Interacting effects of the abiotic and biotic environment on fitness of rainforest *Drosophila*

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Abstract

Interactions within and between species have significant effects on fitness, which are likely to vary across species' ranges. However, empirical tests of this are rare, particularly under naturally varying field conditions. We transplanted 19 656 flies of two Australian tropical rainforest fly species (Drosophila birchii and D. bunnanda) along an elevation gradient in 972 vials at different intraspecific and interspecific densities, to test for effects of abiotic and biotic environmental variation on fitness. We recorded the number of male and female offspring of each species produced after one generation in the field. Productivity (number of offspring per female) of both species declined rapidly with increasing intraspecific and interspecific density, and with elevation. The effect of density was much greater at the warm, low elevation site than at sites higher up the gradient. Surprisingly, increasing interspecific, but not intraspecific, density was also associated with the production of offspring with a strongly male-biased sex ratio in both D. birchii and D. bunnanda. By contrast, in vials where only one of the species was present, the mean sex ratio was equal or slightly female-biased. Comparison of productivity of mixed and single-species vials suggests that higher mortality of female larvae in interspecific competition can partially, but not completely, explain the observed sex ratio difference. There were also differences between the species in the effect of interspecific competition on sex ratio across the elevation gradient, with effects

weakest at the site where each is most locally abundant (low for D. bunnanda and high for D. birchii). These results suggest that biotic interactions, both within and between species, are a critical factor shaping species' distributions and their potential responses to environmental change.

Introduction

Rapid environmental change is driving changes in species' local abundances and distributions, ecological communities, and in the timing of life history events (e.g. Franks *et al.* 2007; Charmantier & Gienapp 2014). While these changes are often attributed to the direct effects of a changing abiotic environment (particularly temperature and moisture), changes to the biotic environment (e.g. competition, parasitism, predation) also have significant effects on fitness (e.g. Milazzo *et al.* 2013; Alexander *et al.* 2015), and these effects are likely to vary under different abiotic conditions (Davis *et al.* 1998; Ørsted *et al.* 2017).

The frequency of interspecific interactions changes if species within an ecological community vary in their responses to climate change, due to differences in their abilities to track a changing environment through migration, or their potential for plastic or evolutionary responses (Gilman *et al.* 2010). However, few studies have tested the consequences of variation in the intensity of within and among species' interactions across the range of environments a species experiences across its range, and biotic interactions are rarely incorporated into models predicting species' responses to environmental change.

In this study, we conducted a field transplant experiment to test the effect of increasing intensity of intraspecific and interspecific interactions on fitness of two closely-related species of Australian tropical rainforest *Drosophila* across an abiotic (elevation) gradient that spans the entire ecological range of these species at this latitude. In a previous experiment, we transplanted one of these species, *Drosophila birchii*, in cages to field sites along two elevation gradients to assess the fitness effects of a changing abiotic environment, and estimated the local abundance of *D. birchii* at sites along multiple elevation gradients (O'Brien *et al.* 2017). We found that *D. birchii* was rare at warm, low elevation sites, and abundance increased with elevation up to ~900m asl before declining sharply above this point. In contrast to patterns of abundance, fitness in field cages (measured as the number of offspring produced by flies mating in cages) was highest at the warmest, low elevation sites and declined linearly with increasing elevation. Therefore, although abiotic constraints (e.g. low temperature) can explain the upper limit to *D. birchii*'s elevational range, we predicted that biotic interactions (which were largely absent from these cages) may limit population growth and persistence at the warm edge of *D. birchii*'s range.

Here, we again transplanted *D. birchii* along an ecological gradient that represents its upper and lower elevation limit. In addition however, we manipulated the density of *D. birchii* and the closely related species *D. bunnanda*, to explore the interacting effects of abiotic and biotic environmental change on the fitness of both species. These species both belong to the montium subgroup of *Drosophila*, are rainforest

specialists, and have partially overlapping distributions. They are similar in size and are expected to utilise similar food resources at sites where their distributions overlap. *Drosophila bunnanda* is most abundant at lower elevations, where it typically outnumbers *D. birchii* ~3:1 in field traps. Its abundance declines with elevation and it is virtually absent above ~500m asl, where abundance of *D. birchii* is greatest (Bridle *et al.* 2009; O'Brien *et al.* 2017). Given these patterns, and the suggestion of stronger biotic effects on fitness at low elevation in O'Brien *et al.* (2017), we predicted that: (1) density would interact with elevation, with biotic interactions (within and among species) having the greatest effect on fitness at low elevations, and (2) the outcome of interactions between these species would vary across the elevation gradient: interspecific interactions would have a greater effect on fitness of *D. bunnanda* than *D. birchii* at high elevations, while the reverse would be true at low elevations.

Methods

Source of flies used in field transplant experiment

We established isofemale lines from field-mated *D. birchii* females collected in April 2016 from two high elevation (~900m a.s.l.) and two low elevation (<100m a.s.l.) sites at each of two gradients: Paluma and Mt Lewis. We placed females individually in 40 ml glass vials containing 10 ml of standard *Drosophila* medium (agar, raw sugar, inactive yeast, propionic acid and methyl-4-hydroxybenzoate), topped with a few grains of live yeast to stimulate egg-laying. We left them to oviposit for 3 – 4 days

and then moved them to a fresh food vial. This was repeated until they ceased laying eggs, and their offspring were left to emerge. We mixed all offspring of the same female together to found the next generation and establish the isofemale line. Ten *D. birchii* isofemale lines per site (80 lines in total) were maintained in the laboratory at 23 °C on a 12:12 hr light:dark cycle for 10 months (~20 generations). At this time, we mixed lines from the same site together to create eight mass-bred populations (see Supplementary Information) for use in cage transplant experiments.

We established isofemale lines of the competitor species, *D. bunnanda*, using the same method as for *D. birchii*. *Drosophila bunnanda* is rare above 400m a.s.l. (Bridle *et al.* 2009; O'Brien *et al.* 2017), therefore these lines all came from low elevation sites at each of the two gradients where *D. birchii* was collected. We maintained five *D. bunnanda* lines from each of Paluma and Mt Lewis (10 *D. bunnanda* lines in total) in the laboratory over the same period and under the same conditions as for the *D. birchii* lines. We then combined them to establish a single mass bred population.

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

Establishment of field transplant experiment

Emergees from mass-bred populations were separated by sex under light CO_2 anaesthesia within 24 hours of emergence every day over seven days and held in single-sex vials (maximum density 10 flies) to ensure they remained unmated. They

were held for a minimum of 72 hours to recover from the effects of CO_2. Such a long collection period was necessary to obtain sufficient flies to run the experiment, but meant that experimental flies varied in age from 3 – 10 days when they were put in field vials. To avoid confounding effects of age, emergees from each population were therefore mixed prior to establishment of field vials.

We transplanted all populations of *D. birchii* and *D. bunnanda* in vials along one of the natural elevation gradients where flies were sourced (Paluma). We only transplanted flies at Paluma, because the two gradients from which lines were sourced had very similar ranges of abiotic conditions (temperature and humidity), which changed in the same way with increasing elevation (O'Brien *et al.* 2017). There were three transplant elevations: Low (80m above sea level (asl)), Mid (450m asl) and High (900m asl). Within each elevation, we divided the site into five sub-sites ('blocks') to account for localized environmental heterogeneity. The low and high transplant elevations included the sites from which the Paluma isofemale lines were sourced.

We transplanted flies in 30ml plastic vials containing 5 ml of standard *Drosophila* media. Vials were closed with a square of muslin secured with a rubber band, which prevented flies from getting in or out, but allowed air exchange with the outside. Vials were placed in cages, constructed from 600 ml plastic bottles with two 135 x 95 mm windows cut out of the sides, such that there was free flow of air around the tops of the vials. We placed two - four vials in each bottle, and suspended bottles from tree branches with builders' twine at a height 1.3 – 1.8 m above the ground. A

26cm plastic picnic plate was suspended upside down on the twine above each bottle to protect vials from rain. A cylinder of 20 mm strong wire aviary mesh was put over each cage to prevent damage by birds and mammals.

We placed a Carbon-51 USB data logger (Sensormetrix, UK) inside each of ten randomly chosen bottles at each transplant location (two per block), which measured temperature and humidity every 10 minutes throughout the period of the transplant experiment. We also monitored ambient temperature and humidity at transplant locations at the same frequency, using a Tiny Tag datalogger attached to a tree trunk in the centre of each site. Mean daily temperatures at the mid and low sites were, respectively, ~3 °C and 6 °C warmer than at the HE site, and therefore simulated different intensities of climate warming.

We used a response surface design to assess the effects of density, where we independently varied the numbers of each species in order to disentangle the effects of intraspecific vs interspecific interactions (Inouye 2001). There were 10 different treatments, each defined by the number of *D. birchii* and *D. bunnanda* in a vial, with a total density of 6, 12, 24 or 48 flies (Figure 1). Within this range, increasing density reduced *D. birchii* productivity under the same food conditions used in this experiment, suggesting it is an appropriate range for detecting competition effects (personal observation). For each species in a vial, there were equal numbers of males and females. Five replicate vials of each Population x Treatment combination were transplanted at each location (one per block).

In total, we transplanted 19 656 flies (11 808 *D. birchii* and 7 848 *D. bunnanda*) in 972 vials. We placed unmated male and female flies (aged 4 – 11 days) in vials less than 24 hours before vials were installed in the field, and flies were left in field vials for 10 days. Therefore, virtually all courtship, mating and egg-laying happened in the field. After 10 days, any surviving flies were removed and discarded to allow us to identify emergees (survival in the parental generation was not recorded). We left vials in the field until emergence began, which was 14 days at low elevation, 17 days at mid elevation, and 21 days at high elevation. On the day that the first emergence was observed, all vials at that transplant elevation were removed to the laboratory to enable daily emergence to be recorded. Vials were held in a constant temperature room set to the same mean temperature as the elevation at which they had been transplanted (as determined from the dataloggers inside cages) on a 12:12 hr light:dark cycle at 60% relative humidity (RH). For the low, mid and high transplant elevations, this mean temperature was 23 °C, 19.5 °C and 17 °C, respectively. Virtually all larvae had pupated by the time vials were brought in from the field. Temperature effects on survival, emergence time and body size are much greater at the larval than at the pupal stage in these species (personal observation). Therefore, the change in environment associated with moving vials to the laboratory was expected to have minimal effect on the traits measured here.

Measuring fitness

We removed and counted the number of emergees of each species and sex from each vial daily for 11 days after emergence began at the site, then every three days for an additional nine days to capture any late emergence. Species identification

was done blind to the treatment or site from which flies emerged. Male *D. birchii* and *D. bunnanda* were distinguished by examining their genital bristles (Schiffer & McEvey 2006). The species of females was identified from 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). All emerging flies were preserved in ethanol for subsequent measurement of body size. For each species (*D. birchii* and *D. bunnanda*) emerging from each vial, we recorded the number of male and female offspring, to give: (1) productivity (total number of offspring per laying female) and offspring sex ratio (number of male offspring as a proportion of the total).

Data analysis

We fitted (generalized) linear mixed models to explore the effects of biotic (intra- and interspecific density) and abiotic (transplant elevation) environmental variation on productivity and offspring sex ratio. Separate models were fitted for each measure in each species. All models were fitted using *lme4* (Bates *et al.* 2015), implemented in R v 3.4.2.

Models included fixed effects of intraspecific (*w*ithin species) density W_i, interspecific (*b*etween species) density B_j, transplant elevation L_k, and all 2-way and 3-way interactions. We additionally included source population $P_{l(ijk)}$ as a random effect. The linear equation for each model was:

$$y_{ijklm} = W_i + B_j + L_k + W_i \times B_j + W_i \times L_k + B_j \times L_k + W_i \times B_j \times L_k + P_{l(ijk)} + e_{m(ijkl)} \quad [1]$$

Where $e_{m(ijkl)}$ is the residual.

For productivity, we first square root transformed data, and fitted linear mixed models assuming a normal distribution. Transformation is not effective for addressing non-normality in binomial data (Warton & Hui 2011), therefore for offspring sex ratio, we fitted Equation (1) using generalized linear models with a binomial distribution and a logit link function.

The significance of fixed effect terms in each model were estimated by comparing the log likelihood of a model with the relevant term removed with that of the full model (minus any higher order interactions involving that variable) using a chi-squared test. Where there were significant differences between elevations, or significant effects of the interaction of elevation with intraspecific and/or interspecific density in either species for a particular measure, we explored these further by fitting separate linear models for each transplant elevation (i.e. low, mid and high), with the remaining fixed and random effects the same as in the full model.

Results

Productivity

We found strong effects of elevation and density on productivity (number of offspring per female) of both species. Productivity was highest at low elevation and density, and decreased significantly with increasing intraspecific and interspecific density, and with increasing elevation (Tables 1, 2; Figure 2). In Figure 2, we show the independent effects of intraspecific and interspecific density on productivity, by plotting the fitted values (± SE of the residuals) and the partial regression line from the linear model against the relevant density. Effects of intraspecific and interspecific density were both strong, and in the same direction, across all elevations for both species (Figure 2).

There was also variation between elevations in the effect of intraspecific and (in *D. birchii*) interspecific density on productivity (indicated by significant interactions of these terms with elevation; Table 1). In *D. birchii*, the reduction in productivity with increasing intraspecific and interspecific density was greatest at low elevation, and this effect decreased at mid and high elevations (Table 2; Figure 2). In *D. bunnanda*, the same was true for intraspecific density, which had a marginally non-significant effect at the high elevation site, despite large negative effects at low and mid elevations (Table 2). Interspecific density had a negative effect on productivity of *D. bunnanda* at all elevations (Table 2), although the magnitude of this effect did not vary significantly among elevations (Table 1).

Offspring sex ratio

We also found strong effects of density on the offspring sex ratio in these species, but here there were very different effects of intraspecific vs interspecific density. Partial regression plots exploring their independent effects are shown in Figure 3. We found a strong effect of interspecific density, but not intraspecific density, on the sex ratio in offspring emerging from field vials in both species. Specifically, the proportion of males increased with increasing interspecific density in both species (Table 1; Figure 3). In *D. birchii,* this effect was consistent across all three elevations where field vials were transplanted. In *D. bunnanda*, offspring sex ratio, and the effect of interspecific density on the sex ratio, also varied between elevations (Table 1). We did not detect an effect of interspecific density on *D. bunnanda* offspring sex ratio at the low elevation site, and the mean proportion of males was also lowest at this site (Table 2; Figure 3). *D. bunnanda* offspring sex ratio was highest (most male-biased) at the mid elevation site, and there was a significant increase in the proportion of males with increasing interspecific density (i.e. density of *D. birchii*) at both mid and high elevations (Table 2; Figure 3).

The greatest difference in the proportion of male offspring as a function of interspecific density was between single-species vials and mixed-species vials, regardless of the relative or total density of the two species. This can be seen clearly in Figure 4, where the mean offspring sex ratio is plotted for different relative proportions of the two species at two total densities (12 and 24 flies), for each species at each elevation. When only one species was present (black squares in Figure 4), mean offspring sex ratios were almost always at or below 0.5 (i.e. equal or female-biased). By contrast, the mean offspring sex ratio for treatments where both

species were present (white, light grey and dark grey squares in Figure 4) was always male-biased, and was up to 0.85 in *D. birchii* and 0.80 in *D. bunnanda* (Figure 4).

Because there was a negative effect of interspecific density on productivity in most cases (see above and Figure 2), it is possible that mortality of female offspring prior to emergence (i.e. eggs or larvae) could explain the observed sex ratio difference. To investigate this possibility, we calculated the predicted sex ratio of offspring from each treatment assuming that the productivity reduction compared with the single-species treatment at the same total density was entirely due to mortality of female larvae (white, light grey and dark grey circles in Figure 4). We calculated these for each of the five blocks within each elevation, and the error bars on points in Figure 4 are standard errors across blocks. For *Drosophila birchii*, these predicted sex ratios for mixed species treatments were similar to the sex ratios seen in the single species treatments at low and mid elevations, with the exception of the treatment with the highest intensity of interspecific competition (25% *D. birchii*; total density 24)(Figure 4). However, at the high elevation site (where interspecific density had less effect on productivity; Table 2; Figure 2), the predicted sex ratios for most mixed species treatments remained strongly male-biased, suggesting female mortality during development cannot entirely account for this observation. In *D. bunnanda*, the opposite pattern was seen: under the assumption that female mortality accounts for change in productivity, predicted sex ratios for mixed species treatments were similar to observed sex ratios in single species treatments at the high elevation site, but generally remained strongly male-biased at low and mid elevations (Figure 4),

where the negative effects of interspecific density on productivity were less (Table 2; Figure 2).

Discussion

We have demonstrated strong, and often interacting, effects of abiotic and biotic environmental variation on the fitness of two tropical rainforest fly species when tested in naturally varying climatic regimes that match the warm and cool limits of their ecological distribution. Our results suggest that the strength and nature of interactions within and among species, and their effects on fitness, are strongly affected by the abiotic environment. Biotic interactions are therefore likely to be critical in determining how individual species respond to climate change, and the resilience of ecological communities.

Productivity of both species declined with elevation (i.e. decreased temperature), consistent with previous results (O'Brien *et al.* 2017), and with predictions for ectotherms, where rates of growth and reproduction are strongly temperature-dependent. However, within elevations, negative effects of density (intraspecific and interspecific) on productivity were sufficient to overcome this difference in both species. For example, for *D. birchii* at the low elevation site, productivity at the highest density was only ~5% of that at the lowest density, and ~16% of productivity at the lowest density at the high elevation site. Responses of these species to a

given thermal environment will therefore be highly dependent on their biotic environment.

Both *D. birchii* and *D. bunnanda* produced offspring with a strongly male-biased sex ratio when in vials with the other species present, but not in single-species vials at the same total density (Figure 3, 4). To our knowledge, this study is the first to demonstrate such different effects of intra vs interspecific competition on sex ratio in insects, and it raises intriguing questions about the mechanism involved. One possibility is that male and female larvae experience interspecific (but not intraspecific) competition differently, such that females bear a greater cost. This could be the case if female larvae of the two species have greater overlap in their resource use (i.e. which part of the food they use) or in their development time than male larvae, resulting in fewer females surviving to emergence. However, while interspecific competition also reduced productivity in both species, we have shown that this cannot completely account for the male-biased sex ratio in mixed-species vials, even if this productivity reduction was entirely due to mortality of female offspring (Figure 4).

An interesting alternative explanation is that mothers (or fathers) manipulate the sex ratio of offspring in response to the presence of an allospecific competitor, but not in response to a high intensity of interactions from their own species. Males are smaller than females in both of these species, and presumably less costly to produce. Therefore, skewing the sex ratio towards males under these conditions may be a strategy for maximizing the number and fitness of offspring produced,

consistent with optimal sex allocation theory (Trivers & Willard 1973). It has been shown that female *Drosophila melanogaster* can adjust the sex ratio of their offspring in response to the age of their mate (Mange 1970; Long & Pischedda 2005), and that this may be adaptive (Long & Pischedda 2005). It is assumed that this is achieved through cryptic female choice among stored sperm (Mange 1970). However, species of the montium subgroup of *Drosophila* (which includes *Drosophila birchii* and *D. bunnanda*) lack spermathecae (Pitnick *et al.* 1999), meaning they have less capacity for sperm storage than *D. melanogaster*, which may limit the opportunity for active adjustment of the offspring sex ratio in these species. We are conducting ongoing experiments to test whether *D. birchii* and *D. bunnanda* adults can manipulate the sex of their offspring at the egg stage, and the potential adaptive significance of this.

Regardless of the mechanism, the observed male-biased sex ratio in response to interspecific competition has important consequences. Since females are the limiting determinant of population growth, populations with a male-biased sex ratio will grow more slowly than populations with an equal or female-biased sex ratio. Therefore, if our observation that increasing interspecific interactions results in a more male-biased sex ratio is a general phenomenon, it may be a critical factor shaping species co-occurrence, as well as determining the position of ecological limits to species, and therefore the structure and resilience of ecological communities.

We have focused here on the effects of abiotic and biotic environment on the mean fitness of our focal species. However, environmental variation can also affect the variance in traits related to fitness, which has consequences for the potential for evolutionary responses to environmental change if the variance has a genetic basis (Hoffmann & Merilä 1999; Charmantier & Garant 2005; Van Heerwaarden & Sgrò 2014). This is being explored in additional analyses to be included in a future version of this manuscript, by examining trait variation within and among source populations under the different abiotic and biotic environmental conditions in our field transplant experiment.

In nature, species interactions will be much more complex than those in our field cages, including multiple potential competitors as well as parasitoids, pathogens and predators. Parasitoid wasps in particular are expected to be important in shaping *Drosophila* species' distributions in this system, both through direct effects on fitness and indirect effects on competitor species. We are currently investigating the effects of the abundance of parasitoids on fitness of the *Drosophila* species used in this study across their elevational ranges, and this will be explored in a future paper.

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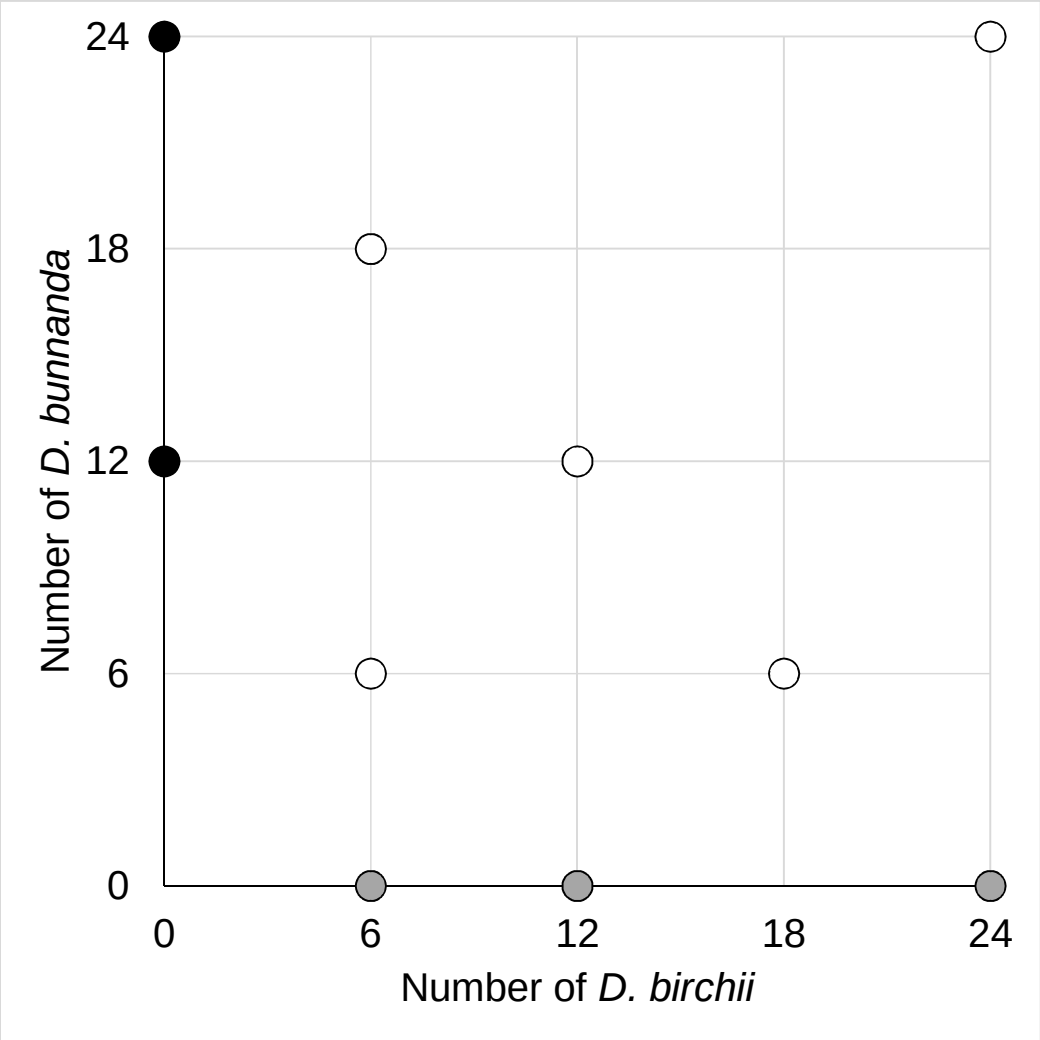
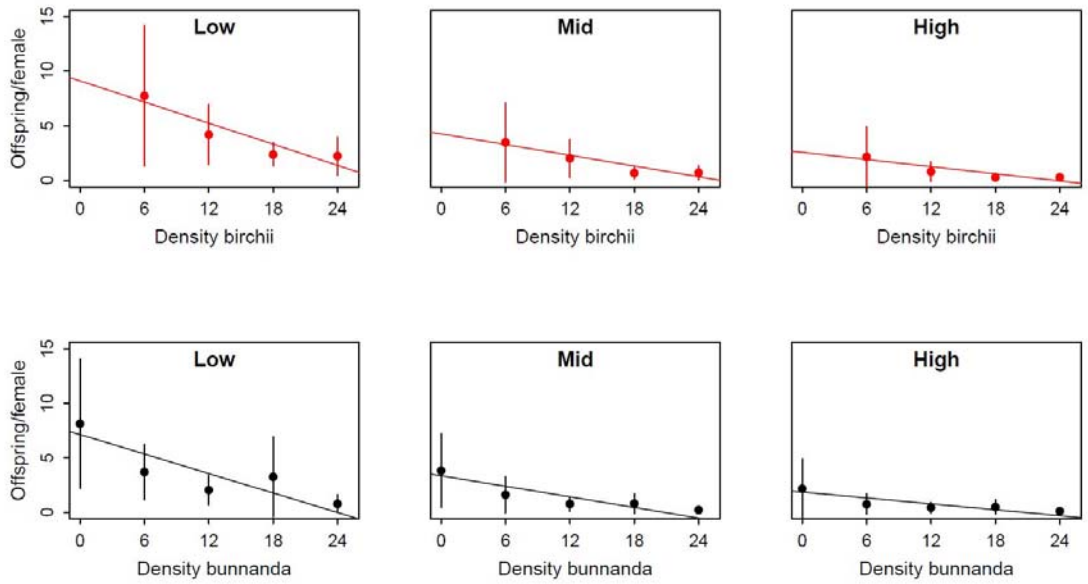


Figure 1. Density treatments in vials, each of which were transplanted to high, middle and low elevation sites at Paluma (average 32 vials/treatment/site). There were 10 treatments, representing different numbers and/or ratios of *D. birchii* and *D. bunnanda*. Grey circles are treatments with only *D. birchii*, black circles are treatments with only *D. bunnanda*, open circles are treatments with a combination of both species.

(A) *Drosophila birchii*



(B) *Drosophila bunnanda*

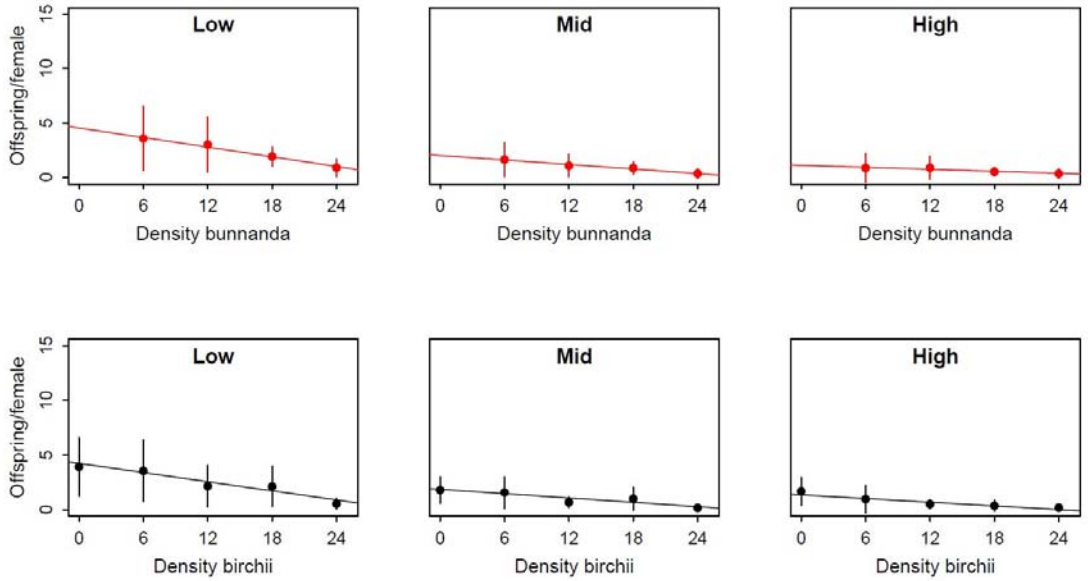
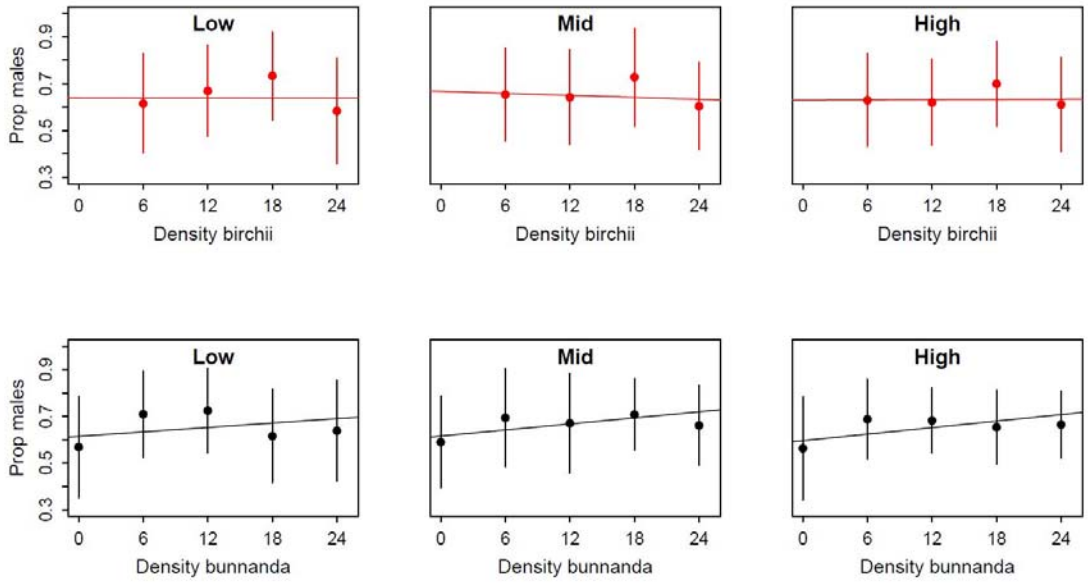


Figure 2. Productivity (number of offspring per female) of (A) *Drosophila birchii* and (B) *D. bunnanda* in field vials at low, mid and high elevation sites at varying intraspecific (top plots, in red) and interspecific (bottom plots, in black) densities. Points are means of predicted values from linear mixed models that included intraspecific density, interspecific density, elevation and all interactions (see Methods and Table 1). 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.

(A) *Drosophila birchii*



(B) *Drosophila bunnanda*

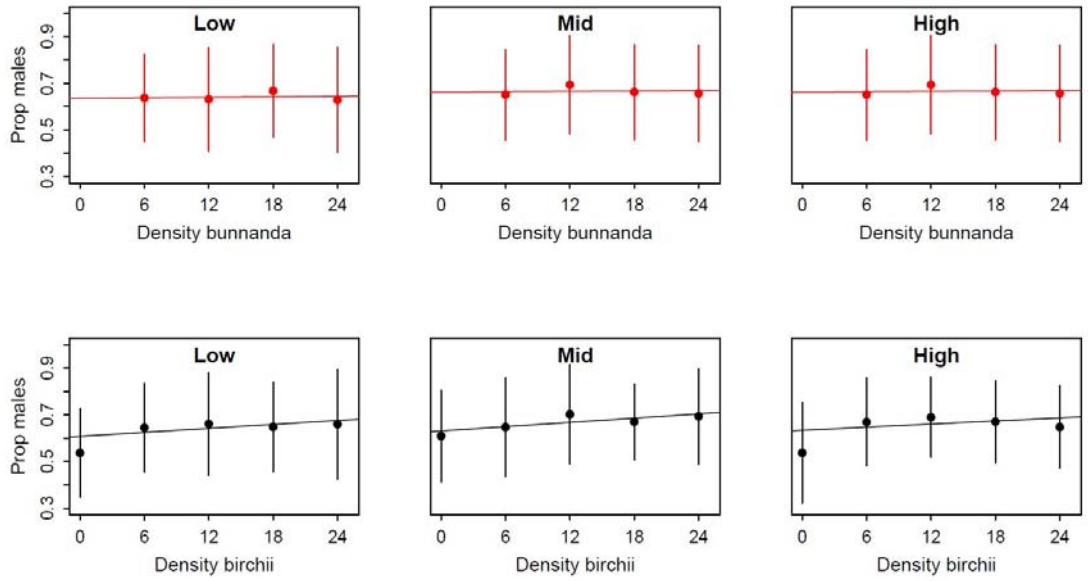
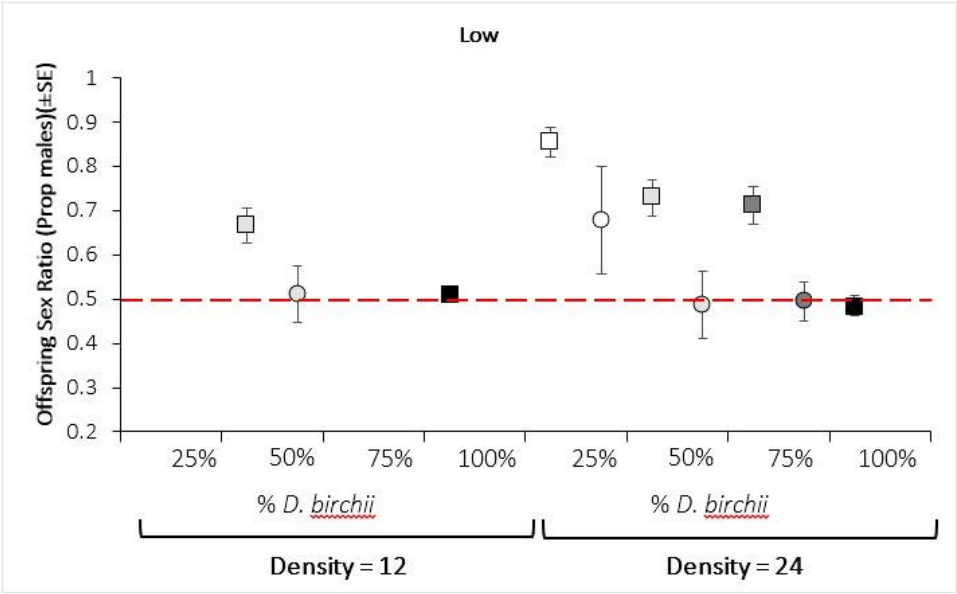
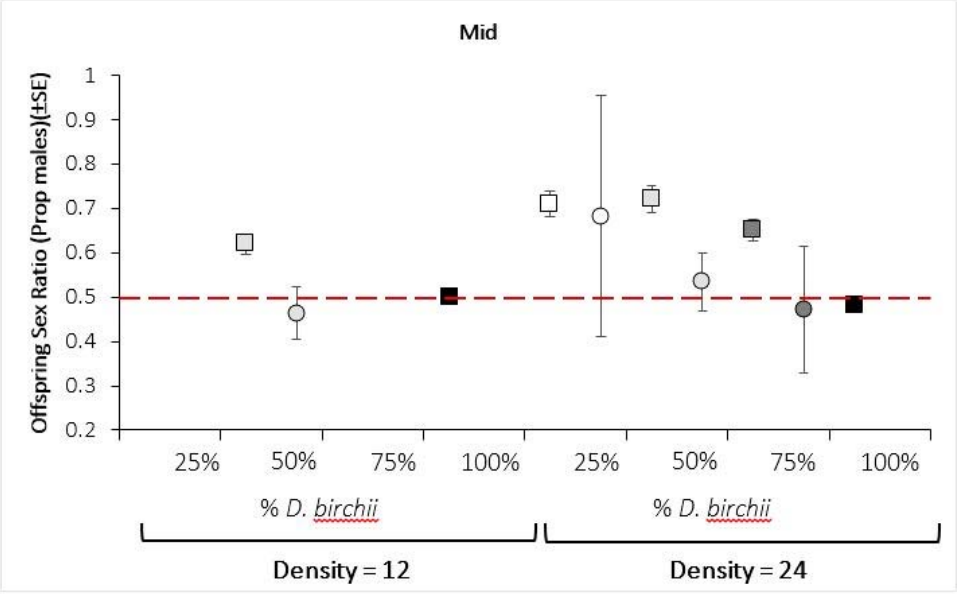
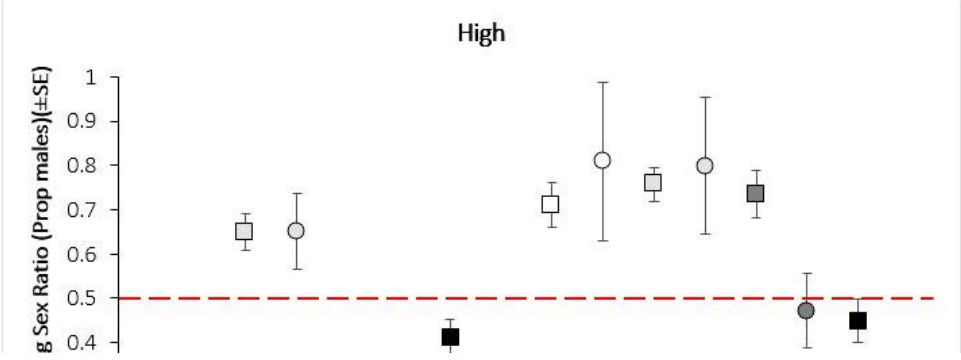


Figure 3. Offspring sex ratio (proportion of emerging offspring that were male) of (A) *Drosophila birchii* and (B) *D. bunnanda* in field vials at low, mid and high elevation sites at varying intraspecific (top plots, in red) and interspecific (bottom plots, in black) densities. Points are means of predicted values from linear mixed models that included intraspecific density, interspecific density, elevation and all interactions (see Methods and Table 1). 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.

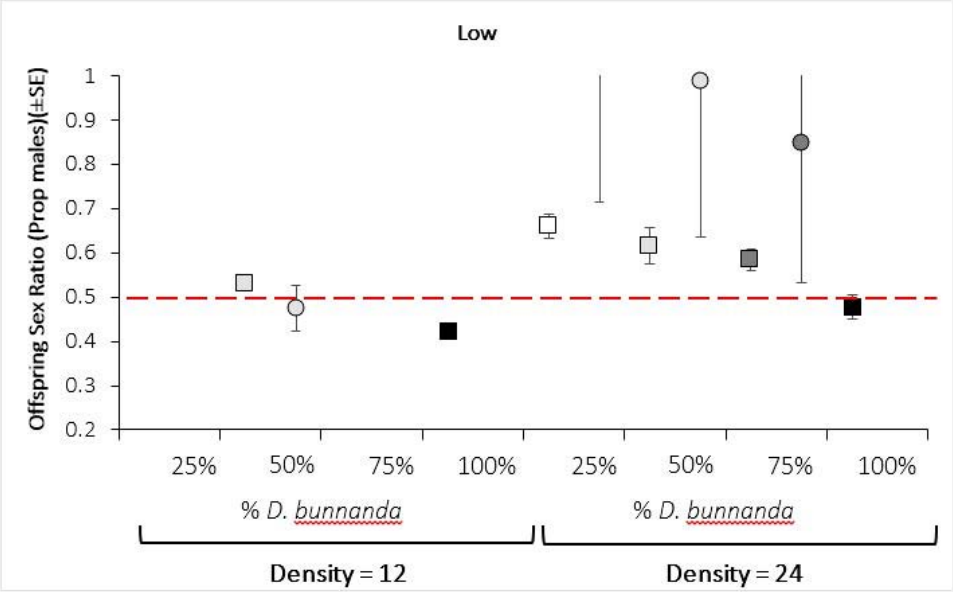
(A) *Drosophila birchii*







(B) ***Drosophila bunnanda***



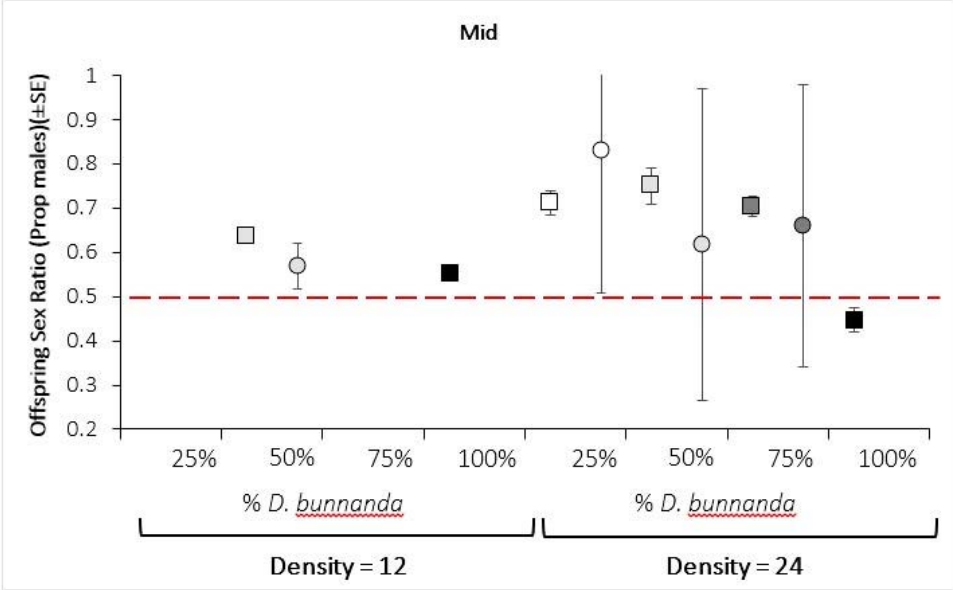


Figure 4. Mean (±SE) sex ratio (proportion male) of (A) *Drosophila birchii* and (B) *D. bunnanda* offspring emerging from field vials at different inter- and intraspecific densities at high, mid and low elevation sites at Paluma. Square markers are observed sex ratios, circles are sex ratios recalculated for mixed – species vials, assuming the reduction in productivity per mother (relative to vials of the same total density with only the focal species) is entirely due to mortality of female offspring. Vials were at one of two total densities: 12 or 24 flies. Percentages within each density indicate the percent of flies of the focal species (*D. birchii* or *D. bunnanda*) initially placed in the vial, with the remaining flies being of the other species. Progressively darker markers indicate increasing percentage of the focal species: 25% (white markers), 50% (light grey markers), 75% (dark grey markers), 100% (black markers). The dashed red line shows the expected 50% sex ratio, therefore values above this line represent a male-biased sex ratio and values below a female-biased sex ratio. Note that for *D. bunnanda* at the low elevation site, productivity in mixed species vials was higher than in vials with only *D. bunnanda* at density=24. This resulted in predicted sex ratios greater than 1 when they were recalculated as described above.

Table 1. Results of generalised linear mixed models testing for fixed effects of intraspecific density, interspecific density, elevation and all 2 and 3-way interactions on (A) productivity (number of offspring per female in a vial) and (B) offspring sex ratio (proportion of offspring that are male) of *Drosophila birchii* and *D. bunnanda* in the field transplant experiment. Separate models were fitted for each trait and species. Population was included as a random factor in all models. *P*-values were obtained for each term by comparing the log likelihood of models with and without the term included using a χ^2 test with degrees of freedom (df) corresponding to those of the relevant term.

(A) Productivity

Species	Fixed effect	df	χ^2	*P*
Drosophila birchii	*Intraspecific density*	*1*	*222.76*	*<2.20 x 10^{-16}*
	Interspecific density	*1*	*329.84*	*<2.20 x 10^{-16}*
	Elevation	*2*	*390.85*	*<2.20 x 10^{-16}*
	Intraspecific density x Interspecific density	*1*	*150.45*	*<2.20 x 10^{-16}*
	Intraspecific density x Elevation	*2*	*11.05*	*0.004*
	Interspecific density x Elevation	*2*	*21.54*	*2.11 x 10^{-5}*
	Intraspecific density x Interspecific density x Elevation	2	1.32	0.516
Drosophila bunnanda	*Intraspecific density*	*1*	*56.22*	*6.49 x 10^{-14}*
	Interspecific density	*1*	*101.31*	*<2.20 x 10^{-16}*
	Elevation	*2*	*184.89*	*<2.20 x 10^{-16}*
	Intraspecific density x Interspecific density	*1*	*41.01*	*1.52 x 10^{-10}*
	Intraspecific density x Elevation	*2*	*18.38*	*1.02 x 10^{-4}*
	Interspecific density x Elevation	2	4.45	0.108
	Intraspecific density x Interspecific density x Elevation	2	2.34	0.311

(B) Offspring sex ratio

Species	Fixed effect	df	χ^2	*P*
Drosophila birchii	Intraspecific density	1	0.177	0.674
	Interspecific density	*1*	*192.14*	*<2.20 x 10^{-16}*
	Elevation	2	5.54	0.063
	Intraspecific density x Interspecific density	*1*	*6.69*	*0.010*
	Intraspecific density x Elevation	2	0.08	0.963
	Interspecific density x Elevation	2	2.69	0.261
	Intraspecific density x Interspecific density x			

	Elevation	2	0.13	0.938
Drosophila. bunnanda	Intraspecific density	1	1.42	0.234
	Interspecific density	*1*	*8.85*	*0.003*
	Elevation	*2*	*21.39*	*2.264 x 10^{-5}*
	Intraspecific density x Interspecific density	1	0.35	0.555
	Intraspecific density x Elevation	2	2.71	0.258
	Interspecific density x Elevation	*2*	*8.64*	*0.013*
	Intraspecific density x Interspecific density x Elevation	*2*	*6.00*	*0.049*

Table 2. Effects of inter and intraspecific competition on (A) productivity and (B) offspring sex ratio of *Drosophila birchii* and *D. bunnanda* at low, mid and high elevations. Estimates of the partial regression coefficient (β) and its standard error (SE) of each effect were obtained from (generalised) linear mixed models fitted separately for each species, trait and elevation. *P*-values for fixed effects were obtained using the same method of log-likelihood comparison as described in Table 1 above. Also shown is the intercept and its SE for each model, back-transformed for both traits so that they are on the original scale. Note that 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.

(A) Productivity

Species	Elevation	Fixed effect	β (SE)	$\chi^2_{(1\ df)}$	*P*
Drosophila birchii	**Low** Intercept (SE) =14.42 (0.01)	*Intraspecific density*	*-0.096 (0.01)*	*92.77*	*<2.20 x 10^{-16}*
		Interspecific density	*-0.130 (0.01)*	*127.76*	*<2.20 x 10^{-16}*
		Intraspecific x interspecific density	*0.004 (0.00)*	*38.26*	*6.20 x 10^{-10}*
	Mid Intercept (SE) =7.68 (0.01)	*Intraspecific density*	*-0.082 (0.01)*	*70.60*	*<2.20 x 10^{-16}*
		Interspecific density	*-0.125 (0.01)*	*129.92*	*<2.20 x 10^{-16}*
		Intraspecific x interspecific density	*0.004 (0.00)*	*70.02*	*<2.20 x 10^{-16}*
	High Intercept (SE) =4.33 (0.01)	*Intraspecific density*	*-0.071 (0.01)*	*62.90*	*2.18 x 10^{-15}*
		Interspecific density	*-0.095 (0.01)*	*79.82*	*<2.2 x 10^{-16}*
		Intraspecific x interspecific density	*0.003 (0.00)*	*48.29*	*3.68 x 10^{-12}*
Drosophila bunnanda	**Low** Intercept (SE) =9.59 (0.04)	*Intraspecific density*	*-0.081 (0.01)*	*31.39*	*2.11 x 10^{-8}*
		Interspecific density	*-0.096 (0.01)*	*42.81*	*6.04 x 10^{-11}*
		Intraspecific x interspecific density	*0.003 (0.00)*	*13.95*	*1.88 x 10^{-4}*
	Mid Intercept (SE) =3.85 (0.02)	*Intraspecific density*	*-0.050 (0.01)*	*27.29*	*1.75 x 10^{-7}*
		Interspecific density	*-0.060 (0.01)*	*38.06*	*6.87 x 10^{-10}*
		Intraspecific x interspecific			

		density	*0.002 (0.00)*	*7.732*	*0.005*
	High	Intraspecific density	-0.048 (0.01)	3.81	0.051
	Intercept (SE) =3.06 (0.02)	*Interspecific density*	*-0.076 (0.01)*	*40.96*	*1.55 x 10^{-10}*
		Intraspecific x interspecific density	*0.003 (0.00)*	*23.66*	*1.15 x 10^{-6}*

(B) Offspring sex ratio

Species	Elevation	Fixed effect	β (SE)	χ^2	*P*
Drosophila birchii	**Low**	Intraspecific density	0.002 (0.004)	0.22	0.641
	Intercept (SE) =0.489 (0.515)	*Interspecific density*	*0.059 (0.009)*	*106.95*	*<2.20 x 10^{-16}*
		Intraspecific x interspecific density	*-0.001 (0.001)*	*4.15*	*0.042*
	Mid	Intraspecific density	0.003 (0.006)	0.02	0.887
	Intercept (SE) = 0.495 (0.525)	*Interspecific density*	*0.075 (0.016)*	*55.76*	*8.17 x 10^{-14}*
		Intraspecific x interspecific density	-0.001 (0.001)	1.90	0.169
	High	Intraspecific density	0.003 (0.009)	0.03	0.872
	Intercept (SE) =0.453 (0.529)	*Interspecific density*	*0.071 (0.021)*	*35.08*	*3.17 x 10^{-9}*
		Intraspecific x interspecific density	-0.001 (0.001)	0.37	0.544
Drosophila bunnanda	**Low**	Intraspecific density	0.022 (0.010)	1.567	0.211
	Intercept (SE) =0.430 (0.542)	Interspecific density	0.034 (0.015)	2.187	0.139
		Intraspecific x interspecific density	-0.001 (0.001)	2.776	0.096
	Mid	Intraspecific density	-0.014 (0.015)	0.503	0.478
	Intercept (SE) =0.592 (0.560)	*Interspecific density*	*-0.0005 (0.023)*	*12.643*	*3.77 x 10^{-4}*
		Intraspecific x			

		interspecific density	*0.003 (0.001)*	*3.963*	*0.047*
	High Intercept (SE) =0.497 (0.579)	Intraspecific density	-0.004 (0.018)	0.919	0.338
		Interspecific density	*0.061 (0.034)*	*6.905*	*0.009*
		Intraspecific x interspecific density	-0.001 (0.002)	0.401	0.527