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Plasticity in male mating behavior modulates female life history in fruit flies

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In many species, intense male-male competition for the opportunity to sire offspring has led to the evolution of selfish reproductive traits that are harmful to the females they mate with. In the fruit fly, *Drosophila melanogaster*, males modulate their reproductive behavior based on the perceived intensity of competition in their premating environment. Specifically, males housed with other males subsequently transfer a larger ejaculate during a longer mating compared to males housed alone. Although the potential fitness benefits to males from such plasticity are clear, its effects on females are mostly unknown. Hence, we tested the long-term consequences to females from mating with males with distinct social experiences. First, we verified that competitive experience influences male mating behavior and found that males housed with rivals subsequently have shorter mating latencies and longer mating durations. Then, we exposed females every other day for 20 days to males that were either housed alone or with rivals, and subsequently measured their fitness. We found that females mated to males housed with rivals produce more offspring early in life but fewer offspring later in life and have shorter lifespans but similar intrinsic population growth rates. These results indicate that plasticity in male mating behavior can influence female life histories by altering females' relative allocation to early versus late investment in reproduction and survival.

KEY WORDS: *Drosophila melanogaster*, life history, male-male competition, phenotypic plasticity, sexual conflict, sperm competition.

Sexual conflict occurs when the reproductive interests of males and females differ (Parker 1979; Chapman et al. 2003; Arnqvist and Rowe 2005). This is predicted to result in the evolution of selfish male traits that are harmful to females, and in response, the coevolution of female traits that resist this harm (Rice 1996; Wigby and Chapman 2004). Empirical and theoretical studies have demonstrated the importance of sexual conflict in driving the evolution of dimorphism between the sexes, variation in mating tactics, and even speciation (Arnqvist 1998; Parker and Partridge 1998; Gavrilets and Waxman 2002; Martin and Hosken 2003). Thus, understanding the ecological and genetic factors that influence the expression of sexually antagonistic traits is of great interest to evolutionary biologists.

Theory predicts that the intensity of conflict between the sexes increases with the degree of promiscuity in a mating system, where the magnitude of promiscuity may be regulated by the optimal mating rate of females or by the amount of male-male competition (Chapman et al. 2003). This theory has been tested by experimentally evolving populations under manipulated levels of sexual selection (Holland and Rice 1999; Hosken et al. 2001; Crudgington et al. 2009). Holland and Rice (1999) generated divergent populations of fruit flies, *Drosophila melanogaster*, by either enforcing monogamy or maintaining promiscuity for 47 generations. Their results suggest that females mated to males descended from populations evolved under enforced monogamy live longer and have a greater reproductive rate compared to their counterparts from populations that evolved under promiscuity. This makes sense, because when monogamy is enforced, selection does not act on harmful male traits integral for success in male-male competition, such as persistent courtship or large investment into the transfer of accessory gland proteins (Acps), resulting in the evolution of male mating phenotypes that are less harmful to females (Chapman et al. 1995; Friberg and Arnqvist 2003; Wigby and Chapman 2005; Hollis et al. 2019). Furthermore,

analysis of the natural genetic variation in male competitive ability reveals a similar trend. Civetta and Clark (2000) compared the relationship between success in male-male competition and male-induced harm across 51 distinct genetic backgrounds, and found that males from genetic backgrounds with higher sperm defensive ability also tended to be more harmful to their mates. In sum, these studies provide evidence that there is a direct relationship between the magnitude of the expression of male traits that influence intrasexual competitive success and the amount of harm inflicted on females via mating.

Although the studies that have explored the relationship between mating system dynamics and male harm are critical for our understanding of the expression of male-induced harm and their consequences for female fitness, they were typically conducted in uniform environments, leaving out the important roles of variation in social and ecological factors (Arbuthnott et al. 2014). Recent theoretical work, however, predicts that phenotypic plasticity in sexually antagonistic traits can either strengthen or weaken the intensity of sexual conflict (McLeod and Day 2017; Day and McLeod 2018). In the past few years, several studies have highlighted the importance of considering these socioecological effects when quantifying the intensity of sexual conflict (Perry and Rowe 2018). These studies suggest that environmental factors such as space availability and complexity, predation risk, and population density can modulate the expression and/or evolution of sexually antagonistic traits and thus the magnitude of male-induced harm (Yun et al. 2017; Gomez-Llano et al. 2018; García-Roa et al. 2019). For example, García-Roa et al. 2019) demonstrated that plasticity in male-induced harm can be modulated by temperature, whereby females exposed to males at 29°C had shorter lifespans and fewer lifetime offspring compared to females that mated with males at 25°C or 21°C. Although these studies highlight the importance of considering a broad range of socioecological factors when measuring the intensity of sexual conflict, to the best of our knowledge, no experimental studies have tested how the modulation by social experience of male reproductive traits influences the magnitude of male-induced harmful effects on females.

In species where males mate multiply, males should be prudent with their degree of investment into mating opportunities because of the costs associated with the production of sperm and other features that aid in sperm competition (Parker et al. 1997; Parker and Pizzari 2010). Thus, males are highly sensitive to cues in their sociosexual environment that indicate the likely number of mating opportunities and/or the intensity of competition (Bretman et al. 2011a). In particular, the presence of rivals in the social environment has proven to increase the expression of traits involved in intrasexual competition (Aragón 2009; Bailey et al. 2010; Bretman et al. 2011b; Kelly and Jennions 2011). These responses are phylogenetically widespread, as species across many taxa (insects, reptiles, birds, and mammals) alter the size and composition of their ejaculates in the presence of a single rival (Kelly and Jennions 2011). In the fruit fly, a model species extensively used to study both behavioral plasticity and sexual conflict, males that are housed with rivals prior to a mating opportunity mate for longer and transfer larger volumes of ejaculate containing more sperm and some Acps during copulation compared to males housed alone (Bretman et al. 2009; Wigby et al. 2009; Fedorka et al. 2011; Moatt et al. 2014). In general, the Acps transferred during mating have positive effects for males and can have both positive and negative effects on female fitness. This plasticity has fitness benefits for males as increased sperm and Acp transfer can result in increased number of offspring produced (Bretman et al. 2009, Bretman et al. 2013), and sometimes also paternity share (in Bretman et al. 2009 but not in Bretman et al. 2013).

Although the increased number of absolute offspring represents a short-term benefit for females, the long-term repercussions of this plasticity for female fitness remain unclear. In a short-term context, certain Acps such as sex peptide and ovulin stimulate offspring production and delay remating rate (Fricke et al. 2009; Wigby et al. 2009). However, exposure to the same Acps reduces female long-term fecundity and lifespan (Johnstone and Keller 2000; Wigby and Chapman 2005), and it is unclear if plasticity in male behaviors can influence these long-term fitness costs.

To address this issue, we tested whether plasticity in male traits that leads to increased siring success under intrasexual competition has a negative long-term influence on the females that they mate with in terms of lifetime offspring production and survival. First, we replicated previous work (Bretman et al. 2009, Bretman et al. 2013) to verify that males alter their expression of sexually antagonistic traits in response to perceived sperm competition. Specifically, we predicted that males under perceived competition would be quicker to mate, mate for longer durations, and delay the remating interval of their mates for longer than males kept alone. Second, we tested whether perceived sperm competition influences the magnitude of harmful effects on the females that males mate with. We predicted that males housed with rivals would reduce their mates' lifetime offspring production and longevity more than males housed alone. Additionally, we predicted that females mated to males that perceive sperm competition risk would have lower intrinsic population growth rates, a rate-sensitive fitness measure that takes into account both lifetime reproductive success and lifespan. To test the robustness of any effects detected, we replicated our test in two distinct populations of fruit flies. Finally, we tested males from multiple genotypes to quantify the degree of genetic variation associated with the plasticity of male response to intrasexual competition and the potential variation in subsequent effects on female fitness.

Methods

FLY STOCKS AND GENERAL

All focal males descended from 28 lines of the *Drosophila genetic reference panel* (Mackay et al. 2012). These lines were derived from wild flies caught in Raleigh, North Carolina, USA, and repeatedly inbred for 20 generations. To alleviate the deleterious phenotypic effects associated with inbreeding, we generated hybrid genotypes by crossing each line to a randomly selected standardized reference line, thereby creating unique hybrids (hereafter referred to as hybrids). Within each hybrid, individuals are genetically identical, but between hybrids, individuals share an identical clonal haplotype inherited from their mother, and a unique clonal haplotype inherited from their father, allowing us to quantify the degree of genetic variation associated with phenotypic differences expressed from this unique haplotype.

Focal females tested in the remating assay (part 1) and the first replicate of the fitness assay (part 2) were descendants of a wild-caught population of flies collected from multiple locations throughout southern Ontario in August 2014 (hereafter ON) (Baxter and Dukas 2017). Since its establishment, we housed this population in two cages each measuring 20 cm × 20 cm × 35 cm and containing several hundred flies maintained with overlapping generations, meaning that each fly lived in the cage until natural death, and had the opportunity to produce multiple generations of offspring.

In the second replicate of the fitness assay, we used females derived from the *Ives* population (hereafter IV) obtained from the Long Lab (Wilfrid Laurier University, Waterloo, Ontario, Canada). The IV population was originally collected in South Amherst, MA, USA in 1975. In 1980, Michael Rose created a lineage of this population that has been maintained at large census size (>1000 adults/generation) and on a standardized culture protocol with nonoverlapping generations (Rose 1984). Since then, this same lineage of IV has been maintained under identical conditions and used extensively as a model for studying longevity and sexual conflict (Rose 1984; Martin and Long 2015; Filice and Long 2016). Unlike the ON population, when the IV females are 4 days posteclosion, they have a single 24-h window to lay their eggs for the next generation.

Competitor males for the focal males in both parts 1 and 2 were descendants of a subpopulation of the IV line that had the *e* (hereafter, ebony) mutation introgressed. This mutation results in a darker body color that is clearly visible with the naked eye, allowing us to easily identify the focal hybrid male during our trials. Although these males tend to be at a competitive disadvantage to wild-type flies, the phenotype is naturally-occurring and confers a selective advantage in some contexts (Pool and Aquadro 2007; Takahashi et al. 2007). Regardless, given that these flies were standard competitors, any competitive disadvantages would be consistently realized across trials and should not impact our results.

We reared all experimental flies at a standardized density of 100 eggs per vial containing ~5 mL of standard fly medium made of water, sucrose, cornmeal, yeast, agar, and methyl paraben, and stored all flies in an incubator at 25°C and 60% relative humidity with a 12:12 h light:dark cycle. We collected newly eclosed virgin flies within 8 h of eclosion under light CO_2 anesthesia. Following their initial collection, we handled all flies using gentle aspiration.

PART 1: EFFECTS OF PERCEIVED COMPETITION ON MATING LATENCIES AND DURATIONS, AND FEMALES' REMATING RATES

We started each replicate by collecting four focal males from each of the 28 hybrid genotypes. We placed two of these males individually into a vial alone, and two individually into a vial each containing two competitor ebony males. Simultaneously, we collected females and housed them in groups of 20 with a dash (~10 mg) of live yeast. Three days later, which is a sufficient amount of time to induce a strong response to rivals (Bretman et al. 2011b), we paired each focal male with a single virgin female in fresh vials containing a dash of live yeast. Observers blind to treatment scored the mating latency and duration to the nearest second. We discarded and replaced pairs that did not mate within 90 min. To prevent multiple matings, we removed males immediately after each mating concluded. We kept females in these vials and returned them to their environmental chamber.

The following morning, we introduced a new wild-type male to each female, and observers blind to treatment measured the latency and duration of all matings. We observed the flies for 4 h and classified the females that did not mate by this point as "not remated." We repeated the entire above procedure in six identical replicates over 12 days, resulting in 12 replicates/male hybrid/treatment, except in the case of missing trials. Missing trials included cases in which we were unable to collect a sufficient number of hybrid males, and instances of female escape or death, resulting in a final sample size of $N = 542$ trials.

PART 2: FITNESS ASSAY

We collected 120 wild-type females and housed them in groups of 20 in vials that contained a dash of live yeast. Simultaneously, we collected 20 focal males from six hybrid backgrounds (a random subset from the original 28 used in part 1). We placed 10 of these males into vials alone, and 10 into vials each containing two competitor ebony males (Fig. 1). Three days later, we placed each female into an individual food vial with a dash of live yeast and paired her with a single male from one of the two experience treatments. Observers blind to male hybrid identity and experience treatment scanned the pairs of flies for 3 h and recorded the latency

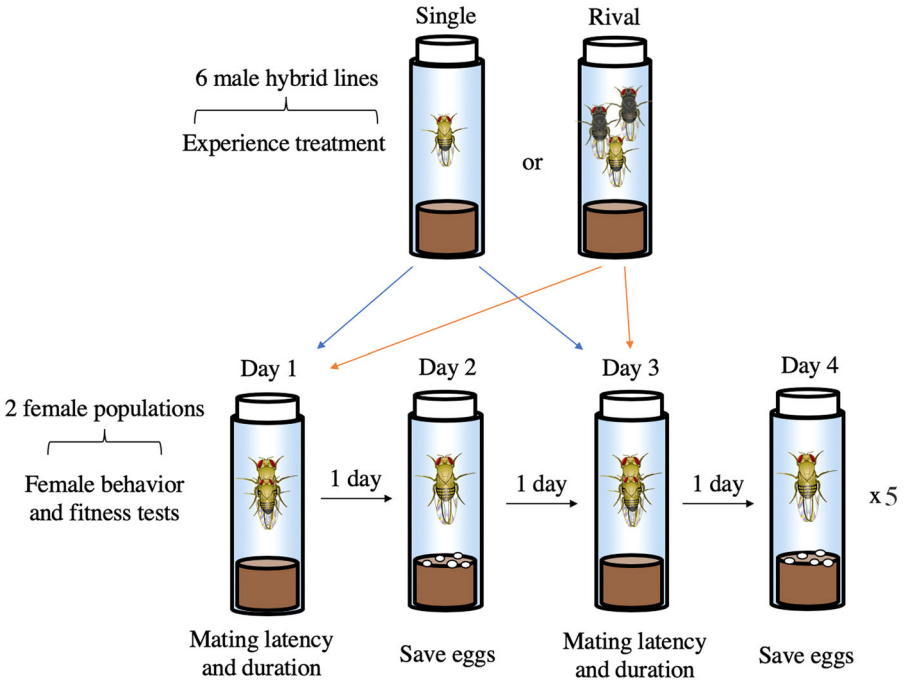

Figure 1. An illustration of female experience in the fitness experiment. Females from one of two different populations were exposed to a focal male that was either previously housed alone or with two competitors. After 3 h, males were removed and females were left alone for a day and then exposed to another male from the same initial treatment. This was repeated a total of five times, resulting in a total of 10 brief exposure periods to males from one of the two treatments.

and duration of each mating. To prevent multiple matings, we removed the males from each vial after the first mating concluded or after 3 h if no mating occurred. We placed the females in the environmental chamber and allowed them to lay their eggs undisturbed for 45 h. Following this period, we moved each female into a fresh food vial with live yeast, and paired her with a new 3-day old male from the same treatment and line combination as before. Again, observers blind to male hybrid identity and treatment recorded the latency and duration of any matings, and we removed males following a mating or after 3 h had passed. We repeated this procedure every other day over 20 days, meaning that each female had ten 3 h opportunities to mate with a male (Fig. 1). After the tenth mating opportunity, we transferred females into fresh vials with live yeast every 5 days until they died. We checked for mortality every morning at the same time, until all females died.

Two weeks following each testing day, observers blind to treatment counted the number of offspring in each vial. Overall then, for each female alive through age 24 days, we had 10 offspring vials. The offspring of females that died before day 24 were only counted up until the day that the females died. In other words, we dropped females from analysis after mortality, rather than counting their offspring production as zero. We performed two replicates of this procedure, one with ON females ($N = 120$) and the other with IV females ($N = 120$).

STATISTICAL ANALYSIS

We conducted all data analysis using R version 3.5.2 (R Core Team 2013). To analyze the effect of perceived competition on male mating behavior, we constructed generalized linear mixed models (GLMMs) using the *lme4* package (Bates et al. 2014). We analyzed mating latency and duration using a Gaussian distribution, and rematings using a binomial distribution. Our maximal models included male treatment as a fixed effect, and hybrid genotype, day of testing, and all possible interactions as random effects. However, we simplified our models until we had no singular fits. In all three cases, our simplified models excluded the interactions with day effect. We calculated the 95% confidence intervals of our random effects by using the *bootMer* function to re-simulate our models 1000 times.

In our fitness analysis, we analyzed female mating frequency using a GLMM with a binomial response variable defined by the total number of matings weighed by the number of mating opportunities. We included male treatment and female population as fixed effects, and male genotype as a random effect. To analyze female offspring production, we constructed a GLMM with a negative binomial response to deal with overdispersion. We included male treatment, female population, and female age as fixed effects, and hybrid genotype with all possible crossed interactions as random effects. To account for repeated measures, we also included individual female identity as a random effect. Similar to

our other GLMMs, we simplified our models until there were no singular fits. In this case, our simplified model excluded the interactions with hybrid genotype. To analyze female lifespan, we constructed a mixed effects Cox survival model. This included lifespan as a survival term, male treatment and female population as fixed effects, and male hybrid genotype as a random effect. Finally, we calculated a measure of fitness for each individual female, intrinsic population growth rate (λ). This is a rate-sensitive measure that gives more weight to offspring produced earlier in life (McGraw and Caswell 1996) and is most relevant in expanding populations (Gilbert and Charlesworth 1981). λ is calculated by placing individual life history data (offspring production and survival) into a Leslie matrix, and calculating the dominant eigenvalue of each matrix (McGraw and Caswell 1996). To analyze differences in λ, we constructed a GLMM with male treatment and female population as fixed effects, and hybrid genotype with all possible crossed interactions as random effects. We calculated the *P*-values of the fixed effects in all of our above models using the *Anova* function from the *car* package (Healy 2005).

Results

PART 1: EFFECTS OF PERCEIVED COMPETITION ON MATING LATENCIES AND DURATIONS, AND FEMALES' REMATING RATES

Males housed with rivals had shorter mating latencies than males housed alone (an average of 7.83 min faster; $\chi^2 = 11.3$ df $= 1$, $P = 0.0007$; Fig. 2A). There was no significant variation in mating latency between different hybrid backgrounds (SD = 111.9), but the interaction between hybrid genotype and experience treatment (SD = 236.7) was significant (Fig. 2A; Table 1). Males housed with rivals mated for significantly longer compared to males housed alone (an average of 2.04 min longer; $\chi^2 = 33.3$, df $= 1$, $P < 0.0001$; Fig. 2B). Males from different hybrid backgrounds (SD = 57.1) varied significantly in their mating duration, but the interaction between genotype and experience (SD = 0) was not significant (Fig. 2B; Table 1). There was a marginally nonsignificant trend whereby females mated to males housed with rivals were less likely to remate a day later (an average of 7.2% less frequently; $\chi^2 = 3.2$ df $= 1$, $P = 0.0727$; Fig. 2C). The effects related to hybrid genotype (SD = 0.342) and the interaction between genotype and experience treatment (SD = 0.204) were both significant in influencing females' likelihood to remate (Fig. 2C; Table 1).

PART 2: FITNESS ASSAY

Male treatment did not have a significant effect on the proportion of times each female remated ($\chi^2 = 0.0057$, df $= 1$, $P = 0.94$), but females from the IV population mated significantly more

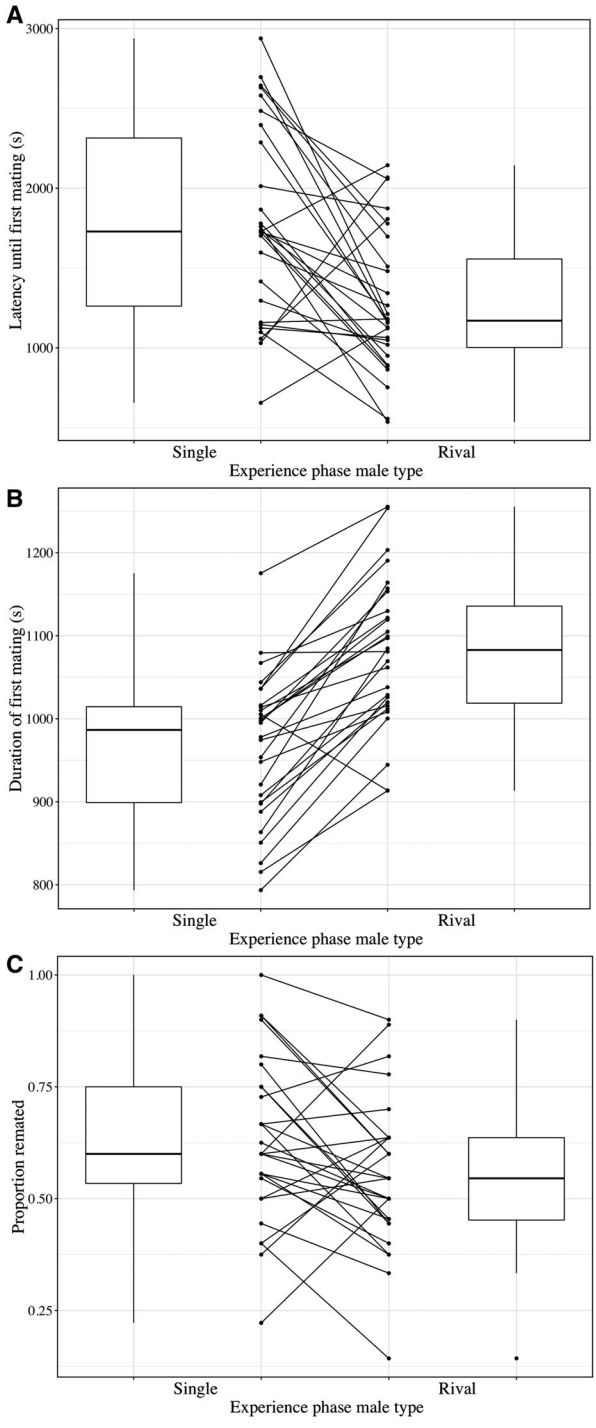

Figure 2. **Effects of previous exposure to rivals on male mating phenotypes. The boxes contain the middle 50% of data (interquartile range [IQR]), and the horizontal lines represent the medians. The whiskers above and below each box represent values within ±1.5 IQR, and any values beyond this are outliers represented by closed circles. The reaction norm plot in the center of each panel depicts the change in the mean of each hybrid genotype across the two experience treatments. (A) Mating latency with virgin females. (B) Mating duration with virgin females. (C) Mating rate of second male with mated females (i.e., effect of first mating on subsequent female receptivity).**

Table 1. Variance components, standard deviation, and 95% confidence intervals estimated using generalized linear mixed models (GLMMs) fit by maximum-likelihood (Laplace approximation) for the mating phenotypes of hybrid males. Males from different genetic backgrounds were randomly assigned as experiencing two rivals or no rivals in the premating environment.

Trait	Source of variance	Variance (SD)	Bootstrapped upper, lower 95% CI	Percent of variance explained
Mating latency	Genotype	12,528 (111.9)	164,436, 0	0.49
	Genotype × treatment	56,019 (236.7)	301,649.7, 0.29	2.2
	Day	137,647 (371)	399,656.5, 4571.8	5.39
	Residual	2,345,257 (1531.4)		
Mating duration	Genotype	3256 (57.1)	10,866.5, 64.9	6.76
	Genotype × treatment	0 (0)	2481, 0	0
	Day	1201 (34.7)	3830.5, 0	2.49
	Residual	43,727 (209.1)		
Remating delay	Genotype	0.117 (0.342)	0.46, 3.08×10^{-24}	8.8
	Genotype × treatment	0.0415 (0.204)	0.49, 0.00042	3.1
	Day	0.171 (0.414)	0.65, 7.98×10^{-11}	12.9
	Residual	1		

frequently than females from the ON population ($\chi^2 = 79.7$, df = 1, $P < 0.0001$; Fig. 3). The interaction between these two factors was not significant ($\chi^2 = 0.043$, df = 1, $P = 0.837$), nor was the random effect of male genotype (SD = 0).

Male treatment did not have a significant effect on the number of offspring females produced over the 10 egg laying periods ($\chi^2 = 0.444$, df = 1, $P = 0.505$). However, the interaction between male treatment and time period was significant ($\chi^2 = 7.84$, df = 1, $P = 0.005$). Over time, the slope in the rival treatment is more negative compared to the slope in single treatment, indicating that females mated and remated to males previously housed with rivals had more offspring early in life and fewer offspring later in life than females mated and remated to males previously housed alone (Fig. 4). The effects of day of egg laying ($\chi^2 = 111.7$, df = 1, $P < 0.0001$) and the population of females being tested ($\chi^2 = 38.9$, df = 1, $P = < 0.0001$) both had a strong effect. The interactions between day and female population ($\chi^2 = 292.5$, df = 1, $P < 0.0001$) were also significant, but the interactions between male treatment and population ($\chi^2 = 2.48$, df = 1, $P = 0.116$) and male treatment × female population × day ($\chi^2 = 0.584$, df = 1, $P = 0.445$) were not. The effect of male genotype on female offspring production was small and not significant (SD = 3.8×10^{-5}).

Females mated to males housed with rivals lived significantly shorter than females mated to males housed alone ($\chi^2 = 4.5$, df = 1, $P = 0.034$; Fig. 5A). Although females from the ON population lived much longer than females from the IV population ($\chi^2 = 78.4$, df = 1, $P < 0.0001$), the interaction between experience and population was not significant ($\chi^2 = 0.137$, df = 1, $P = 0.711$). The random effect of male genotype represented a small, nonsignificant proportion of the variance in female lifespan (SD = 0.098).

Finally, females mated to males from different social treatments did not significantly differ in their fitness when measured in terms of intrinsic population growth rate (λ) ($\chi^2 = 1.17$, df = 1, $P = 0.277$; Fig. 5B), but females from the IV population had significantly higher fitness compared to the ON population ($\chi^2 = 279.6$, df = 1, $P < 0.0001$). The interaction between the two was not significant ($\chi^2 = 0.007$, df = 1, $P = 0.933$), and the amount of variance explained by male genotype was negligible (SD = 9.7×10^{-7}). When looking at the relationship between lifespan and population growth rate, there was a strong negative correlation between the two metrics ($\rho = -0.283$, $S = 2{,}882{,}300$, $P < 0.0001$; Fig. 5C)

Discussion

Here, we replicated the results of previous studies, first by documenting that male fruit flies exposed to rivals mate for longer compared to males housed alone (Fig. 2B; Bretman et al. 2009, 2010, 2011b, 2013; Wigby et al. 2009) and second by finding significant genetic variation in mating duration (Fig. 2B; Fiumera et al. 2007). Our study is the first to report that the changes induced by a male's experience with rivals have a significant effect on the life history of his mates by (1) stimulating early-life reproduction at a cost of decreased late-life reproduction (Fig. 4), and (2) reducing their lifespan (Fig. 5A). However, our estimates of intrinsic population growth rates suggest that the later life costs imposed on females of multiply mating with males that perceive sperm competition risk are balanced out by the early life benefits, contrary to our prediction (Fig 5B). The results from other studies have demonstrated that the context in which mating interactions take place can influence the magnitude of male-induced effects on female fitness (Arbuthnott et al. 2014; Yun et al. 2017;

García-Roa et al. 2019). We add to these findings by documenting that the perception of sperm competition risk in a male's social environment can elicit phenotypic changes in his mating behavior that have significant consequences for their mates' life histories.

When looking at the effect of competitive experience on male mating behavior, our results were mostly consistent in direction with those of similar studies. We found that, on average, males exposed to rivals had shorter mating latencies (Fig. 2A), longer mating durations (Fig. 2B), and tended to reduce the remating rate of their mates compared to males that were not exposed to rivals (Fig. 2C). In fruit flies, mating duration is positively associated with the total amount of seminal fluid transferred (Wigby et al. 2009), and studies have consistently found that increased sperm competition risk results in a greater investment into mating duration (Bretman et al. 2009, 2013; Wigby et al. 2009). This result is intuitive, as males that invest more into the donation of Acps may increase the short-term fecundity of their mates, and have increased success in securing paternity via sperm competition (Hollis et al. 2019). Here, we also identified significant genetic variation in male mating duration, but not in the interaction between genotype and male treatment. In other words, different male genotypes varied in their mating duration, but the change in duration between experience treatments was relatively consistent across genotypes. This is an interesting result, as it suggests that males vary in their investment into the transfer of Acps during mating, but are consistent in adjusting their investment in response to the presence of rivals. Given the evidence for genetic trade-offs in different male reproductive strategies such as between male-male competition and the ability to simulate oviposition (Filice and Long 2018; Nguyen and Moehring 2019), future studies should continue to explore how investment into the production and transfer of Acps are genetically correlated with other male traits.

Unlike the persistent findings about mating duration, the documented effects of perceived competition on mating latency have been mixed. Bretman et al. (2009) found no significant difference in mating latency between males housed alone and with rivals, but Bretman et al. (2013) found that males housed alone were quicker to mate. Here, we reported that males housed with rivals were quicker to mate. It is possible that the mixed results reflect variation in the dominance hierarchies (which are rapidly formed when multiple males are placed in a vial) and the subsequent types of male-male interactions that occur during the male experience phase (Filice and Dukas 2019). In fruit flies, males who have won a previous fight tend to have shorter mating latencies compared to losers (Filice and Dukas 2019). In previous experiments where no difference in latency was found, males housed with rivals were randomly selected out of each vial and then tested (Bretman et al. 2009). Here, we selected from each vial a predetermined focal male that was housed with standard ebony competitors. Thus,

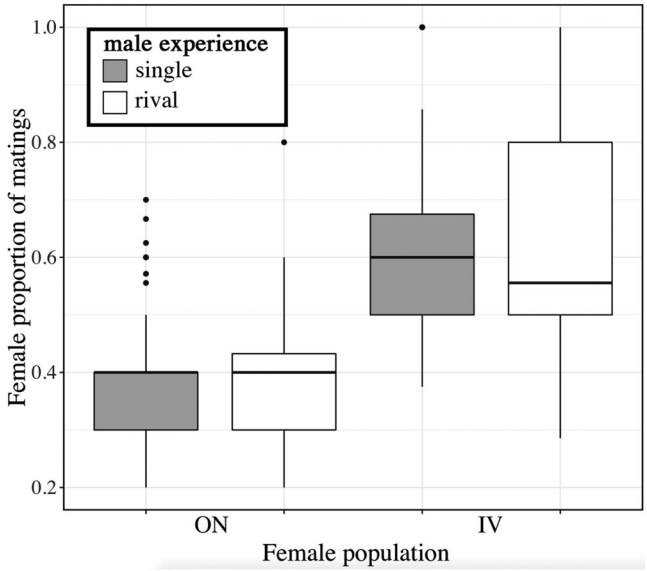

Figure 3. Proportion of times each female from either the ON or IV populations mated out of her number of mating opportunities. The boxes contain the middle 50% of data (interquartile range [IQR]), and the horizontal lines represent the medians. The whiskers above and below each box represent values within ±1.5 IQR, and any values beyond this are outliers represented by closed circles. The shaded boxes represent females mated to males held alone and the white boxes represent females mated to males exposed to rivals.

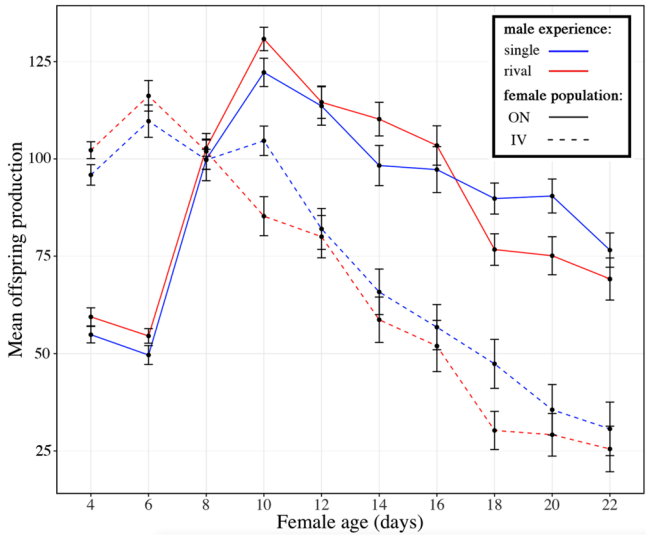

Figure 4. Effect of males' previous exposure to rivals on their mates' offspring production over the first 22 days of the mates' lives. Each point represents the mean number of offspring produced by all the females within each treatment, and the bars above and below each point represent the standard error. The red curves represent females mated to males exposed to rivals, and the blue curves are females mated to males held alone. The solid curves represent females descended from the ON population, and dashed curves are females descended from the IV population.

assuming that each vial has a single dominant male, the variance in male status is higher when there are three or more possible focal males in a vial, whereas in our protocol, each focal male was probably of more similar status when housed with two standard males. It is also possible that the ebony competitors are at a general competitive disadvantage to the focal males, resulting in more frequent winner-effects among our focal flies (Takahashi et al. 2007). This mechanism could also explain the significant interaction that we observed between hybrid genotype and male treatment, if males of some genotypes are more likely to be the dominant males than males of other genotypes. Finally, although it was not significant, males exposed to competitors decreased the sexual receptivity of their mates slightly more than males housed alone, and we add to previous findings by identifying significant genetic variation in this effect (Fiumera et al. 2007). Similar to the genetic variation we identified in mating duration, this result suggests that males vary in the quality and/or quantity of Acps transferred during mating depending on their genetic background (Fiumera et al. 2007). Furthermore, the significant interaction between male treatment and genotype indicates genetic variation in the plasticity associated with experience-dependent manipulative male tactics like the delaying of remating. In other words, some males may switch to strategies that involve delaying remating when there is a risk of sperm competition, whereas males from a different genetic backgrounds may not.

When looking at the effect of male-competitive experience on female fitness, we found that females mated to males previously exposed to competitors invested more into early-life reproduction at a cost of decreased later life reproduction and shorter lifespans. Specifically, females from different experience treatments varied in the amount of offspring they produced over time, characterized by females mated to competitor males having higher production early in life, but lower production later in life (Fig. 4). This result is consistent with the finding that females mated to males housed with rivals tend to lay more eggs in the 24 h following a single mating (Bretman et al. 2009), but offers new insight into a potential late-life reproductive cost associated with this effect. Similar studies to our own have found that females trade-off their lifetime reproduction for early-life reproduction in response to different experiences with males, but we are the first to show that male perception of sperm competition risk can induce this effect (Crudgington et al. 2010; Edward et al. 2011). For example, Edward et al. (2010) found a consistent trend, whereby females with a high exposure to males produced more offspring in the first 8 days of their life but produced fewer offspring throughout the remainder of their lives, compared to females that had a low exposure to males (Edward et al. 2011). In both cases, it is likely that the females received more sex peptide and ovulin, Acps that stimulate egg production. In our study, males exposed to rivals probably transferred a greater volume of Acps (Wigby et al. 2009), and in Edward et al. (2010), females with a high exposure to males mated more often and thus received more Acps, which is consistent with a study that shows males from populations that evolved under high competition deplete their ejaculates faster (Linklater et al. 2007). In our study, males that perceive the risk of sperm competition can benefit from stimulating their mates to produce as many offspring as they can in the short term because of the high likelihood that they will lose paternity to males from subsequent matings.

Although this increase in early-life reproduction appears to be costly for a female's reproductive potential later in life, these later life costs appear to balance out the early-life benefits as females mated to rival-exposed and single males have similar intrinsic population growth rates. This result suggests that females may greatly benefit from a single mating with a male that transfers more egg-stimulating Acps, but repeated exposure to these males may result in long-term costs. As short-term reproduction is highly important for fitness in species with life histories similar to *D. melanogaster*, this may have major implications for female mate choice. In *D. melanogaster*, males that are detrimental to long-term female fitness also tend to be preferred (Friberg and Arnqvist 2003). However, in the study just mentioned and many others, females are consistently housed in small vials, which may result in more harassment and matings than would naturally occur (Pitnick and García-González 2002; Crudgington et al. 2010; Edward et al. 2011). Thus, it may be that in natural settings, the costs associated with prolonged exposure to males that stimulate short-term offspring production may never be realized and males that are deemed as "harmful" in laboratory settings would actually have a net benefit for females in nature. It is therefore important that future studies consider their methodologies when making predictions about the fitness effects of sexually antagonistic interactions (Yun et al. 2017).

It is also interesting to note the large differences in the average number of offspring produced over time and in intrinsic population growth rate between the ON and IV populations, effects that are almost certainly due to the maintenance protocols and consequent evolved life histories of these populations. Our IV population has been maintained with nonoverlapping generations for hundreds of generations, and females of this population only have a single 24-h window when they are 4-day old to lay their eggs for the next generation. Therefore, these flies have been selected to invest as much as they can into early-life reproduction. On the other hand, the ON was recently caught (2014) and has been maintained with overlapping generations, meaning that flies can produce offspring throughout their life. The consequences of this are clear when looking at the average number of offspring produced over time (Fig. 4) and is represented by the significant interaction between population and day. Similar to the interaction between male treatment and day, the large investment into

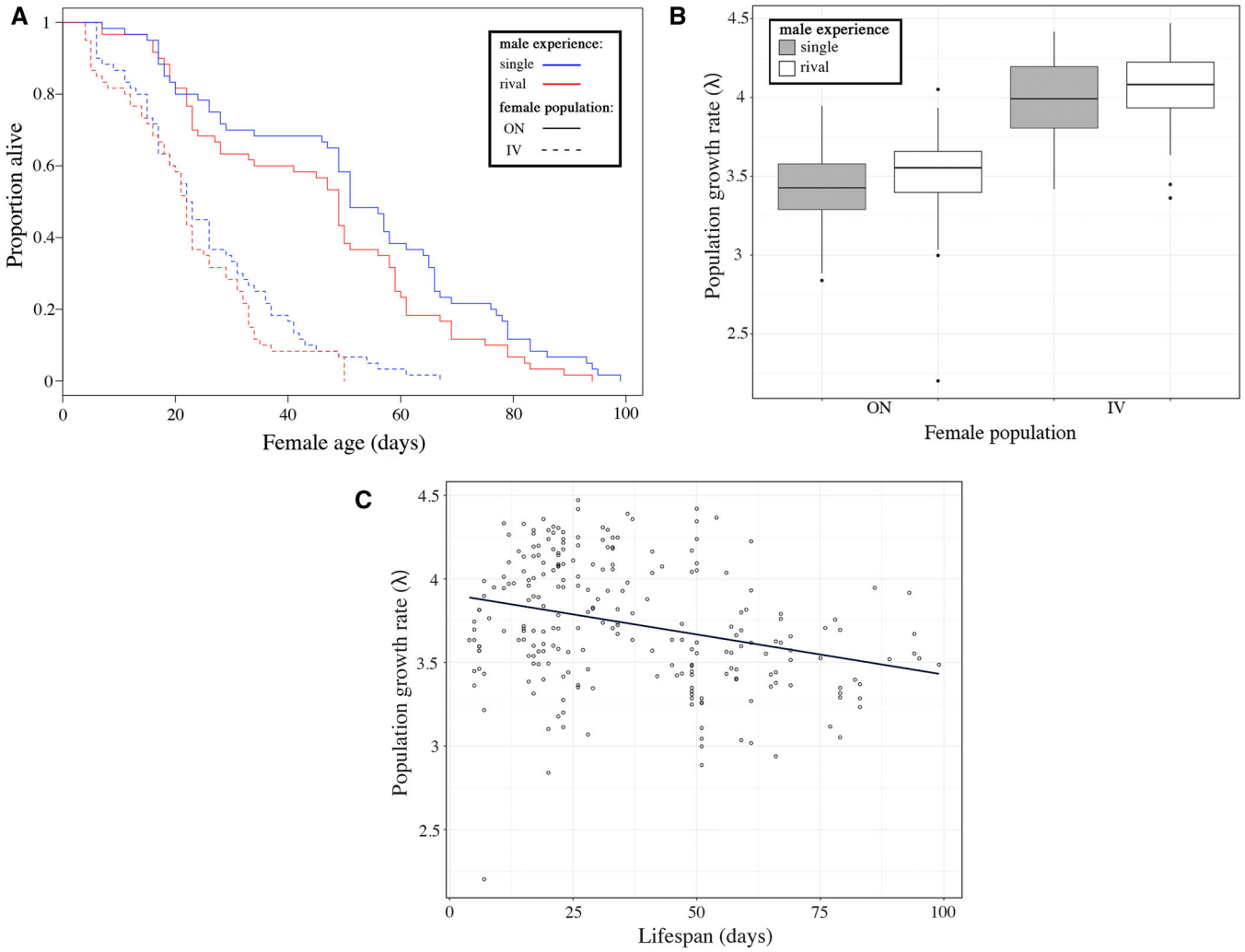

Figure 5. **(A) Effect of males' previous exposure to rivals on their mates' lifespan. Each survival curve represents the proportion of females alive over time. The red curves represent females mate to males exposed to rivals, and the blue curves are females mated to males held alone. The solid curves represent females descended from the ON population, and dashed curves are females descended from the IV population. (B) Effect of males' previous experience on their mates' fitness measured in terms of population growth rate (λ). The boxes contain the middle 50% of data (interquartile range [IQR]), and the horizontal lines represent the medians. The whiskers above and below each box represent values within ±1.5 IQR, and any values beyond this are outliers represented by closed circles. The shaded boxes represent females mated to males held alone, and white boxes are females mated to males exposed to rivals. (C) The relationship between lifespan and population growth rate. Each dot represents a single female tested. The black line represents Spearman's rank correlation, and the shaded region is the 95% confidence interval.**

early-life reproduction of the IV population is associated with decreased later life reproduction, compared to the ON population.

The shorter lifespan of females mated to males exposed to rivals may be explained by a larger donation of Acps during matings from these males, as it is well known that exposure to Acps is associated with reduced longevity in female fruit flies (Chapman et al. 1995; Wigby and Chapman 2005). A potential mechanism is that the increased investment into early reproduction results in an increased rate of senescence (Bretman and Fricke 2019). In fruit flies, females that have a genetic propensity to mate more produce more offspring early in life and die younger (Chapman et al. 1995; Travers et al. 2015), and lineages of the IV population that were artificially selected for increased longevity displayed a decreased investment into early-life offspring production and increased late-life production compared to the base population (i.e., a trend similar to the IV population in Fig. 4; Rose 1984). Furthermore, the negative correlation we identified between lifespan and population growth rate indicates that females who produced the most offspring early on tended to die younger (Fig. 5C). Bretman and Fricke (2019) reported that female longevity and the onset of senescence is not influenced by the receipt of sex peptide, but females with more exposure to males (i.e., more matings) have

reduced longevity and an accelerated expression of senescent decline in traits such as climbing speed and starvation resistance. Although sex peptide on its own appeared to have no detectable effect on female longevity, it may be that other Acps transferred during mating mediate the harmful effects of mating such as those related to stimulating reproduction (Bretman and Fricke 2019). Given that, in our trials, we detected no significant difference in the average number of matings between females exposed to males housed either alone or with rivals, it is likely that the differences we observed in female longevity are due to variation in the total amount of some Acps donated during each mating, or due to variation in other factors such as increased behavioral harassment, risk of transmitting infection, or weaker immune response to infection (Schwenke and Lazzaro 2017). Future studies should continue to investigate the individual and cumulative effects of different Acps to determine their effects on female postmating phenotypes.

Similar to offspring production, we also reported a large difference in lifespan between the two populations we tested, whereby ON females lived much longer than IV females (Fig. 5A). Again, this difference is expected given the maintenance schedule and consequent life histories of these populations. Because the IV population has been maintained with nonoverlapping generations, females have been selected to maximize early-life reproduction, and traits associated with survivorship past 4 days of adulthood are less important. Specifically, the difference in female survivorship and early-life offspring production between the populations may be manifested due to a 50% higher number of matings in the IV than ON females (Fig. 3). Multiple matings in the IV population increase early-life fecundity (Filice and Dukas, unpubl. data), so females likely have a high propensity to mate multiply despite the potential long-term costs that this population has not experienced. In some regard, the survivorship difference we observed between the populations can simply be viewed as a version of the differences that we observed among females mated to males from different experience treatments (Fig. 5A), as both types of differences are probably driven by "live fast, die young" life history strategies.

Taken together, our results have important implications for our understanding of how the context of a social environment influences the life history strategies of males and females, and more broadly the sexual interactions that occur between males and females within a population. Specifically, we found that males that perceive high levels of sperm competition selfishly alter their mating behavior in a way that influences the amount of harm to their mates expressed as reduced survival and late-life reproduction. In terms of lifetime fitness, however, the consequence of this harm appears to balance out due to increased early-life benefits associated with offspring production. We propose that much of the identified male harm to females in sexual conflict research is due to this "produce my offspring fast, die young" manipulation, which varies in intensity depending on a male's experience. Future studies should continue to explore how environmental variation such as the sociosexual landscape influences the expression of mating behavior to improve our understanding of how sexual selection shapes the evolution of behavioral phenotypes as well as how plasticity in sexual behavior affects sexual selection and evolution.

AUTHOR CONTRIBUTIONS

DCSF and RD designed the experiments. DCSF and RB carried out the experiments. DCSF wrote the first draft and performed the statistical analysis. All authors were involved in the revision process.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

Data available from the Dryad Digital Repository: doi:10.5061/dryad.0k6djh9wn.

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