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TWO CONSEQUENCES OF HOMOSEXUAL COURTSHIP PERFORMED BY *DROSOPHILA MELANOGASTER* AND *DROSOPHILA AFFINIS* MALES

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Sexually mature *Drosophila melanogaster* males perform an elaborate series of courtship behaviors in response to conspecific virgin females (Spieth, 1952; Bastock and Manning, 1955). Mature males will also perform vigorous courtship, including attempted copulation, toward young, sexually immature males. This behavior is performed by males from long-established laboratory strains (Cook and Cook, 1975; Jallon and Hotta, 1979), isofemale lines recently derived from natural populations (McRobert and Tompkins, 1983), and males in the field (McRobert and Tompkins, unpubl.).

The evolutionary significance of this homosexual courtship is unclear. It has been suggested that young males that are exposed to courtship stimuli may subsequently perform better courtship themselves when they become sexually mature. Alternatively, mature males that "practice" courting young males may court females more effectively (Tompkins et al., 1980; Siegel et al., 1984). However, neither of these hypotheses has been tested. Accordingly, we observed older *D. melanogaster* males that had courted young conspecific males and young *D. melanogaster* males that had elicited courtship to see whether either performance or elicitation of homosexual courtship affected the males' courtship of females, as indicated by their success in copulating. Our findings provide the first evidence that homosexual courtship benefits the young male.

Mature males, on the other hand, do not benefit from homosexual courtship; their ability to copulate with females is not affected by prior experience with males. Therefore, it is not surprising that a mechanism for minimizing the amount of time that a male spends performing homosexual courtship has evolved in *D. melanogaster*. Briefly, a mature male that courts an immature male will subsequently perform less courtship in response to a second young male. This phenomenon is known as experience-dependent modification of male courtship (Gailey et al., 1982; Vaias and Tompkins, unpubl.).

Drosophila affinis is another species in which mature males court young, sexually immature males (McRobert and Tompkins, 1987). Recently, we have shown that *D. melanogaster* and *D. affinis* interact sexually (McRobert and Tompkins, 1986a). Mature *D. melanogaster* males perform courtship in response to young *D. affinis* males that is quantitatively and qualitatively indistinguishable from the courtship that young conspecific males elicit. In addition, *D. affinis* males court young *D. melanogaster* males as vigorously as they court young conspecific males.

If homosexual behavior is disadvantageous to the courting male, it seems reasonable to assume that a mechanism should have evolved to minimize this behavior in all situations in which courtship of males occurs. Accordingly, we have analyzed the courtship behavior of *D. affinis* to determine whether experience-dependent courtship modification (EDCM) occurs after mature males court young, conspecific males. In addition, we have analyzed the cross-species interactions of *D. affinis* and *D. melanogaster* males to see whether interspecific homosexual courtship is modified by experience.

MATERIALS AND METHODS

Stocks

All flies were maintained on a standard cornmeal-agar-molasses medium, at either 22° or 25°C in a 12:12 LD cycle with lights on at 8 A.M. The *D. mela-*

nogaster stock was a Canton-S (CS) wild-type strain established from a single male-female pair in 1976 (Tompkins et al., 1980) and then maintained in mass culture. A stock producing "isolation males" was derived from the CS stock as follows. In April 1985, a single male-female pair from the CS stock was transferred to a vial. F_1 males were collected as third-instar larvae and then stored individually in vials until they emerged as adults from their pupal cases. Each F_1 male was then transferred to a new vial with 1–3 virgin females from the original CS stock. Subsequent generations were handled in the same way: males were isolated as third-instar larvae and then mated individually to CS virgin females after the males emerged from their pupal cases. Thus, these "isolation males" never encountered other adult males. This stock was maintained for 20 generations before the flies were used in behavioral experiments.

We collected *forked* (*f*) males from a homozygous *f* stock of *D. melanogaster* obtained from the Mid-America Drosophila Stock Center in Bowling Green, Ohio. These mutant flies have short bristles (see Lindsley and Grell, 1968), which made it easy to distinguish them from CS and isolation males.

The *D. affinis* stock was initiated from four fertilized females collected in the Philadelphia area in 1983 (McRobert and Tompkins, 1987).

Behavioral Assays

Effect of Homosexual Courtship on Young Males. — In the first set of experiments, young isolation males were courted by mature *f* males and then tested with CS females. Initially, *f* males were collected within three hours of emergence and then transferred in groups of 10–20 to a bottle with 100–200 CS males and females of various ages so that the *f* males had the opportunity to perform and elicit courtship. Five days later, the *f* males were removed from the bottle. At that time, isolation males were collected within one hour of eclosion. Individual isolation males were then transferred to a vial with 1) 3–5 five-day-old *f* males, 2) 3–5 *f* males whose wings had been surgically removed, or 3) no other flies. Observation of groups 1 and 2 revealed that these isolation males received homosexual courtship from the older *f* males. It is also conceivable that other forms of male-male interaction occurred that could have affected the subsequent courtship of these isolation males. After three days, the isolation males that had been stored with other flies were removed from the vial and then stored alone. Isolation males that had not been stored with other flies were simply left alone for five days before being tested.

To assay their copulation success, the five-day-old isolation males were aspirated individually, without anesthesia, into cylindrical plastic observation chambers (volume $\simeq 0.4$ cm^3), each with one five-day-old virgin CS female. The flies were then observed for 60 minutes or until copulation occurred. For flies that did copulate, the copulation latency (the amount of time elapsing from the beginning of the observation period to the onset of copulation) was recorded.

Effect of Homosexual Courtship on Sexually Mature Males. — In the second set of experiments, mature isolation males were given the opportunity to court young *f* males and then tested for their ability to copulate with females.

TABLE 1. Behavioral assays for the effect of homosexual courtship on young males. Copulation latencies are means $\pm$ SE; $N = 50$ for each data point.

Isolation male's homosexual experience prior to testing	Copulation frequency	Copulation latency (min)
Courted by older males	82%	5.89 $\pm$ 0.6
Courted by wingless older males	80%	9.72 $\pm$ 1.6
None	76%	12.26 $\pm$ 1.9

Isolation males were collected within one hour of eclosion and stored individually for either two or five days. Males that were stored for five days were subsequently tested with females. Males that had been stored for only two days were then transferred individually to vials, each with 3–5 young (3–5 hours old) *f* males. Observation of these vials revealed that the isolation males courted the young *f* males. After 24 hours, the *f* males were removed, and the isolation males were stored individually for 24 hours before being tested with females as described above.

Effect of Experience on the Homosexual Courtship of D. affinis *and* D. melanogaster. — In these experiments, the *D. melanogaster* males were taken from the original CS stock and not from the isolation line. "Mature males," whose courtship was measured, were collected under CO_2 anesthesia within three hours of eclosion, then stored individually in vials for five days. "Training males" and "sex objects" (immature males that can elicit courtship behaviors but not perform them) were collected within three hours of eclosion and stored in groups of 1–10 for 1–6 hours before being paired with experimental males.

For each experiment, a five-day-old mature male was aspirated without anesthesia into a cylindrical plastic chamber (volume $\simeq 0.4$ cm^3), either alone or with a 1–9-hour-old training male. After 30 minutes, the mature male was removed from the chamber and aspirated into a second chamber with a 1–9-hour-old sex object. The pair of flies was then observed at $10\times$ magnification for five minutes. During this period, the courtship index (CI; the percentage of the observation time that the mature male spent performing any of the courtship behaviors; Tompkins et al., 1980), was determined. In response to a sex object, mature *D. melanogaster* males orient, follow, tap, extend and vibrate their wings to produce a courtship song, lick, and attempt copulation (see Tompkins [1984] for a more complete description of these behaviors). Mature *D. affinis* males perform all of these behaviors except licking, although *D. affinis* and *D. melanogaster* males orient and sing differently (McRobert and Tompkins, 1987). Table 3 summarizes the various combinations of training males and sex objects that the mature *D. melanogaster* and *D. affinis* males encountered.

Statistical Analysis

The significance of differences in copulation frequency was determined with a 2×2 contingency chi-square test. The significance of differences in copulation latency was determined by transformation of the data to a natural-log scale and a two-tailed *t* test. The

TABLE 2. Behavioral assays for the effect of homosexual courtship on sexually mature males. Copulation latencies are means ± SE; $N = 50$ for each data point.

Isolation male's homosexual experience prior to testing	Copulation frequency	Copulation latency (min)
Courted young males	74%	9.43 ± 1.4
None	80%	8.27 ± 1.3

transformed data were judged to be sufficiently symmetric to justify the use of t. The significance of differences in mean CI was determined with a two-tailed t test.

RESULTS

Effect of Being Homosexually Courted on Subsequent Heterosexual Mating Success. — Isolation males copulated equally frequently, regardless of whether they had been courted by intact f males, wingless f males, or no males ($P > 0.05$; Table 1). Furthermore, copulations were not significantly faster for isolation males that had been courted by wingless f males than for those that had been stored alone ($P > 0.05$; Table 1), nor were they faster for isolation males courted by intact f males than for those courted by wingless f males ($P > 0.05$; Table 1). However, isolation males that had been courted by intact f males mated significantly faster than those that had been stored alone ($P < 0.001$; Table 1).

Effect of Homosexual Courtship by Sexually Mature Males on Their Own Subsequent Heterosexual Mating Success. — Isolation males that courted young males before being tested with females were no more successful, in terms of copulation frequency ($P > 0.05$) or copulation latency ($P > 0.05$) than isolation males that had no homosexual experience (Table 2).

Experience-Dependent Courtship Modification in D. melanogaster and D. affinis Males Trained and/or Tested with Conspecific Males. — Initially, we confirmed Gailey et al. (1982) and Vaias and Tompkins' (unpubl.) observations that EDCM occurs in D. melanogaster. We observed that mature D. melanogaster males that had courted young D. melanogaster males showed a significant decrease in their mean CI in re-

sponse to conspecific sex objects, in comparison to D. melanogaster males that had not previously courted young conspecific males ($P < 0.001$; Table 3). We also observed that this phenomenon occurred in D. affinis. Mature D. affinis males that had courted young D. affinis males performed significantly less courtship in response to conspecific sex objects than inexperienced D. affinis males ($P < 0.001$; Table 3).

Experience-Dependent Courtship Modification in D. melanogaster *and* D. affinis *Males Trained and/or Tested with Males from the Other Species.* — We observed that EDCM in cross-species courtship occurred in most circumstances. With regard to D. melanogaster, males that had courted either young D. affinis males or young D. melanogaster males performed less courtship in response to D. affinis sex objects than inexperienced D. melanogaster males ($P < 0.001$ and $P < 0.02$, respectively; Table 3). In contrast, D. melanogaster males that had courted young D. affinis males performed as much courtship in response to D. melanogaster sex objects as did inexperienced D. melanogaster males ($P > 0.05$; Table 3).

With regard to D. affinis, we observed that mature males that had courted young D. melanogaster or D. affinis males performed less courtship in response to D. melanogaster sex objects than did inexperienced D. affinis males (both $P < 0.001$; Table 3). D. affinis males also performed less courtship of D. affinis sex objects when they had been trained with D. melanogaster ($P < 0.02$; Table 3), but this decrease is not as striking as when they were trained with D. affinis ($P < 0.001$; Table 3).

DISCUSSION

Homosexual courtship has two consequences for the participants. We have shown that this activity has a beneficial effect on the sex object's ability to mate with a female when he becomes sexually mature. A more immediate consequence of homosexual courtship is experience-dependent courtship modification (EDCM), which affects the courting male. Our results demonstrate that this phenomenon, which was first observed by Gailey et al. (1982) in D. melanogaster, is not limited to that species: it affects D. affinis and D. melanogaster males' courtship of conspecific and heterospecific males.

TABLE 3. Courtship of mature D. melanogaster and D. affinis males. Mature males were five days old when tested; training males and sex objects were 1–9 hours old. See text for details. CI's are means ± SE.

Mature male	Training male	Sex object	CI	N
melanogaster	none	melanogaster	77 ± 3	40
melanogaster	melanogaster	melanogaster	53 ± 4	40
melanogaster	none	affinis	73 ± 3	20
melanogaster	affinis	affinis	46 ± 5	20
melanogaster	affinis	melanogaster	73 ± 3	40
melanogaster	melanogaster	affinis	59 ± 4	20
affinis	none	affinis	38 ± 3	40
affinis	affinis	affinis	13 ± 2	40
affinis	none	melanogaster	43 ± 5	20
affinis	melanogaster	melanogaster	11 ± 2	20
affinis	melanogaster	affinis	27 ± 3	40
affinis	affinis	melanogaster	12 ± 3	20

With regard to the effect of homosexual courtship on sex objects, we have shown that *D. melanogaster* males that are courted when they are young subsequently copulate more quickly with females than do males that never elicited normal courtship. Presumably, it is the males' courtship that is affected by homosexual experience, since a female must be stimulated by a male's courtship before she will open her vaginal plates and allow copulation to occur (Bastock and Manning, 1955). Our findings provide a demonstration that experience at a time when a *Drosophila* male is immature and incapable of performing courtship himself can affect his behavior when he becomes sexually mature. This effect of exposure to an older male's sexual behavior on the young male's subsequent ability to perform those behaviors is reminiscent of the song-learning process that occurs in some bird species (e.g., Marler, 1981).

Assuming that it is the male's courtship that is modified, the question arises as to which courtship behaviors are affected by homosexual experience. Our observations suggest that the courtship song is affected, although it may not be the only component that is modified. Although the copulation latencies of isolation males courted by wingless males were not significantly shorter than those of isolation males that had elicited no courtship, they fell between those of isolation males courted by intact males and isolation males that elicited no courtship.

A mature male that courts a young male wastes time and energy, exposes himself to predation, and loses opportunities to court and inseminate females, without incurring the benefits of sexual reproduction. Furthermore, we have shown that mature males do not benefit from homosexual courtship by becoming more successful in copulating with females in one-on-one encounters. Hence, it is not surprising that a mechanism such as EDCM has evolved to minimize the amount of time a mature male spends courting young conspecific males. This adaptation also confers a fitness advantage in that mature males that have had the opportunity to court young males are more successful in competition for virgin females when young males are also present (Zawistowski and Richmond, 1985). Thus, since EDCM is beneficial to mature males, one might expect to observe this phenomenon in other situations in which homosexual courtship occurs.

We have verified Vaias and Tompkins' (unpubl.) observations that EDCM occurs when the sex objects are intact, active young males (rather than the immobilized flies used by Gailey et al. [1982]). We have also shown that EDCM occurs in *D. affinis* in these conditions. Moreover, unlike *D. melanogaster* males, *D. affinis* males that have courted young males perform less than half as much courtship as naive *D. affinis* males, which indicates that experience with young males has more effect on *D. affinis* males than on *D. melanogaster* males. This may be due to the fact that inexperienced *D. affinis* males perform much less courtship in response to attractive flies than do *D. melanogaster* males (see McRobert and Tompkins, 1986a), suggesting that any experience that modified their courtship behavior might have a greater proportionate effect on their overall CI.

Finally, we have demonstrated that EDCM occurs in most cross-species interactions involving *D. affinis*

and *D. melanogaster* males. This observation suggests that the stimuli provided by young males that mediate the modification of the mature male's courtship are similar in these two species. With regard to *D. melanogaster*, it is known that mature males respond to visual stimuli, aphrodisiac pheromones, and inhibitory pheromones associated with conspecific young males (reviewed by Tompkins [1984]). The observation of Gailey et al. (1982) that mature *D. melanogaster* males perform less courtship in response to young conspecific males if the mature males were previously stored in chambers that had held young *D. melanogaster* males suggests that the relevant stimuli are pheromones, rather than visual cues. Furthermore, if mature *D. melanogaster* males court very young (1–3 hours old) conspecific males, which have not yet begun to synthesize inhibitory pheromones (Curcillo and Tompkins, 1987), they subsequently perform less courtship in response to young males (Vaias and Tompkins, 1988), suggesting that the excitatory pheromone alone may be sufficient for EDCM in *D. melanogaster*.

Although we do not know what stimuli mediate EDCM in *D. affinis*, it is clear that young *D. affinis* males do provide pheromonal cues that can stimulate courtship since, although most mature *D. affinis* males are incapable of courting in the dark, mature *D. melanogaster* males do court young *D. affinis* males in the dark (McRobert and Tompkins, 1987). Thus, it is reasonable to assume that young males of *D. melanogaster* and *D. affinis* may induce EDCM in mature males of either species because the young males produce similar aphrodisiac pheromones.

When a mature *D. melanogaster* male is trained with a young *D. affinis* male and then tested with a young *D. melanogaster* male, the mature male's CI does not decrease. Conversely, while a mature *D. affinis* male that is trained with a young *D. melanogaster* male and tested with a young *D. affinis* male does have a lower CI than a naive male, the mature male still performs more courtship than a mature *D. affinis* male that has been trained and tested with young conspecific males. These observations indicate that experience with a heterospecific male has little or no effect on a mature male's ability to court a conspecific male, suggesting that the stimuli that males perceive when they court young *D. affinis* and *D. melanogaster* males are not identical. Since mature *D. melanogaster* males are less affected by EDCM than are *D. affinis* males, it is not surprising that mature *D. melanogaster* males' courtship of conspecific males does not decrease at all after training with heterospecific males, while *D. affinis* males' courtship of conspecific males is affected to some extent by this experience.

In summary, our findings provide the first evidence that elicitation of homosexual courtship does in fact benefit young *D. melanogaster* males. However, these results are contingent on the assumption that our Canton-S and isolation stocks are representative of the entire *D. melanogaster* species. If this assumption is warranted, then this benefit to young males may have provided the selective pressure for the evolution and maintenance of homosexual behavior. Additionally, we have shown that EDCM occurs in two species that exhibit homosexual courtship, often even when the courtship is interspecific. Since *D. melanogaster* and *D. affinis* males congregate at the same feeding sites in

at least two locations in the eastern United States (McRobert and Tompkins, 1986b, 1986c) and since males of these species have been observed to court each other at these feeding sites (McRobert and Tompkins, 1986a), it seems reasonable to assume that this phenomenon may occur in the wild.

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INSEMINATION REACTION IN *DROSOPHILA*: FOUND IN SPECIES WHOSE MALES CONTRIBUTE MATERIAL TO OOCYTES BEFORE FERTILIZATION

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Patterson (1946) was the first to describe the opaque mass that fills the uterus in certain *Drosophila* species immediately after mating. This insemination reaction results from a massive secretion by the vaginal wall (Patterson, 1946) in response to a nonsperm component of the ejaculate (Lee, 1950). Subsequently, Wheeler (1947) screened a large number of species for the occurrence of this insemination reaction, and the findings were used to assign *Drosophila* species to three categories, depending upon the size and duration of the mass. Class I contains species in which no mass is observed. Species exhibiting a slight uterine reaction following insemination were grouped in Class II. Those species showing a strong reaction and mass of long duration were assigned to Class III. Samples of the species in each group are shown in Table 1.

A number of interpretations have been offered as to the significance of the reaction mass. Because interspecific matings were observed to result in masses of greater size and duration than those produced following intraspecific matings, Patterson (1946) and Patterson and Stone (1952) suggested that the insemination reaction functioned as a postcopulatory reinforcement of reproductive isolation between related species. These same investigators offered the alternative explanations that the reaction is somehow preparatory to fertilization or egg production or that it acts as a copulatory plug. These explanations, however, fail to account for