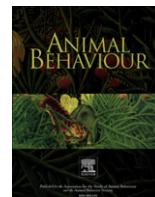


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# Learning in the context of sexual behaviour and danger in female and male *Drosophila pseudoobscura*

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Learning in the context of mate choice is important because it can influence sexual selection and incipient speciation. While fruit flies (*Drosophila* spp.) have been a key model system for research on sexual selection, speciation and learning, much of the research on fruit fly learning in the context of sexual behaviour has focused on males even though females typically control mating decisions. In a series of experiments, we found no evidence that early experience with either only conspecific males or both conspecific and heterospecific males affected the frequency of heterospecific matings by female *Drosophila pseudoobscura*. We conducted further experiments to place our results in perspective. First, we tested whether heterospecific matings do not induce female unreceptivity and thus have little cost, in which case learning would be of little benefit. Contrary to our prediction, we found that females that had initially mated heterospecifically remated conspecifically at a lower frequency than females that had first mated conspecifically. Second, we replicated earlier data indicating that male *D. pseudoobscura* that are rejected by heterospecific females later selectively reduce courtship of heterospecific females. Finally, we tested whether females can learn as well as males in a nonsexual context involving learning to avoid odours associated with danger and found similar learning scores in females and males. Our results indicate that, in spite of the potential benefits from learning in the context of mate choice and possessing a good learning ability in another domain, female *D. pseudoobscura* either do not learn about potential mates or such learning is not as robust as it is in the males.

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Mate choice is a key behaviour that determines the strength and direction of sexual selection in males and females. Furthermore, selective mate choice that results in positive assortative mating can lead to populations diverging into distinct species (Andersson 1994; Coyne & Orr 2004; Andersson & Simmons 2006; Price 2008). While much of the research on sexual selection and speciation focuses on the genetic mechanisms underlying mate choice, learning has been acknowledged to have potentially important effects in both theoretical models (e.g. Beltman & Metz 2005; Servedio et al. 2009) and empirical studies, especially ones involving long-lived vertebrates such as birds (e.g. Clayton 1990; ten Cate et al. 2006). Although the role of learning in mate choice by short-lived invertebrates was traditionally dismissed (Mayr 1974; Roitberg et al. 1993; Alexander et al. 1997), recent research indicates that, in both male and female invertebrates, learning shapes courtship and mating preferences (e.g. Hebets 2003; Dukas 2004; Bailey & Zuk 2009).

By far the leading insect model system for learning in the context of sexual behaviour is the male fruit fly (*Drosophila melanogaster*). While the protocols for quantifying the effects of learning on mate choice were originally developed for investigating the neurogenetics of learning and memory (e.g. Siegel & Hall 1979; McBride et al. 1999; Keleman et al. 2007), we have expanded the scope of that research to examine the ecological and evolutionary aspects of such learning (e.g. Dukas 2005a, 2009). Briefly, in three fruit fly species (*D. melanogaster*, *Drosophila persimilis* and *Drosophila pseudoobscura*), we have found that, although males are initially indiscriminate in choosing their courtship targets, they are quick to selectively decrease courtship of unreceptive female classes that repeatedly reject them, including recently mated females and heterospecific females (Dukas 2004, 2005a, 2009). Importantly, males that learn to avoid females from a partially isolated species show a significant reduction in the frequency of mating with such closely related, heterospecific females, suggesting that learning by the males can contribute to incipient speciation (Dukas 2008; Kujtan & Dukas 2009).

Almost all the work on learning in the context of sexual behaviour in fruit flies has focused on males even though females are the ones that typically control mating decisions (Spieth & Ringo

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1983). Furthermore, the females have ample opportunities to acquire information about potential mates because they are courted frequently by multiple males even prior to reaching sexual maturity (Dukas 2005b). One can readily envision how learning about males would enhance female fitness. The major characteristics that females use to assess male quality, which include body size, song features and odour, vary in time and space (Jallon & David 1987; Ferveur et al. 1996; Savarit & Ferveur 2002). Hence, learning about the specific qualities of the currently available males can allow the females to adjust their mating thresholds to optimally balance the trade off between the benefit of mating with a high-quality male and the cost of delaying mating (e.g. Mazalov et al. 1996; Collins et al. 2006; Dukas 2006).

To further our understanding of the role of learning in mate choice and to correct the strong male bias in the literature on learning in the context of sexual behaviour in fruit flies, we conducted a series of experiments with the closely related, partially isolated species pair *D. persimilis* and *D. pseudoobscura*, which has been a key model system for research on incipient speciation (Mayr & Dobzhansky 1945; Dobzhansky & Powell 1975; Noor 1995; Ortiz-Barrientos et al. 2004). We started by examining the role of experience on females' propensities to mate heterospecifically. Following our failure to document learning in the context of sexual behaviour in the females, we expanded our scope to (1) try to understand the apparent lack of learning, (2) replicate our previous data for learning about mates in the males, and (3) test for learning in the females within a nonsexual context.

## GENERAL METHODS

We used wild stocks of flies kept in  $20 \times 20 \times 35$  cm population cages containing a total of a few thousands individuals of each species. The *D. pseudoobscura* population was initiated from flies collected in Tucson, Arizona, U.S.A., in 2004. The *D. persimilis* population originated from flies collected on Santa Cruz Island, CA, U.S.A., in 2004. The two species were kept in separate environmental chambers at 60% relative humidity on a 12:12 h light:dark cycle with lights on at 1000 hours. *Drosophila pseudoobscura* was kept at a temperature of 25 °C, whereas *D. persimilis* was maintained at its preferred cooler temperature of 22 °C (Dobzhansky & Powell 1975). Each population cage was provided with two standard 240 ml food bottles each containing 50 ml of standard fly medium (corn meal, glucose, yeast, sucrose, agar and methyl paraben).

All flies used for the experiment developed at a low density of approximately 200 larvae per standard food bottle. Unless stated otherwise, we sexed flies anaesthetized with CO<sub>2</sub> within 8 h after eclosion and placed them in single-sex, standard 40 ml vials each containing 5 ml of medium. The female vials also contained a sprinkle of live yeast. We placed 20 flies per vial but transferred the males into individual vials 1 day before they were used for either training or testing. The transfer of males to individual vials ensured vigorous courtship because males housed in single-sex groups habituate to flies and show considerably reduced courtship intensity and mating success (Noor 1997; R. Dukas, unpublished data). We housed the vials in the same environmental chambers as the parental stocks.

The focal flies in this study were either female or male *D. pseudoobscura*. For clarity, we omit the species names throughout the paper and refer to within-species interactions as conspecific and to interactions with *D. persimilis* as heterospecific. We focus on female *D. pseudoobscura* because they mate heterospecifically at a relatively high frequency in the laboratory whereas female *D. persimilis* do not (Mayr & Dobzhansky 1945; Noor 1995). In our populations, we found only 1% heterospecific matings in 3-day-old female *D. persimilis* ( $N = 200$ ; R. Dukas, unpublished data).

## EXPERIMENT SERIES 1: EFFECTS OF EARLY EXPERIENCE

### *Experiment 1a: Experience with Conspecific Males and Heterospecific Matings*

Here we simulated the most likely natural scenario of females encountering conspecific males early in life and heterospecific males later. We expected that conspecific experience would reduce females' tendency to mate heterospecifically.

#### *Protocol*

We followed the general protocol of Dukas (2005b) of letting focal females experience males before the females reached sexual maturity and then testing these females after they reached sexual maturity. This protocol ensured that the experience phase involved no matings because recently mated females typically reject males' mating attempts (see Results of experiment 2 below).

*Experience phase.* On day 1, we sexed females within 4 h of eclosion and placed them 20 per vial in the environmental chamber until 0800 hours on day 2. Preliminary experiments indicated that the females are sexually immature at that age. On the morning of day 2, we placed half the females singly into vials each containing two 4-day-old conspecific males and half the females singly into vials containing no flies. We then observed the vials containing males for 2 h. In a few cases, we replaced males that showed no courtship within 20 min and we interrupted mountings so that no matings occurred during training. At the end of the experience period, we transferred females of each treatment into cages containing a standard food bottle and placed the cages in the environmental chamber until the test phase on the morning of day 3.

*Test phase.* The test phase consisted of four combinations involving (1) an experienced female and two conspecific males, (2) an experienced female and two heterospecific males, (3) a naïve female and two conspecific males and (4) a naïve female and two heterospecific males. We always included two rather than one male per vial to allow females mate choice. The males did not interfere with each other and their primary activity involved courtship and attempted copulations with the females. The test phase lasted 30 min and we recorded all matings and their latencies. A mating was recorded if the male mounted the female for at least 1 min. This criterion was necessary because many males' mating attempts are terminated by the females after a brief mounting. The mating latency was defined as the time elapsed from the session's start until mating initiation. We tested a total of 320 females, 80 per treatment combination.

In addition, we recorded male courtship behaviour in a random sample of vials during the first 15 min of the tests. This resulted in a behavioural data set for 64 of the vials. For these 64 vials, we recorded the start and end of each courtship activity and later summed the total courtship duration in each vial, which included all 'following', 'wing vibration' and 'mounting attempts' (Cobb et al. 1985; Dukas 2009). We also noted the start and end of mating to calculate the mating latencies and durations.

In summary, the experiment consisted of all four combinations of two treatments during the experience phase (courtship by conspecific males versus no experience with males) on day 2, and two treatments (conspecific or heterospecific males) presented during the 30 min test phase on day 3. Observers blind to female experience and male species identity recorded the data. The main statistical analyses involved logistic regression for the mating data, log-transformed mating latencies and arcsine square root proportions of time spent courting, which met ANOVA assumptions, and a nonparametric test of the mating durations, which remained

non-normally distributed after transformations (an ANOVA revealed a similar result).

### Results

Females that experienced courtship by conspecific males before reaching sexual maturity mated heterospecifically at frequencies similar to those of naïve females. Female experience also did not affect the frequency of conspecific matings (logistic regression: Wald test:  $\chi^2_1 = 0.09$ ,  $P = 0.77$  for the interaction between female experience and male species; Fig. 1). Regardless of female experience, mating latencies were longer in heterospecific matings than in conspecific matings ( $402 \pm 304$  versus  $184 \pm 83$  s, respectively; male species:  $F_{1,156} = 17.9$ ,  $P < 0.001$ ; female experience\*male species:  $F_{1,156} = 0.39$ ,  $P = 0.5$ ). Male behaviour could not explain the above results because heterospecific males courted females significantly longer than did conspecific males ( $F_{1,57} = 61$ ,  $P < 0.001$ ) regardless of female experience ( $F_{1,57} = 2.5$ ,  $P = 0.12$ ; female experience\*male species:  $F_{1,57} = 1.9$ ,  $P = 0.17$ ) or trial outcome (female acceptance or rejection:  $F_{1,57} = 0.8$ ,  $P = 0.38$ ). Interestingly, heterospecific matings were significantly shorter than conspecific matings ( $154 \pm 30$  versus  $225 \pm 12$  s, respectively; Mann–Whitney  $U$  test:  $U = 115$ ,  $N_1 = 5$ ,  $N_2 = 28$ ,  $P < 0.05$ ).

#### Experiment 1b: Experience with Conspecific or Heterospecific Males and Heterospecific Matings

Experiment 1a simulated a realistic case where females are initially exposed to conspecific but not heterospecific males. To further examine the effects of experience, we simulated a more extreme scenario in which females experience either conspecific or heterospecific males while immature. We predicted that females would mate less often with heterospecific males if they experienced conspecific rather than heterospecific males.

#### Protocol

**Experience phase.** The protocol was similar to that of experiment 1a except that half the immature females experienced courtship by conspecific males and the other half experienced courtship by heterospecific males for 2 h on day 2.

**Test phase.** The test phase consisted of placing each female with two test males in one of four combinations involving (1) a female

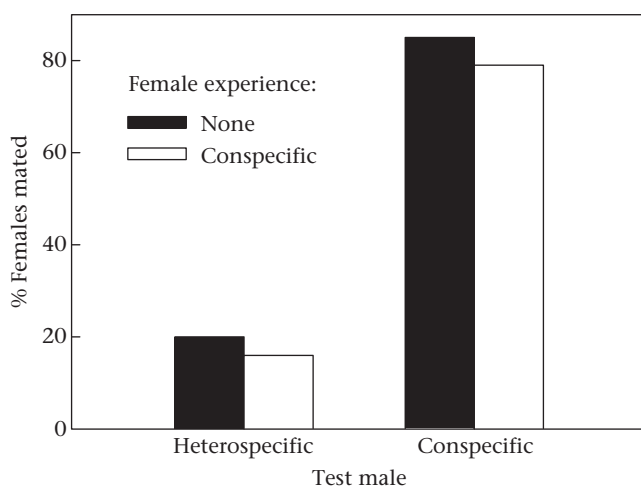
experienced with conspecific males and tested with conspecific males, (2) a female experienced with conspecific males and tested with heterospecific males, (3) a female experienced with heterospecific males and tested with conspecific males and (4) a female experienced with heterospecific males and tested with heterospecific males. The test phase lasted 60 min, and we recorded all matings and their latencies. We tested a total of 337 females.

### Results

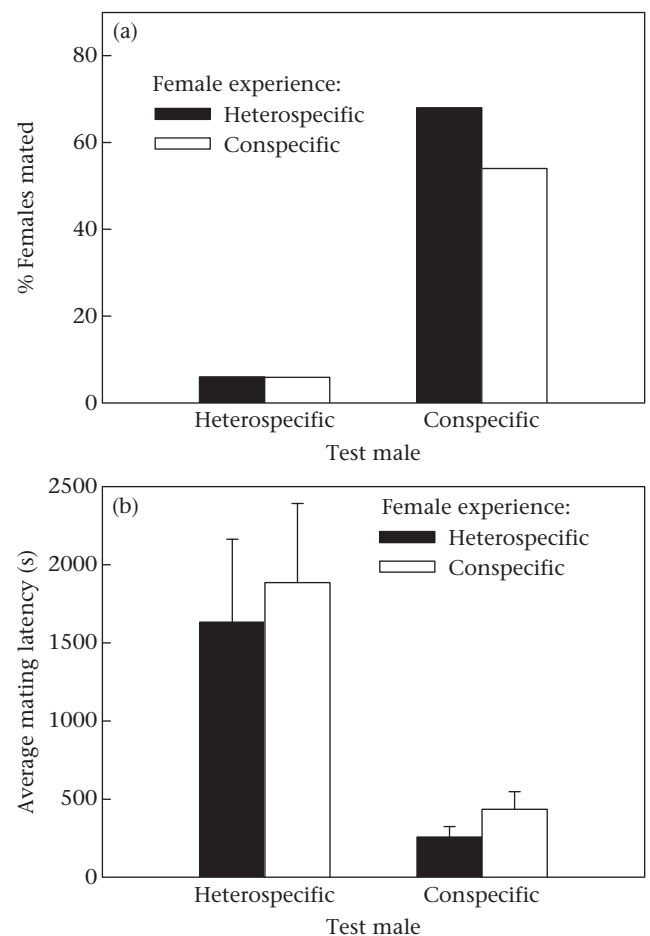
The heterospecific mating frequencies were low regardless of female experience (Fig. 2a), but the conspecific mating frequencies showed a nonsignificant tendency to be higher for females experienced with heterospecific males than for females experienced with conspecific males (logistic regression: Wald test:  $\chi^2_1 = 3.3$ ,  $P = 0.07$ ; Fig. 2a). Furthermore, females experienced with heterospecific males showed a nonsignificant tendency for shorter mating latencies than females experienced with conspecific males ( $F_{1,101} = 3.3$ ,  $P = 0.07$ ; Fig. 2b).

#### Experiment 1c: Experience with Conspecific or Heterospecific Males and Frequency and Latency of Conspecific Matings

Experiments 1a and 1b indicated no effect of females' early experience on their heterospecific mating propensities. Experiment 1b, however, suggested that females mate less often with



**Figure 1.** Average percentage of females ( $N = 320$ ) that mated with heterospecific and conspecific males. Females either had no prior experience with males (filled bars) or had been courted by conspecific males 1 day earlier when sexually immature (open bars).



**Figure 2.** (a) Average percentage of females ( $N = 337$ ) that mated with heterospecific and conspecific males 1 day after experiencing courtship by heterospecific (filled bars) or conspecific (open bars) males. (b) Average + SE mating latencies of females ( $N = 113$ ) that mated with heterospecific or conspecific males 1 day after experiencing courtship by heterospecific (filled bars) or conspecific (open bars) males.

conspecific males and are slower to accept conspecific males if they experience attractive conspecific males rather than unattractive heterospecific males when immature. This result is analogous to our *D. melanogaster* data, which indicated that females were less likely to mate with small males if they had experienced attractive, large males rather than unattractive, small males when immature (Dukas 2005b). We thus conducted another experiment focusing on the effect of early experience on conspecific rather than heterospecific mating parameters. That is, in experiment 1b, we predicted lower heterospecific mating frequencies in females experienced with conspecific males rather than heterospecific males when immature. In addition to tests with heterospecific males, experiment 1b also included tests with conspecific males as a necessary control (Fig. 2). In experiment 1c, we predicted lower conspecific mating frequencies in females experienced with attractive conspecific males rather than unattractive heterospecific males when immature and we tested all males with conspecific females.

#### Protocol

The protocol was similar to that of experiment 1b except that we tested all females with conspecific males.

#### Results

Mating frequencies were nearly identical in females that had previously experienced heterospecific and conspecific males (73% versus 72%). Mating latencies also did not differ significantly between females experienced with heterospecific males and conspecific males ( $305 \pm 48$  s versus  $442 \pm 81$  s, respectively;  $F_{1,124} = 0.7$ ,  $P = 0.4$ ).

### EXPERIMENT 2: REMATING PROPENSITY OF FEMALES MATED CONSPECIFICALLY OR HETEROSPECIFICALLY

Young female *D. pseudoobscura* accept heterospecific males rather readily (Figs 1, 2a) in spite of the species differences in song and odour (Noor & Coyne 1996; Noor & Aquadro 1998). Such matings are costly to the females because they result in only half as many fertile offspring (Dobzhansky & Powell 1975). We recorded, however, significantly shorter mating durations in heterospecific matings than in conspecific matings (experiment 1a). It is thus possible that, unlike conspecific matings, heterospecific matings do not make females unreceptive to males. If this is the case, females mated heterospecifically may subsequently mate with conspecific males and thus incur a smaller fitness cost. Possible reasons for a differential effect of conspecific and heterospecific matings on female receptivity include, first, that heterospecific males transfer less sperm and seminal fluid during the shorter matings (Gromko et al. 1984; Ram & Wolfner 2009). Indeed, in *D. melanogaster*, shorter teneral matings were associated with significantly higher frequencies of rematings 3 days later (Figure 8b in Seeley & Dukas 2011). Second, evolutionary changes in either the male sex peptides or in the female physiological response to them could reduce the effect of heterospecific males on the females (Arnqvist & Rowe 2005; Pitnick et al. 2009; Bono et al. 2011). Finally, another explanation for a differential effect of conspecific and heterospecific matings on receptivity may be that some cues (or the lack of necessary cues) from heterospecific matings may increase the females' tendency to remate. We examined the possible differential effect of conspecific and heterospecific matings on subsequent receptivity by allowing females to mate conspecifically or heterospecifically and then testing their remating propensities 2 days later.

#### Protocol

The general methods were similar to the ones described above. On day 4, we set up vials each with a female and either two

conspecific or two heterospecific males. We recorded the starts and ends of matings and included in the mated category all females that experienced mountings longer than 1 min. We then transferred most of the mated females of each treatment into separate small cages with food and placed them in the environmental chamber. To verify the fertility status of females recorded as mated on day 4, we transferred a sample ( $N = 54$ ) of the mated females individually into numbered food vials and kept them in the environmental chamber until the remating test. We checked these vials for larvae 5 days after the remating session. On day 6, we placed each of the focal females into vials each containing two conspecific males. We recorded matings in all vials during the 30 min trials and all courtship activity during the first 15 min of trials in a sample of the vials. In the sample of females tracked individually, 25% of the females that mated heterospecifically laid no fertile eggs between mating and remating but only 17% of these infertile females remated. In contrast, 13% of the females that mated conspecifically also laid no fertile eggs between mating and remating but 100% of them remated. Overall, we tested 102 females that mated heterospecifically and 120 females that mated conspecifically, and conducted courtship observations during the remating trials of 32 heterospecifically and 32 conspecifically mated females.

#### Results

Females that had mated heterospecifically remated at a lower frequency than females that had mated conspecifically (11% versus 21%, respectively; logistic regression: Wald test:  $\chi^2_1 = 4$ ,  $P < 0.05$ ). The proportion of time that males spent courting females during the remating trials was not affected by the male species that these females had mated with 2 days earlier ( $13.4 \pm 2.4\%$  versus  $10 \pm 1.9\%$  for heterospecific and conspecific first mates, respectively;  $F_{1,62} = 1.1$ ,  $P = 0.29$ ).

### EXPERIMENT 3: EFFECTS OF REJECTION BY HETEROSPECIFIC FEMALES ON MALES

With one exception (Dukas 2009), all the learning data from our work on the closely related species pair *D. pseudoobscura*–*D. persimilis* is on male *D. persimilis* because heterospecific matings are frequent between male *D. persimilis* and female *D. pseudoobscura* but rare in the reverse male–female combination (Noor 1995; R. Dukas, unpublished data). Given our failure to observe learning in the context of sexual behaviour in the females, we attempted to replicate our only positive result for learning in male *D. pseudoobscura*, which indicated that the males reduced heterospecific courtship after experiencing rejection by heterospecific females.

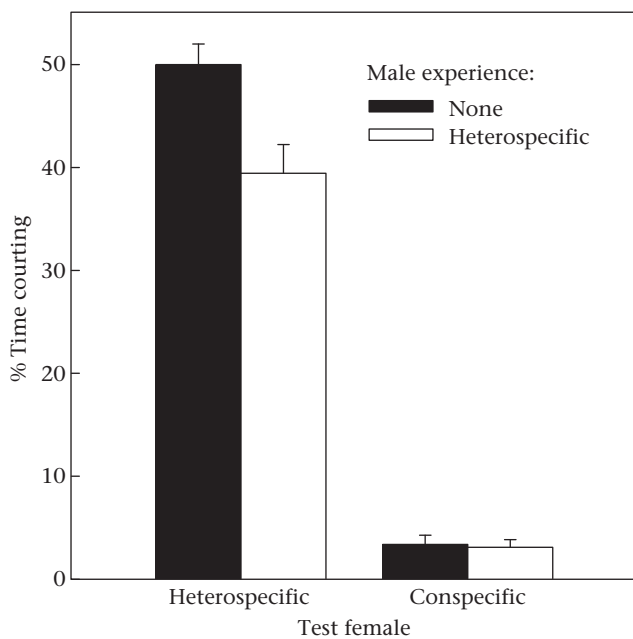
#### Protocol

For the experience treatment, we placed 4-day-old males individually in vials each containing two heterospecific females. We replaced a few males that did not perform courtship during the first 10 min and we interrupted mountings initiated in three vials. For the naïve treatment, we placed 4-day-old males individually in vials. The experience phase lasted 1 h, after which we transferred the males into fresh empty vials for a 15 min break. Then we placed half the males of each treatment each with two conspecific females and the other half each with two heterospecific females.

#### Results

The males experienced with courting heterospecific females directed significantly less courtship towards heterospecific females





**Figure 3.** Average  $\pm$  SE percentage of time that naïve males (black bars) and males experienced at courting heterospecific females (white bars) spent courting heterospecific or conspecific females.  $N = 192$  males.

than the naïve males. In contrast, male experience did not affect courtship durations towards conspecific females (male experience  $\times$  female species:  $F_{1,188} = 7.2$ ,  $P < 0.01$ ; Fig. 3). There were no heterospecific matings and only two experienced males and one naïve male failed to mate conspecifically.

#### EXPERIMENT 4: AVOIDANCE LEARNING IN MALES VERSUS FEMALES

Our results reported above indicated robust learning in the context of sexual behaviour in males but not in females. With a single exception (Dukas 2005b), work on individual learning about prospective mates in fruit flies has focused on males. Furthermore, studies of learning in other contexts have not compared males and females. We thus wished to test whether females can learn as well as males in a nonsexual context. We performed such a test using the established protocol of training flies to avoid odours associated with electric shock (Tully & Quinn 1985).

#### Methods

We tested the ability of sexually mature 3-day-old virgin flies to associate a novel odour with danger. The testing apparatus was similar to that of Tully & Quinn (1985) and consisted of a Plexiglas elevator that moved the flies from a training tube, which was lined with an electrified copper grid, to a point between two choice tubes. A vacuum pump drew air through small, 50 ml flasks, bubbling through the odorants, and then through the training and test tubes and out of the room. We used two odours, 3-octanol (OCT) and 4-methylcyclohexanol (MCH), with one odour designated safe and the other paired with pulses of electric shock. We diluted the odours in heavy mineral oil to concentrations that naïve flies preferred equally in preliminary tests (1:250 OCT and 1:100 MCH). We maintained the air flow at 11 ml/s per tube and kept the vacuum pump on for the entirety of training and testing so that (1) the flies habituated to the noise and (2) clean room air cleared the previous odour between training.

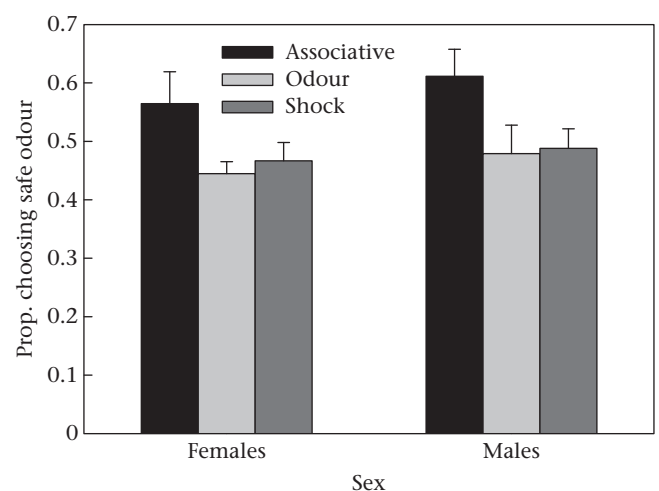
We conducted an associative treatment involving associating odours and shocks and two nonassociative controls involving shocks but no odours, and odours but no shocks. In the associative treatment, we transferred 100 flies to the apparatus and allowed them to acclimate during a 90 s rest period of clean air flow. The flies received 60 s of exposure to the first odour, a 30 s rest with clean air, then 60 s of the second odour, with one of the odours paired with electric shock. The 60 s electric shock trial involved 12 pulses of 80 V DC current each lasting 1.25 s followed by a 3.75 s break. We conducted all training and testing under dim red light and balanced the order of odour and shock presentations across replicates. Immediately after training, we shook the flies into the central elevator chamber, let them rest for 90 s and then moved the elevator into the convergence of the two odour streams for a 60 s choice phase. The entire training and test took less than 8 min. Following the choice phase, we anaesthetized the flies and transferred them into marked vials for a subsequent counting by an observer blind to fly treatment. For each test, we calculated the proportion of flies choosing the safe odour as a single data point.

The control treatments were similar to the associative treatment with the exceptions that no odours were delivered in the shock-only treatment and no shocks were administered in the odours-only treatment. The tests in the control treatment were identical to the one in the associative treatment except that the safe odour was randomly chosen in advance and balanced across replicates. We conducted a total of 48 tests (8 replicates  $\times$  3 treatments  $\times$  2 sexes) while balancing the identity of the safe odour, odour sides and trial order.

We conducted two statistical tests on the arcsine square root proportions of flies choosing each odour. First, we compared the proportion of flies choosing the safe odour in the associative learning versus the two control treatments. Second, we focused on the associative learning trials and compared the proportion of males and females choosing a given odour when it was associated with either safety or danger.

#### Results

The proportion of flies choosing the safe odour was significantly higher in the associative treatments than in the two control treatments (ANOVA:  $F_{1,32} = 29.6$ ,  $P < 0.001$ ; Fig. 4), but the effect of sex was not significant (ANOVA:  $F_{1,32} = 2.8$ ,  $P = 0.1$ ; Fig. 4). A further



**Figure 4.** Average  $\pm$  SE proportion of males and females that chose the odour that was not associated with shock in the associative treatment or the preassigned odour in the odour and shock controls.  $N = 48$  tests.

analysis including only the associative learning trials also indicated no significant difference in the proportion of males and females choosing the safe versus dangerous odour ( $F_{1,8} = 0.9$ ,  $P = 0.3$ ).

## DISCUSSION

We predicted that experience with conspecific and heterospecific males would modify mate choice by female *D. pseudoobscura*, but we found no evidence for such learning in the present study (experiments 1a–c; Figs 1, 2) or during numerous additional attempts over the past 5 years. The logical foundation for our prediction is sound. First, females can benefit from adjusting their mate choice criteria based on learned information about the qualities of the locally available males (Mazalov et al. 1996; Collins et al. 2006; Dukas 2006). Second, we have documented learning in the context of sexual behaviour in female *D. melanogaster* (Dukas 2005b). Third, learning about courtship targets in male *D. melanogaster* has been documented in numerous laboratories (e.g. Siegel & Hall 1979; Dukas 2005a; Ejima et al. 2005). Fourth, we have found that learning decreases heterospecific courtship in male *D. pseudoobscura* (Dukas 2009; Fig. 3) and that it decreases both heterospecific courtship and matings in male *D. persimilis* (Dukas 2008; Kujtan & Dukas 2009). Finally, studies in a few other insect species indicate effects of learning on female mate choice (Bailey & Zuk 2008, 2009; Svensson et al. 2010).

The most obvious thing that females could learn is to avoid heterospecific males after encountering conspecific males. Conspecific and heterospecific males differ slightly in song and odour (Noor & Coyne 1996; Noor & Aquadro 1998). Clearly, females could sense the species differences because they either rejected heterospecific males or were much slower to accept them (Figs 1, 2). Yet, experience did not alter females' frequency of accepting heterospecific males (Figs 1, 2a). A more subtle manifestation of learning could be through females' adjustment of their conspecific mating criteria. That is, based on our results with female *D. melanogaster* (Dukas 2005b), we examined whether experience with the more attractive conspecific males would increase the females' mating criteria compared to experience with the less attractive heterospecific males. Indeed, we found some evidence for such a pattern in experiment 1b, in which females experienced with conspecific males had lower mating frequencies and longer mating latencies than females experienced with heterospecific males (Fig. 2). However, these results were marginally significant ( $P = 0.07$ ), and we failed to replicate them in experiment 1c. Given the large temporal variation in male courtship behaviour and female mating propensities, it is likely that learning has a weak effect on females, which we captured in one experiment but not in another.

To understand the apparent lack of learning in the context of mate choice in the females, we examined how heterospecific matings affected the females' subsequent mate choice. Briefly, our logic, detailed in the rationale for experiment 2, was that heterospecific matings may not be costly if they do not induce low female receptivity to males, thus allowing the females to quickly remate with conspecific males. That is, the value of learning to avoid heterospecific matings may be low if females pay little cost for such matings. Surprisingly, we found a pattern opposite the one we predicted, with females being half as receptive to remating with conspecific males after initially mating heterospecifically as they were after initially mating conspecifically. It thus appears that heterospecific matings are costly not only because they result in half as many fertile offspring owing to sons' infertility, but they also appear to decrease females' propensity to remate. The stronger effect of heterospecific than conspecific matings on female unacceptivity to males may be a consequence of the rapid evolution of male and female sexually antagonistic interactions, which can be

kept in check within but not between species (Rice 1996; Arnqvist & Rowe 2005; Bono et al. 2011).

Given the apparent lack of learning in the context of mate choice in female *D. pseudoobscura*, we wished to substantiate our results by verifying that male *D. pseudoobscura* do indeed learn about mates as we documented only once (Dukas 2009) and by examining whether females can learn as well as males in a nonsexual context. In experiment 3, we replicated our earlier results indicating that males selectively reduce heterospecific courtship after being rejected by heterospecific females (Fig. 3). In experiment 4, we found that females showed learning scores similar to those of males in an avoidance learning task involving electric shock associated with odours (Fig. 4). In short, we can conclude that female *D. pseudoobscura* can learn as well as males in the context of avoiding danger, but not in the context of sexual behaviour, or that such learning in females is not as robust as is shock avoidance learning or learning about mates in males.

While our work adds to the limited data on learning in the context of mate choice in insects, it also contributes some uncertainty about the prevalence of such learning. Learning may have important influences on processes of sexual selection and speciation (e.g. Beltman & Metz 2005; Rebar et al. 2011). However, we must test for such effects through well-replicated studies that, first, establish robust learning in the context of sexual behaviour in convenient model systems such as fruit flies, and, second, move on to quantifying the effects of such learning on the evolution of sexual traits and population divergence. In fruit flies, we currently have a fair understanding of learning about potential mates in males and its possible evolutionary effects (Dukas 2008; Kujtan & Dukas 2009) while data on learning in females are still very limited (Dukas 2005b; Auld et al. 2009; Mery et al. 2009).

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