



Life history of aggression: effects of age and sexual experience on male aggression towards males and females



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Aggression is a prominent behaviour well studied in a large variety of animals, but it has not been well integrated within life history research. To address this shortcoming, we conducted a series of experiments simultaneously quantifying the effects of age and sexual experience on the use of aggression in male fruit flies. We studied three types of aggression relevant to male fruit flies' natural history: fighting in the context of resource defence, forced copulation with newly eclosed females, and coercion of recently mated females. Young, sexually mature flies were initially reluctant to use aggression towards either males or females. Within a few days, however, their use of aggression increased and then plateaued. While sexual experience caused males to decrease aggression towards females, it did not affect their aggression towards males. It is likely that aggression involves some cost, which leads males to favour peaceful over aggressive pursuit of females. However, sexually experienced males are probably highly motivated to deter other males from their apparently attractive resource. Our results highlight the importance of studying multiple types of aggression and the need for careful assessments of the relative fitness benefits and costs of aggression versus peaceful alternatives in shaping the life history of aggression.

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Life history research has been instrumental in identifying key features characterizing animals as they go through the major life transitions of growth, reproduction and aging (Roff, 2002; Stearns, 1992). The major focus of life history studies has appropriately been on topics such as age and size at sexual maturity, the number and size of offspring, trade-offs between current and future reproduction, and senescence (Stearns, 1992). Although aggression is a prominent and well-studied animal behaviour (Hardy & Briffa, 2013; Nelson, 2005), it has received scant attention in life history research. Notable research on aggression includes extensive work on territorial fights in a variety of spider species (Elwood & Prenter, 2013; Riechert, 1986) and butterflies (Davies, 1978; Kemp, 2013), shell fights in hermit crabs, *Pagurus bernhardus* (Briffa & Elwood, 2001; Elwood & Neil, 1991), contests in red deer, *Cervus elaphus*, and other ungulates (Clutton-Brock, Guinness, & Albon, 1982; Jennings & Gammel, 2013), and violence in humans (Baron & Richardson, 2004; Daly, 2016; Daly & Wilson, 1988; Quetelet, 1833).

The two relevant life history models of aggression predict that younger males should be less willing to fight than older males. This is because younger males have a higher residual reproductive value than older males and can thus lose more from injuries (Kemp, 2006; Parker, 1974). Kemp (2006) noted, however, that young individuals should be more willing to fight under a narrow set of conditions where there is a steep decline in resource-holding potential with age and the benefit from holding a resource is relatively small. While the existing models are insightful, it is not clear how relevant they are to species that lack weapons. In such species, males cannot readily inflict injuries on their opponents, so the costs of fighting are not clear. Another limitation of the current models is that they do not consider a broad range of realistic population dynamics parameters, such as growth rate and density dependence, which strongly affect predictions of life history models (Abrams, 1993; Williams, Day, Fletcher, & Rowe, 2006). Indeed, although both Kemp (2006) and others (Hardy & Briffa, 2013; Huntingford & Turner, 1987) highlighted a general pattern of fighting tending to increase with male age in a variety of species, they also noted many exceptions. Two well-studied species in which peak aggression occurs in young rather than older males are the lizard *Anolis aeneus* (Stamps, 1978) and humans (Farrington, 1986; Reiss & Roth, 1993).

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While many studies reported on variation in aggression with age (reviewed in Hardy & Briffa, 2013; Huntingford & Turner, 1987; Kemp, 2006), most did not carefully account for correlates of age that can affect aggression. Such possible confounds include selective mortality (Huntingford & Turner, 1987), mating experience, as well as subtle effects of social housing conditions prior to tests. For example, if subjects are housed in groups, social interactions within the group might determine subsequent aggression. Another limitation of most studies of aggression is that they focus on a single context, most commonly male fighting associated with access to females. It is widely agreed, however, that aggression is not a unitary phenomenon (Huber & Kravitz, 2010; Moyer, 1968). Hence it is highly appropriate that we incorporate multiple, ecologically relevant types of aggression into routine research protocols.

To broaden our knowledge on the life history of aggression, we have adopted fruit flies (*Drosophila melanogaster*) as a model system and focused on three types of aggression. Several factors make fruit flies an ideal species for examining the life history of aggression. The flies are short-lived and cheap to maintain. Ethical difficulties are limited because the flies do not inflict injuries during fights. There are numerous tools for functional and mechanistic research in this species. Finally, fruit flies have recently been adopted for genetic and neurobiological research on aggression (Anholt & Mackay, 2012; Chen, Lee, Bowens, Huber, & Kravitz, 2002; Dierick & Greenspan, 2006; Edwards et al., 2009; Zhou, Rao, & Rao, 2008), which, combined with our evolutionary ecological approach, can help illuminate general features of aggression and its trajectory throughout life.

We focused on three realistic types of male aggression, fighting in the context of resource defence, forced copulation with recently eclosed (teneral) females, and coercion of recently mated females. In settings with small, dispersed fruit and low fly density, capable male fruit flies use aggression to monopolize the attractive patches that females seek for feeding and egg laying (Dow & Schilcher, 1975; Hoffmann, 1987; Markow, 1988). Resource-holding males also rely on aggression to guard females they have recently mated with (Baxter, Barnett, & Dukas, 2015a). By far, fighting in the context of resource defence has dominated the mechanistic literature on fruit fly aggression, although most papers refer to it as territorial aggression (Chen et al., 2002; Edwards, Rollmann, Morgan, & Mackay, 2006; Hoffmann, 1987).

In addition to the male–male aggression protocol, we also examined two types of male aggression towards females. Forced copulation with teneral females has been well characterized in the field and laboratory. Such forced copulation occurs primarily within 2 h post eclosion. During this short period, the vaginal plates of teneral females are still soft. Hence the females cannot physically resist intercourse by males that succeed in mounting them in spite of the females' persistent efforts to dislodge the males. We chose this type of aggression because it occurs at a sufficiently high frequency and thus can have significant effects on both male and female fitness (Dukas & Jongsma, 2012a, 2012b; Markow, 2000; Seeley & Dukas, 2011). While the role of coercion in the remating of recently mated females has not been well addressed, there is ample evidence that such rematings are more likely to occur at high male-to-female ratios, after long periods of persistent male mating attempts, and when females have no refuge from males (Byrne, Rice, & Rice, 2008; Gromko, Gilbert, & Richmond, 1984; Wigby & Chapman, 2004). These features are typically associated with convenience polyandry (Arnqvist & Rowe, 2005; Thornhill & Alcock, 1983), where females give in to males' persistent harassment and remate. We should note that aggression and forced copulation are typically discussed in distinct bodies of literature, so one might question whether it is appropriate to call forced copulation aggression. We feel, however, that when physical force is used by

one actor (the aggressor), which the other actor (the victim) attempts to evade, and when the aggressor inflicts injuries that reduce the expected life span of the victim (Dukas & Jongsma, 2012b), the term aggression is appropriate.

Overall then, we tested the effects of male age and mating experience on three types of aggression. The theoretical models suggest that, generally, male aggression should increase with age (Kemp, 2006; Parker, 1974). Furthermore, we have previously documented that sexually mature, 1-day-old males spent less time than did 4-day-old males courting recently mated females that were unlikely to remate. Males' mate choosiness decreased with age and reached asymptote by age 7 days (Baxter, Barnett, & Dukas, 2015b; Dukas & Baxter, 2014). This suggests that young males are less willing to incur costs associated with access to females than are mature males. We thus predicted that, as with our mate choosiness data (Baxter et al., 2015b), both male–male and male–female aggression would initially increase and then asymptote with male age. Our predictions for the effects of sexual experience were more complex. Previous data indicated a higher mate choosiness in previously mated than in virgin males (Baxter et al., 2015b; Byrne & Rice, 2006). Hence we predicted that, compared to virgin males, sexually experienced males would show less coercion of teneral and recently mated females because such females clearly reject pursuing males. As for male–male aggression in the context of resource defence, we predicted no effect of sexual experience owing to the operation of two opposing factors. On the one hand, we expected sexual deprivation to increase males' motivation to use aggression in order to secure access to females. On the other hand, because encountering and mating with females informs males that they occupy an attractive resource, we expected previously mated males to show a higher motivation to use aggression for resource defence than males that had not encountered females previously.

METHODS

General

We used descendants of wild-caught *D. melanogaster* collected in several southern Ontario localities in August 2014. We housed the flies in population cages containing several hundred flies per cage. We kept the cages in an environmental chamber at 25 °C and 60% relative humidity with a 12:12 h light:dark cycle, with the lights turning on at 1000 hours. We reared the experimental flies at a low density of about 300 eggs per 240 ml bottle containing 50 ml of standard fly medium made of water, sucrose, cornmeal, yeast, agar and methyl paraben. We sexed flies within 4 h of eclosion to ensure virginity and minimal experience with other flies. We used gentle aspiration to sex and transfer males into individual 40 ml vials each containing 5 ml of medium, and CO₂ to sex and place females in groups of 20 per vial, which also contained 5 ml of medium and a dash of live yeast.

We conducted all tests in cylindrical arenas made of Plexiglas (3 cm in diameter and 2.5 cm high). To deter flies from climbing on the arenas' walls and ceilings, we coated the walls with Insect-a-Slip (Fluon, BioQuip, Gardena, CA, U.S.A.) and the ceilings with Surfasil (Sigma Aldrich, Oakville, ON, Canada). We covered the floor of each arena with filter paper, and placed at its centre a circular food patch (1.3 cm in diameter, 1.5 mm high) coated with a suspension made of 3 g of live yeast in 100 ml of grapefruit juice. Throughout the study, we provided flies with ad libitum food optimized for fruit flies' preferences and needs, kept flies at low densities and kept to a minimum the duration of aggressive encounters.

Our experiments comprised two dimensions. First, we examined independently the effects of male age and sexual experience. Second, we assessed the effects of age and experience on three types of aggression: male–male aggression in the context of resource defence, and male–female aggression in the contexts of forced copulation of teneral females and coercive matings with recently mated females. Below we first detail our protocols for manipulating male age and experience, and then present specific methods for each of the three types of aggression.

Effects of Age on Aggression

As individuals age, they gain further experience. To separate the effects of age and experience on aggression, we conducted two sets of experiments. In the first set, we varied male age while holding mating experience constant whereas in the second set, we manipulated male mating experience while holding age constant. In the experiments on male age, we used males that were 1, 4 and 7 days old (Fig. 1a). We housed these males individually in regular food vials until the time of testing. Our previous work indicated that males are sexually mature and have a high mating success and fertility when they are 1 day old (Baxter et al., 2015b; Dukas &

Baxter, 2014). We used males that were 1–7 days old because this represents a realistic age range for wild fruit fly populations. The limited field data suggest a median life span of 3–6 days in *D. melanogaster* (Rosewell & Shorrock, 1987). In the similarly sized antler flies (*Protopiophila litigate*), median life span in the field was 6 days (Bonduriansky & Brassil, 2002). Finally, in a few honeybee field studies, median forager life span was 5–7 days (Dukas, 2008a, 2008b; Dukas & Visscher, 1994). We had to limit the number of male age classes used because it was crucial that we conduct tests of all age groups simultaneously due to day and time of day effects.

Effects of Mating Experience on Aggression

In the experiments on male age and aggression, we equalized males' experience by keeping the males away from females prior to testing. Age and experience, however, were positively correlated, meaning that older males had been deprived of females longer than younger males. We thus conducted another set of experiments in which we manipulated males' experience with females while keeping male age constant. On day 1, we randomly assigned newly eclosed males into either an experienced treatment or a deprived treatment. In the experienced male treatment, we added one 3-

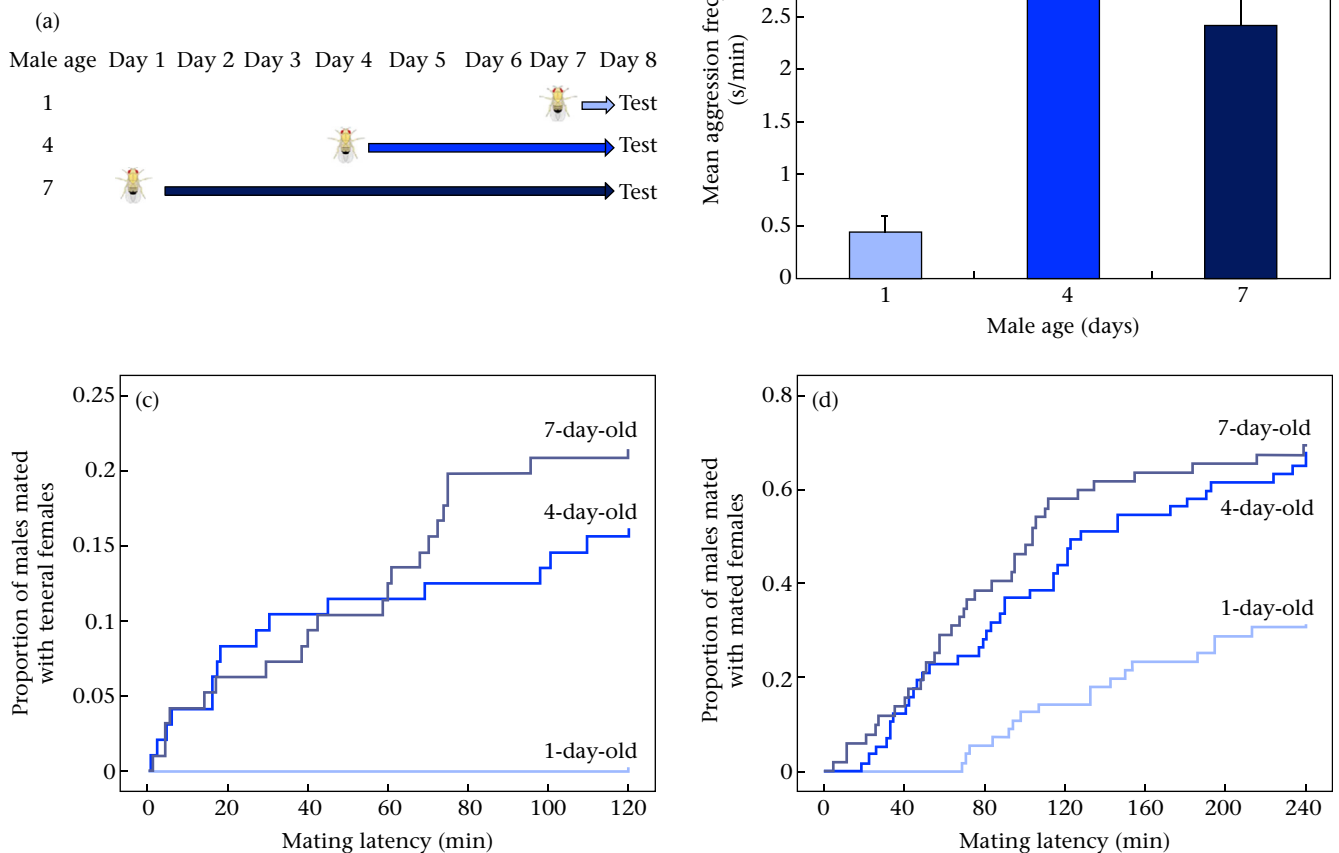


Figure 1. (a) Three treatments for the effects of age on aggression: males were 1, 4 and 7 days old when tested on day 8. (b) Mean + SE aggression frequency per arena in the three male age treatments ($N = 108$ arenas, 36 per treatment). (c) The cumulative proportion of 1-, 4- and 7-day-old males that force-copulated with teneral females across a 120 min trial duration ($N = 288$, 96 per treatment). (d) The cumulative proportion of 1-, 4- and 7-day-old males that mated with recently mated females across a 240 min trial duration ($N = 165$, with 56 1-day-old males, 57 4-day-old males and 52 7-day-old males).

day-old virgin female to each male vial on days 2, 3 and 4, without removing the females added on the previous days (Fig. 3a). This means that each male of the experienced treatment had ample opportunities to court and mate with both virgin and recently mated females. After the experience phase, we checked the food vials for the presence of larvae, which indicated that each male did in fact mate with females in his vial. In the deprived male treatment, we simply left the males alone in their vials, and thus they never had the opportunity to interact with females prior to the test (Fig. 3a). We conducted the tests on day 5, when the males were 4 days old.

Resource Defence

Our basic protocol involved aspirating two males and a 4-day-old, recently mated female into each arena. The female had mated with a nonfocal male 1–3 h prior to the test. After a 10 min habituation, we videorecorded each arena for 15 min using webcams (Logitech HD Pro C920). Later, observers blind to fly treatment scored the videos using Noldus software (Noldus Information Technology, Wageningen, The Netherlands). The observers recorded the total duration of aggression by the two males, which included all occurrences of lunging, wing threat, high-level fencing, charging, holding, boxing and tussling (Chen et al., 2002; Dierick & Greenspan, 2006). We analysed the data with a generalized linear model (GLM) with gamma distribution and log link function (IBM, 2013), and applied sequential Bonferroni corrections to the post hoc comparisons.

In the test for the effect of male age, our three treatments included pairs of males that were 1, 4 or 7 days old ($N = 36$ pairs of males per treatment). We conducted two experiments assessing the effect of sexual experience. The first experiment consisted of our preferred protocol, in which we added to each arena one experienced and one deprived male, and a 4-day-old, recently mated female ($N = 40$ arenas). The female had mated with a nonfocal male 1–3 h prior to the test. After a 10 min habituation, we videorecorded each arena for 15 min. Later, observers blind to male treatment recorded from the videos the total duration of aggression displayed by each male. We analysed the data with a generalized linear model (GLM) with gamma distribution and log link function (IBM, 2013), with male treatment as a repeated measure within each arena.

In our second test for the effect of sexual experience, we replicated the protocol of Yuan, Song, Yang, Jan, and Jan (2014), because their results, unlike ours, suggested that males housed with females are less aggressive towards other males than are males deprived of females. To each arena, we added two males and two 4-day-old virgin females. We had three treatments in which the two contesting males within an arena were both deprived of females, both experienced with females, or one was deprived and one was experienced with females. One male per arena was coloured with pink fluorescent powder, and this colouring was counterbalanced across male experience. After adding the males to the arenas, we observed the arenas for mating. After the matings ended, we videorecorded each arena for 30 min. Later, observers blind to male treatment recorded from the videos the total duration of aggression displayed by each male.

We followed the protocol of Yuan et al. (2014) and discarded all trials in which one of the males did not mate. The sexually experienced males failed to mate in 23 trials, and the sexually deprived males failed to mate in 12 trials. We also excluded eight trials in which the female mated more than once. After these exclusions, we ended up with $N = 16$ experienced versus experienced arenas, $N = 18$ deprived versus deprived arenas and $N = 17$ experienced versus deprived arenas. The need to discard trials in which one of

the males did not mate is an obvious weakness of the Yuan et al. (2014) protocol. Another complication is that matings with virgin females creates a conflicting, unnatural dynamic in which both males mate-guard their recently mated female (see Baxter et al., 2015a). We analysed the data with a generalized linear model (GLM) with gamma distribution and log link function (IBM, 2013), with male treatment as a repeated measure within each arena.

Forced Copulation with Teneral Females

We collected teneral females within a few minutes post eclosion and aspirated one teneral female and one male into each arena. Observers blind to male age continuously scanned the arenas for 2 h and recorded the mating latencies and durations. In the test for the effect of male age, our three treatments included males that were 1, 4 or 7 days old ($N = 96$ arenas per treatment). In the test for the effect of sexual experience, our two treatments were experienced and deprived males ($N = 128$ arenas per treatment). We analysed the data with Cox regressions, and used simple contrasts when comparing three treatments.

Coercive Mating with Recently Mated Females

Evidence for male coercion

To critically assess whether mating with recently mated females is coercive, we compared courtship and matings with recently mated and virgin females. We had three treatments (Fig. 2a): mated females; virgin females matched to the mated female treatment for trial start time (virgin 1 treatment); and virgin females matched to the mated female treatment for approximate mating start time (virgin 2 treatment). This protocol allowed us to simultaneously compare the initial courtship of mated and virgin females, courtship just prior to mating, and behaviour during mating while controlling for time of day given that the mating latency of previously mated females is much longer than that of virgin females (see below). The initial matings of the recently mated females occurred 1 day before the test with nonfocal, 4-day-old males in regular food vials containing a dash of live yeast. After the matings, we discarded the males and kept the females in their individual food vials overnight. We later examined the vials for the presence of larvae to ensure that the initial matings were fertile. Only one female was not fertile, and she was excluded from the analysis.

For the test phase, we placed in each arena a 4-day-old virgin male and a 4-day-old female belonging either to the mated or virgin 1 treatment. We then began videorecording, which continued until mating ended or until 4 h passed. Based on preliminary data, we set up the virgin 2 treatment about 1 h later. This treatment was similar to the virgin 1 treatment except that it started later such that matings occurred at about the same time as matings in the mated treatment (Fig. 2a). Later, we generated three sets of similar-length video clips for analyses (squares in Fig. 2a). The initial courtship consisted of the courtship during the first few minutes of trials with virgin 1 and mated females (purple squares in Fig. 2a). The length of these clips was determined by the short mating latencies of the virgin females. The final courtship consisted of the courtship during the last few minutes prior to mating with virgin 2 and mated females (blue squares in Fig. 2a). Again, the length of these clips was determined by the short mating latencies of the virgin females. Finally, the mating clips (green squares in Fig. 2a) included the full mating by each female as we did not expect a major difference in mating duration between the treatments (Bretman, Fricke, & Chapman, 2009). Separating the courtship and mating portions and equalizing the lengths within each set allowed us to remain blind to female treatment given the obvious differences in mating latencies.

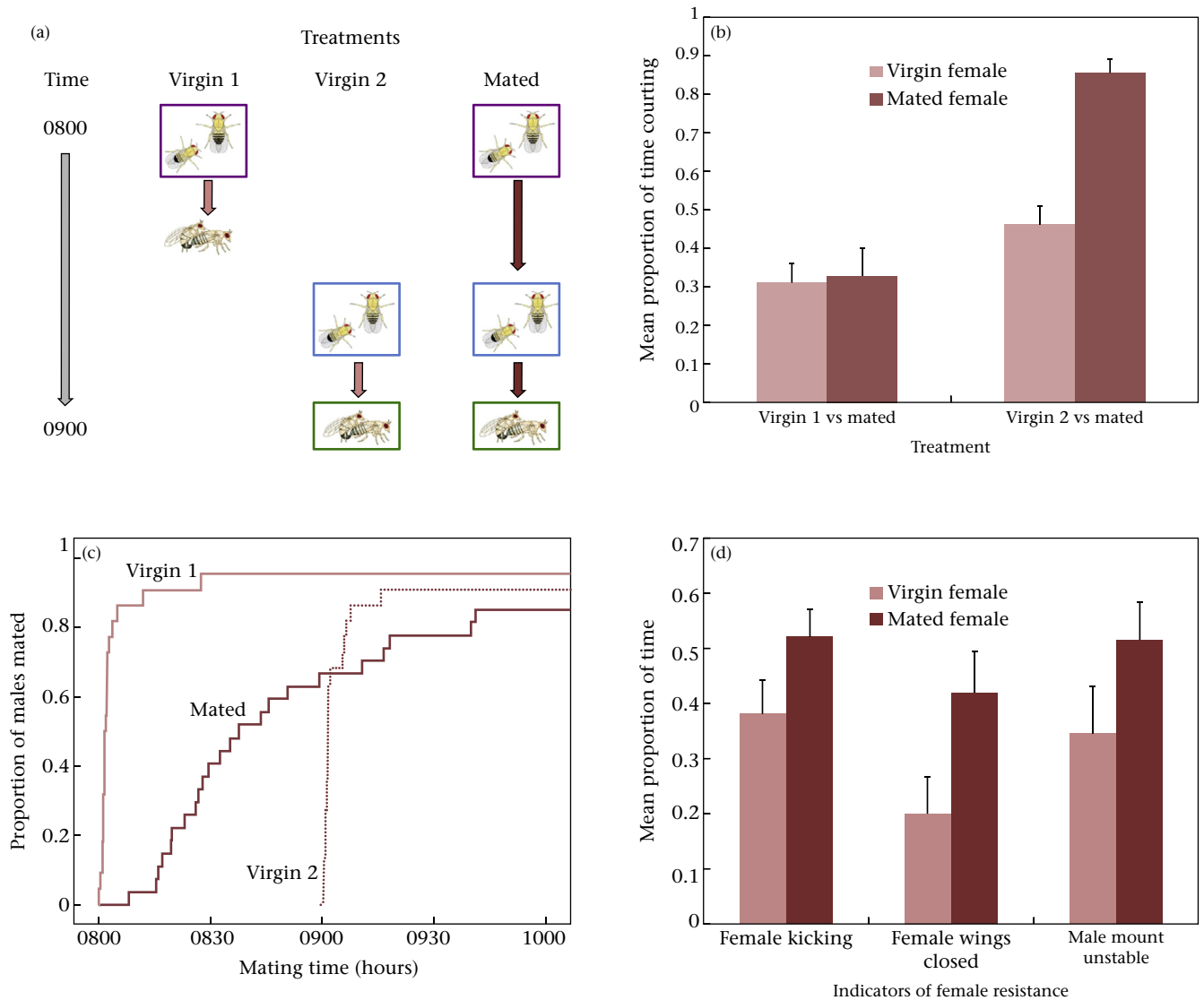


Figure 2. (a) Three treatments for testing whether mating with recently mated females is coercive. The virgin 1 female treatment was matched to the mated female treatment for trial start time (purple squares) whereas the virgin 2 female treatment was matched to the mated female treatment for the approximate times of courtship just prior to mating (blue squares) and mating (green squares). (b) Mean + SE proportion of time males spent courting virgin and mated females at the commencement of trials (virgin 1 versus mated) and just prior to mating (virgin 2 versus mated) ($N = 65$, with 21 virgin 1, 20 virgin 2 and 24 mated female trials). (c) The cumulative proportion of virgin 1, virgin 2 and mated female matings. Note that trials of the virgin 1 and mated females started at 0800 hours while trials of the virgin 2 treatment started at 0900 hours. (d) Mean + SE proportion of time females resisted during mating (through kicking and having their wings closed) and proportion of time males struggled to maintain mounting of females. Matings occurred at approximately the same time of day (green squares in Fig. 2a).

Observers blind to female treatment recorded from each of the courtship clips the duration of male courtship (Dukas & Dukas, 2012). While we also examined a variety of other male and female behaviours during courtship as we have done in the past (Dukas & Scott, 2015; Seeley & Dukas, 2011), we found no substantial differences between the treatments. For the mating clips, we recorded the duration of time that females kicked the males and had their wings closed. We considered closed wings as evidence for coercive mating because, prior to consensual mating, females spread their wings and this allows males to firmly hold the females during copulation (Spieth, 1974). We also recorded from the mating clips the duration of unstable male mounting, which included males struggling to stay mounted on the females or being noticeably at an angle to the females rather than being securely mounted on her dorsal side between her wings. Because these data violated normality assumptions even

after transformations, and showed no good fit to other distributions, we used nonparametric statistics in the analysis of coercive mating behaviours.

Our sample sizes for the virgin 1, virgin 2 and mated female treatments were 22, 22 and 28, respectively. One of the females from the mated female treatment did not produce offspring as a result of her first mating, and therefore was excluded from the analyses. There were also one, two and three trials in the virgin 1, virgin 2 and mated female treatments, respectively, that did not result in mating during the test phase. Therefore, these trials are not included in the analysis of coercive mating behaviours, giving us sample sizes of 21, 20 and 24 for virgin 1, virgin 2 and mated female treatments. However, these trials were still included in mating latency comparisons, as we performed Cox regressions to compare mating latencies between treatments, which take the absence of mating into consideration.

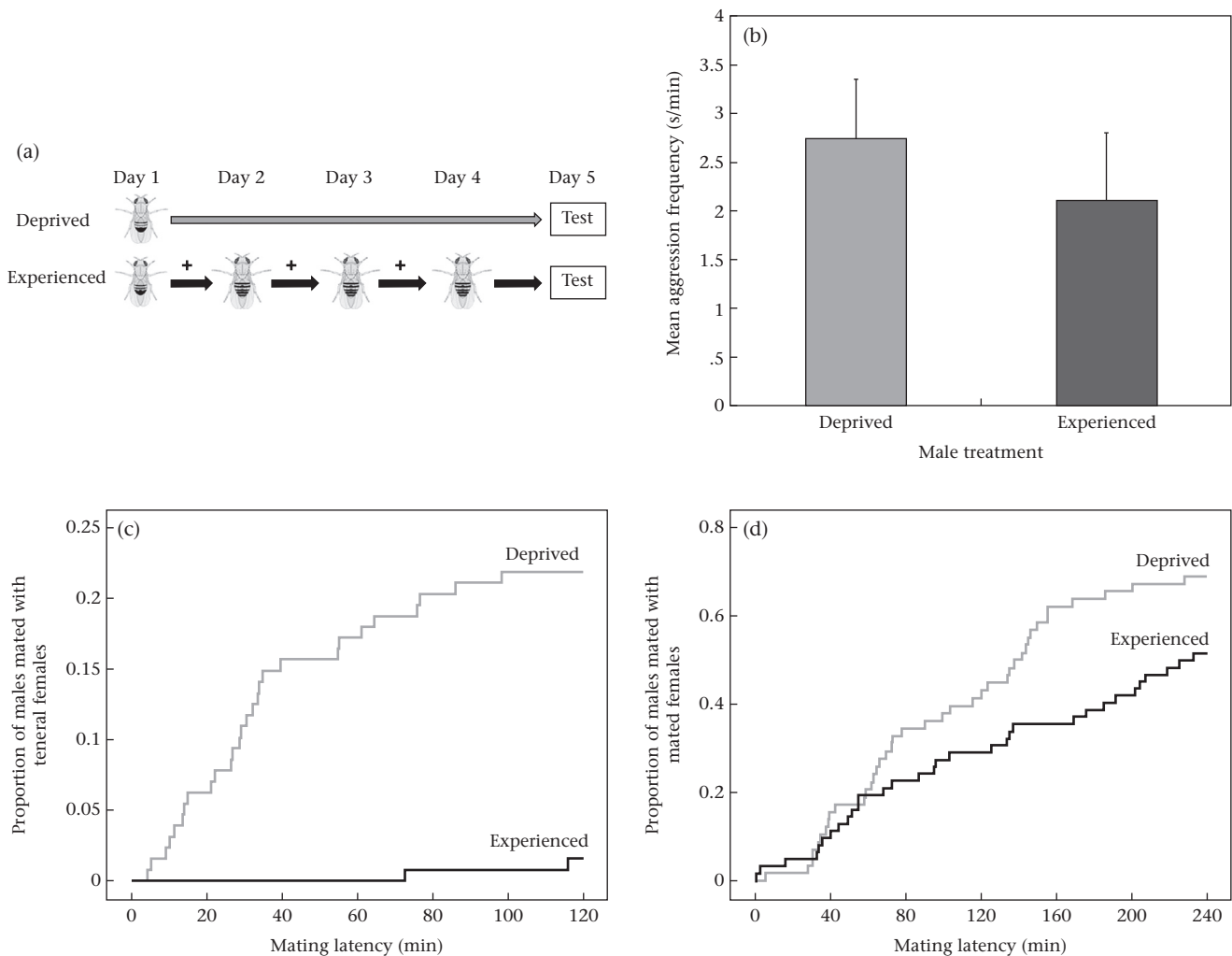


Figure 3. (a) Two treatments for the effects of mating experience on aggression. Males were either housed alone (deprived treatment) or housed with females (experienced treatment) until the test on day 5. One female was added to each experienced male's vial on days 2, 3 and 4. Males of both treatments were 4 days old when tested on day 5. Note that males are smaller than females and have a dark posterior. (b) Mean \pm SE aggression frequency per male for mating-deprived and mating-experienced males, which were paired together in arenas ($N = 40$ arenas). (c) The cumulative proportion of previously mating-deprived and mating-experienced males that force-copulated with teneral females across a 120 min trial duration ($N = 256$, 128 per treatment). (d) The cumulative proportion of previously mating-deprived and mating-experienced males that mated with recently mated females across a 240 min trial duration ($N = 120$, with 58 deprived males and 62 experienced males).

Effects of age

This experiment had two phases, the first involving the initial mating of virgin females and the second being the actual remating test. Theoretically, rematings could be affected by the age of the males that the females had initially mated with. Hence we had three types of mated females that initially mated with virgin, nonfocal males that were either 1, 4 or 7 days old. The initial matings occurred in regular food vials containing a dash of live yeast, and each containing one male and one female that was 3 days old. After the matings, we discarded the males and kept the females in their individual food vials overnight. We later examined the vials for the presence of larvae to ensure that the first matings were fertile and excluded from the analysis the 30 females (15% of the total sample) who were infertile (11 mated to 1-day-old males, 9 mated to 4-day-old males and 10 mated to 7-day-old males).

As before, our three main treatments included males that were 1, 4 or 7 days old ($N = 56$ 1-day-old males, $N = 57$ 4-day-old males and $N = 52$ 7-day-old males, after infertile females were excluded). In addition, we also had three categories within each male age, of females initially mated with a male that was 1, 4 or 7 days old. This generated a total of nine treatment combinations. We aspirated one

recently mated female and one focal male into each arena. Observers blind to fly treatment scanned the arenas continuously for 4 h, recording mating latencies and durations. We analysed the data using a Cox regression with simple contrasts. Note that we recorded forced copulations with teneral females over 2 h and coercive matings with recently mated females for 4 h owing to relevant natural history factors. By definition, the teneral state is transitional, and most forced copulations with teneral females occur within 2 h (Markow, 2000; Seeley & Dukas, 2011). In contrast, the frequency of coercive matings with recently mated females is positively correlated with the duration of persistent male coercive attempts (Gromko et al., 1984).

Effects of experience

This experiment, like the previous one, had two phases. The initial matings occurred during the first phase in regular food vials containing a dash of live yeast, with each vial containing one virgin 4-day-old male and one female that was 3 days old. We discarded 22 females (15% of the total sample) who did not produce larvae after the initial mating. In the second phase, we aspirated one male from either the experienced or deprived treatment and a recently

mated female into each arena ($N = 62$ experienced and $N = 58$ deprived males after infertile females were excluded). Observers blind to male treatment scanned the arenas continuously for 4 h, recording the latency and duration of any matings that occurred. We analysed the data with a Cox regression.

RESULTS

Effects of Age on Aggression

Resource defence

Aggression varied significantly with age (GLM: Wald $\chi^2_2 = 47.7$, $N = 108$, $P < 0.001$; Fig. 1b). Four-day-old males were more aggressive than 1-day-old males ($P < 0.001$), but the 4- and 7-day-old males showed similar levels of aggression ($P = 0.33$).

Forced copulation with teneral females

The frequency of forced copulations varied significantly with male age (Cox regression: Wald $\chi^2_2 = 9.7$, $N = 288$, $P < 0.01$; Fig. 1c). Four- and 7-day-old males force-copulated significantly more frequently than 1-day-old males (Wald $\chi^2_1 = 7.7$, $N = 192$, $P < 0.005$ and Wald $\chi^2_1 = 9.5$, $N = 192$, $P < 0.005$, respectively), but 7-day-old males did not force-copulate significantly more frequently than 4-day-old males (Wald $\chi^2_1 = 0.76$, $N = 192$, $P = 0.38$).

Coercive mating with recently mated females

We first tested whether remating involved male coercion. While males spent similar proportions of time courting virgin and previously mated females during the initial few minutes of trials (purple squares in Fig. 2a; Mann–Whitney U test: $U = 227$, $N_1 = 21$, $N_2 = 24$, $P = 0.57$; Fig. 2b), they showed significantly more intense courtship of the previously mated than of the virgin females in the few minutes just prior to mating (blue squares in Fig. 2a; $U = 45$, $N_1 = 20$, $N_2 = 24$, $P < 0.001$; Fig. 2b).

Mating latency varied significantly with female treatment (Cox regression: Wald $\chi^2_2 = 20.6$, $N = 71$, $P < 0.001$; Fig. 2c). The mating latencies in the virgin 1 and virgin 2 treatments (means \pm SEs of 3.5 ± 1.3 and 3.0 ± 0.8 min, respectively) were much shorter than those in the mated female treatment (48.1 ± 8.3 min; Cox regression: Wald $\chi^2_1 = 17.4$, $N = 49$, $P < 0.001$ and Wald $\chi^2_1 = 13.4$, $N = 49$, $P < 0.001$, respectively; Fig. 2c). During mating, the previously mated females spent a larger proportion of time kicking and with their wings closed (Mann–Whitney U test: $U = 177$, $N_1 = 20$, $N_2 = 24$, $P = 0.095$ and $U = 144$, $N_1 = 20$, $N_2 = 24$, $P < 0.05$, respectively; Fig. 2d). Consequently, the males were more likely to have an unstable hold of the previously mated than of the virgin females ($U = 168.5$, $N_1 = 20$, $N_2 = 24$, $P = 0.063$; Fig. 2d). The mating durations in the mated female treatment (14.8 ± 0.58 min) were significantly longer than in the virgin 2 female treatment (12.7 ± 0.46 min; $U = 129$, $N_1 = 20$, $N_2 = 24$, $P < 0.01$).

Next, we assessed the effects of male age on the frequency of matings with recently mated females. Mating frequency varied significantly with male age (Cox regression: Wald $\chi^2_2 = 20.2$, $N = 165$, $P < 0.001$; Fig. 1d). Four- and 7-day-old males mated with recently mated females more frequently than did 1-day-old males (Cox regression: Wald $\chi^2_1 = 14.4$, $N = 113$, $P < 0.001$ and Wald $\chi^2_1 = 18.7$, $N = 108$, $P < 0.001$, respectively). There was no significant difference in the mating frequency between 4- and 7-day-old males (Cox regression: Wald $\chi^2_1 = 0.50$, $N = 109$, $P = 0.48$). The age of the first-mating male in each treatment did not affect the likelihood of remating (Wald $\chi^2_2 = 2.3$, $N = 165$, $P = 0.32$).

The trials for coercive remating with recently mated females were 240 min in duration; however we also analysed the frequency of remating after 120 min to allow for direct comparisons between these results and the results for forced copulation of teneral

females. We found that after 120 min, mating frequency varied significantly with male age (Cox regression: Wald $\chi^2_2 = 8.5$, $N = 165$, $P < 0.05$; Fig. 1d). Four- and 7-day-old males mated more frequently than 1-day-old males (Cox regression: Wald $\chi^2_1 = 3.2$, $N = 113$, $P < 0.073$ and Wald $\chi^2_1 = 8.1$, $N = 108$, $P < 0.005$, respectively). The mating frequencies of 4- and 7-day-old males did not vary significantly (Cox regression: Wald $\chi^2_1 = 2.3$, $N = 109$, $P = 0.13$). The age of the first-mating male in each treatment did not affect the likelihood of remating (Wald $\chi^2_2 = 0.78$, $N = 165$, $P = 0.68$).

Effects of Mating Experience on Aggression

Resource defence

In the first experiment with a single mated female in each arena, the experienced and deprived males showed similar levels of aggression (GLM: Wald $\chi^2_1 = 0.34$, $N = 40$ arenas, $P = 0.56$; Fig. 3b). In the second experiment with two virgin females in each arena, the experienced and deprived males showed similar levels of aggression (GLM main effect of experience: Wald $\chi^2_1 = 1.4$, $N = 49$ deprived and 47 experienced males, $P = 0.24$; Fig. 4).

Forced copulation with teneral females

Males of the deprived treatment force-copulated with teneral females significantly more frequently than males from the experienced treatment (Cox regression: Wald $\chi^2_1 = 14.3$, $N = 256$, $P < 0.001$; Fig. 3c).

Coercive mating with recently mated females

Deprived males were significantly more likely to mate with recently mated females than were experienced males after the 240 min trial duration (Cox regression: Wald $\chi^2_1 = 4.2$, $N = 120$, $P < 0.05$; Fig. 3d). We also analysed the frequency of remating after 120 min and found that the difference between deprived and experienced males approached, but did not reach, significance (Cox regression: Wald $\chi^2_1 = 2.2$, $N = 120$, $P = 0.14$).

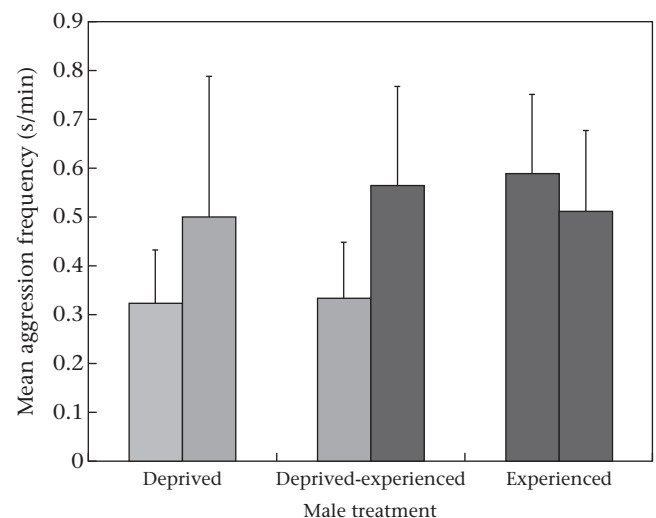


Figure 4. Mean \pm SE aggression frequency per male for mating-deprived and mating-experienced males. There were three arena combinations that males could be placed in: two deprived males ($N = 17$ arenas), one deprived and one experienced male ($N = 16$ arenas), or two experienced males ($N = 16$ arenas).

DISCUSSION

Our major results were, first, that for all three aggression types, aggression initially increased with age and then plateaued (Fig. 1). Second, sexually experienced males were less aggressive towards females but not towards males than were virgin males (Figs. 3 and 4). The distinct effects of sexual experience on aggression towards females and males underline the importance of studying multiple types of aggression. That is, given the complexity of the proximate and ultimate mechanisms underlying aggression (Huber & Kravitz, 2010; Moyer, 1968), we should not expect different types of aggression to covary. We should note that, because we did not subdivide aggression into distinct categories (e.g. wing threat and lunging), we cannot judge whether there was age-specific variation in qualitative aspects of aggression.

We studied aggression using three protocols representing distinct features of fruit fly natural history. As noted in the Introduction, the role of aggression in resource defence and forced copulation had been well established. Furthermore, Hoffmann (1990) documented that 3- and 4-day-old males are more likely to be resource holders when competing with 1- and 2-day old males, respectively. This is consistent with our direct data on aggression, which indicate increased aggression between age 1 and 4 days. The role of aggression in female remating, however, had not been studied. We thus compared male–female interactions during courtship and mating in pairs consisting of a male and a virgin female versus pairs consisting of a male and a mated female. The most obvious difference between the female categories was an average mating latency of about 3 min with the virgin females and 45 min with the mated females. Intriguingly, males courted the previously mated females much more intensely than they courted virgin females in the few minutes just prior to mating (right bars in Fig. 2b). This novel observation will require future elaboration because it might help explain males' mating success with reluctant females. The longer mating latencies and higher frequencies of kicking and lack of wing spreading with recently mated than with virgin females (Fig. 2d) are consistent with the hypothesis that rematings by recently mated females are caused by male coercion (Arnqvist & Rowe, 2005; Gromko et al., 1984; Thornhill & Alcock, 1983). Nevertheless, the issue of rematings in fruit flies as well as in other species requires further examination because it is not fully clear why females that can control rematings succumb to male coercion (Boulton & Shuker, 2016). We discuss the effects of age, sexual experience, and their interactions on the three types of male aggression below.

Effects of Age on Aggression

The life history of age-specific aggression within and among species is not well understood. The limited theory focuses on male–male aggression and assumes significant injury risks. Under these conditions, models generally predict peak fighting later in life (Kemp, 2006; Parker, 1974). While there are excellent data about the cost of aggression in animals with the capacity to inflict injury (Table 3.1 in Huntingford & Turner, 1987), the cost of aggression in species in which participants do not get wounded is unknown. In a variety of species, fighting is associated with physiological costs that increase mortality rate (Huntingford & Turner, 1987). In male fruit flies, pursuit of females is associated with increased mortality rate (Cordts & Partridge, 1996). It is thus likely that the major cost of male–male and male–female aggression in fruit flies is increased mortality rates associated with heightened activity. Another possible cost is the increased tendency to lose a fight after a previous loss (Hsu, Earley, & Wolf, 2006; Trannoy, Penn, Lucey, Popovic, & Kravitz, 2016). Such loser effects may vary in

magnitude with age and experience (Fawcett & Johnstone, 2010). In nature, another cost of aggression may be elevated predation rates caused by a combination of increased detection rates by visual predators, reduced attention devoted to approaching predators (Dukas, 2002; Dukas & Kamil, 2000) and reduced flight initiation distance (Brick, 1998; Cooper, 1999; Jakobsson, Brick, & Kullberg, 1995; Ydenberg & Dill, 1986).

Assuming that aggression, even with no injury risk, increases mortality rate, then the reluctance of young male fruit flies to engage in aggression towards either males or females can be explained by their higher residual reproductive value compared to that of older males (Kemp, 2006; Parker, 1974). It is likely that experience plays a role as well. Under this scenario, the default strategy of young males is to initially seek reproductive opportunities that do not involve aggression. After failing to secure matings with the peaceful tactic, the males gradually increase their motivation to fight with males over high-quality resources and to coerce females. Our experiments on the effect of sexual experience indeed indicate that it plays a key role in shaping the trajectory of age-specific aggression.

Effects of Sexual Experience on Aggression

Assuming that aggression is more costly than peaceful alternatives, males should avoid fighting and coercion if they can gain copulations using peaceful options. The obvious peaceful tactics are direct pursuit of females without engaging in aggression with males (i.e. scramble competition; Andersson, 1994; Spieth, 1974; Thornhill & Alcock, 1983) and quick departure from females that provide clear rejection signals (Connolly & Cook, 1973; Dukas & Scott, 2015; Spieth, 1952). The relative fitness costs to males of the aggressive and peaceful strategies, however, are unknown. Nevertheless, our results are consistent with the notion that aggression in fruit flies is more costly than peaceful options because males that had had access to virgin females were much less likely to engage in forced copulation with teneral females or to engage in coercion of recently mated females (Fig. 3c and d). A nonmutually exclusive alternative is that the fitness benefit from coercive matings is lower than that from consensual matings with virgin females. This is indeed the case for forced copulations with teneral females, which result in lower paternity than do consensual matings with virgin females (Dukas & Jongsma, 2012b). Owing to last-male precedence, however, paternity from coercive matings with previously mated females may be comparable to that from consensual matings with virgin females because the latter females may end up remating as well (Gromko et al., 1984).

Two studies have documented that previously mated male fruit flies exercise more stringent mating criteria than do virgin males as indicated by their stronger preferences for large over small females and virgin over mated females (Baxter et al., 2015b; Byrne & Rice, 2006). These data agree with our interpretation that males consider aggressive pursuit of females as a lesser option than seeking sexually receptive females. Consequently, males that have encountered and mated with virgin females are less likely to engage in coercive matings than are virgin males of the same age.

The effect of sexual experience on male–male aggression is more complicated than its effect on male–female aggression. As with male–female aggression, we expected previously mated males to be less willing than virgin males to engage in presumably costly fighting with other males. The major function of male–male aggression in fruit flies, however, is securing an attractive resource frequented by females seeking nutritious food and egg-laying sites. Hence males successful at mating probably perceive a food resource as more valuable than do virgin males. Such higher estimates of a resource value by mated males can lead to a greater motivation to

defend it via aggression. At least one other study documented that experience with females increased males' motivation to fight. In the speckled wood butterfly, *Pararge aegeria*, males exposed to females in the territory persisted in fighting against intruders longer and were more likely to win the contest than were males that did not encounter a female (Bergman, Olofsson, & Wiklund, 2010). Data from house crickets, *Acheta domesticus*, however, have been inconsistent, with one study suggesting decreased aggression (Brown, Smith, Moskalik, & Gabriel, 2006) and another indicating increased aggression by sexually experienced males (Killian & Allen, 2008).

Overall then, sexual experience may not affect male–male aggression in the context of resource defence because both female-deprived and mated males should have a high motivation to fight. Indeed we observed no effects of sexual experience on male–male aggression in two experiments (Figs. 3a and 4). We conducted another experiment assessing the effect of experience on male aggression in the context of resource defence in order to resolve the disagreement between our results (Fig. 3b) and those of Yuan et al. (2014), who reported decreased aggression in males previously housed with females. Yuan et al. (2014), however, employed a novel protocol in which each male had to mate with a virgin female just prior to the recording of contest. A weakness of this protocol is that, if one of the two males did not mate, the trial was discarded. If a male's reduced probability of mating is associated with his aggressiveness, this can lead to biased results. Furthermore, we have shown that males in the presence of their recent mates show heightened aggression associated with adaptive mate guarding (Baxter et al., 2015a). Nevertheless, even when we employed the protocol of Yuan et al. (2014), we unequivocally failed to find decreased aggression in males previously housed with females (Fig. 4). We cannot resolve this discrepancy at this point.

Conclusions

Our simultaneous assessment of the effects of age and sexual experience on three types of aggression allows us to draw a general picture of the life history of aggression in fruit flies. This depiction may be relevant for many other weaponless species with a plastic mating system of resource defence polygyny under low density and dispersed, defendable resources, or scramble competition otherwise. Young males shun aggression towards both males and females most likely because it inflicts costs that decrease expected life span. Males that succeed in matings may maintain their low aggression levels. Males that fail at acquiring mates gradually increase their motivation to persist in pursuing both teneral females for forced copulations and previously mated females, which may be coerced into remating. It is likely that, in settings where most matings occur at resources defended by capable males, males' motivation to fight increases with age so that they can either acquire or maintain an attractive resource. In that setting, we do not expect males to ever decrease their level of aggression because matings inform them of the high attractiveness of the resource they currently defend. While the divergent effects of sexual experience on aggression towards females versus other males is clearly adaptive, it suggests distinct underlying mechanisms that can be examined in future work.

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