

Male fruit flies learn to avoid interspecific courtship

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Experimental data suggest, and theoretical models typically assume, that males of many fruit flies (*Drosophila* spp) are at least partially indiscriminate while searching for mates, and that it is mostly the females who exert selective mate choice, which can lead to incipient speciation. Evidence on learning by male *D. melanogaster* in the context of courtship, however, raises the possibility that the initially indiscriminate males become more selective with experience. I tested this possibility by comparing the courtship behavior of male *D. melanogaster* experienced at courting females of the closely related species, *D. simulans*, and inexperienced males. I found that compared with the inexperienced males, the males experienced with courting *D. simulans* females showed significantly lower courtship toward female *D. simulans*. Both male treatments, however, showed virtually identical courtship durations with female *D. melanogaster*. These results indicate that male fruit flies adaptively refine their courtship behavior with experience and suggest that the males contribute more to assortative mating and incipient speciation than is commonly assumed. **Key words:** courtship, *Drosophila*, fruit flies, learning, mate choice, speciation. [*Behav Ecol* 15:695–698 (2004)]

Fruit flies (*Drosophila* spp) have served as a prime model system in research on the effect of mate choice and assortative mating on speciation (Coyne and Orr, 1989; Korol et al., 2000; Powell, 1997; Rice and Hostert, 1993). In fruit flies, as well as many other species, female reproduction is primarily limited by her rate of egg production, whereas male reproduction is limited by the number of females he inseminates (Bateman, 1948). This, and the consequent high ratio of sexually active males to fertilizable females, causes the males to be rather indiscriminate and the females to exert careful mate choice (Andersson, 1994; Emlen and Oring, 1977). Indeed, several studies have documented that male fruit flies of various species readily court interspecific females, showing either complete or partial failure to discriminate interspecific from intraspecific females (Noor, 1996; Spieth and Ringo, 1983; Wood and Ringo, 1980), although other studies have documented male discrimination (for review, see Noor, 1996). Empirical research suggests that in many *Drosophila* species, only the females can be responsible for selective mate choice, leading to incipient speciation, which is indeed the assumption typically made in theoretical models (Kirkpatrick and Ravigne, 2002).

Research on assortative mating typically involves inexperienced virgin males and females that are kept in isolation from the other sex and other species before testing. Research on the cellular biology of learning, however, has established a protocol involving learning by male *D. melanogaster* in the context of courtship. The males are first paired with unreceptive fertilized females and later tested with anesthetized virgin females. The experienced males court the anesthetized females significantly less than do control inexperienced males. Although the use of anesthetized females is artificial and evolutionary irrelevant, it allows researchers investigating neurobiological and genetic aspects of associative learning to focus on changes in the males while elegantly eliminating confounding effects of the females (McBride et al., 1999; Siegel and Hall, 1979; Tompkins et al., 1983).

Given the existing data about learning in male fruit fly, it is relevant to ask whether such learning affects evolutionary relevant behavior such as mate choice. For example, will initially indiscriminate male fruit flies learn to avoid courting interspecific females that invariably reject their mating attempts? I addressed this question with the two closely related species, *D. melanogaster* and *D. simulans*.

Human observers cannot distinguish members of the two species with the naked eye, although males can be distinguished under microscopic examination based on morphological difference in their genitals. The two species differ in their courtship songs and pheromonal profiles. The interpulse interval, a key parameter of male song, is about 50% shorter in *D. melanogaster* than in *D. simulans* (Kawanishi and Watanabe, 1980; Schilcher and Manning, 1975). Female *D. simulans* lack the cuticular hydrocarbon, 7,11-heptacosadiene, a major pheromone in female *D. melanogaster*. In contrast, female *D. simulans* possess higher levels of 7-tricosene than do female *D. melanogaster* (Jallon and David, 1987). *D. melanogaster* and *D. simulans* have similar global geographic distributions. Although they commonly occur sympatrically, *D. simulans* tends to occur farther from human habitations than does *D. melanogaster* (Ashburner, 1989; Carracedo and Casares, 1985; Gromko and Markow, 1993; Markow, 2000). Natural hybridization between *D. simulans* and *D. melanogaster* females is rare, but sterile hybrids have been occasionally produced in the laboratory (Carracedo and Casares, 1985; Jamart et al., 1993; Wood and Ringo, 1980). Previous studies indicate that male *D. melanogaster* readily court female *D. simulans* (Manning, 1959; Wood and Ringo, 1980), and my preliminary data showed nearly identical courtship durations by male *D. melanogaster* toward female *D. simulans* and *D. melanogaster*. Hence, the *D. melanogaster*–*D. simulans* species pair is ideal for studying effects of experience on interspecific courtship.

Here I tested whether, compared with inexperienced males, male *D. melanogaster* with experience courting female *D. simulans* would subsequently show shorter courtship duration with female *D. simulans* than with female *D. melanogaster*. I predicted that the males experienced with courting female *D. simulans* would show selective reduction in courtship toward female *D. simulans* but not toward female *D. melanogaster*.

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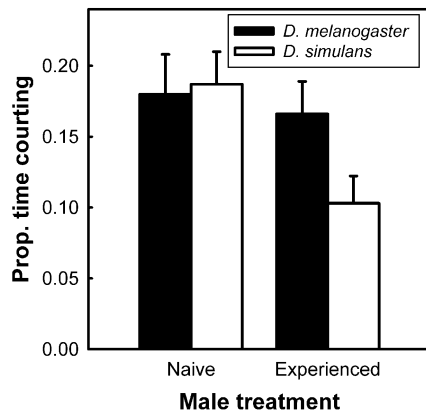


Figure 1
The proportion of time either naïve males or males experienced at courting female *D. simulans* spent courting either two female *D. melanogaster* (black bars) or two female *D. simulans* (white bars). Each bar depicts the mean \pm 1 SE for 48 males, with a total of 192 males.

METHODS

General

I used wild stocks of flies kept in $20 \times 20 \times 35$ -cm population cages containing a total of a few thousand individuals of each species. The two species were kept in separate environmental chambers at 25°C, 70% relative humidity (RH), on a 16-h light/8-h dark cycle with lights on at noon. The *D. melanogaster* population was initiated from wild flies collected in Vancouver, British Columbia, in early summer 2001. The *D. simulans* population was from the Florida City line, which was established around 1985. Each population cage contained two standard 240-ml food bottles, each containing 50 ml of standard fly medium containing sucrose, corn meal, yeast, agar, benzoic acid, methyl paraben, and propionic acid.

All flies used for the experiment developed at a low density of approximately 300 larvae per standard food bottle. Before virgin collection, which occurred less than 7 h after eclosion, I mixed flies from approximately 12 bottles in a single cage in order to randomize between-bottle effects. I then anesthetized small numbers of flies with CO₂, sexed them, and placed individual males and groups of 15 females each in standard 40-ml vials containing 5 ml medium. The vials were housed in the same environmental chambers as the parental stocks for 3 days before the experiment, which was always conducted from 0800–1200 h. That is, the experiments were conducted in the flies' predawn because previous research (Hardeland, 1972) and my experience indicated peak courtship activity during that period. All flies used in the experiments were virgin, and each fly was used only once. All males had no experience with other flies after they were sexed and placed individually in the vials.

The experiment had four replicates, each conducted on a different day. Each replicate consisted of six successive sessions, with each session including eight males. Each session, the males were randomly assigned two per each of four conditions outlined in the two sections below. Hence, I tested a total of 192 males, 48 per condition. All fly transfers during the experiment were done with gentle aspiration.

Experience phase

Each session, the four males randomly selected for the "experienced" treatment were placed individually in empty vials. Then I added to two female *D. simulans* to each of these

four vials. The four males randomly selected for the "naïve" treatment were placed individually in empty vials. I watched males of the experienced treatment for about 10 min, and 95% of them were observed courting a female *D. simulans* during that period. The experience phase lasted 1 h. I chose to use inexperienced males rather than males experienced with *D. melanogaster* females as a control to eliminate the confounding effect involved in some of these males having mating experience.

Test phase

When the experience phase ended, I transferred each of the eight males into a fresh empty vial. Then (1) two males of the experienced treatment each received two female *D. melanogaster*; (2) two males of the experienced treatment each received two female *D. simulans*; (3) two males of the naïve treatment each received two female *D. melanogaster*; and (4) two males of the naïve treatment each received two female *D. simulans*.

Two observers trained to eliminate interobserver differences conducted the observations. The results indeed indicated no observer effect ($F_{1,184} = 0.03$, $p > .8$). Each observer received four randomly selected vials, one each with (1) an experienced male and two female *D. melanogaster*; (2) an experienced male and two female *D. simulans*; (3) a naïve male and two female *D. melanogaster*; and (4) a naïve male and two female *D. simulans*. I recorded the start and end of each courtship activity and later summed the total courtship duration for each male, which included all "following," "wing vibration," and "mounting attempts" (Cobb et al., 1985). Courtship duration has been widely used in studies on the effect of experience on male courtship (see McBride et al., 1999; Siegel and Hall, 1979). In a previous study, I found that courtship duration was highly positively correlated with other measures such as the number of courtship bouts, wing vibrations, and mounting attempts (Dukas and Mooers, 2003). Hence, in this study, I only recorded overall courtship activity. In addition to courtship duration, I also recorded the start of mating if it occurred. The test phase lasted 10 min.

In sum, the experimental protocol consisted of all four combinations of two male treatments during the experience phase (experienced and inexperienced), which lasted 60 min, and two female species (*D. melanogaster* and *D. simulans*) presented during the subsequent 10-min test phase. The experiment was conducted in a blind fashion, meaning that during the test phase, the observers did not know either the male treatment or the female species (which are visually identical). All the data were recorded via laptop computers programmed in C, with the vials identified by random numbers subsequently linked by the computer to prerecorded fly identity.

Analyses

The main statistical analysis was done on arcsine-transformed proportions of the time spent courting out of the total time available. In trials with no matings (all the trials with female *D. simulans* and 66% of the trials with female *D. melanogaster*), the time available was 10 min. In trials with matings (34% of the trials with female *D. melanogaster*), the time available was the duration before mating.

RESULTS

In the test phase, male experience significantly affected courtship duration directed at females (Figure 1). Naïve males spent similar proportions of time courting female *D. melanogaster* and female *D. simulans*, whereas males experienced

at courting female *D. simulans* spent about 40% shorter times courting female *D. simulans* than female *D. melanogaster* (ANOVA, $F_{1,180} = 4.6$, $p < .05$ for the interaction between male experience and female species).

Three of the other factors included in the ANOVA, replicate ($F_{3,180} = 2.1$, $p > .1$), male treatment ($F_{1,180} = 3.7$, $p > .05$), and female species ($F_{1,180} = 0.8$, $p > .3$), were nonsignificant, whereas session effect was significant ($F_{5,180} = 2.8$, $p < .05$), with longer courtship duration in early morning than late morning sessions. No matings occurred with female *D. simulans*. Both the frequency of matings and mating latencies were similar between the male treatments. Thirty-one percent of the naïve males and 31% of the experienced males mated with female *D. melanogaster* during the 10-min test period, and the mating latencies were 404 ± 32 s (mean \pm 1 SE) with naïve males and 371 ± 40 s with experienced males ($t_{28} = 0.6$, $p > .5$).

DISCUSSION

Male *D. melanogaster* with no prior experience with *D. simulans* females spent about equal durations courting *D. melanogaster* and *D. simulans* females. In contrast, male *D. melanogaster* experienced with courting *D. simulans* females spent much shorter times courting *D. simulans* than *D. melanogaster* females (Figure 1). Note that reduced motivation alone cannot explain the results because the effect of experience on the males was selective, reducing their courtship toward female *D. simulans* but not *D. melanogaster*. This result challenges traditional findings of either no discrimination or partial discrimination by male fruit flies and suggests that some of these findings may be based on experimental protocols that did not allow for male learning before mate-choice trials. It is thus possible that male fruit flies actually become quite selective in their courtship efforts with experience. Such experienced individuals can magnify the degree of assortative mating compared with levels obtained in the traditional tests involving naïve males. This possibility is especially relevant because female *D. melanogaster* prefer mating with males that are a few days old over younger males (Long et al., 1980). Such older males would typically be more experienced in courting various classes of intra- and interspecific females than young males.

Several studies have documented habituation and learning by male *D. melanogaster* in the context of courtship. First, male *D. melanogaster* experienced with unreceptive fertilized females subsequently reduce courtship of anesthetized virgin females (McBride et al., 1999; Siegel and Hall, 1979; Tompkins et al., 1983). Second, Gailey et al. (1982, 1985) found that inexperienced males initially court but quickly habituate to recently eclosed immature males.

In addition to the above well-controlled and replicated studies, there has been conflicting information about environmental effects of mixed culturing during the preadult stages, with Manning (1959) reporting no effects and Eoff (1973) documenting that the frequency of hybridization between *D. melanogaster* and *D. simulans* was higher when adults eclose in mixed-species cultures than in single-species cultures.

Learning in the context of courtship is also known in a few species of solitary bees, in which the males learn to selectively avoid unreceptive conspecific females based on individually distinct odors (Barrows, 1975; Smith, 1983; Wcislo, 1992). In vertebrates, the most relevant research involves the cowbird (*Moluthrus ater*), in which the males learn to selectively retain song features based on feedback from the females (Freeberg et al., 2002; West and King, 1988). Other research involving learning in the context of sexual behavior include extensive work by Domjan and colleagues (reviewed in Mills et al., 1997) on the Japanese quail (*Coturnix japonica*), research indicating

that male ground squirrels (*Spermophilus tridecemlineatus*) learn which females are about to become receptive and can later relocate these females (Schwagmeyer, 1995), and studies indicating that male fish (*Trichgaster trichopterus*) can learn to anticipate female arrival based on associated cues (Hollis et al., 1997).

The traditional scenario in which males of many species, including some fruit flies, are assumed to be indiscriminate can readily be justified by both theory and data. A male *D. melanogaster* typically encounters individual females with large genetically and environmentally based variation, which may obscure between-species differences (Carracedo and Casares, 1985; Jallon and David, 1987; Kawanishi and Watanabe, 1980). There is a high ratio of sexually active males to receptive females, and the cost of courtship and sperm is relatively low (but see Cordts and Partridge, 1996). Under such challenging conditions, a male may incur a lower cost from courting a fly that is not a receptive conspecific female than from missing the relatively rare opportunity of fertilizing an intraspecific female. However, it is also possible that males have a greater mating success if they learn to avoid courting distinct classes of unreceptive females than if they remain indiscriminate (Barrows et al., 1975). Learning to avoid unreceptive female classes is most likely to increase a male's fitness if he encounters a sizable proportion of interspecific and unreceptive conspecific females and if he can learn to correctly reject only or mostly unreceptive females.

The evidence presented in the present study, together with previous research (Gailey et al., 1985), indicates that experience can affect mate choice in *D. melanogaster*. The effect of experience on flies must therefore be considered in future experimental protocols and theoretical analyses involving mate choice, assortative mating, and incipient speciation.

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