Original Article

Previous inter-sexual aggression increases female mating propensity in fruit flies

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Female mate choice is a complex decision making process that involves many context-dependent factors. Understanding the factors that shape variation in female mate choice has important consequences for evolution via sexual selection. In many animals including fruit flies, *Drosophila melanogaster*, males often use aggressive mating strategies to coerce females into mating, but it is not clear if females' experience with sexual aggression shapes their future behaviors. Here, we used males derived from lineages that were artificially selected to display either low or high sexual aggression toward females to determine how experience with these males shapes subsequent female mate choice. First, we verified that males from these lineages differed in their sexual behaviors. We found that males from high sexual aggression backgrounds spent more time pursuing virgin females, and had a shorter mating latency but shorter copulation duration compared with males from low sexual aggression backgrounds. Next, we tested how either a harassment by or mating experience with males from either a high or low sexual aggression backgrounds influenced subsequent female mate choice behaviors. We found that in both scenarios, females that interacted with high sexual aggression males were more likely and faster to mate with a novel male one day later, regardless of the male's aggression level. These results have important implications for understanding the evolution of flexible polyandry as a mechanism that benefits females.

Key words: aggression, behavioral plasticity, *Drosophila melanogaster*, fruit fly, mate choice, sexual conflict.

INTRODUCTION

Animals can respond to experiences in their environment by altering some of their physiological, morphological and/or behavioral traits, a process known as phenotypic plasticity (West-Eberhard 1989). In the case of behavioral plasticity, animals tend to exhibit striking variation in their mating behaviors in response to environmental cues (Mery and Burns 2010; Dingemanse and Wolf 2013; Dukas 2013). Given the importance of mating behaviors for successful reproduction to occur, plasticity in mating behaviors is thought to represent adaptations that help animals make decisions that maximize their evolutionary fitness across changing environmental contexts (Rodríguez et al. 2013). For example, females can benefit from having flexible mate choice thresholds that allow them to accommodate variation in factors including predation risk, time and energetic costs of mate choice, and abundance of potential mates (Qvarnström 2001). Mate choice encompasses two major components: choosiness (the investment into discriminating between different mates), and mating preference (the

rank function of preferred stimuli exhibited by potential mates) (Widemo and Sæther 1999). Given that the outcomes of female mate choice can have a major influence on the evolution of male traits via sexual selection, understanding the factors that shape flexible mate choice is an essential task for biologists (Andersson 1995; Kokko et al. 2003).

Recently, the study of behavioral plasticity in female mate choice has made swift progress using invertebrate models, due to precise and effective environmental and genetic control techniques (Kelly 2018). For example, in the field cricket, *Teleogryllus oceanicus*, a species where females rely on male acoustic signals to locate and choose mates, females reared in silent environments are less choosy of male song calling compared with females reared in environments with acoustic songs (Bailey and Zuk 2008). The authors suggest that this may be an adaptive tactic to compensate for the reduced availability of male sexual signals. Similar studies have shown that females can modulate their mate choice based on ecological factors such as cues of mate availability (Scott et al. 2020), intrinsic condition (Hunt et al. 2005), and previous courtship and/ or mating experience (Dukas 2005; Rebar et al. 2011; Travers et al. 2016; Filice and Long 2017). Despite our growing understanding of the importance of mating experience in shaping flexible mate

choice, there have been few attempts to incorporate the importance of sexual conflict theory when generating hypotheses and interpreting results.

In many species, the optimal reproductive interests of males and females conflict, resulting in the selection of traits that are antagonistic towards the opposite sex (Parker 1979; Arnqvist and Rowe 2005). In the fruit fly, *Drosophila melanogaster*, intense male-male competition and asymmetries in optimal mating rates between the sexes has led to the evolution of male phenotypes that inflict harm on females. Some examples of male-induced harm include genital damage during copulation (Kamimura 2007), wing damage during sexual pursuit (Dukas and Jongsma 2012), and the toxic side effects of accessory gland proteins (Acps) transferred in the ejaculate during insemination (Chapman et al. 1995). Often, this harm directly translates into reduced female fitness in terms of both longevity and lifetime fecundity (Chapman et al. 2003). Arguably, the most extreme form of sexual conflict is forced copulation, which essentially allows males to bypass female mate choice (Thornhill 1980; Mckinney et al. 1983; Smuts and Smuts 1993; Clutton-Brock and Parker 1995). In fruit flies, forced copulation may result in direct physical harm to the female, such as wing damage, as well as lower reproductive success and higher mortality rates (Dukas and Jongsma 2012). Baxter et al. (2019) documented wide natural variation in male sexual aggression, where more sexually aggressive males were characterized by higher forced copulation frequencies and spending more time pursuing and mounting females compared with less sexually aggressive males, who displayed these behaviors at lower frequencies. In a follow up study, Dukas et al. (2020) artificially selected males that were the least and the most successful in forced copulation with sexually immature females (0.14 proportion in sample of males selected for low forced copulation, 0.31 in sample of males selected for high forced copulation, after 20 generations). It is exclusively costly for sexually immature females to be pursued by males because sexually immature females that are forced to copulate experience increased mortality and lower fertility (Dukas and Jongsma 2012). Hence from the perspective of sexually immature females, any male pursuit can be viewed as harassment. Given the potential costs for females that experience sexual harm, we predicted that selection should favor females that utilize flexible mate choice based on the levels of sexual aggression in their environments in order to resist these costs. In other words, females that utilize the social information gained from aggressive sexual encounters may have higher reproductive success compared with those that do not. Although others have hypothesized that the avoidance of harmful male phenotypes can act as a mechanism to offset the direct costs of sexual harm (Holland and Rice 1998; Gavrilets et al. 2001; McLeod and Day 2017), empirical studies that test this hypothesis are lacking (Filice and Long 2017).

To address this hypothesis, we tested how experience with males that vary in their expression of sexual aggression influences subsequent female mate choice. Specifically, we were interested in comparing the effect of exposure to males selected for either high or low sexual aggression on a female's mating propensity and preferences in 1) a scenario where immature females experience prior harassment from males, and 2) a scenario where mature females experience prior pursuit and mating with males. The rationale behind testing these two separate scenarios was to attempt to parse out any differences in female behavior that occurred due to pursuit experience versus any changes that also occurred due to the receipt of male seminal fluid via mating. First, we needed to verify that our distinct male lineages varied in their expression of mating behaviors. Based on previous results using these lineages (but in petri dishes instead of vials) (Dukas et al. 2020), we predicted that males from lineages selected for high sexual aggression would spend more time coercing immature females to mate than males from lineages selected for low sexual aggression. We also predicted that males from the high sexual aggression lineages would have shorter mating latencies and longer mating durations with sexually mature females. We predicted shorter latencies due to the increased amount of pursuit, and longer durations due to its association with increased success in fertilization success, which may be an important trait for males that have evolved to use sexually coercive strategies (Bretman et al. 2009). Next, we tested female behavior after experiencing these scenarios and predicted that in both contexts, experience with a high sexually aggressive male would result in a lower mating receptivity in order to offset the physical costs induced by a sexually aggressive partner. We also predicted that females would express a preference for the type of male they did not previously have experience with. In other words, females that were previously paired to a low aggression male would mate more frequently with a high aggression male, and vice versa. This is an empirical prediction that is based on a previous study that observed this trend in female behavior using males from hemiclonal lines with high- and low-harm phenotypes (Filice and Long 2016).

METHODS

Fly stocks and general

All focal females were 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, a lineage of these flies was established at large census size (>1000 adults/generation) on a standardized culture protocol with non-overlapping generations (Rose 1984). Because then, this same lineage of IV has been maintained under identical conditions and used extensively as a model for studying evolutionary fitness and sexual conflict (Rose 1984; Martin and Long 2015; Filice and Long 2016).

All the males in our experiments were derived from six artificially selected lineages descended from 500 wild-caught females collected in Hamilton, ON in 2018. The artificial selection lasted for 20 generations. In three of these lineages, Dukas et al. (2020) selected for males that did not forcibly copulate with teneral females within a 2 h period. In the other three lineages, they selected for males that did forcibly copulate within a 2 h period. Hence, the former three lineages consisted of males low in sexual aggression and the latter three lineages had males high in sexual aggression. Behaviorally, males from the high sexual aggression lineages spent more time pursuing and mounting females and were more likely to mate with mature females than males from the low sexual aggression lineages (Dukas et al. 2020). Owing to the high demands on time and space, Dukas et al. (2020) always conducted selection on one low and one high lineage per day over three successive days. This generated three sets, each including one low and one high lineage.

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 all flies as virgins (within 8 h of eclosion) under light CO_2 anesthesia. After their initial collection, we handled all flies using gentle aspiration. All pre-test conditions and subsequent mating tests took place in 25×95 mm vials.

Experiment 1: harassment and post-harassment tests

In this experiment, we relied on the fact that forced copulation of sexually immature females occurs only during the first couple hours post female eclosion. At this time, the immature females cannot fly to evade males and their soft cuticle prevents them from resisting forced copulation. After this short period, immature females can avoid male advances until they reach sexual maturity about 20 h later, and will typically not mate prior to this period (Markow 2000; Seeley and Dukas 2011). This lack of mating allowed us to set up conditions that isolated the effects of male pursuit, rather than also including the potential effects of mating owing to sperm and seminal fluid proteins. On the first morning of each replicate, we collected 100 virgin females from the IV population within 1 h of eclosion and placed them into individual vials for 4 h to allow them to mature to a stage in which forced copulation can no longer occur. We then placed a single male from a high sexual aggression lineage into half of these female vials, and a single male from a low sexual aggression lineage into the other half. Within each replicate, all males came from one of the three sets each consisting of one low and one high lineage. These males were collected upon eclosion three days prior and were housed in groups of three within vials. Housing the males in small groups ensured standard social experience, as social isolation has been shown to influence the expression of aggressive behavior (Wang et al. 2008). During the first 10 min of each pairing, an observer blind to male treatment recorded to the nearest second the duration of male harassment of the sexually immature females using the *Drosophila Assay Assistant* app on an iPod Touch. Behaviors that counted as harassment included chasing, courtship (singing, observed as wing vibration), and mounting attempts. After this initial observation period, each pair of flies was left undisturbed for an additional 4 h but were systematically scanned to ensure that no matings occurred. After this 4 h period, the males were removed and females remained in the food vials in isolation. On the after morning, 17 h after being isolated, each female was introduced to a new male from either a high or low lineage in a fully reciprocal design (Figure 1). In other words, half of the original females from each treatment were paired with a new male from a low lineage, and the other half were paired with a new male from a high lineage. In this post-harassment test, two observers blind to both female experience and male identity systematically scanned all 100 vials and recorded the latency and duration of all matings to the nearest second. Mating latency was the duration from the start of the trial until the commencement of mating. Pairs that did not mate within 2 h were considered to have not mated in our data. We replicated this procedure across the three sets of low and high lineages twice each. Hence, our total sample size for the harassment tests was $N = 600$. However, 15 females died or escaped in between the harassment and post-harassment test, resulting in a total N of 585 (for each of the three pairs of lineages, $N = 193$, 195, 197).

Experiment 2: mating and post-mating tests

On day 1 of each replicate, we collected 80 newly-eclosed IV females and placed them in individual vials with a dash (~5 mg) of live yeast. Simultaneously, we collected 40 newly-eclosed males from a low lineage and placed them into groups of three and did the same with 40 males from a high lineage. On day 2, we collected an additional 40 males from each of the high and low lineages and
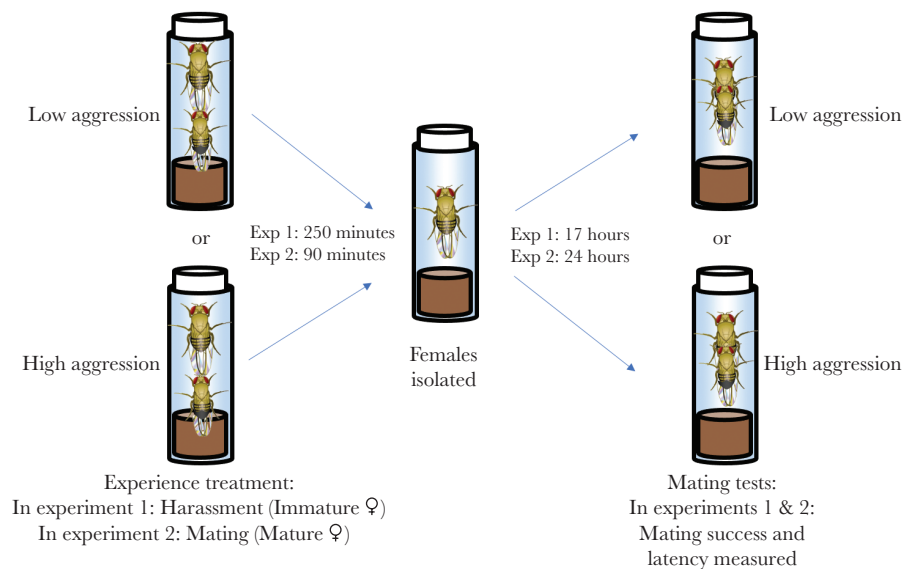

Low aggression

or

Exp 1: 250 minutes
Exp 2: 90 minutes

Females isolated

Exp 1: 17 hours
Exp 2: 24 hours

Low aggression

or

High aggression

High aggression

Experience treatment:
In experiment 1: Harassment (Immature ♀)
In experiment 2: Mating (Mature ♀)

Mating tests:
In experiments 1 & 2:
Mating success and
latency measured

Figure 1

An illustration of the experimental design for both our experiments. In experiment 1, sexually immature females were exposed to either a high or low sexual aggression male for 250 min, and then housed in isolation for an additional 17 h. In experiment 2, sexually mature females mated with either a low sexual aggression or high sexual aggression male within a 90 min period, and then housed in isolation for the next day. In both experiments, after each experience phase, females were paired with a new male from either a high or low sexual aggression background and their mating behaviors were scored.

placed them into vials in groups of three. Similar to experiment 1, within each replicate, the males belonged to one of the three sets, each including one low and one high lineage. On day 3 we placed a single male collected on day 1 from either a low or high lineage into each female vial. Two observers blind to fly treatment systematically scanned each pair for 90 min and noted the latency and duration of each mating. Trials where the pair did not mate within 90 min were excluded from further analysis. After this 90 min period, we removed and discarded all males, and placed the females back into the chamber for 24 h. On day 4, we individually placed all females into fresh food vials, and then added a single male collected on day 2 from either a high or low lineage. Similar to experiment 1, our design was fully reciprocal, so half of the females from each initial treatment were paired with a low lineage male, and the other half were paired with a high lineage male (Figure 1). Two observers blind to fly treatment systematically scanned the vials for 3 h and recorded the latency and duration of each mating. Pairs that did not mate within 3 h were considered to have not mated in our data. We replicated this procedure across each of the six selection lineages two times each. We set up 480 trials but had to omit the trials in which females did not mate during the first mating experience ($N = 53$ from low treatment, $N = 18$ from high treatment) so our final sample size was $N = 409$ (for each of the three pairs of lineages, $N = 124, 138, 147$).

Statistical analysis

We conducted all data analyses using R version 3.5.2 (R Core Team 2013). For our data collected during the experience treatments (harassment in experiment 1 and mating in experiment 2), we constructed generalized linear mixed models using the lmer function from the lme4 package (Bates et al. 2014). We treated all our dependant factors (harassment, mating latency, and mating duration) as gaussian response variables and verified all model fits by visually inspecting plots of model residuals. We included the selection treatment identity of the experience male (low or high sexual aggression) as a fixed effect, and the lineage set as a random effect in these models. For our data collected during the post-experience tests (post-harassment and post-mating tests), we constructed Cox proportional hazard mixed models, using the coxme function from the coxme package (Therneau and Grambsch 2000). For both the post-harassment and post-mating results, we constructed a model that took into account the binomial outcome of mating success and the latency of successful matings as a survival term. These models included the selection treatment identity of the experience male, the selection treatment identity of the post-experience male, and the interaction between these two factors as fixed effects, and the lineage set as a random effect. We calculated the significance of the fixed effects using a log-likelihood ratio χ^2 test using the Anova function from the car package (Fox et al. 2014).

RESULTS

Experiment 1: harassment tests and post-harassment experience tests

Males descended from high sexual aggression lineages displayed significantly more harassment toward sexually immature females compared with males descended from low lineages (Wald χ^2 = 62.7, df = 1, $P < 0.0001$; Figure 2). On the day after harassment
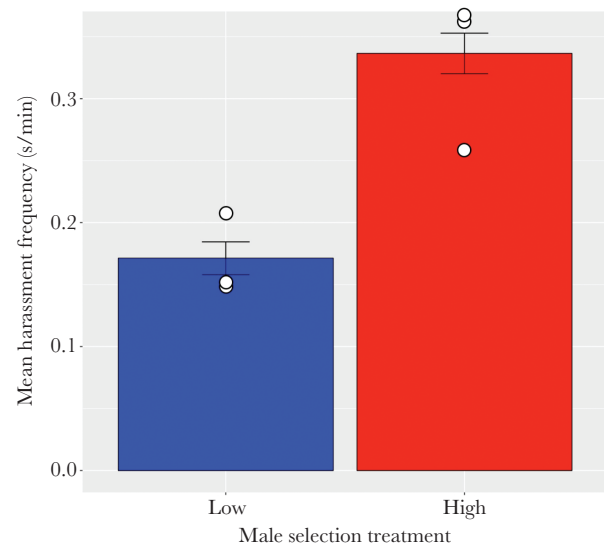

Figure 2
The mean harassment frequency (calculated as total time spent displaying harassment in seconds divided by total observation time in minutes) displayed by males towards sexually immature females during a 10 min observation period for males derived from lineages selected for low (blue bar) and high (red bar) sexual aggression. The main bars represent the means of all individual samples, the error bars represent one standard error of each sample, and the open circles represent the mean of each male lineage.

experience, females that were previously exposed to males from the high lineages mated quicker and had a higher overall proportion of matings compared with females previously exposed to males from the low lineages (Wald χ^2 = 6.9, df = 1, $P = 0.0085$; Figure 3). However, when looking at the identity of the second male, females from both groups had a similar mating latency and proportion whether their second partner was from a low or high lineage (Wald χ^2 = 2.6, df = 1, $P = 0.1$; Figure 3). The interaction between the identity of the first and second male was not significant (Wald χ^2 = 0.41, df = 1, $P = 0.52$). The identity of the first male that females had experience with did not significantly influence her future mating duration (Wald χ^2 = 1.7, df = 1, $P = 0.19$). However, the identity of the second male (the male that mated with the female) did, where males from the low aggression lineages mated for significantly longer compared with males from high aggression lineages (Wald χ^2 = 7.3, df = 1, $P = 0.007$). The interaction between identity of the first and second male was not significant (Wald χ^2 = 0.24, df = 1, $P = 0.62$).

Experiment 2: mating and post-mating tests

In the experience phase, females paired with males from high sexual aggression lineages displayed significantly shorter mating latencies (Wald χ^2 = 17.8, df = 1, $P < 0.0001$; Figure 4a), and mated for shorter durations (Wald χ^2 = 8.9, df = 1, $P = 0.0028$; Figure 4b). On the day after mating experience, females that previously mated with males from the high sexual aggression lineages remated quicker and had a higher overall proportion of rematings compared with females previously exposed to males from the low sexual aggression lineages regardless of who they were paired with to remate (Wald χ^2 = 5.1, df = 1, $P = 0.024$; Figure 5). When looking at the identity of the second male, females remated significantly faster and at a greater
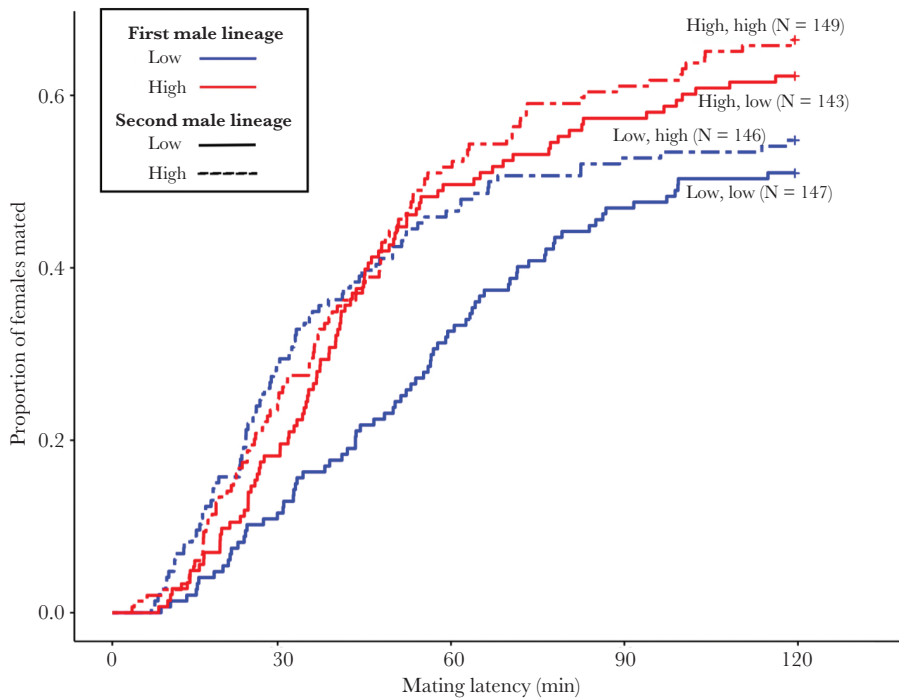
Figure 3

Mating latency as a function of prior harassment experience. The effect of immature females' experience with males from either low (blue) or high (red) sexual aggression lineages on their subsequent mating propensity with a new male from either a low (solid) or high (dashed) sexual aggression lineage. Each cox-regression curve represents the proportion of females that mate per treatment group over time.
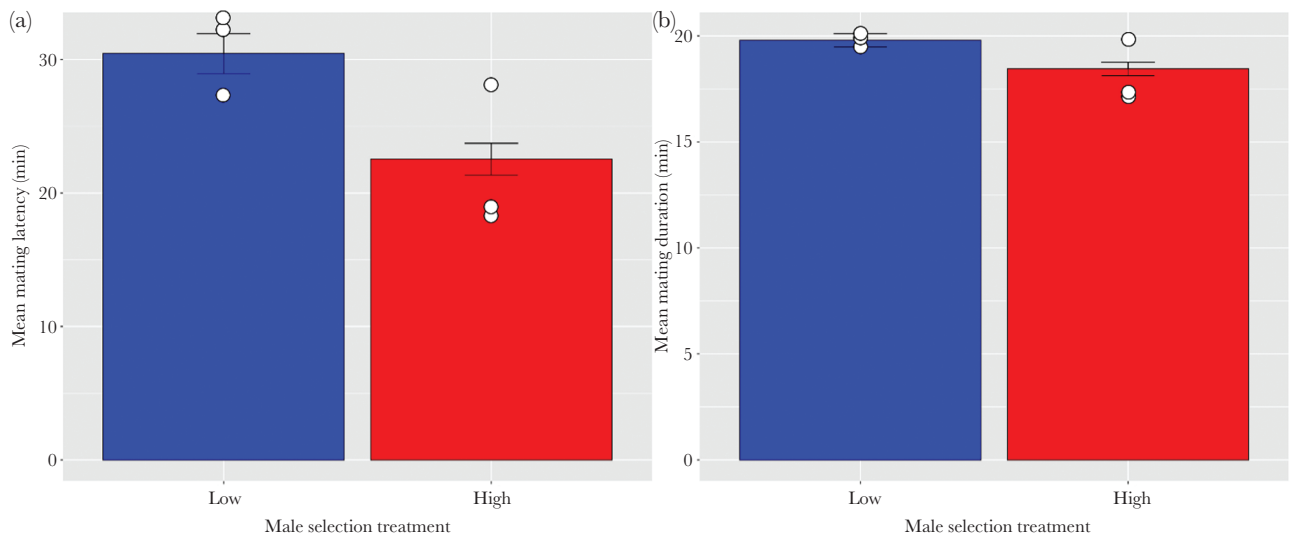

Figure 4

The mean mating latency (A) and duration (B) of females during the experience phase of experiment 2. Males were derived from lineages selected for low (blue bars) and high (red bars) sexual aggression. The main bars represent the means of all individual samples, the error bars represent one standard error of each sample, and the open circles represent the means of each male lineage.

proportion with males from the high lineages compared with females paired with males from the low lineages regardless of who they were initially paired with on the first mating (Wald χ^2 = 4.2, df = 1, P = 0.04; Figure 5). The interaction between the identity of the first and second male was not significant (Wald χ^2 = 2.5, df = 1, P = 0.11).

The identity of the first male, second male, and the interaction between the two did not significantly influence the remating duration of females (First male: Wald χ^2 = 1.1, df = 1, P = 0.3; Second male: Wald χ^2 = 2.9, df = 1, P = 0.09; Interaction Wald χ^2 = 0.09, df = 1, P = 0.76).
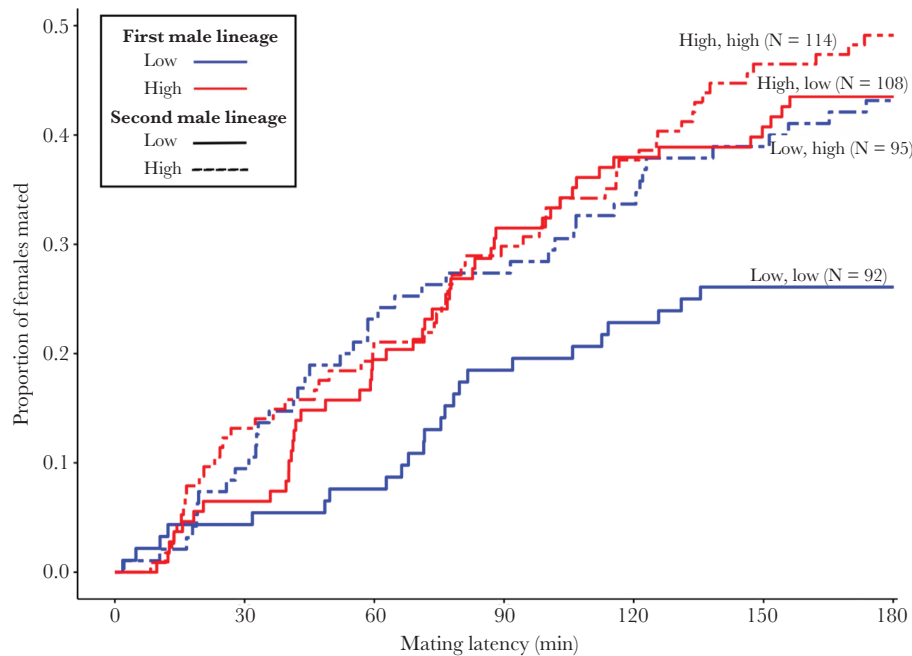
Figure 5

Mating latency as a function of prior mating experience. The effect of mature females' experience with mating with males from either low (blue) or high (red) sexual aggression lineages on their subsequent mating propensity with a new male from either a low (solid) or high (dashed) lineage. Each cox-regression curve represents the proportion of females that mate over time.

DISCUSSION

Previous sexual experience such as exposure to males with different mating characteristics is well known to shape subsequent female mate choice behavior (Dukas 2006; Rodríguez et al. 2013). Here, we add to our understanding by demonstrating that male phenotypes that vary in sexual aggression are one source that shape this plasticity. First, we verified that males derived from lineages selected for high and low sexual aggression differ in their sexual behaviors in attempt to replicate the findings of Dukas et al. (2020). As expected, males from high aggression lineages displayed more harassment and mated quicker, compared with males from low aggression lineages. Contrary to our prediction, the high sexual aggression males also had shorter mating durations. Next, we tested how experience with males from either a low or high sexual aggression background influences subsequent female mating behavior and found that females that experience harassment and/or mating from a male descended from a high sexual aggression lineage display higher mating propensities and have shorter mating latencies when paired with a subsequent male. Overall, these results advance our understanding of how sexually antagonistic traits can shape plasticity in mating behaviors.

The main motivation of this study was to investigate how the differences in the male phenotypes described above influence female mate choice in terms of mating propensity and preference. Opposite to what we predicted, we found that females who experienced harassment and mating from a high sexual aggression male were more receptive when presented with a mating opportunity on the after day. There are several possible explanations for these unexpected outcomes. First, in the case of the harassment experience for immature females, the increased harassment experienced by females may have caused them to develop sexual maturity quicker than females that experienced less harassment. It is well known that environmental factors can influence

the development of sexual maturity in many species (Papaj 2000), but to the best of our knowledge, no one has directly tested the effects of early life harassment on development. In nature, female fruit flies will experience harassment from males immediately after they eclose (Markow 2000; RD unpublished data). Because the female fruit flies in our study were derived from a population that was selected for early-life reproduction as a consequence of lab maintenance (and fruit flies generally benefit from a life-history strategy that focuses on early reproduction in expanding populations (Edward et al. 2011)), it would be ecologically relevant to invest into maturity as quickly as possible if mating opportunities are available (*see* Filice et al. 2020). Furthermore, female fruit flies become sexually receptive about 1 day after eclosion (Manning 1967). In our study, the harassment phase took place on the morning of day 0 when females were recently eclosed, and the post-harassment mating test took place on the after morning of day 1, meaning that some aspect of the experience phase may influence the timing of sexual maturity. Future studies should continue to investigate this by looking at the developmental and physiological consequences of early-life exposure to sexual harassment.

In the case of the remating tests for the mature females, the difference in female mating propensity may be explained by differences in the post-copulatory male manipulation expressed between the two male treatments. In other words, it could be that males from the low sexual aggression treatment transfer different volumes and/or compositions of accessory gland proteins in their ejaculate that result in their mates having a decreased mating receptivity (Wigby et al. 2009). This is consistent with our above hypothesis regarding the evolution of trade-offs in pre- and post-copulatory traits, as these males may have evolved different strategies to compensate for the traits selected against during artificial selection. It is also consistent with evidence that suggests that longer mating durations tend to positively associate with reduced

female remating receptivity (Bretman et al. 2009). However, another and more intriguing possibility is that females are flexibly controlling their own mating rate to gain direct and/or indirect benefits (Arnqvist and Nilsson 2000; Parker and Birkhead 2013). If, for example, males from the high sexual aggression treatment stimulated less oviposition or were of poorer genetic quality compared with males from the low sexual aggression treatment, females could benefit via remating to increase their fecundity, or to "trade-up" genetic quality respectively (Long et al. 2010; Sutter et al. 2019). Future studies should continue to link the outcomes of flexible female mating behavior to their fitness outcomes in order to disentangle when this plasticity is a female adaptation and when it may simply be male manipulation that benefits males.

In terms of mating preferences, we found that female preferences did not differ between females exposed to high or low sexual aggression males (as characterized by the non-significant interaction terms) in the mating tests from both experiments 1 and 2. In both experiments, females had higher mating frequencies with males from a high sexual aggression background regardless of the identity of their previous experience. This is consistent with a result from a similar test in Dukas et al. (2020), and suggests that in general, males from the high sexual aggression treatment have higher pre-copulatory mating success. This increased success is likely due to some behavioral component that was selected for during the artificial selection such as higher frequencies of pursuit and mounting attempts. Nonetheless, it may be that in this particular case, the effects of the second male consistently overrode the effects of the first male on female mating preferences.

When looking at the behavioral differences between males from low and high sexual aggression lineages, it was not surprising to see that males from lineages selected for high sexual aggression displayed a greater frequency of harassment behaviors and faster mating latencies. During the artificial selection regime, males from high sexual aggression lineages were always sired by males that were successful in forceful copulation with a sexually immature female. Because forced copulation success requires persistent pursuit in the face of rejection, it may be that this more frequent pursuit led to quicker matings. Although we predicted that high sexual aggression males would mate for longer durations, it is possible that the longer mating durations observed in males selected for low sexual aggression represents a trade-off between pre- and post-copulatory mating strategies (Simmons et al. 2017; Filice and Dukas 2019). Pre-copulatory strategies include elements such as persistent pursuit, courtship and male weaponry, and post-copulatory strategies include traits that enhance sperm competitive success. In many cases, factors such as genetics, development, and natural selection can generate conditions whereby the expression of a pre-copulatory trait is constrained by the expression of a post-copulatory one (or vice versa) (Simmons et al. 2017). Given that the males used in our studies still had to compete amongst each other for matings after artificial selection occurred (Dukas et al. 2020), it would make a lot of sense that traits targeting post-copulatory success would be selected for in order to compensate for the forced mating success traits which were being selected. In other words, males selected for low forced copulation success would largely depend on post-copulatory success to secure paternity. One study observed a similar outcome in the horned beetle, *Onthophagus nigriventris*, where males that were prevented from developing horns, a characteristic important for pre-copulatory competition

and mate choice, tended to develop larger testes, which may assist in successful post-copulatory competition (Simmons and Emlen 2006). In another study, male fruit flies from genetic backgrounds that had high mating success in pre-copulatory scramble competition tended to have lower success in stimulating egg production in their mates (Filice and Long 2018). Although these are examples of developmental and genetic trade-offs respectively, future studies should continue to utilize artificial selection techniques to investigate how selection can act as a source of trade-offs between pre- and post-copulatory mechanisms.

Taken together, our results are important for our understanding how females' experience with different levels of sexual aggression may influence their subsequent mating behavior. Specifically, we found that females that experience harassment and mating from males artificially selected for high sexual aggression are more willing to mate on the after day compared with females that experience males selected for low sexual aggression. We propose that this may represent an adaptive mechanism to gain direct and/or indirect benefits, but future studies should continue to investigate the link between female plasticity and fitness in order to determine the specific conditions for flexibility in polyandrous behavior to be adaptive.

AUTHOR CONTRIBUTIONS

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