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Temperature-Dependent Competitive Outcomes between the Fruit Flies *Drosophila santomea* and *Drosophila yakuba*

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ABSTRACT: Changes in temperature associated with climate change can alter species' distributions, drive adaptive evolution, and in some cases cause extinction. Research has tended to focus on the direct effects of temperature, but changes in temperature can also have indirect effects on populations and species. Here, we test whether temperature can indirectly affect the fitness of *Drosophila santomea* and *Drosophila yakuba* by altering the nature of interspecific competition. We show that when raised in isolation, both *D. santomea* and *D. yakuba* display similar variation in relative fitness across temperatures of 18°, 22°, and 25°C. However, *D. santomea* has higher fitness than *D. yakuba* when experiencing interspecific competition at 18°C, while the inverse is true at 25°C. Patterns of fitness across thermal and competitive environments therefore indicate that the outcome of interspecific competition varies with temperature. We then use a coexistence experiment to show that *D. santomea* is rapidly (within eight generations) extirpated when maintained with *D. yakuba* at 25°C. By contrast, *D. santomea* remains as (or more) abundant than *D. yakuba* over the course of ~10 generations when maintained at 18°C. Our results provide an example of how the thermal environment can affect interspecific competition and suggest that some species may become more prone to extinction under scenarios of climate change through indirect effects of the thermal environment on competitive advantages between species.

Keywords: species' ranges, competition, climate change, temperature, context dependent.

Introduction

Species vary in their physiological tolerance and behavioral preference for different thermal environments (Calosi et al. 2010; Kellermann et al. 2012). Temperature is therefore an important abiotic factor that can drive local adaptation (McNab 1971; Freckleton et al. 2003; Campbell-Staton et al. 2016, 2018; Stager et al. 2016; Delhey 2017, 2019) and shape species' ranges (Soberón 2007; Calosi et al. 2010; Early and Sax 2011; Kellermann et al. 2012). However, a species' range and response to variation in climate are also shaped by biotic interactions. Competition is one outcome of biotic interactions that, like temperature, can drive phenotypic and ecological divergence (i.e., character displacement; Pfennig and Pfennig 2009; Stuart and Losos 2013), the maintenance of intraspecific variation (Roughgarden 1972; Bolnick 2001; Harris et al. 2008), speciation (Polechová and Barton 2005; Winkelmann et al. 2014), extinction (Park 1954; Davis et al. 1998*a*; Alexander et al. 2015), and global biogeographic patterns (Pianka 1966; Willig et al. 2003). The majority of biotic interactions, such as competition, occur across a range of abiotic conditions (either temporally or geographically). To understand how biotic interactions, such as interspecific competition, affect a species' abundance and evolution, it is important to understand the outcome of those interactions across different abiotic conditions (e.g., thermal environments; Davis et al. 1998*a*; Alexander et al. 2015).

The outcome of interspecific competition has been shown to vary with temperature for a number of species inhabiting different environments. For example, experimental work in communities of algae (Goldman and Ryther 1976; Hillebrand 2011), beetles (Park 1954; Wilson et al. 1984), fungi (Carreiro and Koske 1992), alpine plants (Klanderud and Totland 2007; Alexander et al. 2015), and fruit flies (Davis et al. 1998*a*, 1998*b*) has shown how temperature can indirectly affect a species' relative abundance through competition. However, direct effects of climate and competition have also been reported: experimental communities of tussock tundra plant communities show no noticeable interaction between temperature and competition (Hobbie et al. 1999). Therefore, while the majority of studies point toward temperature as an important abiotic control on the fitness consequences of interspecific

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competition, more examples are required to understand its generality.

One aspect of temperature-mediated competitive outcomes that remains underexplored is their prevalence in closely related (e.g., sibling) species that inhabit partially overlapping or adjacent ranges that are coincident with a gradient of thermal environments. Species that have diverged recently and come into secondary contact can share more aspects of their ecology than distantly related species, potentially leading to strong competition (Schluter and McPhail 1992; Grant and Grant 2006). Species pairs that display overlapping or adjacent (parapatric) geographic ranges that differ in temperature or humidity therefore provide useful systems to test how interspecific competition might contribute to those species' realized geographic ranges.

Drosophila santomea and *Drosophila yakuba* are sibling species of fruit flies that inhabit adjacent ranges on the island of São Tomé in the Gulf of Guinea (Lachaise et al. 2000). Previous research on temperature-mediated competitive outcomes using drosophilid flies has focused on nonsibling species with broadly overlapping geographic ranges (Davis et al. 1998*a*). *Drosophila santomea* and *D. yakuba* therefore provide a system in which temperature-mediated competitive outcomes can be tested between closely related species that have evolved differences in their thermal niches: *D. santomea* is endemic to the cool tropical forest on São Tomé, while *D. yakuba* is a broadly distributed generalist species, regularly found in association with human-modified habitats across sub-Saharan Africa, including on São Tomé (Lachaise et al. 1988; Cooper et al. 2018). In laboratory experiments, adult *D. santomea* show a behavioral preference for moderate temperatures (22°C), and larval survival and egg hatchability drop at temperatures above 25°C (Matute et al. 2009). In nature, populations of *D. yakuba* are found in environments subject to a much wider range of temperatures than populations of *D. santomea*, and *D. yakuba* can tolerate an overlapping but broader range of temperatures than *D. santomea* in the lab (Matute et al. 2009; Cooper et al. 2018). On the island of São Tomé, the distributions of *D. santomea* and *D. yakuba* are adjacent, with *D. santomea* typically occurring at forested habitats above 800 m and *D. yakuba* occurring at lower-elevation open habitats (Llopart et al. 2005*a*; Comeault et al. 2016). The two species form a narrow hybrid zone between low and high elevation that has been relatively stable for more than 20 years and occurs as lowland agricultural fields give way to upland rain forest habitats (Lachaise et al. 2000; Llopart et al. 2005*b*; Matute 2010*b*; Comeault et al. 2016; fig. 1). Where the species' ranges overlap on São Tomé, a small proportion of hybrid individuals can be found (fig. 1*D*). Male F_1 offspring between *D. yakuba* and *D. santomea* are sterile, and females show reduced fertility (Lachaise et al. 2000; Coyne et al. 2002). The fact that *D. santomea* and *D. yakuba* show differences in their realized niche in nature yet display broadly overlapping thermal tolerances in the lab raises the question of how interspecific competition may contribute to the maintenance of their distinct ecological niches.

Here, we test how the outcome of competition between *D. santomea* and *D. yakuba* varies across an ecologically relevant range of thermal environments. We predict that if competition contributes to their realized niches in nature and is indirectly affected by the thermal environment, we would observe a significant interaction between temperature and competition on relative fitness. We first study the distribution of *D. santomea* and *D. yakuba* on the island of São Tomé and confirm that temperature and seasonality differ between areas where these two species are found. We then manipulate temperature and the opportunity for competition in the lab to show that both species display maximal performance at moderate temperatures but that *D. santomea* has higher fitness when maintained in the presence of *D. yakuba* at low temperatures, while the opposite is true at warmer temperatures. We then use a multigenerational coexistence experiment to show that the relative abundances of *D. santomea* and *D. yakuba* are strongly affected by the thermal environment: at low temperatures, *D. santomea* and *D. yakuba* maintain viable populations when kept together, while at higher temperatures, *D. santomea* is rapidly extirpated when kept with *D. yakuba*. Our results show that the interaction between temperature and competition helps shape ecological differences between these two species and, disturbingly, suggest that with warming temperatures, *D. yakuba* will be able to outcompete *D. santomea*, potentially contributing to extinction of this island endemic species.

Material and Methods

The Thermal Niche of Drosophila santomea *and* Drosophila yakuba *on São Tomé*

We qualitatively describe the thermal niche of *D. santomea* and *D. yakuba* found on the island of São Tomé using a previously published data set of occurrence records (Comeault et al. 2016) and climate data from the WorldClim database (http://www.worldclim.org; bioclim variables 1–19; table S1; tables S1–S3 are available online; Booth et al. 2014). For each site, we extracted values for bioclim variables 1–19 at a resolution of 2.5 arc degrees using the raster R library (Hijmans et al. 2019). Because the transect on São Tomé covers a relatively small geographic area—it spans only four unique sets of bioclim variables—we report the range of mean annual temperatures (bioclim variable 1),

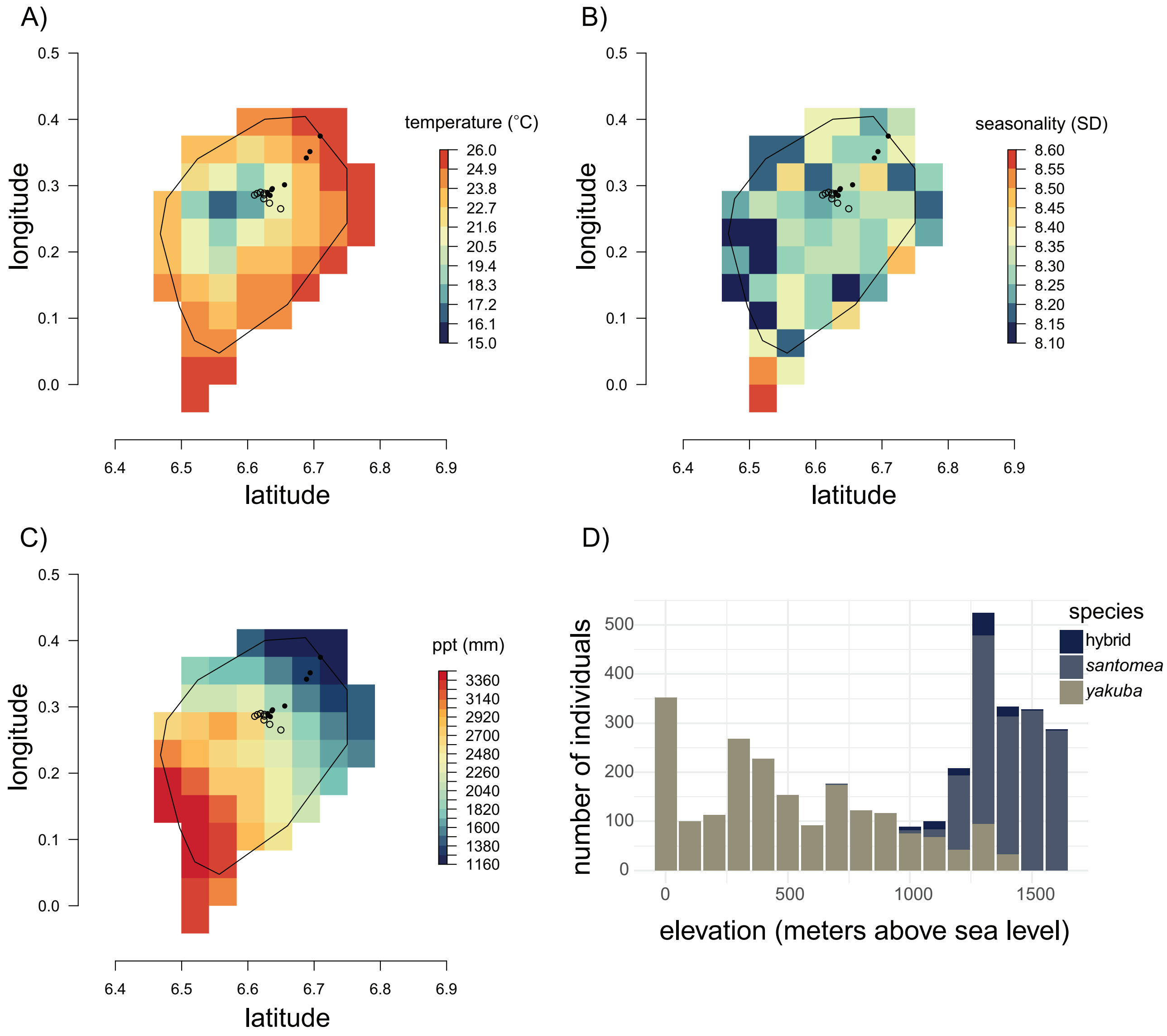

Figure 1: Mean annual temperature (*A*), temperature seasonality (*B*), and mean annual precipitation (*C*) on the island of São Tomé. The black line outlines the coast of São Tomé, and white space indicates the Atlantic Ocean. Filled circles denote sites where *Drosophila yakuba* was the more abundant species, and open circles denote sites where *Drosophila santomea* was more abundant. *D* gives abundances of *D. santomea*, *D. yakuba*, and their hybrids along an elevational transect on São Tomé from sea level to 1,600 m (data from Comeault et al. 2016).

temperature seasonality (bioclim variable 4), and annual precipitation (bioclim variable 12) for sites where either *D. santomea* or *D. yakuba* is the more abundant species according to relative abundances reported in figure S1 of Comeault et al. (2016) rather than summarize climatic variation using a typical decomposition-based method (e.g., principal components analysis). These three variables were chosen because mean annual temperature was strongly correlated with all thermal bioclim variables ($r > 0.9$) except isothermality ($r = 0.11$) and seasonality ($r = 0.36$), and annual precipitation was negatively correlated with temperature ($r = -0.99$).

We also used data loggers (Senonics Minnow 1.0TH; http://senonics.com/) to record temperature every 10 min at eight sites on São Tomé. These sites ranged from an elevation of 100 to 1,933 m and spanned a transition from human-dominated environments to montane rain forests. Data were recorded for a minimum of 43.5 h except for the lowest elevation site, where we recorded data for 11.5 h. We tested for a correlation between the mean temperature at a site and elevation, as *D. yakuba* is typically found at low-elevation open habitats, while *D. santomea* is found at high-elevation forested habitats.

Details of Populations Used for Experiments

To measure performance under different temperatures and competitive environments, we generated genetically diverse laboratory populations by combining five male and five female offspring from each of 20 isofemale lines established from inseminated females collected on São Tomé. Females were collected between February 1 and February 14, 2015, at the sites lake7 (for *D. santomea*) and monte7 (for *D. yakuba*), and the populations used in the experiments described below were created on March 12 and March 19, 2015, after approximately two to four generations in the lab. We used lines that were recently collected from single locations in nature to minimize effects of adaptation to the laboratory environment and to approximate genetic diversity observed in the two species on São Tomé. The two resulting populations (*D. santomea*: san_lake_7S; *D. yakuba*: yak_monte_7S) were maintained at large population sizes spread over three to five 175-mL polypropylene bottles (Genesee Scientific, Morrisville, NC) for between five and ten overlapping generations before experiments were initiated.

Temperature's Effect on Competition between D. santomea *and* D. yakuba

We tested the relative performance of *D. santomea* and *D. yakuba* at each of three biologically relevant temperatures (18°, 22°, and 25°C) when maintained in isolation or together. To initiate this experiment, we placed six 1–9-day-old female flies from the stock populations into individual 30-mL vials containing standard cornstarch medium. Sampling females from stock populations in this way results in >95% of the females being inseminated and actively laying viable eggs (see app. I; apps. I, II are available online). To quantify performance when individuals experienced only intraspecific competition, six females of either *D. santomea* or *D. yakuba* were added to the vials. To quantify the effect of interspecific competition, three females of each species were added to the same vial. Therefore, the total number of laying females remained constant between the isolation and competition treatments, and adult flies and larvae experienced intraspecific competition only in the former treatment, while they experienced both intra- and interspecific competition in the latter treatment. We created a total of 30 replicate vials containing only *D. santomea* and only *D. yakuba* and 30 competition replicates containing both species. We then randomly assigned 10 replicates of each of the three resulting treatments—*D. santomea* in isolation, *D. yakuba* in isolation, or competition—to each of the three temperature treatments. Females were allowed to lay eggs for 7 days and were then removed from the vials. When removing the females, we added a dampened (0.5% propionic acid) Kimwipe (Kimberly-Clark no. 34155) as a pupation site and to inhibit the growth of fungi. As a measure of performance across temperatures and in different competitive environments, we counted the total number of each sex of adult fly that eclosed from each vial over the following 23 days. While we counted the total number of flies, we focused on males because male *D. santomea* and *D. yakuba* are easily and unambiguously identified according to pigmentation: *D. santomea* males are a solid yellow, and *D. yakuba* males have black pigmentation on the last three tergites of their abdomen (females show some variation in color). Despite the potential for misclassification of females, we found no difference in sex ratio across treatments or temperatures (binomial generalized linear model [GLM]; fig. S1; figs. S1–S7 are available online) and summarize the results when analyzing all offspring in "Results" and figure S2. We stopped counting emerging flies after 23 days because this duration spanned peak eclosion for both species across all temperatures (fig. S3).

To test whether temperature affected the outcome of competition between *D. santomea* and *D. yakuba*, we first modeled the mean (per-female) number of male flies that eclosed from an experimental replicate as a function of temperature, competition treatment (three levels: *D. santomea*, *D. yakuba*, or interspecific competition), and the interaction between temperature and competition. This model was fitted using the glm function in R (R Development Core Team 2017) assuming Poisson-distributed error. To determine whether there was a significant interaction between temperature and competition treatment, we conducted a likelihood ratio test (LRT) that compared the model described above with one that lacked the interaction term using the anova function in R. Because this analysis identified a significant interaction between temperature and competition (see "Results") on the mean number of male offspring produced, we also fitted independent GLMs, splitting the data by temperature treatment. We then used Tukey's post hoc tests as implemented with the glht function in the multcomp R package (Hothorn et al. 2008) to test for significant pairwise differences in performance between competition treatments for each temperature treatment. Finally, for replicates that contained both *D. santomea* and *D. yakuba* (i.e., the competition treatment), we tested for differences in performance between these species at different temperatures by conducting dependent-samples sign tests (these data are naturally paired by replicate) as implemented by the SIGN.test function in the bsda R package (Arnholt and Evans 2017). Because *D. santomea* on the island of São Tomé are found in cooler environments than *D. yakuba*, we predicted that *D. santomea* would outcompete *D. yakuba* at

18°C and *D. yakuba* would outcompete *D. santomea* at 22° and 25°C.

Competition and Coexistence at Different Temperatures

We found a significant interaction between temperature and competition treatments in the single-generation experiment described above. Building on this result, we next tested whether competition between *D. santomea* and *D. yakuba* at different temperatures could lead to one species competitively displacing the other. To test this, we created two-species experimental communities in mesh cages (24.5 cm × 24.5 cm × 24.5 cm; https://www.bugdorm.com) and maintained them at either 18° or 25°C (four replicates at each temperature). Each cage was prepared by adding approximately 2.5 cm of dampened coconut fiber as a substrate to help maintain a relative humidity between ~40% and 80%. We then added two 175-mL polypropylene bottles (Genesee Scientific, Morrisville, NC) containing ~30 mL of standard cornmeal medium and a dampened Kimwipe to each cage along with 24 adult males and 24 adult females of both *D. santomea* and *D. yakuba* (96 flies founded each experimental community; ~3 days old when introduced to the cages). Every 2 weeks, we added two fresh bottles containing cornmeal medium, and bottles were removed after they were in the cages for at least 4 weeks (for details, see table S2). We randomly sampled a subset of flies from each experimental cage at 34, 57, 90, 113, and 148 days after initiating the experiment (148 days is ~10 generations at 18°C and ~12 generations at 25°C for both species; see fig. S3). During sampling, we scored individuals as being *D. santomea*, *D. yakuba*, or potential hybrids. At any one time, 0%–10.5% of individuals sampled were identified as potential hybrids (mean = 2.1%), and we found no evidence that the proportion of hybrids increased over the course of the experiment. Because we were interested in competition between the two parental species, we excluded hybrids from the analyses described below. To test for consistent changes in the relative abundance of the two species within each cage, we conducted Mann-Kendall trend tests on the proportion of *D. santomea* within each cage. We expect a significant (and consistent) change in the proportion of *D. santomea* within each cage if it was either at a competitive advantage (increase) or disadvantage (decrease) over *D. yakuba*. We also fitted generalized linear mixed models (GLMMs), assuming Poisson-distributed error, to test for consistent differences between the relative numbers of *D. santomea* and *D. yakuba* within each cage over the course of the experiment. We fitted these models on the number of males sampled (response variable) and included species as the fixed effect with cage and sample date as random effects.

Results

The Thermal Niche of Drosophila santomea *and* Drosophila yakuba *on São Tomé*

We found that along the altitudinal transect on São Tomé, *D. santomea* is more abundant than *D. yakuba* at cooler sites, where mean annual temperatures range from 17.8° to 20.6°C and seasonality ranges from 8.24 to 8.29 (standard deviations in temperature; fig. 1). By contrast, *D. yakuba* is more abundant at sites experiencing higher mean annual temperatures (20.6°–25.5°C) and stronger seasonality (8.27–8.35; fig. 1). There is also a general trend of higher precipitation toward the center and southwest of São Tomé, sites where *D. santomea* is more abundant than *D. yakuba*, and lower precipitation in the northeast, where *D. yakuba* is more abundant (figs. 1*C*, 1*D*, S4). Finally, we found that short-term temperature trends (mean temperature) at eight locations on São Tomé were negatively correlated with elevation ($r = -0.97$, $P < .0001$) and spanned the range of temperatures we tested competition across (mean temperature across sites ranged from 15.6° to 31.1°C; fig. S5).

Temperature's Effect on Competition between D. santomea *and* D. yakuba

When the species were raised in isolation, performance, measured as the number of emergent male offspring, varied with temperature for both *D. santomea* and *D. yakuba* (GLMs: for *D. santomea*, $\chi^2 = 14.26$, $P = .0008$; for *D. yakuba*, $\chi^2 = 8.54$, $P = .014$). *Drosophila santomea* produced significantly more male offspring when maintained at 22°C (mean = 8.1, SD = 0.5) compared with when they were maintained at 18°C (mean = 4.8, SD = 0.3; Tukey's honestly significant difference [HSD]: $Z = 2.5$, $P = .03$) or at 25°C (mean = 4.5, SD = 0.3; Tukey's HSD: $Z = 3.6$, $P = .001$). *Drosophila yakuba* also produced significantly more male offspring when maintained at 22°C (mean = 8.0, SD = 0.5) compared with when they were maintained at 25°C (mean = 5.1, SD = 0.3; Tukey's HSD: $Z = 2.9$, $P = .012$), but there was not a significant difference (Tukey's HSD: $P > .1$) in performance either between 22° and 18°C (mean = 5.9, SD = 0.4) or between 25° and 18°C. These results suggest that both *D. santomea* and *D. yakuba* perform best at temperatures in the low 20s (°C), which is consistent with previous results (Matute et al. 2009).

In addition to temperature, the interaction between temperature and competitive environment had a strong effect on performance (LRT: $\chi^2 = -28.69$, $P < 1.0 \times 10^{-4}$; fig. 2*A*). When maintained together (i.e., experiencing both intra- and interspecific competition) at 18°C, *D. santomea* had higher fitness than *D. yakuba* (Tukey's HSD:

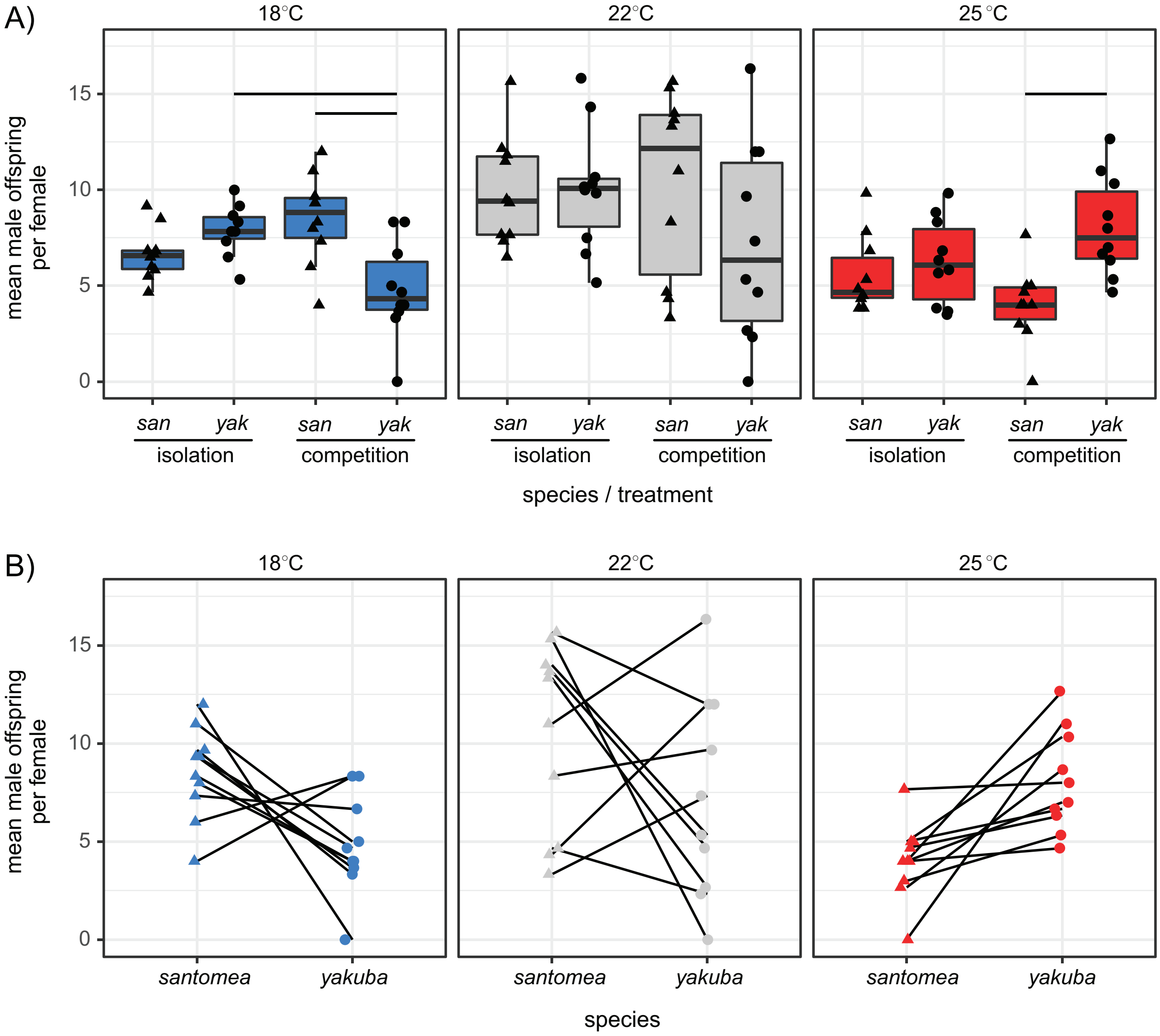

Figure 2: Effect of temperature and competition on performance. *A*, Mean number of offspring per female when *Drosophila santomea* (san) and *Drosophila yakuba* (yak) were raised with the opportunity for intraspecific competition only (isolation) or with both intra- and interspecific competition (competition). Groups that showed a significant difference in the mean number of offspring produced are indicated by horizontal bars (Tukey's pairwise contracts; generalized linear models run separately for each temperature). When maintained at 18°C and in competition with *D. santomea*, *D. yakuba* showed a significant reduction in fitness (*left*), while the opposite is true at 25°C (*right*). At 22°C, there is a large variance in the number of offspring that emerged, and we did not detect a difference between *D. santomea* and *D. yakuba*. See figure S6, available online, for data presented grouped by competition treatment rather than by temperature. *B*, Competition data presented in *A* but presented as paired data. Lines connect the number of male *D. santomea* and *D. yakuba* for each of 10 replicates conducted at each temperature.

$Z = 3.09$, $P = .01$), and *D. yakuba*'s fitness was lower than when maintained in isolation (Tukey's HSD: $Z = 2.65$, $P = .04$; fig. 2*A*). When maintained together at 25°C, *D. yakuba* outperformed *D. santomea* (Tukey's HSD: $Z = 3.55$, $P = .002$), but there was not a significant difference in *D. santomea*'s fitness when maintained in isolation or together with *D. yakuba* at 25°C (Tukey's HSD: $Z = 1.42$, $P = .48$). At 22°C, there was no significant effect of competitive environment on per-female performance in either *D. santomea* or *D. yakuba* (Tukey's HSD: all $P > .05$). The mean number of male offspring produced per *D. santomea* female at 18°C did not differ when maintained either with or without *D. yakuba* (Tukey's HSD: $Z = 1.38$, $P = .51$), and the same was true for *D. yakuba* when maintained at 25°C (Tukey's HSD: $Z = 1.41$, $P = .49$). This result suggests that either intraspecific competition at our experimental densities was weak relative to interspecific competition or intraspecific competition in the competition treatment was weak relative to interspecific competition. We report results from parallel analyses including both female and male offspring in appendix II and figure S1. The only difference we observed when analyzing total offspring (i.e., male and female offspring both included) was that *D. yakuba* produced fewer offspring when they experienced interspecific competition at 22°C than when they were maintained in the absence of interspecific competition at 22°C (Tukey's HSD: $Z = -2.724$, $P = .0325$; fig. S2).

When we treated the interspecific competition replicates as paired data, *D. santomea* tended to produce more offspring than *D. yakuba* at 18°C, resulting in a marginally significant effect ($S = 8$, $P = .055$; fig. 2*B*, *left*). At 18°C, there was also a strong negative correlation between the number of male *D. santomea* and male *D. yakuba* produced across the 10 replicates ($r = -0.86$, $P = .0013$), a pattern consistent with strong interspecific competition. At 25°C, *D. yakuba* consistently produced more offspring than *D. santomea* ($S = 0$, $P = .001$; fig. 2*B*, *right*), and there was no consistent difference in the number of offspring produced by either species at 22°C ($S = 9$, $P = .828$; fig. 2*B*, *middle*).

Competition over Multiple Generations in Different Thermal Environments

Consistent with the results we observed over the course of a single generation, when the two species were maintained together at 25°C, the proportion of *D. santomea* monotonically decreased over the course of the experiment (Mann-Kendall trend tests: $P = .036$, $P = .036$, and $P = .013$, respectively, for the three cages that maintained viable populations over the entire course of the experiment; GLMM with fixed effect of species: $Z = 18.11$, $P < .0001$). At 25°C, *D. santomea* became extinct in three of four cages maintained at 25°C after 57 days (four or five generations; in the fourth cage, only one of 67 sampled individuals was *D. santomea*; figs. 3, S4). By contrast, when maintained at 18°C, the proportion of *D. santomea* in each cage did not monotonically change over the course of the experiment (Mann-Kendall trend tests: $P > .1$ for each replicate), and *D. santomea* was frequently more abundant than *D. yakuba* (fig. 3); however, this trend was not statistically significant (GLMM with fixed effect of species: $Z = -1.314$, $P = .19$). Data underlying all results and figures have been deposited in the Dryad Digital Repository

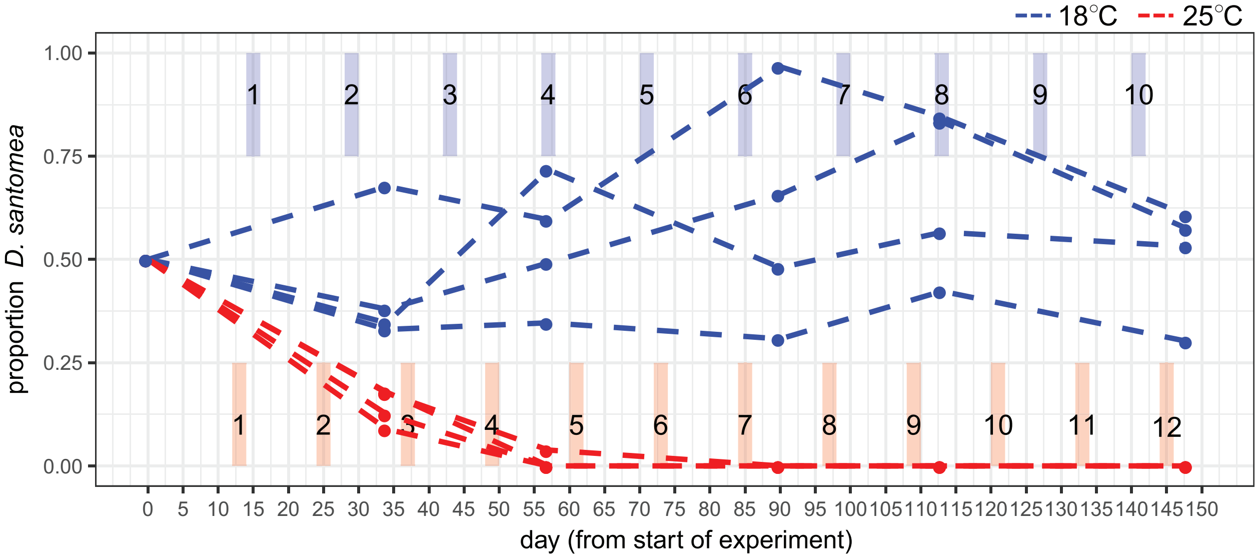

Figure 3: Multigeneration effect of temperature on the coexistence of *Drosophila santomea* and *Drosophila yakuba*. When *D. santomea* and *D. yakuba* are maintained together in cages at 18°C (blue lines), the proportion of *D. santomea* within the cages tends to be near or above 0.5. By contrast, when maintained at 25°C (red lines), *D. santomea* is competitively excluded from the cages within approximately four to seven generations. The approximate number of generations is indicated for both temperature treatments by shaded rectangles (blue indicates generation times when maintained at 18°C; red indicates generation times when maintained at 25°C).

(https://doi.org/10.5061/dryad.bk3j9kd8t; Comeault and Matute 2020).

Discussion

Research is continually uncovering how effects of interspecific interactions are shaped by the environmental context under which those interactions take place (Park 1954; Goldman and Ryther 1976; Wilson et al. 1984; Carreiro and Koske 1992; Davis et al. 1998*a*, 1998*b*; Russell and Moran 2006; Klanderud and Totland 2007; Hillebrand 2011; Alexander et al. 2015; Germain et al. 2020). Our results add to these studies and show how the thermal environment modifies competitive outcomes between the sibling species *Drosophila yakuba* and *Drosophila santomea*. We find that in the absence of interspecific competition, both species display the highest performance at a moderate temperature of ~22°C (fig. 2*A*). However, when they experience interspecific competition at some stage of their life cycle (our experiments do not allow us to identify the proximate mechanism of competition), their competitive dominance hierarchy varies across thermal environments: at higher temperatures, *D. yakuba* outcompetes *D. santomea*, while at lower temperatures, *D. santomea* outcompetes *D. yakuba* (fig. 2*A*). The direction of competitive advantage therefore depends on the thermal environment and is consistent with the thermal niche these two species inhabit in nature (fig. 1). These results suggest that the temperature-dependent outcome of competition between these two species is contributing to the maintenance of their narrow band of sympatry at midelevations on the island of São Tomé.

Temperature and Competition's Role Defining Species' Ranges

We did not observe a symmetrical effect of competition at the low and high temperatures that we tested, as *D. santomea* did not competitively exclude *D. yakuba* within ~10 generations of being maintained together at 18°C (fig. 3). While *D. santomea*'s competitive advantage at lower temperatures meant that it tended to be the more abundant species in experimental enclosures maintained at 18°C, this effect was minor (figs. 3, S7). One explanation is that the strength of competition was weaker in cages maintained at 18°C than in those maintained at 25°C. Generation times, population growth rates, and rates of resource uptake for ectothermic organisms are all predicted to increase with temperature, thereby increasing the pace of interspecific interactions such as competition (Brown et al. 2004). While we did not track population growth rates, observation of the cages indicated that population sizes tended to be lower at lower temperatures. Therefore, while our single-generation competition experiment controlled for the number of individuals (six females per vial) and found that interspecific competition increased or decreased the relative fitness of *D. santomea* and *D. yakuba* to a similar degree, but in opposite directions, at low and high temperatures (fig. 2), competition could have been weak in cages that we maintained at 18°C. This could help explain the coexistence of the two species when maintained at 18°C, and future work is needed to identify the specific mechanisms contributing to coexistence under our experimental conditions.

Alternatively, if *D. yakuba* is able to maintain high fitness at relatively low temperatures, even in the presence of *D. santomea*, what stops *D. yakuba* from moving to higher-elevation habitats on São Tomé? One variable that our experiments do not account for is behavioral preferences for different environments. Laboratory experiments have shown that *D. santomea* displays a behavioral preference for cooler environments compared with *D. yakuba* (Matute et al. 2009). *Drosophila yakuba* is also considered to be a broadly distributed generalist species found across sub-Saharan Africa (Lachaise et al. 1988; Yassin et al. 2016), while *D. santomea* is primarily found in association with figs on the island of São Tomé (Lachaise et al. 1988, 2000). More research into the particular ecology of these two species in their natural habitats is needed; however, we have observed both *D. santomea* and *D. yakuba* eclosing from figs collected on São Tomé (table S3), indicating that the opportunity for direct competition occurs on shared host fruits in nature. It is therefore likely that multiple ecological factors, including temperature, humidity, habitat type, and diet, affect the realized distributions of *D. santomea* and *D. yakuba* on São Tomé. Future studies testing competition and performance on different diets and at temperatures below 18°C (the lowest we tested) are needed to more fully understand the factors defining the ranges of *D. santomea* and *D. yakuba*. Our results show that differences in competitive ability across thermal environments is one factor that affects the realized niches of these two species.

Context-Dependent Responses to Climate Change

Climate change exposes species to warmer mean annual temperatures, and their demographic and evolutionary responses to warmer temperatures will depend on both direct and indirect effects of the thermal environment. For example, species may show direct responses to different thermal environments through the evolution of novel or different physiological traits (Eliason et al. 2011; Cooper et al. 2012; Campbell-Staton et al. 2020). In the context of climate change, a species will be able to persist and maintain demographically viable populations only if the benefit of direct evolutionary responses to warmer (or more variable) temperatures is not outweighed by negative changes in biotic interactions. Previous studies have shown how changes in the thermal environment and/or the community of interspecific competitors can lead to lower fitness or rapid extirpation (Davis et al. 1998*a*; Alexander et al. 2015). Competitive exclusion has therefore been discussed as an important outcome of climate change in a number of species (Finstad et al. 2011; Bulgarella et al. 2014), even including between anatomically modern humans and Neanderthals (Banks et al. 2008).

One of the predictions generated from our results with respect to competition between *D. santomea* and *D. yakuba* is that under warmer mean daily temperatures, the highland forest endemic species, *D. santomea*, may be challenged by competitive exclusion by *D. yakuba*. There are three caveats to consider when interpreting this prediction. First, the multigenerational experiment we conducted that showed competitive exclusion of *D. santomea* by *D. yakuba* at 25°C (fig. 3) did not include appropriate experimental controls, where the two species were maintained in isolation. We therefore assume that *D. santomea* is capable of maintaining viable populations at 25°C under the same experimental conditions in the absence of competition. Two lines of evidence support this assumption. First, when we raised *D. santomea* in isolation at 25°C over a single generation, each female produced an average of 5.6 male and 6.5 female offspring (fig. 2), suggesting that they can maintain positive population growth at this temperature. Second, previous work on thermal performance traits in *D. yakuba* and *D. santomea* shows that males and females of both species remain fertile and reproductively active at 24°C (Matute et al. 2009). We therefore interpret the multigenerational experiment summarized in figure 3 as providing evidence for competitive exclusion of *D. santomea* at 25°C. This result highlights the importance of considering both direct and indirect effects when estimating the impacts that environmental change will have on biodiversity (Lashomb et al. 1987; Russell and Moran 2006; Vale et al. 2008; Baker et al. 2018; Germain et al. 2018).

Second, we did not account for frequency- or density-dependent processes that may alter competitive advantages over the course of the multigenerational experiment. For example, if *D. santomea* exhibited strong competitive dominance over *D. yakuba* at 18°C, one prediction is that they would consistently be the more abundant species across the course of the experiment. While there was a weak trend in our data to suggest that *D. santomea* tended to be more abundant than *D. yakuba* at 18°C, this trend was not statistically significant (GLMM: $P = .19$; fig. S7). Because we used a nonstandardized approach and sampled a subset of individuals within each experimental cage to estimate relative abundances, we were not able to test density-dependent effects. Future work is therefore needed to understand the density dependence of competitive outcomes between *D. santomea* and *D. yakuba* across different thermal environments.

Third, we did not test whether local adaptation to the experimental environment (biotic and abiotic) altered the competitive interaction between *D. santomea* and *D. yakuba*. Studies of local adaptation in these and other species of drosophilid flies have shown that they can show evolutionary responses to selection in as few as 10 generations (Koopman 1950; Higgie et al. 2000; Matute 2010*a*, 2010*b*; Bergland et al. 2014; Tobler et al. 2014; Comeault et al. 2016; Behrman et al. 2018). Local adaptation in *D. santomea*, *D. yakuba*, or both species could have altered the nature of competition or population growth rates over the course of our experiment. Future experiments that either control for evolution or explicitly measure evolution's effects on species interactions under different environmental conditions are needed and have the potential to greatly increase our understanding of species' responses to environmental change (e.g., Germain et al. 2020).

A Role for Biotic Interactions in Range Shifts Associated with Climate Change

An often-discussed (and observed) response that species have to climate change is a poleward or upslope shift in their range (Parmesan 2006; Colwell et al. 2008; Lenoir et al. 2008; Schuetz et al. 2019). Range shifts can be driven by a species tracking favorable abiotic conditions, such as temperature, but the rate and extent of range shifts are likely to vary among species, resulting in "community reorganization" (Van der Putten 2012). Climate-associated range shifts are therefore likely to affect interspecific interactions in at least two ways. First, they can change the identity of the interacting members of a community and, in the case of interspecific competition, alter competitive dominance hierarchies (Alexander et al. 2015). Second, they can change the environmental context of the interaction—such as when tracking one environmental variable results in a change in a second—and alter the outcome of specific interactions (Bronstein 1994; Davis et al. 1998*a*; Tylianakis et al. 2008; Chamberlain et al. 2014; Harrower and Gilbert 2018). The results we have presented here provide an example of the latter and point to the importance of quantifying interspecific interactions under different environments to better predict responses to climate change. This may be particularly important for tropical endemic species threatened by the invasion of sibling species that can displace them through competitive exclusion.

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Statement of Authorship

A.A.C. and D.R.M. designed the study, collected the data, and wrote the manuscript. A.A.C. analyzed the data, and D.R.M. obtained funding.

Data and Code Availability

Raw data tables and scripts used to analyze data and generate figures have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.bk3j9kd8t; Comeault and Matute 2020).

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Montane rain forest habitat of *Drosophila santomea* on São Tomé at an elevation of ~1,500 m above sea level. Photo: A. A. Comeault.