Genetic variation in sexual aggression and the factors that determine forced copulation success

Carling M. Baxter [1], Janice L. Yan [2], Reuven Dukas*

Animal Behaviour Group, Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, ON, Canada

Sexual conflict is common in nature and sometimes results in sexual aggression. An extreme case is forced copulation, where one individual forcibly mates with another individual who resists the mating. To understand what makes some males sexually aggressive, we established an experimental system that allowed us to quantify the characteristics that contribute to males' forced copulation success. In fruit flies (*Drosophila melanogaster*), sexually mature females can choose to accept or reject courting males; however, males can forcibly copulate with newly eclosed, sexually immature, teneral females. We tested males from 59 genotypes and found significant genetic variation in forced copulation success, with a broad-sense heritability of 0.16. We then chose three genotypes with the lowest and three genotypes with the highest forced copulation success rates and compared the behaviour of males from these two groups. Males from genotypes with high forced copulation success were more persistent in their pursuit of teneral females and mounted them more frequently than did males from the low-success genotypes. Males of the two categories, however, were similar in their attractiveness to both teneral and sexually mature females. Our results suggest that males vary in their pursuit strategies. Some males respond to female rejection signals by giving up and searching for receptive females, while other males persist in pursuit and coercion in spite of female objection. Our work highlights the practicality of using forced copulation in fruit flies as a model for further research on the mechanisms affecting variation in sexual coercion and forced copulation success and their evolutionary consequences.

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Sexual reproduction was once thought to involve cooperation between males and females in their mutually beneficial endeavour to create offspring. However, it has long been understood that the optimal reproductive strategies for males and females differ, creating an opportunity for conflict between the sexes (Parker, 1979). Studies of sexual conflict have since greatly influenced our understanding of evolution via sexual selection (Arnqvist & Rowe, 2005).

Sexual conflict can be quite subtle, such as males exploiting pre-existing sensory biases in females for their own reproductive advantage. An example of this is seen in guppies, *Poecilia reticula*, where females are attracted to males with orange spots due to a general, innate attraction to orange objects, which is common to both sexes (Rodd, Hughes, Grether, & Baril, 2002). However, conflict can also be overt, such as when males aggressively coerce, force or intimidate females into mating. Males can use harassment and aggression to coerce unreceptive females into accepting a mating, as seen, for example, in water striders (Rowe, Arnqvist, Sih, & Krupa, 1994). In the water strider *Gerris gracilicornis*, females are under greater predation risk than males. Males exploit this higher vulnerability by creating ripples that attract predators while mounting females, and only stop this activity once mating begins (Han & Jablonski, 2009, 2010). Aggression can be used to directly forcibly copulate with females, such as in Lake Eyre dragons, *Ctenophorus maculosus* (McLean, Chan, Dickerson, Moussalli, & Stuart-Fox, 2016; Olsson, 1995), waterfowl (Mckinney, Derrickson, & Mineau, 1983; McKinney & Evarts, 1997) and wolf spiders (*Schizocosa ocreata*) (Johns, Roberts, Clark, & Uetz, 2009). In such cases, males can attack and pin down a female, causing injury to the female, and forcibly mate with her while she resists the mating. Finally, males can also use aggression as a long-term sexual intimidation tactic, where their aggression towards females is temporally decoupled from their mating, and leads to increased mating success in chacma baboons, *Papio ursinus* (Baniel, Cowlishaw, & Huchard, 2017), and increased paternity in Kasekela chimpanzees, *Pan troglodytes schweinfurthii* (Feldblum et al., 2014).

* Correspondence: R. Dukas, Animal Behaviour Group, Department of Psychology, Neuroscience & Behaviour, McMaster University, 1280 Main Street West, Hamilton, ON, L8S 4K1, Canada.
  E-mail address: dukas@mcmaster.ca (R. Dukas).
  [1] baxtercm@mcmaster.ca
  [2] yanj12@mcmaster.ca

Fruit flies (*Drosophila melanogaster*) are an important model system for studying sexual conflict, with a strong focus on post-mating conflict (Chapman, Liddle, Kalb, Wolfner, & Partridge, 1995; Filice & Long, 2016; Laturney & Billeter, 2016; Rice et al., 2006). As for examples of sexual coercion in fruit flies, it has been shown that females are more likely to remate after long periods of persistent male pursuit, when females have no refuge from males, and particularly when the ratio of males to females is high, which suggests that males are harassing females into remating (Byrne, Rice, & Rice, 2008; Wigby & Chapman, 2004). Recently mated females are also more likely than virgin females to resist during mating by kicking the males and keeping their wings closed, hence reducing the ability of the males to mount securely (Baxter & Dukas, 2017). However, it can be difficult to distinguish between female resistance to male coercion and female mate screening, where females reject all males initially and then only accept males who demonstrate they are of high quality by persisting in their mating attempts. Since mate screening relies on the ability of a female to exercise control over the occurrence of mating, situations in which the female cannot prevent copulation can provide us with unambiguous examples of male coercion. Female fruit flies are typically able to reject males and prevent intromission. Therefore, although they can still be coerced into mating, using them as a model to study male coercion presents the difficulty of ruling out possible mate screening. In the first 2 h posteclosion, however, females are in a teneral stage where their wings are still curled, their cuticle is soft and their vaginal plates have not yet hardened. Since teneral females cannot fly or physically prevent intromission by males who have mounted them, they can be forcibly copulated by sexually mature males, and these forced copulations occur both in nature and under laboratory conditions (Markow, 2000). Teneral females attempt to flee from males who persistently pursue them, and females who are forcibly copulated suffer significant costs, including wing damage, early mortality and reduced reproductive success, while males benefit from these matings because they father some offspring (Dukas & Jongsma, 2012a; Seeley & Dukas, 2011). Because matings with sexually immature, teneral females are unambiguously forced, they provide us with a clear model system for experimental research on aggressive manifestations of sexual conflict.

In laboratory studies with fruit flies, approximately 20–40% of males forcibly copulate when presented with a teneral female (Dukas & Jongsma, 2012b; Markow, 2000; Seeley & Dukas, 2011). So, why is it that some males aggressively force copulate while others do not? One potential explanation is that males who cannot obtain consensual matings resort to aggressively coercing females to mate, such as in scorpion flies (*Panorpa latipennis*), where males who have not obtained the nuptial gifts necessary for consensual mating will attempt to aggressively force-copulate with females (Thornhill, 1980). However, this is certainly not true in all cases; for example, in waterfowl, it is generally the males who are already pair-bonded who engage in forced copulations (Mckinney et al., 1983; McKinney & Evarts, 1997). And in wolf spiders, only approximately 30% of males who have been rejected by a female end up forcibly copulating with her (Johns, 2007; Johns et al., 2009), so not all males faced with rejection perform forced copulations. What contributes to the variation in forced copulation rates across males who are faced with the same circumstances?

Our goal was to examine the behavioural determinants of forced copulation success. We first assessed the genetic variation in forced copulation success across 59 male genotypes (using a standard genotype for all teneral females), allowing us to estimate the broad-sense heritability of forced copulation success. Based on these results, we chose three genotypes with the highest and three genotypes with the lowest forced copulation rates to perform follow-up experiments with detailed behavioural observations of forced copulation trials. We predicted that 'high-success' male genotypes would be more persistent and perform more coercive actions when pursuing teneral females than 'low-success' male genotypes. Additionally, we quantified teneral females' behaviour while being pursued by males and predicted that females would be less resistant to high- success male genotypes, thus facilitating these males' higher forced copulation rates. Finally, we tested males' mating success with mature virgin females, who have full control over mating. If higher-quality males are more likely to overcome teneral female resistance and thus succeed in forced copulations, then we would expect that these males would also have higher mating success with mature virgin females. However, if lower-quality males are more motivated to perform forced copulations, then we would expect such males to have lower mating success with mature virgin females.

GENERAL METHODS

We used a subset of 60 Wolbachia-free lines from the *Drosophila* Genetic Reference Panel (DGRP). Mackay et al. (2012) generated these lines by collecting wild mated females in Raleigh, North Carolina, U.S.A. and then inbreeding their offspring for 20 generations of full-sibling mating. We housed all lines in standard fly vials with 5 ml of our standard food medium (1 litre = 90 g of sucrose, 75 g of cornmeal, 10 g of carrageenan, 32 g of yeast and 2 g of methyl paraben dissolved in 20 ml of ethanol). We maintained all flies in an environmental chamber at 25 °C and 50% relative humidity with a 12:12 h light:dark cycle with the lights turning on at 1000 hours.

Zwarts et al. (2015) found that the majority of DGRP lines suffer from neurological defects, which they attributed to the fixation of recessive mutations affecting the architecture of the mushroom bodies. Their analyses indeed identified 24 genes directly linked to mushroom body defects. To lessen such deleterious effects, we crossed males from 59 distinct DGRP lines to females from a single line (DGRP-83) to create F1 hybrid flies. While such crossing generally reduces inbreeding depression (Charlesworth & Willis, 2009), its specific effect on the mushroom body has not been quantified. To generate the hybrids, we collected virgin females of DGRP-83 within 8 h of eclosion using light CO_2 anaesthesia and housed them in groups of 15 per food vial sprinkled with live yeast to stimulate egg laying. Once females were 3–5 days old, we transferred eight DGRP-83 females and five young males (1–4 days old) from each one of 59 DGRP lines into food vials with live yeast. We transferred these parental flies of the 59 hybrid crosses to new food vials with live yeast daily and scraped excess eggs from the vials to ensure a consistent rearing density across lines. Eleven days after egg laying, we collected by gentle aspiration the F1 hybrid flies (hereafter hybrid genotypes) within 8 h of eclosion to ensure virginity and aspirated them into individual food vials. We tested these males when they were 4 days old.

We conducted all trials within ±2 h of the flies' subjective dawn. This period is associated with the morning peak of fly activity in most studies (Green et al., 2015) and has been verified in our laboratory (R. Dukas, personal observation). We tested all flies in polystyrene petri dishes that were 35 mm in diameter and 8 mm high. We covered the floor of each arena with a piece of filter paper and coated the walls and ceilings of the arenas with Surfasil (Sigma Aldrich, Oakville, ON, Canada). Surfasil is a siliconizing agent that, when dry, forms an odourless slippery film that the flies cannot walk on, thus restricting the flies to the base of the arena.

Since we wished to test for genetic variation in male propensity to forcibly copulate, males from the above-mentioned 59 hybrid genotypes were our focal individuals. To reduce variation in the response of the teneral females, we used females of a single

reference line. We chose to use females of DGRP-83 as our teneral females, as they would be equally related by descent to males of each hybrid genotype. It is likely that females also possess genetic variation in susceptibility to forced copulation and that there are male–female interactions, but these topics are beyond the scope of our research.

Ethics Statement

Our research complied with all applicable laws and did not require approval from an ethics committee.

GENETIC VARIATION IN FORCED COPULATION SUCCESS

Methods

We collected teneral females of DGRP-83 within a few minutes of eclosion and aspirated one teneral female and one 4-day-old hybrid male into each arena. Observers blind to the focal males'

Figure 1. (a) Genetic variation in male forced copulation success shown as the proportion ±1 SE of trials that resulted in forced copulation for 59 hybrid genotypes. The points are ordered along the *X* axis by increasing proportion and are labelled according to the paternal *Drosophila* Genetic Reference Panel (DGRP) line of each hybrid genotype. The three 'low' and three 'high' forced copulation success genotypes used in the determinants of forced copulation success experiment are coloured blue and red, respectively. (b) Proportion ±1 SE of trials that resulted in forced copulation for the chosen three low-success and three high-success genotypes (shown in blue and red, respectively).

hybrid genotypes continuously scanned the arenas for the presence of mating for a maximum of 2 h. Trials occurred on 13 test days within a 15-day period. We tested two to four males per hybrid genotype per day, resulting in a final sample size of 43–46 males per hybrid genotype, with the exception of hybrid genotype 894, for which we were only able to collect and test 34 males.

Statistical Analysis

We used a generalized linear mixed-effects models (GLMM) in R v.3.3.2 (R Core Team, 2016) with the package 'lme4' v.1.1–12 (Bates, Maechler, Bolker, & Walker, 2015). We used a binomial distribution to model the occurrence of forced copulation during the 2 h trials, with hybrid genotype and test day as random factors. To assess the significance of the random effect of hybrid genotype, we report *P* values calculated as the fraction of parametric bootstrapped likelihood ratio test (LRT) statistics (with 10 000 iterations) that were larger than the observed LRT values, using the package 'pbkrtest' v.0.4–7 (Halekoh & Højsgaard, 2014).

We also calculated broad-sense heritability (H^2) as $V_G / (V_G + V_E)$, where V_G is the genetic variance (i.e. the among-hybrid genotype variance) and V_E is the environmental variance (i.e. residual variance from the model). We multiplied the among-hybrid genotype variance by two because all hybrid genotypes had genetically identical mothers from DGRP-83.

Results

We found significant variation in forced copulation success across hybrid genotypes (LRT: $P < 0.01$; Fig. 1a). The broad-sense heritability of forced copulation success was 0.16.

DETERMINANTS OF FORCED COPULATION SUCCESS OF 'LOW' AND 'HIGH' HYBRID GENOTYPES

Methods

To perform detailed video observations of the males' behaviours leading up to forced copulation, we chose to test a subset of six genotypes and shorten the trial duration to 1 h. Based on the data from the previous experiment, we chose three hybrid genotypes that had the lowest and three genotypes that had the highest forced copulation rates within 1 h (marked in Fig. 1a in blue and red, respectively, but note that Fig. 1a depicts mating rates over 2 h). Our previous work on sociability (Scott, Dworkin, & Dukas, 2018) and aggression (C. M. Baxter, J. L. Yan, & R. Dukas, personal observation) with the same DGRP hybrid genotypes indicated moderate repeatability of behavioural scores over time. We thus decided a priori that we would only score the videos from the hybrid genotypes that showed forced copulation rates consistent with their assignment into the low and high forced copulation rate categories. We considered the forced copulation rate of a genotype to be consistent if it was among the top three forced copulation rates for the high-success genotypes, or among the bottom three for the low-success genotypes.

We reared flies of the three low-success and three high-success hybrid genotypes and collected and housed males as described above. We again aspirated one 4-day-old focal male and one DGRP-83 newly eclosed, teneral female into each arena. We then immediately began videorecording for 1 h using Logitech HD Pro c920 webcams and iPods. Trials occurred on 6 test days, where we tested four males per hybrid genotype per day, resulting in a sample size of 24 males per hybrid genotype.

Later, observers blind to the focal males' hybrid genotypes observed the videos using BORIS observation software (Friard &
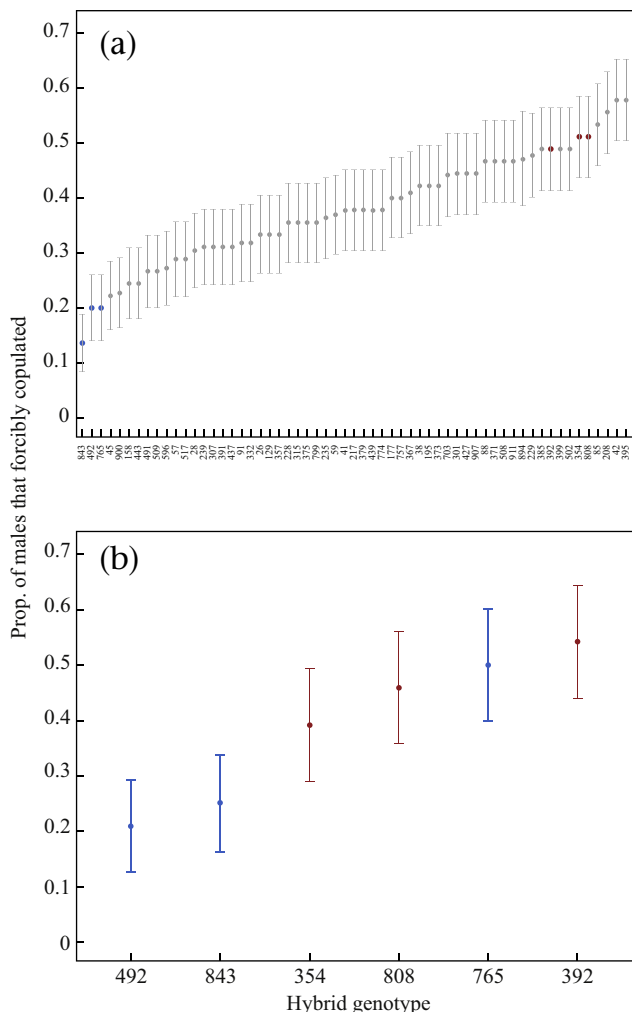
Gamba, 2016). For each arena, an observer recorded the duration that each male pursued the female and the number of times he mounted the female as indicators of persistence and coercive actions, respectively. Observers also recorded the duration that the female spent running away from the male while he pursued her as an indication of resistance. We excluded two trials with hybrid genotype 354. In one trial, mating occurred at trial commencement, so we did not have behavioural observations. In the other trial, the male spent 43% of the trial duration on the ceiling of the arena and never interacted with the female. No other trial had a fly on the ceiling for more than 20% of the trial duration.

Statistical Analysis

We again used the 'lme4' package to perform linear mixed-effects models (LMM) and generalized linear mixed-effects models (GLMM). All models included hybrid genotype and test day as random factors and forced copulation success group ('low' versus 'high') as a fixed factor. We report Wald χ^2 values generated with the 'Anova' function from the 'car' package v.2.1–4 (Fox & Weisberg, 2011) for tests of the forced copulation success grouping (i.e. 'low' versus 'high'). We used a binomial distribution to model the occurrence of forced copulation during the 1 h trials with low- and high-success hybrid genotypes. To assess males' persistence, we log-transformed the duration that each male pursued the female to increase the normality of the distributions and added 1 s of pursuit to all males to eliminate three (out of 114) zeros. We used a LMM on the log-transformed durations and included the log of trial duration as an offset (since males experienced different trial durations depending on if/when they force-copulated). For coercive actions, we modelled the number of mounts and mounting attempts males performed with a negative binomial distribution and included the log of the pursuit duration as an offset. Finally, to assess female resistance, we analysed the duration that females spent running from males while they were being pursued. We log-transformed both female running duration and male pursuit duration to increase the normality of the distributions and added 1 s of running to all females to eliminate nine (out of 114) zeros. We used an LMM to assess female running duration and included male pursuit duration as an offset. We verified model fits by visually inspecting plots of model residuals.

As a secondary analysis, we grouped males based on whether or not they force-copulated during the trial. The logic being that, if the behavioural differences between our low and high genotypes are representative of what varies between individual males who fail or succeed to forcibly copulate, then we would see similar trends when comparing between 'unsuccessful' and 'successful' males as we see when comparing between males from low and high genotypes. We used binomial logistic regressions to assess whether forced copulation success varied with the proportion of pursuit, mounting frequency or female evasion attempts. In each model, we also included hybrid genotype and test day as random factors.

Results

Only five of the six hybrid genotypes showed consistent forced copulation rates in the screening (Fig. 1a) and follow-up experiments (Fig. 1b). Following our protocol, we thus excluded hybrid genotype 765 from the video scoring. The two remaining hybrid genotypes with low forced copulation rates had half the forced mating success as the three hybrid genotypes with high forced copulation rates (GLMM: $\chi^2_1 = 6.58$, $N = 119$, $P < 0.05$; Fig. 1b).

Males from the high-success hybrid genotypes spent significantly more time pursuing teneral females than did males from the low-success hybrid genotypes (LMM: $\chi^2_1 = 5.35$, $N = 118$, $P < 0.05$;

Fig. 2a). Males from the high-success hybrid genotypes also performed significantly more mounting attempts while accounting for pursuit duration (GLMM: $\chi^2_1 = 12.9$, $N = 118$, $P < 0.001$; Fig. 2b). Females showed a nonsignificant tendency to spend more time running from high-success hybrid genotype males than from low-success hybrid genotype males when accounting for pursuit duration (LMM: $\chi^2_1 = 1.68$, $N = 118$, $P = 0.20$; Fig. 2c).

When comparing males grouped by forced copulation success, we found that both male pursuit and mounting frequency significantly predicted forced copulation success (GLMM: pursuit: $z = 4.4$, $N = 118$, $P < 0.001$; mounting: $z = 3.9$, $N = 118$, $P < 0.001$). However, the proportion of time that a female spent attempting to evade a male did not significantly predict whether or not she would be forcibly copulated (GLMM: $z = 0.50$, $N = 118$, $P = 0.62$).

MATING SUCCESS OF 'LOW' AND 'HIGH' HYBRID GENOTYPES WITH MATURE VIRGIN FEMALES

Methods

Simultaneous with the forced copulation trials described in the previous section (Figs 1b, 2a–c), we also tested males of the same hybrid genotypes with mature virgin females. We followed the same procedure as above, except that we placed each male in an arena with a 2-day-old virgin female of DGRP-83 instead of a teneral female. We used 2-day-old virgin females because they are sexually mature but choosier than older virgin females (C. M. Baxter, J. L. Yan, & R. Dukas, personal observations). We tested 48 males per hybrid genotype.

Observers who were blind to hybrid line identity scanned the mature female arenas and recorded whether or not the males mated within a 1 h trial.

Statistical Analysis

We used a binomial distribution to model the occurrence of mating during the 1 h trials with the low-success and high-success hybrid genotypes (fixed factor). The model also included test day and hybrid genotype as random factors. We only analysed the five hybrid genotypes included in the analyses of the previous experiment.

Results

Males from the low-success and high-success genotypes mated at similar rates with mature females (GLMM: $\chi^2_1 = 2.55$, $N = 240$, $P = 0.11$; Fig. 2d).

DISCUSSION

In our initial screen of males from 59 hybrid genotypes we found significant variation in forced copulation success across genotypes (Fig. 1a). Such genetic variation is expected, and our laboratory has already documented genetic variation in sociability and activity levels (Scott et al., 2018), mating success, latency and duration (Filice & Dukas, 2019), and both male–male and female–female aggression (C. M. Baxter, J. L. Yan, & R. Dukas, personal observations) in these same hybrid genotypes derived from DGRP lines. Many other studies using flies from the DGRP lines have also documented genetic variation in a variety of behaviours such as courtship (Gaertner et al., 2015), same-sex sexual behaviour (Hoskins, Ritchie, & Bailey, 2015), male–male aggression (Edwards et al., 2009; Shorter et al., 2015), odour aversion (Swarup, Huang, Mackay, & Anholt, 2013), behavioural plasticity (Saltz, Lymer, Gabrielian, & Nuzhdin, 2017), punishment and relief memory
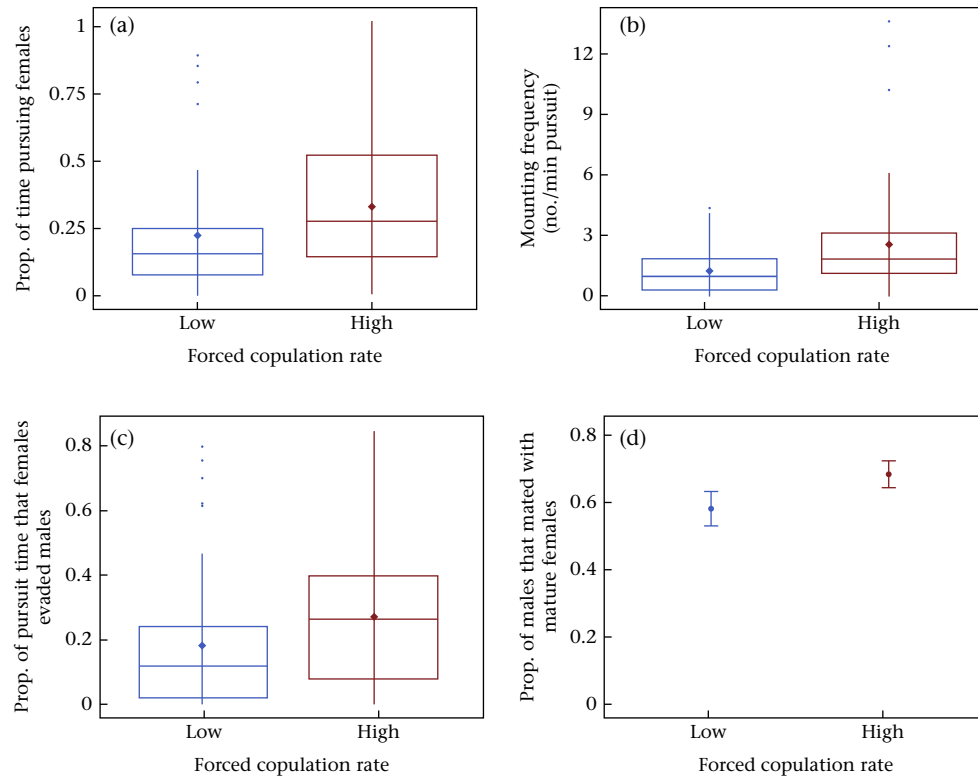
Figure 2. Comparing males from 'low' and 'high' forced copulation success genotypes (shown in blue and red, respectively). (a) Male persistence, shown as the proportion of time that males spent pursuing teneral females during the trials. The bold horizontal lines indicate the medians, the boxes represent the interquartile range (IQR) between the first and third quartiles, and the vertical lines extend to the minimum and maximum values. Outliers are indicated by small dots, and the means are indicated by the larger diamond shapes. (b) Male coerciveness, shown as the number of times males mounted teneral females per minute of pursuit. (c) Teneral female resistance, shown as the proportion of male pursuits that females ran away from the males. (d) Male mating success with mature females, shown as the proportion ± 1 SE of trials that resulted in matings with mature virgin females.

(Appel et al., 2016) and startle response (Mackay et al., 2012). Since we used teneral females from a single genotype in our screen, we can confidently attribute the variation in forced copulation success to genetic variation across the male genotypes, implying that males with different genetic backgrounds are predisposed to distinct mating strategies.

To examine the behavioural determinants of forced copulation success, we chose three male genotypes with the lowest forced copulation rates within 1 h and three male genotypes with the highest forced copulation rates within 1 h (note that Fig. 1a shows forced copulation rates within 2 h trials). When we retested these six genotypes, we found that their forced copulation rates were generally consistent with what we found in the 59-genotype assay, with the exception of one of the low-success genotypes (765) which, in this replication, had the second highest forced copulation success rate (Fig. 1b). This inconsistency across experiments is most likely due to gene–environment interactions that may have specifically affected hybrid genotype 765 in this case. We have observed such variation in behaviour over time in our previous work with the DGRP hybrids (C. M. Baxter, J. L. Yan, & R. Dukas, personal observations; Scott et al., 2018). Since we wanted to compare males from high- and low- success genotypes, we chose to focus on the five genotypes that were consistent over time. As we predicted, males from the high-success genotypes were more persistent (Fig. 2a) and mounted more frequently (Fig. 2b) than males from the low-success genotypes. When we compared 'unsuccessful' to 'successful' males, we found that male pursuit duration and mounting frequency positively predicted forced copulation success. The fact that these results are in the same direction indicates that the behavioural differences we found

between high- and low-success genotypes are representative of what varies between individual males who either succeed or fail to forcibly copulate.

The results comparing high- and low-success genotypes support the idea that heritable behavioural differences across males contribute to their variation in forced copulation success. At the same time, we found no significant difference in how females responded to males from low- and high-success genotypes with respect to running away from them during pursuit (Fig. 2c). This indicates that the variation in forced copulation success between male genotypes was not driven by variation in female resistance. This could be because males from high-success genotypes persist in their pursuit despite rejection signals from teneral females, which parallels what we have found in potentially coercive matings with recently mated females (Figure 2 in Baxter & Dukas, 2017). Recently mated females typically reject males, and do so by extruding their ovipositor, which is a rejection signal specific to mated females (Bastock & Manning, 1955; Connolly & Cook, 1973). Due to their low receptivity, mating latencies with recently mated females are significantly longer than those with virgin females. Males who succeed in mating with recently mated females not only spend a greater cumulative amount of time pursuing them despite their clear rejection behaviour, but also spend a significantly greater proportion of time in active pursuit in the few minutes prior to mating than males who mate with virgin females (Baxter & Dukas, 2017). This suggests that male persistence, in spite of clear rejection signals, plays a role in the potentially coercive mating of recently mated females, and may also play a similar role in the forced copulation of teneral females.

Finally, when we paired males with mature virgin females (who can prevent unwanted matings), we found no significant difference in mating success between males from low- and high-success genotypes (Fig. 2d). We did find that males from high-success genotypes had nonsignificantly greater mating success with mature females, and, if this trend is indicative of a true difference, it would imply that males with high forced copulation success also have high mating success with mature virgins. However, further experiments are needed to clarify whether this is a true biological difference and, if so, what male behaviours and/or female preferences are contributing to it.

For this study, we chose to focus on behavioural differences that contribute to variation in forced copulation success. Morphological variation, however, may also be a contributing factor. For example, larger males may be able to more easily forcibly copulate with smaller females. Larger male-to-female body size ratios increase the likelihood of mating after a premating struggle in the seaweed fly (*Coelopa ursina*) (Crean & Gilburn, 1998), and correlate with increased durations of forced copulations in Lake Eyre dragons (Olsson, 1995). Variation in the morphology of specific body parts could also aid male fruit flies in overcoming female resistance, like in water striders (*Gerris odontogaster*), where males with longer abdominal processes can more firmly grasp females who attempt to dislodge them, and as a result have greater mating success than males with shorter abdominal processes (Arnqvist, 1989). A potential candidate structure in fruit flies (*D. melanogaster*) are the sex combs, an array of bristles on the forelegs of males, which can vary in the number and length of bristles across individuals (Ahuja, de Vito, & Singh, 2011; Snook, Gidaszewski, Chapman, & Simmons, 2013). Males that have had their sex combs removed have drastically reduced mating success compared with intact males, despite displaying persistent courtship and mounting attempts (Cook, 1977; Hurtado-Gonzales, Gallaher, Warner, & Polak, 2015; Ng & Kopp, 2008), demonstrating that sex combs are important for mating success. While no studies have examined how variation in sex comb structure relates to forced copulation success, it is possible that either the structure of the sex combs, or other physical traits affect a male's ability to overcome teneral female resistance. We will examine this possibility in our ongoing research on the mechanisms underlying forced copulation success.

We consider the forced copulation of teneral female fruit flies to be an aggressive act since males inflict harm, such as wing damage, upon females, and females who are forcibly copulated while teneral are more likely to experience early mortality than females who consensually mate once mature (Dukas & Jongsma, 2012a). So, given that forced copulation is an aggressive act, are males who perform forced copulations simply more aggressive in general? Note, however, that aggression is not a unitary trait, but a broad categorization of behaviours that can occur under different contexts with distinct goals (such as gaining access to mates, defending territory, protecting offspring, etc.), and that different forms of aggression may have distinct genetic, neurobiological and physiological underpinnings (Moyer, 1968). Despite this, aggressive behaviours under different contexts are sometimes positively correlated. For example, in western bluebirds, *Sialia mexicana*, males who aggressively defend their nests against heterospecifics are also more aggressive in conspecific male–male competition (Duckworth, 2006). In mice, transgenic males who lack the monoamine oxidase A (MAOA) enzyme are more aggressive towards males they are housed with and towards intruder males, and they are more sexually aggressive towards unreceptive females (Cases et al., 1995). Additionally, St John and Corning (1973) found a positive correlation between male and female aggression levels across inbred mouse lines. However, Hyde and Ebert (1976) found that lines selected for increased female aggression did not show

increased male aggression, and Gammie, Hasen, Rhodes, Girard, and Garland (2003) did not find support for an association between male–male and maternal aggression. In fruit flies, we have found that older males are more aggressive towards other males and also have higher forced copulation rates than younger males (Baxter & Dukas, 2017), which could be taken as support for a positive correlation between male–male aggression and male sexual aggression towards females. However, in the same series of experiments, we also found that males with previous mating experience showed reduced forced copulation rates compared with mating-deprived males, while showing similar levels of aggression towards other males, indicating that prior experience can have distinct effects on sexual aggression and male–male aggression. Therefore, it is not obvious whether males who are genetically predisposed to engage in high levels of sexual aggression would also display more aggression under different contexts, making this a valuable avenue for future studies.

Given the prevalence of sexual coercion and aggression in nature, we feel it is vital to gain a better understanding of the genetics, neurobiology and evolutionary biology of sexual aggression and forced copulations. However, studying aggressive sexual coercion can be complicated by many factors. First, it may not always be possible to reliably generate sexual coercion under controlled laboratory settings for certain species. Additionally, unless it is clear that the female cannot prevent an unwanted mating, it may not be possible to fully disentangle female mate screening from male sexual coercion. Finally, for many species, it is not feasible to subject females to males who may sexually coerce them. Our goal for these experiments was not only to assess genetic variation and behavioural differences contributing to forced copulation success, but to demonstrate that the forced copulation of teneral females is an excellent model for studying aggressive sexual coercion. In particular, given that fruit flies are a well-established model organism, this lesser studied model of forced copulation with teneral females provides many opportunities for future studies to take advantage of the numerous resources and tools that exist for examining sexual conflict and its evolutionary consequences in fruit flies.

Acknowledgments

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