



## Dynamics of learning in the context of courtship in *Drosophila persimilis* and *D. pseudoobscura*

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### ARTICLE INFO

#### Article history:

Received 27 July 2008

Initial acceptance 10 September 2008

Final acceptance 7 October 2008

Published online 17 November 2008

MS. number: A08-00492

#### Keywords:

assortative mating

*Drosophila persimilis*

*Drosophila pseudoobscura*

fruit fly

learning

speciation

Recent data indicating that male fruit flies adaptively reduce courtship of heterospecific females, which typically reject them, suggest that learning could contribute to reduced levels of matings between individuals from diverging populations with partial premating isolation. To further examine the robustness of learning in the context of courtship in fruit flies, I wished to broaden the types of experience provided to males prior to testing. In both *Drosophila persimilis* and *D. pseudoobscura*, alternating trials of mating with conspecific females and rejection by heterospecific females produced the strongest reduction in heterospecific courtship. Trials of rejection by heterospecific females produced equally strong reduction in heterospecific courtship in *D. persimilis* but not in *D. pseudoobscura*, whereas trials of mating with conspecific females did not reduce heterospecific courtship at all. The pattern of strong reduction in heterospecific courtship was also replicated when I simulated the likely natural scenario in which males interact with conspecific females since eclosion and later encounter and experience rejection by heterospecific females. The results indicate that a variety of relevant experiences cause a rapid decrease in the time that male fruit flies spend courting heterospecific females. Such learning in partially reproductively isolated populations could contribute to speciation.

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There has recently been a resurgence in research on the mechanisms leading to speciation (Schluter 2000; Coyne & Orr 2004; Noor & Feder 2006; Grant & Grant 2008; Price 2008). While much of the current effort involves work at the genetic and ecological levels, there has also been renewed interest in the effects of learning on population divergence. The diverse theoretical work clearly indicates that learning could be an important factor contributing to speciation (Lachlan & Servedio 2004; Beltman & Metz 2005; Verzijden et al. 2005; Servedio et al., in press). The empirical research, however, is somewhat lagging. Sexual imprinting in birds has been a prime target of research linking learning to speciation (Grant & Grant 1996; Irwin & Price 1999; Slagsvold et al. 2002; Qvarnstrom et al. 2004; ten Cate et al. 2006). However, with the exception of the unusual brood parasitic indigobirds (*Vidua* spp.) (Payne et al. 2000; Sorenson et al. 2003), no study has explicitly linked learning to speciation in birds. Other taxa with limited data suggesting a role of learning in speciation include mammals (Kendrick et al. 1998), fish (Magurran & Ramnarine 2004, 2005; Verzijden & ten Cate 2007) and spiders (Hebets 2003).

Recently, fruit flies (*Drosophila* spp.) have been added to the list of taxa in which learning may have contributed to incipient speciation. First, I documented that male and female fruit flies (*D. melanogaster*) rely on learning to adaptively modify courtship (Dukas 2004, 2005b) and mate choice (Dukas 2005a). Second, in the sibling species, *D. persimilis* and *D. pseudoobscura*, males learned to decrease heterospecific courtship and this resulted in lower levels of heterospecific mating (Dukas 2008). The findings linking learning to increased levels of assortative courtship and mating are intriguing because fruit flies have been a leading model system in research on speciation (Coyne & Orr 2004; Mallet 2006; Noor & Feder 2006). Moreover, unlike birds and fish, large populations of fruit flies can readily be maintained in the laboratory for the necessary critical experiments linking learning to population divergence. First, however, one has to establish whether learning in the context of assortative courtship and mating is a sufficiently robust phenomenon to influence incipient speciation.

In all my previous experiments with male fruit flies mentioned above, I used an avoidance learning protocol in which males previously inexperienced with females were allowed to court and experience rejection by heterospecific females and were later tested with either heterospecific or conspecific females. The selective reduction in heterospecific but not conspecific courtship in experienced compared to inexperienced males indicated learning. Although successful in critically testing for learning, the avoidance learning protocol is unrealistic because it implies that mature males never encounter conspecific females prior to

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encountering only heterospecific females. Hence I wished to enrich the types of experience available to males prior to testing to examine the strength of learning in the context of courtship. Specifically, I predicted that mating with conspecific females and rejection by heterospecific females would result in stronger heterospecific avoidance than only rejection by heterospecific females, and that these two treatments would produce stronger heterospecific avoidance than either only acceptance by conspecific females or no experience with any female, with the latter two treatments showing no heterospecific avoidance. I also expected a decline in courtship duration in successive training trials with heterospecific females but no change in courtship duration in successive training trials with conspecific females. Finally, I expected that males that spent a few days with conspecific females before encountering heterospecific females would also show a strong reduction in heterospecific courtship with experience.

## GENERAL METHODS

I used stocks of *D. persimilis* and *D. pseudoobscura* provided by the *Drosophila* Tucson Stock Center (Tucson, Arizona, U.S.A.). The *D. pseudoobscura* population was initiated from flies collected in Tucson, Arizona in 2004. The *D. persimilis* population originated from flies collected on Santa Cruz Island, CA, U.S.A., in 2004. The *D. pseudoobscura* population I used is from outside the geographical range of *D. persimilis*, which only occurs in sympatry with *D. pseudoobscura* along the Pacific Coast (Dobzhansky & Powell 1975; Markow & O'Grady 2005). The flies were maintained in my laboratory since spring 2006 in large cages containing standard fly media kept inside distinct environmental chambers. Flies used in the experiments were sexed within 8 h of eclosion and the females were placed in groups of 20 in single-sex vials. In experiments 1 and 2, males were also placed 20 per vial and transferred into individual vials 1 day before the test because such isolation increases their courtship intensity and mating success (Noor 1997; R. Dukas, unpublished data). Flies in experiments 1 and 2 were 4 days post eclosion. Fly handling and ages in experiment 3 are detailed below. All flies were used only once.

Members of the closely related species pair, *D. persimilis* and *D. pseudoobscura*, are visually indistinguishable but differ in their cuticular hydrocarbons and male courtship song. Inexperienced males of the two species indiscriminately court hetero- and conspecific females but the females prefer to mate with conspecific males. In the laboratory, heterospecific mating is more frequent between male *D. persimilis* and female *D. pseudoobscura* than between the alternate pairing. The hybrid daughters are fertile whereas hybrid sons are infertile (Mayr 1946; Noor 1995; Machado et al. 2002; Ortiz-Barrientos et al. 2004; Dukas 2008). Heterospecific courting is costly for males because they waste time and energy courting females that typically reject them, and infrequent matings produce only half as many fertile offspring. Hence, learning in the context of sexual behaviour could be adaptive in male *D. persimilis* and *D. pseudoobscura*.

## EXPERIMENT 1: *D. PSEUDOBSCURA*

### Methods

#### Training

Males were randomly assigned to each of four treatments, positive/negative, negative, positive and none (Table 1). All males placed with females were observed continuously and the courtship activity of some of the males was recorded for the duration of the training trials (see below). The positive trials involved the completion of mating with a conspecific female. I verified that all males mated during these trials and replaced females in several

vials where matings did not occur within 10 min. The trials were terminated upon the completion of mating and were followed by a 1 h break. This long break was designed to diminish effects of sensitization following mating, which are associated with a short-term indiscriminate increase in males' courtship activity (Broughton et al. 2003; Dukas 2005b). The negative trials involved courtship and rejection by heterospecific females, but I included in the analyses the four males that each mated heterospecifically once. Removing these males from the analyses did not alter the results. The negative trials lasted 30 min and were followed by 10 min breaks. Males of all treatments were transferred to new vials at the start of each trial and placed next to each other in the same vial rack to control for the effects of experimenter's handling.

#### Test

At the end of the break following the last training session, each male was placed with two heterospecific females and males' courtship behaviour was recorded for 15 min as in my previous studies (e.g. Dukas 2004, 2008). Only three males, all belonging to the positive treatment, mated during the test. All the behavioural recordings during training and tests were conducted with observers blind to fly treatment and female species.

#### Analyses

I trained and tested 128 males, of which I recorded the behaviour of 66 during the training trials. For half the males, the negative trial was first, as in Table 1, and for the other half, the order was reversed. Preliminary analyses indicated no order effects ( $F_{1,120} = 1.5$ ,  $P = 0.2$ ), which are not discussed further here. The main behavioural parameter was the total time spent courting (Dukas & Mooers 2003). I calculated for all trials the proportion of time spent courting out of the total time available, which was the trial duration in all trials with no matings and the mating latency in all conspecific trials and the several heterospecific trials that ended with matings. To test my first set of predictions involving the test phase, I conducted three planned comparisons between (1) the positive/negative versus negative males, (2) the negative versus positive and none males, and (3) the positive versus none males. To test the second set of predictions involving the training phase, I ran two repeated measures ANOVAs, one for the negative trials of the positive/negative and negative males, and the other for the positive trials of the positive/negative and positive males. Analyses were conducted on arcsine-square-root-transformed proportions and log-transformed latency data and the transformed values met ANOVAs assumptions.

### Results

#### Test

The type of experience significantly affected heterospecific courtship by male *D. pseudoobscura* (ANOVA:  $F_{3,124} = 7.6$ ,  $P = 0.01$ ; Fig. 1). The positive/negative males had significantly shorter courtship durations than the negative males ( $t_{124} = 3.2$ ,  $P = 0.002$ ). The negative males did not court significantly less than the positive and none males ( $t_{124} = 1$ ,  $P = 0.3$ ), and there was no significant difference between the positive and none males ( $t_{124} = 0.5$ ,  $P = 0.6$ ).

#### Training

Both the positive/negative and negative males showed a similar reduction in courtship duration between the first and second heterospecific trials (repeated measures ANOVA: within-subject effects:  $F_{1,42} = 40.4$ ,  $P < 0.001$ ; between-subject effects:  $F_{1,42} = 0.02$ ,  $P = 0.9$ ; Fig. 2a). In contrast, both the positive/negative and positive males showed no reduction in courtship duration between the first

**Table 1**The four experience treatments for male *Drosophila pseudoobscura* in experiment 1 and male *D. persimilis* in experiment 2

Treatment	Experience	Trial 1	Trial 2	Trial 3	Trial 4	Test (all treatments)
1	Positive/negative	Rejected by 2 H females	Mated with C female	Rejected by 2 H females	Mated with C female	Tested with 2 H females
2	Negative	Rejected by 2 H females		Rejected by 2 H females		
3	Positive		Mated with C female		Mated with C female	
4	None					

Half the sets experienced the schedule given in the Table, and the other half had the order of presentation reversed, with conspecific females encountered first. Each treatment included 32 males. C = conspecific; H = heterospecific.

and second conspecific trials (within-subject effects:  $F_{1,42} = 0.25$ ,  $P = 0.6$ ; between-subject effects:  $F_{1,42} = 2.9$ ,  $P = 0.1$ ; Fig. 2b).

## EXPERIMENT 2: *D. PERSIMILIS*

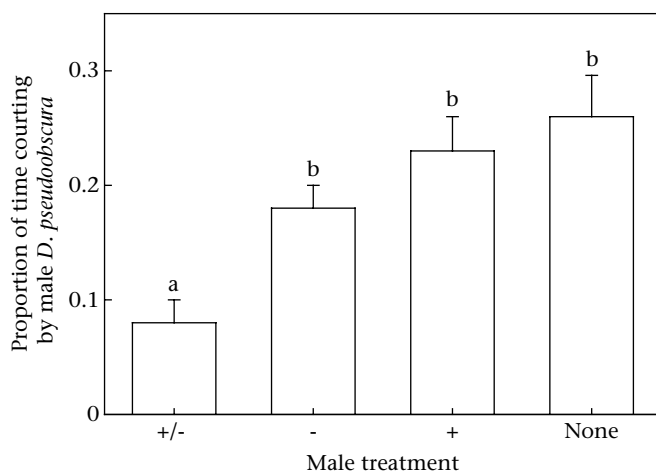
### Methods

The experiment was similar to experiment 1 with the species switched. Because heterospecific matings are more likely to occur between male *D. persimilis* and female *D. pseudoobscura* than the other pairing combination, the negative and test trials included only a single heterospecific female. Mountings occurring in 17 training trials were interrupted and the female was replaced with another one so that all the negative trials consistently involved courtship and rejection by heterospecific females. Six of the tests (1 of the negative experience, 2 of the positive experience and 3 of the no experience) ended with heterospecific matings. During the positive trials, I replaced the conspecific females in 20 vials in which no mating occurred within 10 min. Seven of the vials belonged to the positive/negative treatment and 13 were from the positive treatment. I trained and tested 128 males and I recorded the behaviour for 48 of these males during the training trials. As before, the trial order was alternated, with half the males having the negative trial first and half the males starting with the positive trial, and as before, order effect did not significantly effect male courtship ( $F_{1,120} = 0.8$ ,  $P = 0.4$ ).

### Results

#### Test

The types of experience significantly affected heterospecific courtship by male *D. persimilis* (ANOVA:  $F_{3,124} = 4.3$ ,  $P < 0.01$ ;

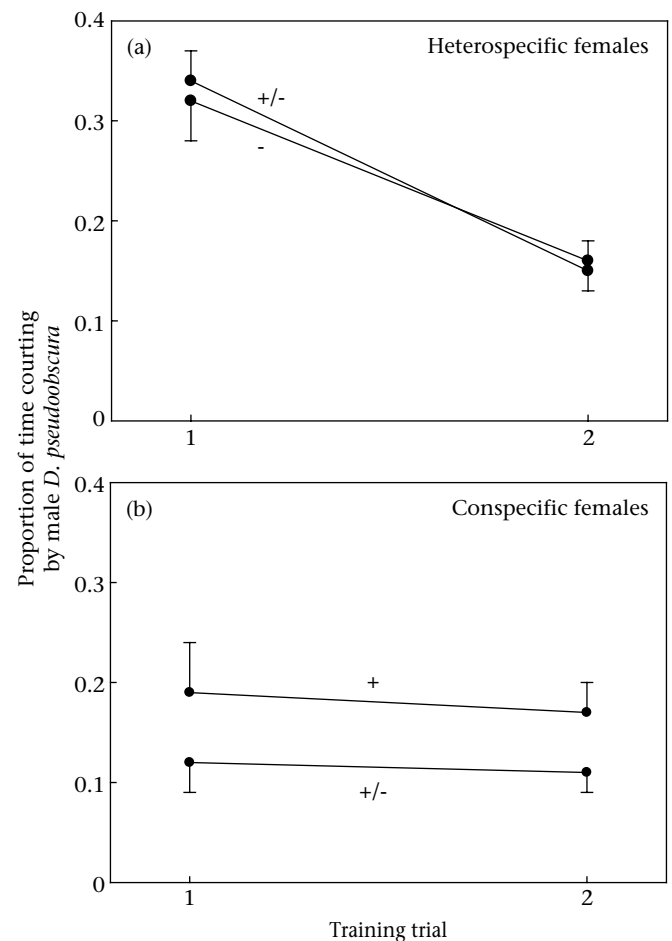


**Figure 1.** Mean + SE proportion of time that male *D. pseudoobscura* with distinct experience spent courting heterospecific females during the test. Males experienced mating with conspecific females and rejection by heterospecific females (+/-), rejection by heterospecific females (-), mating with conspecific females (+) and no encounter with females (none).  $N = 32$  males per treatment. Distinct letters above bars indicate statistically different values.

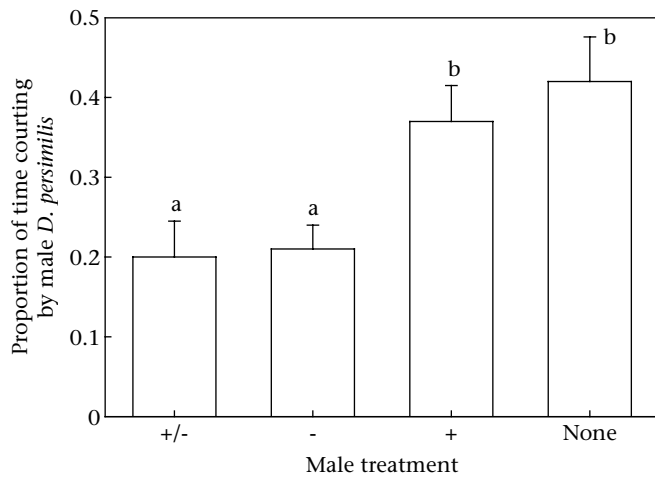
Fig. 3). Here, however, the positive/negative males did not have shorter courtship durations than the negative males ( $t_{124} = 0.44$ ,  $P = 0.7$ ). As predicted, the negative males courted significantly less than the positive and none males ( $t_{124} = 2.6$ ,  $P < 0.01$ ), and there was no significant difference between the positive and none males ( $t_{124} = 0.6$ ,  $P = 0.6$ ).

### Training

Both the positive/negative and negative males showed a similar reduction in courtship duration between the first and second heterospecific trials (repeated measures ANOVA: within-subject effects:  $F_{1,30} = 20.8$ ,  $P < 0.001$ ; between-subject effects:  $F_{1,30} = 2$ ,  $P = 0.16$ ; Fig. 4a). In contrast, there was a significant trial by treatment interaction, indicating a reduction in courtship duration between the first and second conspecific trials for the positive/



**Figure 2.** Mean and SE proportion of time that male *D. pseudoobscura* spent courting (a) heterospecific or (b) conspecific females during training. Males experienced mating with conspecific females and rejection by heterospecific females (+/-), rejection by heterospecific females (-), or mating with conspecific females (+).  $N = 32$  males per treatment.



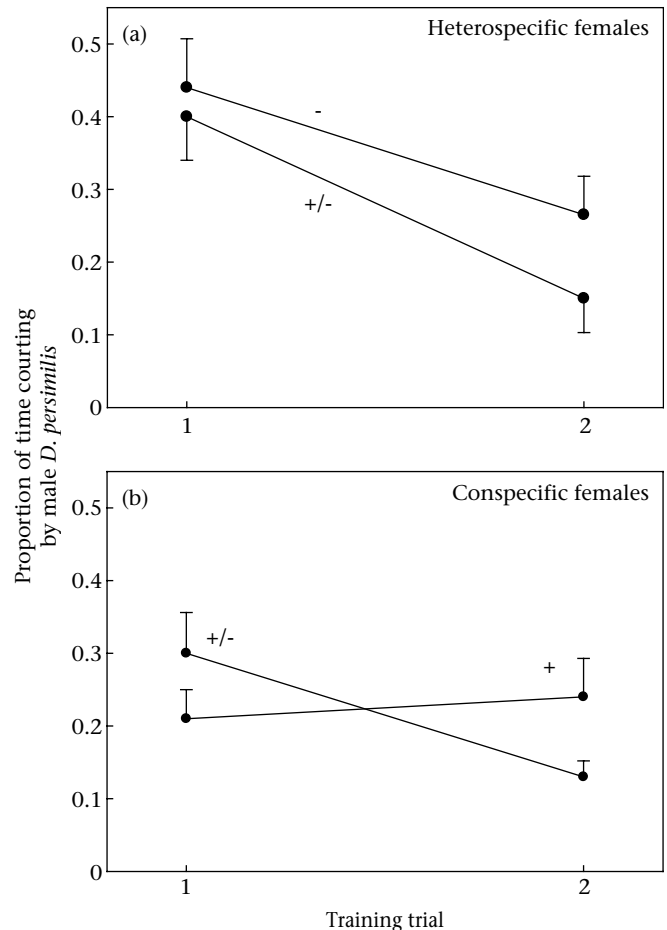
**Figure 3.** Mean  $\pm$  SE proportion of time that male *D. persimilis* with distinct experience spent courting heterospecific females during the test. Males experienced mating with conspecific females and rejection by heterospecific females (+/-), rejection by heterospecific females (-), mating with conspecific females (+), and no encounter with females (none). Distinct letters above bars indicate statistically different values.  $N = 32$  males per treatment.

negative males but not for the positive males ( $F_{1,30} = 5.2$ ,  $P < 0.05$ ; Fig. 4b). The courtship and mating latencies in the conspecific trials, however, were not significantly different between the positive/negative and positive males ( $F_{1,30} = 2.3$ ,  $P = 0.14$  and  $F_{1,30} = 1.3$ ,  $P = 0.3$  for the between-subject effects of courtship and mating latencies, respectively;  $F_{1,30} = 2.3$ ,  $P = 0.14$  and  $F_{1,30} = 0.46$ ,  $P = 0.5$  for the respective interactions).

### EXPERIMENT 3: EFFECTS OF EARLY EXPERIENCE IN *D. PERSIMILIS*

#### Rationale

The standard procedure in fly experiments is to sex flies soon after eclosion and keep them in single-sex vials until use. In all my previous experiments, I also kept males isolated from females until training (Dukas 2004, 2005b, 2008). In nature, however, males can interact with females soon after eclosion. One could argue that such early experience may be crucial for males' proper sexual development (Technau 1984; Heisenberg et al. 1995; Dukas & Mooers 2003). On the other hand, the males would mostly experience rejection from females because most females are recently mated and hence unreceptive (Spieth 1974; Dukas et al. 2006). So it could be that males in natural settings learn little from numerous rejections by any female category. To examine the effects of early experience, I allowed males in this experiment to interact with conspecific females for 4 days from eclosion until the experiment. I expected males to court all three classes of conspecific females, experience persistent rejection by immature and recently mated females, and engage in a single mating with a mature, receptive female. Furthermore, to avoid the complication introduced by rapid female acceptance and subsequent matings in conspecific test trials, in this test I used 1-day-old females, which are sexually unreceptive but still attract considerable courtship (Dukas 2005b, unpublished data). I predicted that, in the test following training, experienced males would show reduced courtship towards heterospecific females, but not towards immature conspecific females, relative to inexperienced males. Additionally, I predicted that experienced males tested with immature conspecific females would show an increase in courtship, whereas experienced males



**Figure 4.** Mean and SE proportion of time that male *D. persimilis* with distinct experience spent courting (a) heterospecific or (b) conspecific females during training. Males experienced mating with conspecific females and rejection by heterospecific females (+/-), rejection by heterospecific females (-), or mating with conspecific females (+).  $N = 16$  males per treatment.

tested with heterospecific females would show a decrease in courtship from the second training trial to the test.

#### Methods

##### Training

Newly eclosed males were randomly assigned to either the experienced or naïve treatment. I placed all the males individually into vials and then added to each vial of the experienced treatment two conspecific females, one was newly eclosed and the other was 2 days old and hence about to become sexually receptive (Snook & Markow 2001; R. Dukas, unpublished data). The flies were then housed in the environmental chamber, and I removed the females from the experienced males' vials 30 min before the start of the second experience phase on day 5. Later examination of the experienced males' vials revealed that 97% contained larvae, indicating that the males in these vials mated with at least 1 female.

The second experience phase consisted of (1) placing each experienced male in a vial with two heterospecific females and allowing the males to court for 30 min, (2) moving these males to new vials for a 15 min break, (3) adding two heterospecific females to each vial of the experienced males for 30 min, and (4) a final 15 min break during which each male was alone in a new vial. The inexperienced males were always placed alone in vials and moved between new vials in parallel with the experienced males.

### Test 1

For test 1, I placed half of the experienced males and half of the naïve males each with two heterospecific females and the other half of each treatment each with two immature conspecific females. The test lasted 30 min rather than 15 min as in the previous experiments, so I could compare the behaviour of the experienced males in the training and test phases. Four inexperienced males and one experienced male mated during the test, all with heterospecific females.

### Test 2

Based on early results, I added a second test for a subset of the males. After these males completed test 1 and a 15 min break spent alone in a new vial, I added to each vial two sexually mature conspecific females to test for the effect of experience on males' propensity to court sexually receptive females. I expected that male experience would not affect the time that males spent courting sexually mature conspecific females and that most males would mate within a few minutes. Each test was terminated after 15 min.

### Analyses

I tested 128 males in test 1 and 40 males in test 2. I also recorded the behaviour of 32 experienced males during the second training phase. Half of these males were tested with heterospecific females and the other half with immature conspecific females. The test data were analysed with ANOVAs and the combined training and test data for the experienced males was analysed with repeated measures ANOVA focusing on the within-subject contrasts.

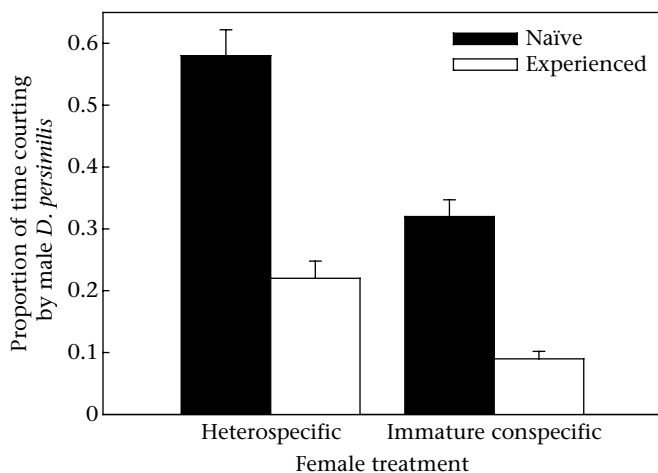
### Results

#### Test 1

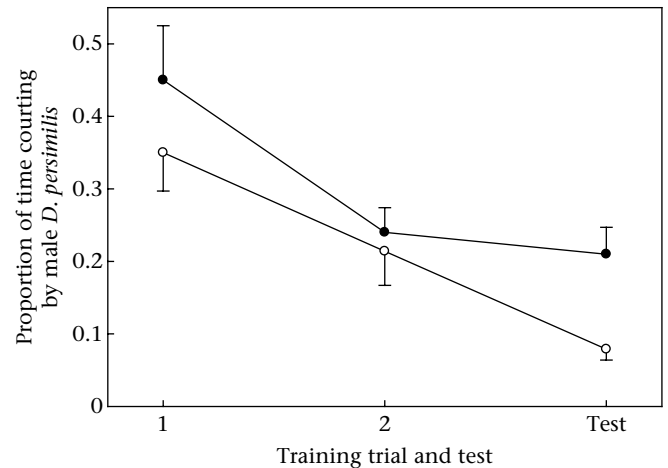
Experienced male *D. persimilis* showed reduced courtship of both heterospecific females and immature conspecific females compared to naïve males (ANOVA: effect of experience:  $F_{1,124} = 89$ ,  $P < 0.001$ ; interaction between experience and test females:  $F_{1,124} = 1$ ,  $P = 0.3$ ; Fig. 5). Unexpectedly, regardless of experience, the males spent more time courting heterospecific females than they did immature conspecific females ( $F_{1,124} = 37$ ,  $P < 0.001$ ).

#### Test 2

In the second test with mature conspecific females, males' experience during training did not affect the proportion of time



**Figure 5.** Mean + SE proportion of time that naïve and experienced *D. persimilis* males spent courting heterospecific and immature conspecific females. Experienced males had early experience courting and mating with conspecific females, and subsequently experienced rejection by heterospecific females.  $N = 32$  males per treatment.



**Figure 6.** Mean and SE proportion of time that *D. persimilis* males with early experience courting and mating with conspecific females spent courting females during training and test. All males experienced rejection by heterospecific females during the two training trials. Half of these males were subsequently tested with heterospecific females (solid circles) and the other half with immature conspecific females (open circles).  $N = 16$  males per treatment.

spent courting (mean ± SE: experienced males:  $0.17 \pm 0.04$ ; naïve males:  $0.18 \pm 0.04$ ;  $F_{1,32} = 0.02$ ,  $P = 0.88$ ). The female treatment in test 1 did not affect males' courtship duration either ( $F_{1,32} = 0.19$ ,  $P = 0.66$ ). In addition, mating latencies were not affected by these parameters (experience:  $F_{1,32} = 2.1$ ,  $P = 0.16$ ; female treatment in test 1:  $F_{1,32} = 0.33$ ,  $P = 0.57$ ). All naïve males and 90% of experienced males mated during test 2.

#### Training

The experienced males dramatically reduced courtship of heterospecific females from the first to second training trial (repeated measures ANOVA: within-subject contrast:  $F_{1,30} = 10.4$ ,  $P < 0.01$ ; Fig. 6). The experienced males further reduced courtship duration from the second training trial to the test (within-subject contrast:  $F_{1,30} = 7$ ,  $P < 0.015$ ), but much of that reduction occurred in males tested with immature conspecific females rather than in males tested with heterospecific females (interaction for within-subject and between-subject contrast:  $F_{1,30} = 3.1$ ,  $P = 0.09$ ; Fig. 6).

### DISCUSSION

In all three experiments, males of both fruit fly species showed the basic pattern of strong reduction in heterospecific courtship after only 30 min of experiencing rejection by heterospecific females (Figs 2, 4, 6). These results substantiate my earlier, more limited data indicating that male fruit flies rely on experience to reduce courtship of rejecting heterospecific females (Dukas 2004, 2008). Many but not all of my predictions were supported by the data. *Drosophila pseudoobscura* males that experienced conspecific matings and heterospecific rejections indeed showed better learning than males in the other three treatments (Fig. 1). With *D. persimilis*, however, males with only negative experience behaved similarly to males with positive and negative experience (Fig. 3).

As predicted, positive experience alone was insufficient to elicit significant avoidance of heterospecific females (right two bars in Figs 1, 3). Unexpectedly, the negative experience in *D. pseudoobscura* males produced only a nonsignificant reduction in heterospecific courtship in the test, but the more powerful within-subject test in the training phase actually indicated strong effects of training, which was similar in males with negative and positive experience and males with only negative experience (Fig. 2a).



A similar pattern of a large decrease in courting heterospecific females by males with negative and positive experience and males with only negative experience was also seen in *D. persimilis* (Fig. 4a). As predicted, *D. pseudoobscura* did not reduce courtship of conspecific females from the first to second training trial (Fig. 2b). In *D. persimilis*, however, males with positive and negative experience, but not males with only positive experience, showed a significant reduction in conspecific courtship from the first to second training trial (Fig. 4b). The only difference between these two treatments was that males in the positive and negative treatment experienced periods of rejection by heterospecific females whereas males of the positive treatment spent the concurrent periods alone. Thus it appears that rejection by heterospecific females had a nonselective effect on *D. persimilis*. Nevertheless, the nonselective courtship reduction in the positive and negative males did not adversely affect their courtship and mating latencies with mature conspecific females.

Male *D. persimilis* that court heterospecific females typically receive a consistent feedback of rejection, whereas males that court conspecific females receive a variable message ranging from instant acceptance to persistent rejection. In an attempt to eliminate this variation, I tested males in experiment 3 with immature conspecific females, which are known to attract considerable courtship (Dukas 2005b, unpublished data). My effort was only partially successful because, although no mating occurred with the immature females, even naïve males devoted less courtship towards immature conspecific females than towards mature heterospecific females (Fig. 5). Nevertheless, males in experiment 3, which had early experience with immature and mature conspecific females in addition to later exposure to heterospecific females, showed the consistent pattern of reduced courtship of heterospecific females. That is, long-term experience with conspecific females did not alter my major findings from experiments 1 and 2 and from previous studies (Dukas 2004, 2008), in which males were isolated from females until the day of training and testing. The experienced males in experiment 3, however, did not show reduced courtship of mature conspecific females in test 2, indicating that their experience produced graded responses to the three classes of females encountered. Specifically, compared to the naïve males, the experienced males devoted less time to courting the two classes of unreceptive females but equal time to courting receptive females.

The results reported here are consistent with an alternative explanation that long periods of courting heterospecific females exhaust the males. The males then reduce their courtship durations, which are sufficient for achieving the typically rapid conspecific matings but translate into low proportions of courtship duration in trials with heterospecific females, in which most females reject the males. I attempted to address this issue in experiment 3 by testing the males with immature females, but, regardless of experience, the males found such females unattractive. In a recent set of experiments (L. Kujtan & R. Dukas, unpublished data), courtship durations of male *D. persimilis* were compared in trials conducted after the males had courted and either copulated or failed to mate with female *D. pseudoobscura*. The males that had positive heterospecific experience subsequently spent about 50% more time courting new virgin heterospecific females than did males with negative heterospecific experience. These results agree with the specific-experience explanation rather than the male-fatigue explanation because males in both treatments spent similarly long durations courting heterospecific females.

There has been little systematic research on the effects of learning on courtship in any species. A notable exception is work on the brood parasitic cowbirds (*Molothrus ater*), which are unusual among birds because young do not have the opportunity to learn song features from their parents. Rather, both sexes rely on innate behaviour and learning from conspecifics in flocks. Additionally,

the males learn to selectively retain song features based on feedback from the females they court (West & King 1988; Freeberg et al. 2002). More limited data from bees and fish suggest a pattern similar to the one I have documented for fruit flies, of males selectively reducing courtship directed towards classes of females that reject them (Barrows et al. 1975; Wcislo 1992; Magurran & Ramnarine 2004).

My focus on males raises the obvious question about the role of females, which predominantly control mating decisions (Spieth & Ringo 1983). So far, my research indicates only limited effects of experience on female mate choice in the sibling species *D. persimilis* and *D. pseudoobscura*, but work in progress attempts to further elucidate the possible role of learning in the females.

Overall, my results indicate that males' negative experience with heterospecific females causes a rapid reduction in time devoted to courting such females. Because work in several neurogenetic laboratories have solidly established associative learning in the context of courtship in fruit flies (Siegel & Hall 1979; Ejima et al. 2005; Keene & Waddell 2007), my research has focused on the ecological and evolutionary consequences of such learning rather than on critically testing for associative learning. I can thus conclude that, using a variety of ecologically realistic protocols, I have consistently documented adaptive effects of learning on courtship in male fruit flies of three of the species most commonly used by evolutionary biologists, *D. melanogaster*, *D. persimilis* and *D. pseudoobscura*. These results confirm that learning in the context of courtship in fruit flies is a robust, readily replicable feature, which can influence patterns of assortative mating leading to speciation.

## Acknowledgments

I thank Meinas Elmusharaf, Zhaowei Gong, Lara Kujtan and Corrine Seeley for dedicated assistance, Kevin Abbott, Lauren Dukas, M. Noor, M. Scott and an anonymous referee for comments on the manuscript, and staff of The Tucson *Drosophila* Stock Center for providing me with proper flies. This study was supported by the Natural Sciences and Engineering Research Council of Canada, Canada Foundation for Innovation and Ontario Innovation Trust.

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