Learning affects mate choice in female fruit flies

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Learning in the context of mate choice can influence sexual selection and speciation. Relatively little work, however, has been conducted on the role of learning in the context of mate choice, and this topic has been mostly ignored in insects even though insects have served as a prime model system in research on sexual selection and incipient speciation. Extending recent work indicating apparently adaptive learning in the context of sexual behavior by male fruit flies (*Drosophila melanogaster*), I tested for the effect of learning on mate choice by female fruit flies. Compared to young virgin females that experienced courtship by large males, young virgin females that experienced courtship by small males were more likely to mate with small and large males in a test conducted a day after the experience phase. These results, which are the first clear empirical demonstration of learning in the context of mate choice by female insects, lay the foundation for research on the role of learning in insect sexual selection and speciation. *Key words:* courtship, *Drosophila*, fruit flies, learning, mate choice, speciation. *[Behav Ecol 16:800–804 (2005)]*

Research in the past few decades has established that learning influences mate choice by vertebrates (e.g., Collins, 1995; Domjan, 1992; Magurran and Ramnarine, 2004). It is still unclear, however, to what extent learning affects mate choice in invertebrates. Conceptually, there is no reason to assume that learning is not involved in insect mate choice. However, no single unambiguous experiment has demonstrated that learning influences mate choice in female insects, and only one study has documented learning affecting mate choice in female spiders (*Schizocosa uetzi*) (Hebets, 2003). Quantifying the role of learning in mate choice by female insects is important because learning may be pivotal in processes determining sexual selection and incipient speciation (Beltman et al., 2004; Gibson and Langen, 1996; Irwin and Price, 1999; Lachlan and Servedio, 2004; ten Cate and Vos, 1999), and many research programs on these processes have used insects as model systems (Andersson, 1994; Coyne and Orr, 1989, 2004; Rice and Hostert, 1993).

It has been known for more than two decades that male fruit flies (*Drosophila melanogaster*) learn in the context of courtship (Siegel and Hall, 1979) and, recently, the evolutionary relevance of such learning has been elucidated. First, male *D. melanogaster* can learn to avoid courting females of the closely related *Drosophila simulans*, which typically reject all mating attempts by male *D. melanogaster* (Dukas, 2004). Second, male *D. melanogaster* experienced with courting recently mated, unreceptive females learn to selectively avoid recently mated females but not receptive virgin females (Dukas, 2005). Learning by male insects in the context of courtship has also been documented in solitary bees (Barrows et al., 1975; Smith, 1983; Wcislo, 1992).

Female *D. melanogaster*, as well as other insects, have ample opportunities to learn in the context of mate choice. Most notably, female *D. melanogaster* typically reach sexual maturity only when they are 24- to 40-h old (Manning, 1967). During that period, however, the immature, unreceptive females are vigorously courted by males (Dukas, 2005; Manning, 1967). Furthermore, although recently mated female *D. melanogaster* typically become unreceptive and less attractive to males for

several days, the females are still courted frequently (Cook R and Cook A, 1975; Dukas, 2005; Manning, 1967). The experience during this second unreceptive period could be used when the females remate. In sum, female *D. melanogaster* typically experience courtship by numerous males before they reach sexual maturity, they then may encounter several males before they choose to mate, and finally, long-lived females may gain considerable experience, which could be employed in their subsequent mate choices. The question then is whether female fruit flies rely on learning when choosing their mate.

What can female fruit flies gain from learning in the context of mate choice? It is well established that females in a variety of species attain direct and indirect benefits from mate choice (Andersson, 1994; Moller and Alatalo, 1999; Moller and Jennions, 2001). However, the mean and variance of male quality may vary widely in time and space. Hence, females who are too choosy for a given environment may experience lower reproductive success if they postpone mating and egg laying longer than necessary. It is thus likely that females under time constraint can choose better males if they rely on learning in the context of mate choice than if they ignore their experience. For example, females encountering many high-quality males can adopt a higher male acceptance criterion than females encountering only low-quality males (e.g., Janetos, 1980; Reid and Stamps, 1997).

To test for learning in the context of mate choice by female fruit flies, I allowed young virgin female *D. melanogaster* to experience courtship (but no mating) by either small or large conspecific males. Large *D. melanogaster* males have higher mating success than small males (Partridge and Farquhar, 1983; Partridge et al., 1987a,b). On the next day, half the females of each experience group were introduced to novel, inexperienced small males, and the other half of the females were exposed to novel, inexperienced large males. If learning affects mate choice, than females experiencing small males should adopt a lower male acceptance threshold than females experiencing large males. Specifically, I predicted that, compared to females experienced with large males, females experienced with small males would show (1) a higher percentage of mating with small males and (2) either a similar or slightly higher percentage of mating with large males. Finally, I also expected a higher percentage of mating with large than small males regardless of female experience based on the previously published data (Partridge and Farquhar, 1983; Partridge et al., 1987a,b).

METHODS

General

I used a stock of *D. melanogaster* initiated from wild flies collected in Vancouver, British Columbia, in early summer 2001. The flies were kept in $20 \times 20 \times 35$-cm population cages containing a few thousand individuals. Each population cage contained two standard 240-ml food bottles, each containing 50 ml of standard fly medium consisting of sucrose, dextrose, corn meal, yeast, agar, benzoic acid, antibiotics, and propionic acid. The population cages were maintained in an environmental chamber at 25°C and 70% relative humidity on a 16:8 h light:dark cycle with lights on at 0010 h.

All females and large males developed at a low density of approximately 300 larvae per standard food bottle. The small males developed at a high density of approximately 500 larvae per bottle. Because female *D. melanogaster* are larger than males, the females were larger than the males of either treatment. Although I focused on male size, it is likely that the variation in larval density affected other male characteristics including male behavior. Virgin collection occurred about 8 h after eclosion. I anaesthetized flies with CO_2, sexed them, and placed groups of 30 small males, 30 large males, and 20 females each in standard 40-ml vials containing 5 ml medium. Males were categorized into distinct size groups based on appearance, and wings of randomly selected males were measured after the experiment. The fly vials were housed in the same environmental chamber as the parental stock. All flies used in the experiment were virgin with no prior experience with the other sex since sexing.

Wing measurements were done on randomly selected samples of 12 large and 12 small males. The right wing of each male was mounted on a microscope slide and photographed with a digital camera through a stereoscopic microscope at a magnification of 40×. Measurements were done with the Scion Image software. Wing length was measured as the linear distance between the humeral-costal break and the end of the third longitudinal vein (Gilchrist and Partridge, 1999). The small males had significantly shorter wings compared to the large males, 1.50 ± 0.014 mm versus 1.84 ± 0.015 mm, respectively (ANOVA, $F_{1,22} = 264$, $p < .001$).

The experiment had eight replicates, each consisting of an experience phase on day 1 followed by a test phase on day 2. The females were 1 and 2 days old during the experience and test phase, respectively, and the males were 4 days old. That is, 4-day-old males were used for training, and new, inexperienced 4-day-old males were used for the test on the next day. Two pairs of replicates were conducted successively on each day. Each replicate included 40 females randomly assigned 10 per each of four treatment combinations (sessions) outlined in the two sections below. Hence, I tested a total of 320 females, 80 per each treatment combination. All fly transfers during the experiment were done with gentle aspiration.

Experience phase

For each replicate (1) 20 randomly selected females were placed individually in vials, each containing two small males and (2) 20 randomly selected females were placed individually in vials, each containing two large males. Because females were chosen randomly, I assumed no size difference between females in each treatment. I then observed the vials for 2 h. I replaced the males in a few vials in which the original males did not display courtship within 20 min. I also interrupted all mountings so that no matings occurred during training. At the end of the experience period, I transferred the females into a standard food bottle and placed the bottle in the environmental chamber until the test phase on the next morning.

Test phase

The test phase of each replicate consisted of the following four session types conducted at random, counterbalanced order. This means that each session type had a similar probability of being presented early and late in the replicates in order to control for time effects. (1) Ten randomly selected females experienced with small males were placed individually in vials, each containing two small males, and (2) 10 randomly selected females experienced with small males were placed individually in vials, each containing two large males. Similarly (3), 10 randomly selected females experienced with large males were placed individually in vials, each containing two small males, and (4) 10 randomly selected females experienced with large males were placed individually in vials, each containing two large males. The test phase lasted 30 min, and I recorded all matings and their latencies. Mating latency was defined as the time elapsed from the session's start until mating initiation.

In addition, I recorded male courtship behavior in a random sample of four vials during the first 15 min of two sessions in each replicate. This resulted in a behavioral data set for 64 of the vials, 16 per session type. For these 64 vials, I recorded (1) the courtship latency, defined as the time elapsed from the session's start until the first courtship by either male and (2) the start and end of all successive courtship activities. I later summed the total courtship duration for each vial according to a published protocol (Dukas, 2004; Dukas and Mooers, 2003).

In sum, the experiment consisted of all four combinations of two treatments during the experience phase (small or large males), which lasted 2 h, and two treatments (small or large males) presented during the 30-min test phase on the subsequent day. The experiment was conducted with observers blind to female experience during the test phase. All the data were recorded via laptop computers programmed in C, with the vials identified by numbers subsequently linked to prerecorded fly identity. The main statistical analyses were done on arcsine-transformed proportions of matings per sessions and log-transformed mating and courting latencies.

RESULTS

Female experience significantly affected the percentage of mating during the test phase. Compared to females experienced with large males, females experienced with small males had a 30% higher percentage of mating with small males and a 15% higher percentage of mating with large males (ANOVA, $F_{1,27} = 10.6$, $p < .005$; Figure 1). In addition, females were more likely to mate with large than small males ($F_{1,27} = 33.2$, $p < .001$; Figure 1), but there was no significant interaction between the male types encountered during the experience and test phases ($F_{1,27} = 0.07$, $p > .5$).

The mating latencies were not affected by female experience ($F_{1,229} = 0.05$, $p > .5$; Figure 2). The mating latencies, however, were shorter with large than small males ($F_{1,229} = 6.7$, $p < .01$) especially for the females experienced with small males, as indicated in the almost significant interaction between the experience and test phases ($F_{1,229} = 2.9$, $p = .09$; Figure 2). The courting latencies were shorter with large than small males ($F_{1,59} = 8.6$, $p < .005$; Figure 3), but there was no effect of female experience ($F_{1,59} = 0.3$, $p > .5$). Finally, the proportion of time spent courting was similar among treatments ($F_{1,59} = 1.8$, $p > .1$ for female experience and $F_{1,59} = 2.7$, $p > .1$ for the test male).
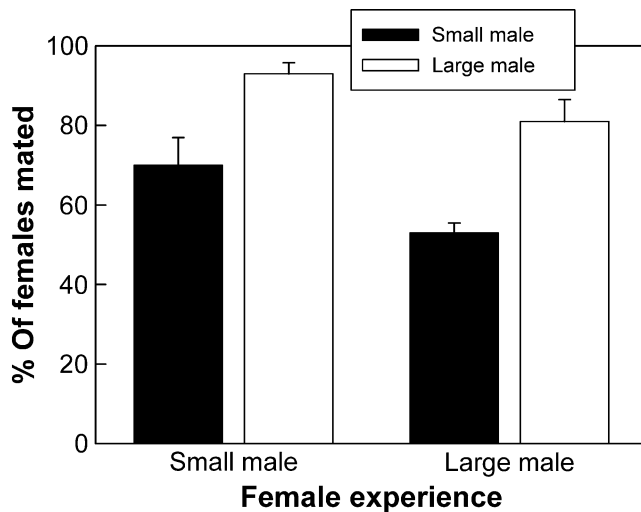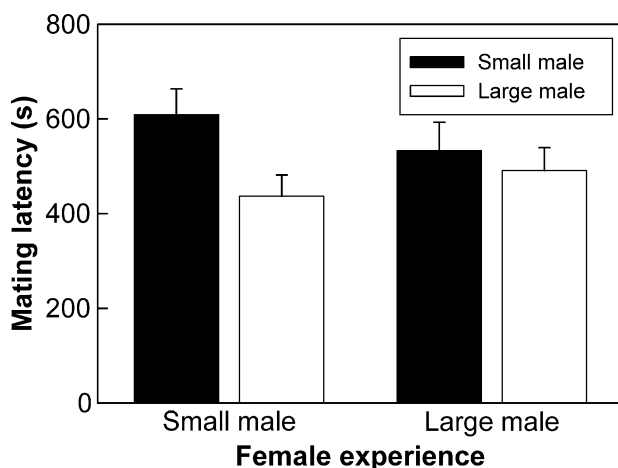
Figure 1
Mean (±1 SE) percentages of females that mated with small males (filled bars) and large males (open bars) after experiencing courtship by either small or large males (*n* = 320 trials).

DISCUSSION

Female fruit flies that experienced courtship by small males were more likely to mate with small and large males than females that experienced courtship by large males (Figure 1). This result suggests that the females employed learning to refine their mate choice. A few alternatives to learning can be ruled out. First, no mating occurred prior to the test, so differential effects of matings by small and large males cannot explain the results. Second, the females did not merely respond to the experience of either a larger variation in male sizes during the experience and test phases or a novel male size encountered during the test phase but not during the experience phase. This is because the females' percentage of mating with large males was high even after the females experienced large males, and their percentage of mating with small males was high only after experiencing small males.

The females' use of learning appears adaptive. In fruit flies as well as many other species, large males have higher

Figure 2
Mean (±1 SE) mating latencies of females that mated with small males (filled bars) and large males (open bars) after experiencing courtship by either small or large males (*n* = 234 trials with matings).
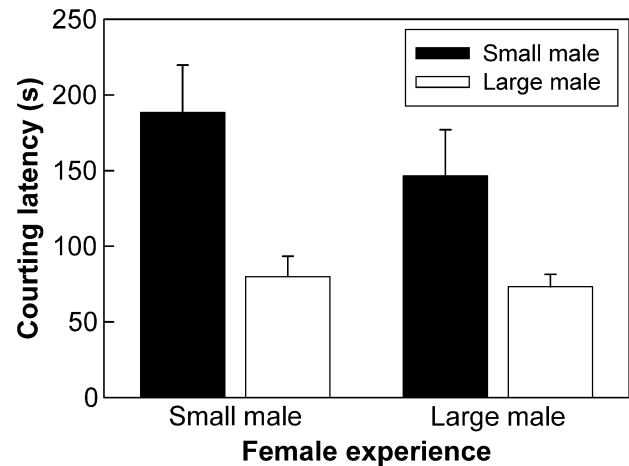
Figure 3
Mean (±1 SE) courtship latencies of small males (filled bars) and large males (open bars) that courted females that had experienced courtship by either small or large males (*n* = 64 trials with behavioral observations).

mating success than small males (Partridge and Farquhar, 1983; Partridge et al., 1987a,b). The mating advantage of large males was also evidenced in my study, in which large males had a higher mating success than small males regardless of female experience (Figure 1). Because the mean and variance of male body size typically vary in time and space, learning allows females to adjust their acceptance threshold and mate with better males relative to the ones currently available. Fruit flies have been used as a key model system for research on the genetics and neurobiology of learning for more than 30 years (Dubnau et al., 2003; Dubnau and Tully, 1998; Quinn et al., 1974; Siegel and Hall, 1979; Waddell and Quinn, 2001), but this is the first empirical evidence that female fruit flies learn in the context of mate choice. Furthermore, this is probably the first clear empirical demonstration of learning affecting mate choice in a female insect.

Earlier studies on the roles of males and females in determining the higher mating success of larger male fruit flies only established that differential male behavior is important (Partridge et al., 1987a). My results also indicate differential male behavior, with large males having shorter courtship latencies than small males (Figure 3). The higher mating success and shorter mating latencies of large males (Figures 1 and 2) cannot be attributed exclusively to either male or female roles. However, there was clearly a significant female role in accepting males of distinct sizes because female experience partially determined the mating success of small and large males (Figure 1).

Learning in the context of mating may be rather common among female insects as suggested by a few other studies, which did not critically test for learning. First, in a field study with bark beetles (*Ips pini*), females in patches with high male quality had higher acceptance thresholds than females in patches with low male quality (Reid and Stamps, 1997). Second, female crickets (*Gryllus lineaticeps*) typically prefer male calling songs with high than low chirp rates. Experimental females showed lower responses to male calls with low chirp rates after listening to calls with high chirp rates than after listening to calls with low chirp rates (Wagner et al., 2001). Third, female crickets (*Gryllus bimaculatus*) who had mated first with small males accepted large males as second mates in more than 90% of the trials. In contrast, females who had mated first with large males accepted small males as second mates in less than 50% of the trials (Bateman et al., 2001).

Finally, a few studies on sexual isolation in various *Drosophila* species suggested that mating experience influenced the frequency of matings between partially isolated species (e.g., Dobzhansky and Koller, 1938; O'Hare et al., 1976; Pruzan, 1976). It should be noted that some studies documented no effects of experience on mate choice by female insects. For example, experience had no effect on mate choice in female seaweed flies (*Coelopa frigida*) (Shuker and Day, 2002). The apparent between-species variation in the use of learning for mate choice requires further evaluation.

The potential role of learning in mate choice by insects has received little attention even though such learning can influence sexual selection and processes leading to speciation (Agrawal, 2001; Irwin and Price, 1999; Kirkpatrick and Dugatkin, 1994; Laland, 1994). The relative neglect of learning in the context of mate choice reflects a few prevailing misconceptions. First, a few authors asserted that short-lived insects should invest little in learning because they have little time for gaining from that investment (Alexander et al., 1997; Mayr, 1974; Staddon, 1983). Such assertions are misleading because the investment in learning should reflect the expected net fitness benefits from learning regardless of an individual's lifespan (Dukas, 1998; Dukas and Visscher, 1994). Extensive data indeed indicate that short-lived animals exhibit rather elaborate learning abilities (Papaj and Lewis, 1993; Waddell and Quinn, 2001). Second, as detailed in the Introduction, suggestions that females have no opportunities to learn about potential mates before mating (Alexander et al., 1997) are incorrect for many *Drosophila* spp. Similarly, females in a variety of other insect species may also have chances of encountering males before sexual maturity, after sexual maturity but before accepting a mate, and before accepting successive mates. I know, however, of no study evaluating the opportunities for learning about males in female insects. Finally, the notion that learning in the context of mate choice is unlikely to be valuable in female insects who may mate only once (Alexander et al., 1997; Roitberg et al., 1993) is unsatisfactory. First, the single mating decision may be made after the female has experienced numerous courting males, and, second, even a few learning trials can significantly affect insect behavior (e.g., Dubnau and Tully, 1998; Siegel and Hall, 1979).

In sum, both female (Figure 1) and male (Dukas, 2004, 2005) fruit flies rely on learning to make apparently adaptive mate choice decisions. Thus, integrating learning into research on the processes underlying sexual selection and speciation, in which fruit flies have served as a central model system, can improve our understanding of such processes.

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