MALE COURTSHIP IN DROSOPHILA: THE CONDITIONED RESPONSE TO IMMATURE MALES AND ITS GENETIC CONTROL

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ABSTRACT

Experimentally naive male Drosophila melanogaster respond to sexually immature males with intense courtship. However, this response decreases markedly in a short period of time, and "experienced" males then avoid further courtship with immature males for 4 hr. This subsequent inhibition of the courtship response is specific to immature males; the response to virgin females remains intact. This experience-dependent modification in courtship behavior is designated as "conditioned courtship." Seven mutant strains isolated for their inability to express avoidance conditioning (on criteria independent of courtship) were all found to be mutant with respect to expression of conditioned courtship. The potential application of this phenomenon to mosaic analysis of these mutations is posed. Other results indicate that immature males constitutively release a chemical signal that is sufficient for the expression of conditioned courtship. The interpretation of conditioned courtship as a component of fitness is discussed.

SEXUALLY immature male Drosophila melanogaster have the surprising capacity to stimulate mature males to court them and to attempt copulation with them (Cook and Cook 1975; Jallon and Hotta 1979). Soon after emergence from the pupal case, males do not court or copulate with females and, hence, may be described as "immature." Many features of the courtship activity between a mature male and an immature male are reminiscent of the more familiar intersexual encounter. For example, courtship behavior (Bastock and Manning 1955) occurs in virtually all isolated pairs, and within the first 10 min of pairing, the mature male spends about half the time in active courtship (Tompkins, Hall and Hall 1980). Immature males respond to this courtship with wing flicks and attempted escape. Although elucidation of the benefit of such courtship remains problematic (Hall 1981), it seems reasonable to suppose that protracted courtship bouts with immature males could be disadvantageous in terms of fitness.

We begin by presenting evidence for the existence of a mechanism whereby males limit further courtship with immature males. In brief, the intensity of the courtship response of a mature male toward an immature male decreases in a short period of time, and for the next several hours such an experienced male

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cannot be induced as before to court an immature male. During this time there is a normal courtship response to a virgin female. The behavioral difference between a male that has previously courted an immature male and an experimentally naive male is striking: only naive males court when paired with immature males. This difference is easily induced, is reliably reproduced, and can be readily quantified. For these reasons the phenomenon offers an especially sensitive and facile assay for the effects of allelic substitutions on behavior. Results to be described deal in part with the effects of specific mutations on the capacity of flies to express this behavioral change. As such they provide the starting point for other studies dealing with the gene control of higher behaviors, particularly mosaic analyses, which can potentially give insight on the organization of complex behaviors within the central nervous system (CNS) (cf. VON SCHILCHER and HALL 1979).

Finally, our new results invite comparisons with earlier studies of gene-controlled effects on males that have courted fertilized females. For in that situation as well, male courtship is behaviorally rejected and copulation is avoided, courtship activity soon diminishes, and there occurs a modification in the courtship response lasting 2–3 hr (SIEGEL and HALL 1979). We discuss the possibility that there have evolved two similar mechanisms that serve to limit males’ persistent courtship of flies unreceptive to copulation.

MATERIALS AND METHODS

Fly stocks, culture, collection and test conditions

Normal mature males were generated from a stock of the Canton-S strain (hereafter called wild type) provided by J. C. HALL; immature males were also generated from this stock. Males representing a wild population of D. melanogaster were generated from “gel-16,” a stock established in the laboratory in 1979 from a single inseminated female trapped near Gelendzik, USSR; provided by C. TAYLOR.

Mutant males were generated from the following stocks. dnc¹ and dnc²—X-linked alleles independently derived from the Canton-S strain on the basis of inability to associate a chemical odorant with electric shock (DUDAI et al. 1976). dnc alleles map to the 3D region of the X chromosome (BYERS, DAVIS and KICER 1981). The dnc¹ strain is maintained homozygous; dnc² females are sterile, and this strain is, therefore, maintained by crossing mutant males to C(I)DX, yf females (hereafter called attached-X). Dunce mutants have increased levels of cyclic AMP compared with wild type due to defect in the enzyme cyclic-AMP phosphodiesterase (BYERS, DAVIS and KICER 1981).

dnc³M¹—an independently derived allele of dnc maintained on an X chromosome that carries the additional recessive markers y (yellow cuticle 1-0.00), cv (crossveinless wings 1-13.7), v (vermilion eye color 1-33.0) and f (forked bristles 1-56.7). This dnc allele was isolated on a female sterility criterion and subsequently found to perform poorly in the shock-odorant avoidance conditioning test (BYERS, DAVIS and KICER 1981).

y cv v f—an X-chromosome mapping stock carrying the same genetic markers as the dnc³M¹ stock, but with the dnc¹ allele.

cabbage (cab, genetically unmapped), rutabaga (rut, 1-46; M. LIVINGSTONE, unpublished results), turnip (tur, near carnation, 1-62.5; BOOKER and QUINN 1981)—mutations derived from the Canton-S strain and isolated on the basis of inability to associate a chemical odorant with electric shock (ACEVES-PiNA and QUINN 1979; QUINN, SZIBER and BOOKER 1979; DUERR and QUINN 1982); rut flies have decreased levels of cyclic AMP compared with wild type (M. LIVINGSTONE, personal communication), amnesiac (amm, near f)—a mutant that conditions as wild type in shock-odorant tests but “forgets” rapidly (QUINN, SZIBER and BOOKER 1979).

Females used in tests for male courtship were attached-X.
Flies were maintained at 25°C on a medium of cornmeal, sucrose, dextrose, wheatgerm, yeast and agar. All males and females (except as noted for immature flies) were collected under light ether anesthesia 0-10 hr after eclosion; males were isolated in food vials and females grouped ten per vial. Flies were kept for 5 days in a room maintained at 25 ± 1°C on a cycle of 12-hr light and 12-hr dark and then used in experiments.

Immature wild-type males were collected by aspiration and utilized in experiments within 6 hr after eclosion. Certain courtship tests required the use of sexually immature females, which stimulate high levels of courtship but do not copulate (MANNING 1967). Newly emerged, attached-X females were collected under light ether anesthesia and aged 14-20 hr before use in such tests.

Experiments were conducted in the room described and were completed within the first 8 daylight hours of the light-dark cycle. All observations were made on flies that had been gently aspirated into the cylindrical chambers (0.4 cm³) of a “mating wheel” (HOTTA and BENZER 1976). Since relative humidity was not controlled in the observation room, a circlet of 42 Whatman filter paper dampened with a droplet of distilled water was placed in each chamber to provide a standard, moist environment during experiments.

Quantification of male courtship: responses and interstrain comparisons

Courtship response of each test male was quantified as a courtship index (CI) (e.g., SIEGEL and HALL 1979; TOMPKINS, HALL and HALL 1980), formulated as the percentage of total time in active courtship behavior by a male within an observation period. Courtship was assessed visually and recorded on a stopwatch. In tests for conditioned courtship, CIs were determined as the response of test males to ether-immobilized immature males; for each such test the observation period was terminated when the etherized fly regained mobility (about 8-10 min). Courtship-stimulating flies that do not move most reliably reveal conditioning effects (discussed in SIEGEL and HALL 1979). An index of conditioned courtship ($\lambda_c$) was determined for each strain as a ratio formed by the mean CI determined for a first set of males courting immature males for the first time, and the mean CI of a second set of males that had previously courted immature males, given by:

$$\lambda_c = \left( \frac{1 - CI_2}{CI_1} \right) \times 100$$

where $CI_1 =$ mean CI for 20 males in their initial courtship experience with immature males (after pairing with other mature males); $CI_2 =$ mean CI for 20 males in their second courtship experience with immature males (after previous pairing with immature males); $\lambda_c =$ index of conditioned courtship response toward immature males.

$\lambda_c$ values near 100 indicate a $CI_1$ much larger than the corresponding $CI_2$ and are, thus, interpreted as effective conditioned courtship. Conversely, $\lambda_c$ values near zero indicate virtually no conditioned courtship.

The components of $\lambda_c$ were determined as follows. $CI_1$ was always established by pairing, in 20 separate trials, a courtship-naive test male with a mature wild-type male for a 30-min period; these males were distinguishable from test males since their wing tips had been clipped during collection. Each test male was then separated from the marked male by gentle aspiration, transferred to a clean chamber, and then CI with an ether-immobilized immature male determined. The mean value of these tests measures a strain’s initial, hence, control response to immature males ($CI_1$). To test for a modified courtship response, individual CIs with immobilized immature males were again recorded after pairing a second group of 20 males with immature males for 30 min. The mean value for these tests quantifies a strain’s subsequent, hence, experimental response to immature males ($CI_2$). $\lambda_c$ values thus obtained for mutant strains were then compared with the $\lambda_c$ values for wild type.

Statistics

Comparison of responses for significant difference, whether between CIs or $\lambda_c$ values was accomplished by Student’s two-tailed t-test. All values are reported as mean ± SEM. The SEM for a $\lambda_c$ value was determined from a formula for the variance of a parameter $z$ (i.e., $\lambda_c$), as a function of $x$ and $y$ (i.e., $CI_1$ and $CI_2$) with known variances $V_x$ and $V_y$ (equation A.9.14 of CROW and KIMURA 1970).
Duration of modified courtship behavior toward immature males

To determine the time period conditioned males remained unresponsive to immature males, mature males were paired with immature males for 30 min and then transferred to fresh chambers of a mating wheel and allowed to "rest" in isolation for predetermined periods of time. These males were then tested as described.

Characterization of the cues leading to modified behavior toward immature males

A preliminary experiment was carried out to test whether the rejection behavior of an immature male brings about courtship modification in a mature male. A special λ, was determined for normal and mutant males as described but with the following difference. Rather than the usual pairing with intact, freely moving males, test males were instead placed individually with single males whose CNS had been disrupted. This disruption was effected just prior to pairing by first immobilizing males with CO₂ and then crushing their heads with fine forceps (such preparations remain viable for hours and can demonstrate conditioned behavior; BOOKER and QUINN 1981). "Headless" immature males continue to elicit courtship from mature males but perform no overt rejection behavior in response to this courtship. CI, and CIp in this case were determined as the response to immature males after first pairing with either headless mature males or headless immature males.

A final experiment was undertaken to ascertain whether immature males liberate chemicals sufficient to elicit courtship and/or conditioned response in wild-type males. Immature males were isolated for 30 min in observation chambers containing the usual circlet of moist paper, as well as a ringlet of 42 Whatman paper covering the vertical wall. They were then removed, and pairs of mature wild-type males were placed in each chamber, and the total time the males courted each other in a 10-min period was recorded. For other tests, mature wild-type males were isolated into such chambers for 10 min and then moved to fresh chambers and tested in the usual way for courtship with immature males. Results of both sets of tests were compared with those from other tests in which the filter-papered chambers had housed mature males, rather than immature males.

RESULTS

Experience-dependent modification of male courtship: the response to immature males

Twenty wild-type males were singly paired with immature males for a 30-min period, and courtship behavior was recorded. Within the first 10 min after pairing, each of the mature males had directed the standard components of courtship behavior at an immature male. In accord with previous results, the mean CI for this first 10 min interval was 67 ± 4 (cf. JALLON and HottTA 1979; TOMPKINS, HALL and HALL 1980). We observed, however, that courtship activity rapidly decreased with time, such that in the final 10-min interval the CI was 17 ± 4; half the males displayed virtually no courtship at all. A second group of mature males was paired for 30 min with immature virgin females; for this group there occurred no significant decrement in the mean CI between the first and last 10-min intervals (76 ± 3 and 73 ± 3).

Direct experimental evidence for behavioral modification as a result of courtship with immature males was provided when mature males were first isolated with immature males for 30 min and then tested for courtship activity with an immobilized immature male. The results (Table 1) reveal a dramatic effect on the subsequent behavior of the mature males. The CI for this group was 1 ± 0.3; only six of 20 males courted immature males during this final test. During these tests, we noticed that it was not the performance of the individual courtship activities and their sequence that was changed, but rather it was the
duration of individual courtship bouts and their frequency that was decreased. In comparison, males that had courted immature males expressed no decrease in the quantity of courtship time directed toward subsequent virgin females, a result that demonstrates the specificity of the effect; there is no evidence for a general inhibition of the courtship response. Finally, data in the top rows of Table 1 strongly suggest that neither the mere presence of another fly nor confinement in an observation chamber for 30 min are stimuli sufficient to bring about a noticeable modification in courtship behavior. Instead, these results support the idea that courtship with an immature male is an experience that can predictably cause a specific change in subsequent courtship response. For simplicity, we shall refer to this altered response as “conditioned courtship.”

Mutations known to disrupt shock-avoidance conditioning and its retention also disrupt the expression of conditioned courtship

To gain initial insight on the basis for the conditioned courtship response to immature males, we compared the wild-type expression of this phenotype with that in mutant strains known to be defective with respect to the ability to associate a chemical odorant with electric shock (see MATERIALS AND METHODS). Since major factors leading to wild-type response in both tests appear to be chemosensory (see next section and DISCUSSION), it seemed reasonable to predict a high correlation of mutant phenotype in these strains when tested for capacity to display conditioned courtship, should there be common elements of sensory processing. Assessment and comparison of λc values for the various strains revealed just such a close parallel in response. All shock-avoidance mutants (with the exception of cab) displayed significantly different λc values when compared with wild type (Table 2). Moreover, the λc values obtained for the mutant strains indicate a gradation of response. We observed that those mutant strains that demonstrated a λc value significantly greater than zero also showed a decline in response to active immature males during the 30-min conditioning period (results not presented). On the other hand, males from the dnc' and dnc² strains showed no significant decline in response to active immature males (results not presented) and their resultant λc values were essentially zero.

It is noteworthy that there was virtually no expression of conditioned courtship by males carrying any of three mutant dnc alleles, maintained in strains of varying genetic background (see MATERIALS AND METHODS). Thus, the y cv v f
strain, unselected for any defect in conditioning, demonstrated conditioned courtship that was equivalent to wild type; however, substitution of the dnc$^+$ allele by dnc$^{M14}$ resulted in the disruption of this capacity to display conditioned courtship. The fact that mutations known to disrupt avoidance conditioning so often also affected the expression of conditioned courtship suggested that the present phenomenon might also exemplify avoidance conditioning. Results to be presented next address this point.

Mutant amn flies are comparable to wild type in that they respond in a normal way to avoidance conditioning, but tests carried out after "training" show that memory is abnormally short (QUINN, SZIBER and BOOKER 1979). However, amn males are clearly mutant with respect to conditioned courtship (Table 2). The cabbage strain (originally isolated as defective in acquisition of avoidance conditioning, ACEVES-Piña and QUINN 1979) was found to be equivalent to wild type in courtship conditioning (Table 2). But the conclusion that cab and wild type are alike was based solely on results of tests carried out just after the initial experience with immature males. Determination of how long conditioned males remained unresponsive to immature males revealed a significant difference between the two strains. Whereas the "experienced" wild-type males avoided courtship of immature males for 4-5 hr, cab males had a high CI within 30 min (Figure 1). Males from the y cv v f strain were also tested for duration of conditioned courtship and were found to be similar to wild type. In summary, shock avoidance studies show that amn can achieve the wild-type level of avoidance conditioning but memory is defective, whereas cab is conditioning defective. Just the converse of these relationships is true for these two mutant strains with respect to conditioned courtship. The fact that mutations at certain loci can disrupt both avoidance conditioning and conditioned courtship suggests that gene-controlled processes or structures are common to both phenomena.

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**Table 2**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cl$_1$ ± SEM</th>
<th>Cl$_2$ ± SEM</th>
<th>λ ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton-S</td>
<td>35 ± 5</td>
<td>1 ± 0.3</td>
<td>98 ± 1</td>
</tr>
<tr>
<td>y cv v f</td>
<td>24 ± 7</td>
<td>4 ± 2</td>
<td>83 ± 8 (NS)</td>
</tr>
<tr>
<td>y dncM14 cv v f</td>
<td>46 ± 6</td>
<td>37 ± 5</td>
<td>21 ± 11$^a$</td>
</tr>
<tr>
<td>dnc$^+$</td>
<td>43 ± 6</td>
<td>36 ± 6</td>
<td>17 ± 15$^a$</td>
</tr>
<tr>
<td>dnc$^+$</td>
<td>62 ± 6</td>
<td>65 ± 7</td>
<td>−5 ± 13$^c$</td>
</tr>
<tr>
<td>amn</td>
<td>60 ± 7</td>
<td>39 ± 5</td>
<td>34 ± 10$^f$</td>
</tr>
<tr>
<td>cab</td>
<td>36 ± 8</td>
<td>4 ± 3</td>
<td>90 ± 9 (NS)</td>
</tr>
<tr>
<td>rut</td>
<td>35 ± 7</td>
<td>14 ± 4</td>
<td>59 ± 11$^f$</td>
</tr>
<tr>
<td>tur</td>
<td>59 ± 5</td>
<td>29 ± 5</td>
<td>51 ± 8$^f$</td>
</tr>
</tbody>
</table>

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$^a$ Twenty males were in each group. NS = not significant, $P$ greater than 0.05 when compared with Canton-S (t-test).

$^b$ Significant at the 10$^{-4}$ level when compared with y cv v f (t-test).

$^c$ Significant at the 10$^{-4}$ level when compared with Canton-S (t-test).
Chemical substances released by immature males can induce both courtship response and conditioned courtship

Immature males with disrupted CNS (see MATERIALS AND METHODS) are courted in a normal manner by mature males but do not behaviorally reject this courtship with the usual wing flicks (cf. Tompkins, Hall and Hall 1980). If conditioned courtship is independent of this behavioral response, then mature males that have courted “unresponsive” immature males should thereafter avoid courtship with other immature males. In agreement, wild-type and cab males give \( \lambda_c \) values in Table 3 statistically no different from those in Table 2. Dunce males consistently fail to express conditioned courtship, whether paired with active or immobilized immature males (Tables 2 and 3). The next experiment provided a test of the notion that chemical substances play a role in conditioned courtship.

Mature, wild-type males were isolated into chambers that had previously contained either immature males or other mature males. After 30 min, each male was tested for courtship response to an immature male. The CI for males “rested” in chambers previously occupied by other mature males was not statistically different from other control values measured for wild type (CI\(_1\) = 37 ± 7). In contrast, males “rested” in chambers that had been occupied by
immature males thereafter courted immature males at a reduced level (CI = 12 ± 3). Because the resultant \( \lambda_c = 69 \pm 9 \) is a value not statistically different from that based on pairings with behaviorally inactive males (Table 3), these results support the hypothesis that chemosensory factors alone are the necessary and sufficient basis for the subsequent modification of behavior toward immature males.

The foregoing result led us to wonder whether we might demonstrate the release of courtship-stimulating substances by immature males. If so, then two mature males which do not ordinarily court each other would be induced to do so when placed in a chamber previously occupied by an immature male. When pairs of males were placed in chambers that had contained individual immature males, the result was striking: a high level of courtship interaction was observed in all males (CI = 19 ± 4, \( n = 10 \) pairs). Control pairs, introduced into chambers previously housing single mature males, courted very little (CI = 1 ± 1).

**DISCUSSION**

Courtship in Drosophila has been described as an invariant sequence of behaviors, beginning with recognition between courting male and prospective mate and culminating in copulation. We have demonstrated that elicitation of this response can be modified and is, therefore, not rigidly fixed, for mature males that have courted immature males then avoid further such courtship for a 4- to 5-hr period. Moreover, results obtained with the mutant strains demonstrate that this experience-dependent courtship avoidance depends on gene-controlled processes and/or anatomical features.

Siegel and Hall (1979) described a decrease in courtship response to virgin females after experience with fertilized females (which stimulate courtship but are unreceptive to copulation). The most striking result of this experience is the prolonged avoidance to court other fertilized females, lasting 13–15 hr (D. A. Gailey, unpublished results); the reduced response to virgin females is transient by comparison. As we have demonstrated, during the period in which conditioned males no longer court immature males, the response to virgin females remains unaltered. Thus, it seems a likely prediction that within a population, males that express these courtship avoidance mechanisms may enhance their probability of locating and copulating with receptive females. If so, then con-
ditioned courtship may be interpreted as a fitness component. We have obtained the following experimental results that are in support of this interpretation. (1) Mature males representing a wild population (see MATERIALS AND METHODS) initially court immature males intensely ($C_1 = 64 \pm 6, n = 20$) but are readily conditioned to avoid courtship with subsequent immature males of that strain ($C_2 = 2 \pm 1, n = 20; \lambda_c = 97 \pm 2$); conditioned males still court virgins at the usual, high level ($C_I = 68 \pm 5, n = 20$). (2) Courtship-naive, wild-type males placed for 30 min in small "populations" of nine immature males and one virgin female show about a ninefold greater $C_I$ with the immature males; the time to copulation is much protracted compared with males individually paired with virgin females. This is in striking contrast to the response of wild-type males that had previously courted immature males. Experienced males spend much less time courting immature males and achieve copulation much more rapidly. On the other hand, dunce males, whether naive or experienced, continue to court immature males with persistence and take as long to achieve copulation as naive, wild-type males (D. A. GAILEY, unpublished results).

Although similar in a general way, the conditioned responses to immature males and to fertilized females differ in important details. First, the response to virgin females remains intact for males that have courted immature males but not among males that have courted fertilized females. Second, our results suggest that immature males constitutively liberate a chemical cue that can inhibit the courtship of mature males; fertilized females condition male courtship only upon the courtship-induced release of a necessary cue (D. A. GAILEY and R. W. SIEGEL, unpublished results). Third, the two conditioned courtship responses are differentially disrupted by gene mutation. The cab mutation upsets the acquisition and $amn$ the retention of female-determined courtship modification (SIEGEL and HALL 1979; D. A. GAILEY and R. W. SIEGEL, unpublished results); these two mutations have exactly the reverse effects with respect to male-determined courtship modification.

To explain the results in hand and as a guide to further analysis of the underlying basis for the conditioned response to immature males, we pose the following working hypothesis: that immature males constitutively release courtship-excitative pheromone (cf. TOMPKINS, HALL and HALL 1980) and that mature males habituate to this chemical cue. We distinguish habituation, or the CNS-mediated decline in response to a stimulus (e.g., PINSKER et al. 1970), from adaptation, or decline in response to a stimulus by peripheral sensory cells (cf. SEABROOK 1978). The remainder of the discussion deals with observations that support this hypothesis and with observations that might provide decisive tests.

Volatile compounds extracted from females and from immature males can induce courtship between mature males, but the gas chromatographic profiles of such extracts are very different (TOMPKINS, HALL and HALL 1980). In full accord, we have shown that substances that are constitutively released by immature males are sufficient to stimulate abnormally high levels of courtship between two mature males. In addition, the courtship response to virgin females does not decline in time as does the response to immature males, nor is the modified courtship response to immature males accompanied by an altered
response to virgin females. These findings are incompatible with the notion that females and immature males produce identical pheromones.

It has been suggested that fertilized females present simultaneously to courting males both excitatory and inhibitory courtship signals (Siegel and Hall 1979) that are putative pheromones (Tompkins and Hall 1981a). Furthermore, only males that carry out courtship behavior in the presence of these pheromones express conditioned courtship (R. W. Siegel, D. A. Gailey, L. Tompkins and J. C. Hall, unpublished results). Thus, the modified response to females appears to have as its basis an association established in courting males between constitutive, excitatory pheromone and inducible, inhibitory pheromone.

The situation with respect to immature males appears less complex. Our results indicate that substances constitutively released by immature males not only induce males to court but are sufficient to bring about a subsequent decrement in response to immature males; this conditioning cue need not be induced by courtship. Although Tompkins and Hall (1981b) have presented genetic evidence that mature males release a courtship-inhibitory pheromone, we found no evidence to suggest that this putative substance plays a significant role in conditioned courtship, for mature males that were first paired with other mature males expressed the same courtship response to immature males as did mature males maintained in isolation (Table 1). Although an inducible cue and courtship per se are necessary components in the conditioned response to females, they are not essential elements in the conditioned response to immature males. These basic differences in the two phenomena strongly suggest that their underlying mechanisms differ, and as such, favor the provisional conclusion that the conditioned courtship response to immature males is a habituation-like process. However, rigid interpretations are now premature. We do not exclude the alternative and more complex hypothesis that the conditioned response to immature males involves an association established between excitatory pheromone and a constitutively released, inhibitory cue.

Long-term sensory adaptation to courtship pheromone has been reported in the male silkworm moth Bombyx mori; Kaissling (1972) has shown by electroantennogram (EAG) analysis that after initial adaptation to courtship pheromone there is a 4-hr refractory period before antennal sensory nerves completely recover EAG responses. Adaptation as the mechanism for our phenomenon could be ruled out by utilizing fate-mapping analysis (e.g., Hotta and Benzer 1972) to identify primary anatomical correlates of conditioned courtship response. Application of the Drosophila associative conditioning mutations to mosaic analysis would also be of particular interest in light of the following discoveries. (1) The mutations dnc, rut and tur, isolated for their inability to express associative conditioning, are also deficient in habituation (Duerr and Quinn 1982). (2) The conditioning defects in the strains dnc and rut are associated with altered levels of cyclic AMP (Byers, Davis and Kiger 1981; M. Livingstone, personal communication), which may be a significant factor in learning processes (Klein and Kandel 1980). Such experiments require tests on individual mosaic flies (in contrast to flies tested en masse in shock-odor experiments, e.g., Dudai et al. 1976). Although Booker and Quinn (1981) have demonstrated that individual flies can be conditioned to flex or extend a leg to
avoid electrical shocks, the high frequency (20-45%) of mutant (cab, dnc, tur) individuals that express normal conditioning poses a difficulty for mosaic analysis. On the other hand, virtually all wild-type males express conditioned courtship; dnc males do not. Moreover, the phenotype is easily demonstrated with intact, freely moving flies. Thus, we stress the potential application of conditioned courtship to the further investigation of the Drosophila-conditioning mutants; stocks that will generate males mosaic for dnc and wild-type tissues are currently being prepared.

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LITERATURE CITED


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