Drosophila Males Produce a Pheromone which Inhibits Courtship

Laurie Tompkins * and Jeffrey C. Hall

Department of Biology, Brandeis University, Waltham, MA 02254

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Normal *Drosophila* males produce a volatile pheromone which inhibits courtship. This chemical cue is not identical to the pheromone made by mated female flies, which also inhibits male sexual behavior. Mutant *olfC* males, which fail to respond to several organic compounds, are not inhibited by the pheromone made by males; hence, unlike normal sexually mature males, *olfC* males court each other vigorously.

Most identified insect pheromones are chemical cues produced by females which stimulate males to court or copulate [1]. Examples of pheromones which inhibit sexual behavior are less numerous, although inhibitors produced by females have been described in a number of insect species [2]. The inhibitory pheromone made by female *Drosophila melanogaster,* a dipteran fly, is of particular interest, since it is found exclusively in females which have copulated [3], suggesting the possibility that the inhibitor transferred from the male to the female during mating. Courtship-inhibiting pheromones produced by males have previously been identified in three species of coleopteran and lepidopteran insects [4–6]. In this report, we show that *D. melanogaster* males also make a chemical inhibitor which is not, however, identical to the inhibitory pheromone found in mated females. Besides being the first inhibitor observed in a dipteran male, this pheromone is the first inhibitor identified by observations of the behavior of a genetically mutant animal.

Flies expressing a mutation in the *olfC* gene on the X chromosome fail to respond to aldehydes and acetone, which attract normal flies from a wild-type Canton-S strain [7–8]. Furthermore, *olfC* males, unlike Canton-S, do not court each other more vigorously in the presence of volatile compounds extracted from virgin females than they do in the absence of an extract from sexually attractive flies (Table I). In this respect *olfC* males behave like males expressing another X-linked mutation, *smellblind,* which also affects responses to organic chemicals [9] and sex-stimulating pheromones [10], but is not allelic to *olfC* (L. Tompkins and J. C. Hall, unpublished). However, *olfC* males court each other vigorously regardless of the presence of extract from virgin females (Table I), a phenotype not observed in *smellblind* males [10]. Unlike mutant *fruitless* or very young Canton-S (wild-type) males, which produce sex-stimulating pheromones [10], *olfC* males are not courted by Canton-S males (Table II). In contrast, *olfC* males do court Canton-S males (Table II), suggesting that the mutant males are unable to respond to an inhibitor produced by male flies.

To test this assumption, the effect of volatile compounds extracted from normal males on the courtship stimulated by a virgin female fly was ascertained (Table II). Canton-S males tested without extract court virgin females vigorously. In con-

Table I. Courtship between two males. Stocks of Canton-S ("wild-type") and *olfC* flies were maintained under standard conditions [10]. Males were collected from bottle cultures as young adults (1–8 h post-eclosion), aged individually in culture vials for 3–4 days, then transferred without etherization to small plastic mating chambers [11] (volume ca. 0.2 cm^3). The courtship index (C.I.), defined as the percent of time during which either or both males performed any courtship behavior [10], was determined for a 10 min period while the flies were observed at 10× magnification. For experiments to ascertain the effects of volatile compounds on courtship, Canton-S virgin females were collected as young adults, aged for 3–6 days, then frozen at −20 °C in methylene chloride. 1.5–2 grams of flies were boiled in distilled water with simultaneous distillation and extraction of volatile compounds into 500 ml of methylene chloride [12]. The distillate was evaporated to ca. 1 ml, then diluted 1:500 with ethanol to obtain a concentration which is maximally effective in stimulating courtship between normal males [10]. Five microliters of the diluted extract was applied to a square of filter paper, which was airdried for 1–2 min, then placed on the bottom of the mating chamber immediately before flies were introduced into the chamber.

Genotype of males	Extract	Number of tests	C.I. + S.E.M.
wild-type	none	20	2 ± 1
wild-type	virgin female	10	14 ± 1
olfC	none	10	16 ± 2
olfC	virgin female	20	12 ± 2

* *Present address:* Department of Biology, Temple University, Philadelphia, PA 19122.

Reprint requests to Dr. L. Tompkins.

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Table II. Courtship of individual males. Canton-S ("wild-type") and *olfC* males were collected, aged, and tested as described in the legend to Table I. The courtship index (C.I.) was determined for one male in the presence of another fly, the stimulus. If the stimulus fly was male, it was marked with a small clip on one wing to distinguish it from the male whose behavior was observed. Canton-S virgin females were 18–24 h old when used as stimulus flies, since young virgin females elicit vigorous courtship but do not copulate [13], making it possible to base all courtship indices on 10 min observation periods. Canton-S mated females for behavior tests were collected as virgins, aged for 4 days, observed to copulate with Canton-S males, then tested 24 h later. Volatile compounds were extracted from Canton-S males, 3–6 days old, as described in the legend to Table I. Concentrated extract was diluted 1:5 with ethanol to yield a concentration which is maximally effective in inhibiting courtship (L. Tompkins and J. C. Hall, unpublished). Extract from mated females was prepared from Canton-S female flies collected from bottles in which equal numbers of males and females were maintained for 3 days after being transferred 0–2 days post-eclosion. Over 99% of females maintained according to this procedure are fertile [3]. Concentrated extract from mated females was diluted 1:5 with ethanol to yield a concentration which is maximally effective in inhibiting courtship [3]. Solvent is 1:5 methylene chloride:ethanol.

Genotype of male	Stimulus fly	Extract	Number of tests	C.I. + S.E.M.
wild-type	*olfC* male	none	20	2 ± 0
wild-type	virgin female	none	20	88 ± 2
wild-type	virgin female	male	20	9 ± 2
wild-type	virgin female	mated female	20	49 ± 5
wild-type	virgin female	solvent	20	87 ± 1
wild-type	mated female	none	20	20 ± 5
olfC	wild-type male	none	20	14 ± 2
olfC	virgin female	none	20	46 ± 4
olfC	virgin female	male	20	48 ± 4
olfC	virgin female	mated female	20	11 ± 2
olfC	virgin female	solvent	20	53 ± 3
olfC	mated female	none	20	9 ± 2

trast, normal males in the presence of volatile compounds extracted from males show a 10-fold reduction in the amount of courtship performed. Although *olfC* males court virgin females for less time than normal males, presumably because the mutant males are unable to respond to courtship-stimulating pheromones produced by female flies, their courtship is unaffected by the presence of extract from males. Since solvent without volatile compounds has no effect on the courtship of either Canton-S or *olfC* males, these results demonstrate that males produce volatile courtship-inhibiting pheromones to which *olfC* males do not respond.

As previously described [3], normal males court females which recently mated for less than half the time that they court virgin females. Furthermore, extract from mated females stimulates less than half as much courtship between males as volatile compounds from virgin females [3], indicating that the decrease in courtship observed with mated females is primarily a response to chemical cues. After copulation, females produce less sex attractant and also begin to make an inhibitory pheromone [3]. To determine whether *olfC* males respond to the inhibitor from mated females, the mutant males were observed with females which had recently copulated (Table II). Like Canton-S males, *olfC* males court mated females for much less time than they court virgins. Since the mutant males are not stimulated by the sex attractant made by female flies (Table I), the decrease in courtship observed with mated females is a response to the inhibitor, rather than a reaction to the decrease in the amount of sex attractant produced after copulation. To confirm this, *olfC* and Canton-S males were tested with virgin female flies and volatile compounds extracted from mated females (Table II). Normal males court virgin females for less time in the presence of mated female extract [3]. *olfC* males also show a decrease in courtship, indicating that the mutant males are able to respond to the inhibitor produced by mated females.

Since *olfC* males are inhibited by mated female pheromones but are unaffected by volatile compounds from males, the two inhibitors are not the same compound. Experiments are in progress to identify the chemical nature of the inhibitory materials and determine their interactions with the sex-stimulating pheromones made by *Drosophila* males and females.

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