundances of *D. birchii* at low and high elevations, whereas field observations show much higher abundance of this species at high than at low elevation sites (Figure 1; Figure S3A).

562

563

(3) Selection on competitive ability drives local adaptation within species within a limited part of the elevational range

565

566

Evidence for adaptive divergence between high and low elevation source populations of *D. birchii* was limited. Source elevations did not show overall differences in their trait means (no significant effect of Source elevation; Table 1), nor in their fitness responses to the abiotic or competitive environment (Elevation x Source elevation and Intraspecific/Interspecific density x Source elevation interactions were not significant for productivity or body size; Table 1).

574

High and low elevation source populations of *D. birchii* did differ in the effect of intraspecific competition on wing size, but only at the high elevation transplant site (see Intraspecific density x Source elevation interactions in Table S1; Figure 4). At this site, the reduction in size of male offspring from low elevation source populations as a function of intraspecific density ($\beta_{\text{Intraspecific}} \pm \text{SE} = -0.006 \pm 0.002$; $P = 1.95 \times 10^{-4}$) was three times greater

581 than that of offspring from high elevation source populations ($\beta_{\text{Intraspecific}} \pm \text{SE} =$
582 -0.002 ± 0.001 ; $P = 0.114$) (Table S1; Figure 4), suggesting local adaptation
583 in competitive ability at high vs low elevation, in terms of its effects on body
584 size, at the site where natural density (and therefore intensity of intraspecific
585 interactions) was highest. In females emerging at this site, the difference was
586 even more striking: Intraspecific density reduced mean wing size of female *D.*
587 *birchii* from low elevation source populations ($\beta_{\text{Intraspecific}} \pm \text{SE} = -0.009 \pm$
588 0.002 ; $P = 5.52 \times 10^{-6}$) by nine times as much as it did wing size of high
589 elevation source populations ($\beta_{\text{Intraspecific}} \pm \text{SE} = -0.001 \pm 0.002$; $P = 0.439$)
590 (Table S1; Figure 4). Source elevations did not differ in their fitness
591 responses to interspecific competition at any of the transplant elevations
592 (Table S1).

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Discussion

599 ***Competition reduces fitness more at the warm margin of species'***
600 ***ranges***

601 Biotic interactions are thought to be a more important determinant of fitness,
602 and therefore species' range limits, at lower latitudes (e.g. Coley & Barone
603 1996; Schemske et al 2009) and elevations (e.g. Davis et al. 1998; Pearson &
604 Dawson 2003). However, tests of this hypothesis have yielded inconsistent
605 results (Moles & Ollerton 2016; Grant et al. 2018). Our results suggest that
606 competitive interactions limit population growth more at the low elevation
607 (warm) edge of the ranges of *D. birchii* and *D. bunnanda*, than at their high

608 elevational limits. The low elevation site also had the highest mean
 609 productivity overall. Therefore, for a given interaction density (here
 610 determined by varying the number of flies introduced to a cage), larval
 611 competition was greatest at the low elevation site, meaning food resources
 612 would be depleted more rapidly.

613

614 The large reductions in productivity as intra and interspecific competition is
 615 increased in cages transplanted to the low elevation site will be compounded
 616 by the increased proportions of male offspring at these sites, which will reduce
 617 future population growth rate, and therefore evolutionary potential at this
 618 range margin (Bridle et al. 2019). Although the effects on sex ratio were
 619 subtle, the most prevalent competitive interactions at this site (interspecific for
 620 *D. birchii*; intraspecific for *D. bunnanda*) both increased the proportion of
 621 offspring that were male. Males are smaller than females in both of these
 622 species (Figure 3), and presumably less costly to produce. Therefore,
 623 skewing the sex ratio towards males when faced with competitive stress may
 624 be a strategy for maximising the number and fitness of offspring produced,
 625 consistent with optimal sex allocation theory (Trivers & Willard 1973). It has
 626 been shown that female *Drosophila melanogaster* can adjust the sex ratio of
 627 their offspring in response to the age of their mate (Mange 1970; Long &
 628 Pischedda 2005), and that this may be adaptive. It is not known whether *D.*
 629 *birchii* and *D. bunnanda* are also able to actively manipulate the sex ratio of
 630 their offspring, or whether the effect on sex ratio we observe is due to higher
 631 survival of male offspring during development. This will be explored further in
 632 a future study.

633

634 ***Competition shapes the relative distributions of D. birchii and D.***

635 ***bunnanda***

636 The extent to which competitive interactions shape species' distributions, and
637 under what conditions, is a longstanding question in ecology (e.g. Wisz et al.
638 2013; Godsoe et al. 2015). Our results demonstrate that both the abiotic
639 environment and the intensity of intra and interspecific competition determine
640 the fitness of *Drosophila birchii* and *D. bunnanda* transplanted in cages across
641 their entire elevational ranges. The effects of competition intensity on
642 productivity in cages varied across the elevation gradient in ways that were
643 consistent with the species' relative distributions, and with the predictions of
644 the "Tangled Bank" theory of community assembly (REF): each species
645 suffers a greater loss of fitness (productivity) due to intraspecific competition
646 within the centre of its range, and due to interspecific competition at its
647 ecological margins, where the competitor species is more abundant in nature.

648

649 We used the site-specific intra and interspecific competition effects estimated
650 from our field transplant experiments to predict relative abundance of *D.*
651 *birchii* and *D. bunnanda* along the gradient to test for evidence that
652 competitive interactions limit the distributions of these species. Including both
653 types of competition resulted in predicted relative abundances that much
654 more closely matched observed abundance in the field, particularly when
655 compared with predictions made assuming constant intraspecific competition
656 and no interspecific competition (Figure S3). This is consistent with
657 conclusions from a previous transplant study in *D. birchii* that the abiotic

environment alone cannot explain its elevational distribution, and that biotic interactions are an important limit to population growth, particularly at low elevations (O'Brien et al. 2017). However, our best predictions still over-estimated the relative abundance of *D. birchii* at the low and mid elevation sites, suggesting that additional factors are required to explain the lower range limit of this species, potentially including other competitors, availability of food resources, pathogens and parasitoids. It is for example known that rates of parasitism by parasitoid wasps on *Drosophila* species in these communities increase at lower elevations (Jefferies et al. 2020), and the effect of this on fitness is being assessed in ongoing work.

Evidence for local adaptation is strongly dependent on the abiotic and competitive environment

Despite the very large fitness effects of the abiotic and competitive environments tested in these transplant experiments, evidence for local adaptation in *D. birchii* along the elevational gradient could only be detected under a limited set of abiotic and biotic conditions. Increasing within-species (intraspecific) competition in cages had a negative effect on productivity and wing size of *D. birchii* at all transplant elevations. However, at the high elevation transplant site, the effect on wing size varied according to the elevation from which isofemale lines had been sourced, with flies from low elevation source populations showing a much greater (3x in males and 9x in females) reduction in mean wing size than high elevation flies in response to increasing intraspecific competition. Given that the natural abundance of *D.*

683 *birchii* increases with elevation, this result is consistent with local adaptation of
 684 high elevation flies to higher intraspecific density (e.g. by the evolution of
 685 increased efficiency at extracting nutrient resources in the presence of
 686 conspecifics). In female *Drosophila melanogaster*, large body size has been
 687 shown to be strongly predictive of fitness (survival and lifetime productivity),
 688 but only when tested under cool (not warm) conditions (McCabe & Partridge
 689 1997). If the same is true in *D. birchii*, selection may favour the maintenance
 690 of large body size at the cool edge of the range, even in the presence of high
 691 intraspecific competition.

692

693 In a study of damselflies, Siepielski et al. (2016) also found that source
 694 populations differed in their susceptibility to the negative effects of
 695 intraspecific density. In contrast to our result, they observed that populations
 696 transplanted to their local site showed more, not less, reduction in fitness in
 697 response to intraspecific competition. They attribute this to local populations
 698 being better adapted to the abiotic environment at the transplant site and
 699 therefore more productive, which exacerbated the intensity of competition
 700 (Siepielski et al. 2016). However, in our study we did not find any difference
 701 in mean productivity of populations from elevational extremes.

702

703 Detecting local adaptation in *D. birchii* is therefore strongly dependent on both
 704 the abiotic and biotic environment in which it is measured. This likely explains
 705 why neither population divergence nor local adaptation was detected in a
 706 previous experiment where *D. birchii* sourced from the same elevational range
 707 was transplanted at very low density (O'Brien et al. 2017). Our finding that

708 intraspecific competition can strongly affect the likelihood of detecting
709 population divergence (and possible local adaptation) contrasts with that of
710 Hargreaves et al. (2020), who found in a meta-analysis that maintaining biotic
711 interactions in field transplant experiments did not increase the likelihood or
712 strength of local adaptation detected, compared with studies where biotic
713 interactions were excluded. However, it may be that variation among sites in
714 the extent to which biotic interactions reveal local adaptation, such as we
715 observed here, means that such effects are not detectable when averaged
716 over a wide range of environments.

717

718 Some of the most compelling evidence for effects of biotic interactions on
719 adaptation comes from experimental evolution studies of microbial
720 communities (e.g. Lawrence et al. 2012; Fiegna et al. 2015; Jousset et al.
721 2016; Hall et al. 2018; Scheuerl et al. 2020). Such studies have shown that
722 competitive interactions can constrain adaptive responses to the abiotic
723 environment (e.g. Hall et al. 2018) and that individual species evolve at a
724 slower rate when they are maintained in diverse communities than when
725 evolved alone (Fiegna et al. 2015; Scheuerl et al. 2020). This may be
726 because negative biotic interactions such as competition reduce population
727 sizes and therefore evolutionary potential, or due to trade-offs between
728 adaptation to multiple interacting species or between biotic and abiotic
729 adaptation (Barraclough 2015). It is not yet known whether the tendency for
730 species interactions to reduce evolutionary responses generalises to
731 communities of other types of organisms, but if it does, we would expect local
732 adaptation to be weaker at low latitudes and elevations, where species

733 diversity is typically higher (e.g. Rahbek 1995; Hillebrand 2004; Schemske et
734 al 2009). The greater population divergence of *D. birchii* in response to
735 competition at the high elevation site (compared with the low elevation site)
736 appears to support this pattern.

737

738

739 **Conclusions**

740

741 Using a novel field transplant design, we assessed the fitness effects of
742 competitive interactions between two species of tropical rainforest *Drosophila*
743 (*D. birchii* and *D. bunnanda*) at sites along an elevation gradient spanning the
744 full climatic extent of their distributions. Consistent with expectations from
745 patterns of biodiversity along elevational and latitudinal gradients, we found
746 that fitness effects of both intra and interspecific competition increased
747 towards the warm, low elevation range margin in both species. In each
748 species, intraspecific competition reduced fitness more than interspecific
749 competition at the centre of the species' distribution, whereas the reverse was
750 true at the margins where the competitor species becomes more abundant,
751 consistent with adaptation to the abiotic environment inhabited by each
752 species. We also detected adaptation to the local competitive environment
753 within *D. birchii*, the more widespread species, but only at the high elevation
754 (cold) end of its distribution, suggesting evolutionary responses are contingent
755 upon both the abiotic and biotic environment. Our findings highlight the
756 importance of considering biotic interactions when investigating limits to
757 species' distributions and predicting ecological and evolutionary responses to
758 environmental change. This will be particularly important with climate change,
759 which is expected to have profound effects not just on the abiotic environment

but on community composition, and therefore the type and frequency of interactions between organisms (Lurgi et al. 2012).

Word count: 6995

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Figure 1. Abundance of *Drosophila birchii* (white bars) and *D. bunnanda* (grey bars) males caught in field traps at Paluma between March – June 2017, including the period when the caged transplant experiment was conducted. Bars indicate the mean number of males of each species caught per trap per day, with error bars indicating standard errors among days.

Figure 2. Density treatments in vials, each of which were transplanted to high, middle and low elevation sites at Paluma (average 32 vials/treatment/site). There were 10 treatments, representing different numbers and/or ratios of *D. birchii* and *D. bunnanda*. White circles are treatments with only *D. birchii*, black circles are treatments with only *D. bunnanda*, and grey circles are treatments with a combination of both species.

(i) *Drosophila birchii*

(A) Productivity

(B) Sex ratio

(C) Emergence time

Males

Females

(D) Wing size

Males

Females

(ii) *Drosophila bunnanda*

(A) Productivity

(B) Sex ratio

(C) Emergence time

Males

Females

(D) Wing size

Males

Females

Figure 3. Effect of intraspecific competition (red markers and lines) and interspecific competition (black markers and lines) on productivity, sex ratio, emergence time and wing size of (i) *Drosophila birchii* and (ii) *D. bunnanda* at elevations spanning the abiotic environmental range of both species. Points are means of predicted values from (generalised) linear mixed models that included intraspecific density, interspecific density, elevation and all interactions. Error bars are standard deviations. Lines are the partial regression lines estimated from these models, and

therefore represent the independent effect of each type of competition, after accounting for other sources of variation. Where relevant, predicted values have been back-transformed so they are on the original scale. Dashed lines on plots of sex ratio represent an equal sex ratio in the offspring (proportion males = 0.5), therefore values above this line indicate a male-biased sex ratio and values below the line a female-biased sex ratio. The shaded regions on wing size plots show the area bounded by 1 standard deviation either side of the mean wing size of field-caught flies of the relevant sex and species at that elevation.

Figure 4. Effect of intraspecific competition (given by the density of *D. birchii*) on wing size of *Drosophila birchii* males (top) and females (bottom) from low elevation (red points) and high elevation (black points) source populations, when transplanted to the low, mid and high elevation sites. Points are means of predicted values from linear mixed models that included intraspecific density, interspecific density, source elevation and all interactions, fitted separately for males and females. Error bars are standard deviations. Lines are the partial regression lines estimated from these models, and therefore represent the independent effect of intraspecific competition, after accounting for other sources of variation. Intraspecific competition caused a significant reduction in wing size of both males and females at all transplant sites. However, the response of low and high elevation source populations only differed at the high elevation site, where intraspecific competition caused a greater reduction in the wing size of flies sourced from low elevation than those from high elevation in both males and females.

Table 1. Summary of effects of competition (Intraspecific and Interspecific density) and transplant site (Elevation) on productivity, sex ratio, emergence time and wing size in *Drosophila birchii* and *D. bunnanda*. For *D. birchii*, we additionally tested for divergence between flies sourced from high and low elevation sites (Source elevation). These effects and all 2-way interactions were fitted as fixed factors in (generalised) linear mixed models for each species and trait. For emergence time and wing size, separate models were fitted for each sex. Population was included as a random factor in all models, and vial was additionally included as a random factor in models analysing variation in emergence time and wing size. Values shown for effects on each trait are χ^2 values obtained from a comparison of the log likelihood of models with and without that fixed effect term included. Within each species, we used a Bonferroni-corrected significance threshold of $P = 0.013$ to test each fixed effect, to account for testing of multiple traits. Tests where the P -value for a trait was below this threshold are highlighted and in bold italics. Symbols indicate the range within which P -values fall. See legend beneath table.

Species	Effect	df	Trait					
			Productivity	Sex ratio	Emergence time		Wing size	
					Males	Females	Males	Females
<i>Drosophila birchii</i> (847 vials)	Intraspecific density	1	293.2***	0.016	14.72**	9.077*	122.4***	69.51***
	Interspecific density	1	297.5***	20.65***	19.18***	30.72***	36.88***	14.82**
	Elevation	2	390.8***	8.785†	523.4***	680.9***	510.9***	340.9***
	Source elevation	1	0.354	0.347	2.475	4.129†	1.429	1.794
	Intraspecific x interspecific density	1	135.8***	0.217	0.070	9.495*	6.598†	13.05**
	Intraspecific density x Elevation	2	14.84**	0.542	8.795†	1.348	1.663	0.170
	Intraspecific density x Source elevation	1	1.402	0.025	1.497	10.79*	2.037	3.885†
	Interspecific density x Elevation	2	33.23***	8.376†	0.837	0.049	0.027	13.40*
	Interspecific density x Source elevation	1	0	0.037	0.123	0.056	0.645	1.327
	Elevation x Source elevation	2	2.535	2.005	0.951	1.218	0.683	0.745
<i>Drosophila bunnanda</i> (572 vials)	Intraspecific density	1	166.9***	9.315*	5.609†	535.8***	25.19***	48.29***
	Interspecific density	1	89.92***	2.326	0.105	3.706	25.54***	12.90**
	Elevation	2	267.6***	6.762†	542.8***	539.6***	293.6***	170.6***

Intraspecific x interspecific density	1	36.58^{***}	2.408	3.235	0.729	1.916	0.177
Intraspecific density x Elevation	2	36.81^{***}	2.503	8.451 [†]	7.244 [†]	2.135	10.35[*]
Interspecific density x Elevation	2	1.238	5.710	0.407	5.569	8.347 [†]	5.714

^{***} $P < 0.0001$; ^{**} $0.0001 \leq P < 0.001$; ^{*} $0.001 \leq P < 0.013$; [†] $0.013 \leq P < 0.05$

Table 2. Effects of intraspecific and interspecific competition on productivity, sex ratio, emergence time and wing size in *Drosophila birchii* and *D. bunnanda* at each transplant elevation. Shown are estimates of the partial regression coefficients (β) for effects of intraspecific and interspecific density (i.e. holding the other variable constant) on each trait, and its standard error (SE). These were obtained from (generalised) linear mixed models fitted separately for each species, trait and elevation, and separately for males and females for emergence time and wing size. Also shown is the intercept and its SE for each model, back-transformed where necessary so that all values are on the original scale. For productivity and sex ratio, the expected change in the trait value with respect to each predictor variable described by the coefficients (β) is on the transformed/link scale used in the model (square root for productivity, log for sex ratio), and so should be interpreted accordingly. The final column gives the ratio of the two partial regression coefficients, which we use to evaluate the relative effect of intraspecific vs interspecific competition on each trait at each elevation. This was calculated as $\beta_{\text{Intraspecific}} / \beta_{\text{Interspecific}}$, therefore absolute values greater than 1 indicate a stronger effect of intraspecific competition, and absolute values less than 1 a stronger effect of interspecific competition. Negative values indicate that the two types of competition had opposite effects (i.e. one type increased the value of the trait, while the other decreased it). Within each species, we used a Bonferroni-corrected significance threshold of $P = 0.013$ to test each fixed effect, to account for testing of multiple traits. Cases where the partial regression coefficient was significant at this threshold are highlighted and in bold italics. Tests where $P < 0.05$ but not below the corrected threshold are in italics. Symbols indicate the range within which P -values fall. See legend beneath table.

	Elevation	Trait	Intercept (SE)	$\beta_{\text{Intraspecific}} (\pm \text{SE})$	$\beta_{\text{Interspecific}} (\pm \text{SE})$	$\beta_{\text{Intraspecific}} / \beta_{\text{Interspecific}}$
<i>Drosophila birchii</i>	Low (278 vials)	Productivity	17.13 (0.02)	-0.109 (0.009)***	-0.126 (0.011)***	0.865
		Sex ratio	0.462 (0.52)	0.001 (0.005)	0.027 (0.009)***	0.037
		Emergence time				
		Males	28.33 (0.69)	-0.123 (0.043)***	-0.062 (0.059)*	1.980
		Females	28.22 (0.64)	-0.171 (0.040)†	-0.220 (0.058)**	0.777
		Wing size (x 10 ⁻³)				
		Males	1.708 (0.013)	-5.31 (0.712)***	-4.93 (0.981)***	1.077
		Females	1.808 (0.017)	-4.71 (0.937)***	-2.93 (1.37)	1.608
	Mid (288 vials)	Productivity	8.827 (0.01)	-0.084 (0.006)***	-0.111 (0.008)***	0.757
		Sex ratio	0.498 (0.50)	-0.003 (0.008)	0.008 (0.014)	0.375
		Emergence time				

<i>Drosophila bunnanda</i>	High (281 vials)	Males	30.92 (0.72)	-0.092 (0.049)	-0.066 (0.074) [†]	1.394
		Females	32.30 (0.74)	-0.188 (0.048)	-0.252 (0.070) [*]	0.746
		Wing size (x 10 ⁻³)				
		Males	1.739 (0.015)	-3.91 (1.01) ^{***}	-3.38 (1.50) ^{**}	1.157
		Females	1.841 (0.019)	-4.70 (1.25) ^{***}	-7.12 (1.92) ^{**}	0.660
		Productivity	4.227 (0.01)	-0.070 (0.007) ^{***}	-0.070 (0.009) ^{***}	1.00
		Sex ratio	0.416 (0.54)	0.011 (0.012)	-0.008 (0.018)	-1.375
		Emergence time				
		Males	35.70 (0.83)	0.010 (0.054)	-0.060 (0.079) [*]	-0.167
		Females	36.30 (0.74)	-0.022 (0.050)	-0.102 (0.070) [*]	0.216
	Low (188 vials)	Wing size (x 10 ⁻³)				
		Males	1.842 (0.022)	-2.41 (1.31) ^{***}	-2.08 (1.88) [†]	1.159
		Females	1.963 (0.027)	-2.02 (1.59) ^{**}	-7.19 (2.12) ^{**}	0.281
		Productivity	10.98 (0.03)	-0.090 (0.011) ^{***}	-0.070 (0.012) ^{***}	1.286
		Sex ratio	0.397 (0.54)	0.019 (0.010) [*]	-0.013 (0.013) [†]	-1.462
		Emergence time				
		Males	27.48 (1.05)	-0.163 (0.064) [*]	-0.055 (0.085)	2.964
		Females	26.74 (0.92)	-0.137 (0.058) [†]	0.002 (0.071) [†]	-68.50
		Wing size (x 10 ⁻³)				
		Males	1.567 (0.019)	-2.49 (1.09) ^{**}	-2.26 (1.41) ^{**}	1.102
		Females	1.748 (0.025)	-6.89 (1.54) ^{***}	-4.87 (1.81) ^{**}	1.415
	Mid (194 vials)	Productivity	5.354 (0.02)	-0.064 (0.008) ^{***}	-0.061 (0.010) ^{***}	1.049
		Sex ratio	0.502 (0.56)	-0.002 (0.015)	-0.030 (0.021)	0.067
		Emergence time				
		Males	29.92 (0.94)	-0.032 (0.058)	-0.054 (0.081)	0.593
		Females	28.54 (1.00)	0.041 (0.060)	0.038 (0.079)	1.079
		Wing size (x 10 ⁻³)				

High (190 vials)	Males	1.603 (0.025)	-4.23 (1.53)[*]	-3.84 (2.08)	1.102
	Females	1.758 (0.026)	-7.99 (1.60)^{***}	-5.61 (2.19)	1.424
	Productivity	3.500 (0.02)	-0.052 (0.008)^{***}	-0.061 (0.009)^{***}	0.852
	Sex ratio	0.497 (0.57)	-0.014 (0.016)	-0.025 (0.027)	0.560
	Emergence time				
	Males	38.47 (0.95)	-0.089 (0.057)	-0.174 (0.086)	0.511
	Females	36.26 (0.88)	0.005 (0.052)	-0.020 (0.074)	-0.250
	Wing size (x 10 ⁻³)				
	Males	1.750 (0.021)	-3.33 (1.24)	-8.15 (1.88)^{***}	0.409
	Females	1.858 (0.033)	-4.50 (2.01)	-8.83 (2.70) [†]	0.510
*** $P < 0.0001$; ** $0.0001 \leq P < 0.001$; * $0.001 \leq P < 0.01$; † $0.01 \leq P < 0.05$					