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NEUROGENETICS OF COURTSHIP IN *DROSOPHILA*

(courtship and mating, behavioral mutants, Drosophila *gynandromorphs, visual and olfactory defects, circadian rhythms, learning and memory, neurotransmitter mutants)*

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SUMMARY

Courtship behavior in Drosophila *is programmed by the action of genes that control the development and function of the nervous system. Several genes have been identified that appear to be involved in reproduction, because the mutations at these loci have specific defects in courtship. The mutations affect steps in the courtship pathway ranging from the early stage of wing vibration song to the final stage of copulation. Other single-gene mutants, not isolated on the basis of defective courtship, have been used to dissect further the behavioral pathway. All of these behavioral mutants exhibit particular defects in courtship, including expected abnormalities of general reproductive abilities in mutants that cannot see or smell, and more subtle changes caused by other mutations. The latter have revealed connections between courtship and biological oscillations or experience-dependent phenomena. These higher behavioral mutations are likely to affect the central nervous system.*

Other experiments on genetic variants affecting the central nervous system have involved gynandromorphs. From courtship tests of these mosaics, the presence of male tissue in a portion of the brain has been correlated with performance of the initial stages of courtship. If a gynandromorph is to exhibit later steps of courtship, such as normal song or attempted copulation, there must be male neurons in the thoracic nervous system as well as in the brain. Neural tissues of different sex-chromosome genotype have been identified in these mosaics, using enzyme mutations that mark internal cells. Some of these mutations, in addition, lead to specific neurochemical defects in the metabolism of the neurotransmitter acetylcholine. When such mutations are expressed in the mosaics, they not only mark

tissues of different genotype, but they can also lead to specific defects in courtship behavior.

INTRODUCTION

Reproductive behavior in *Drosophila* has been studied genetically for many decades, beginning with the experiments of STURTEVANT (1915). Recently, genetic approaches toward dissecting courtship and mating have involved specific, genetically tractable variants that affect, or are likely to affect, excitable cells and tissues. Previous work tended to concentrate on mutations affecting the external morphology or on genetically complex strain differences and interspecific comparisons (e.g. MANNING 1965, EWING & MANNING 1967, GROSSFIELD 1975, EHRMAN 1978).

In this paper we discuss results from genetic dissection of *Drosophila* reproduction involving (1) single-gene mutants isolated on the basis of *defective courtship*; (2) mutants with *defective sensory perception or processing*; (3) mutants with known or strongly suspected *abnormalities in the structure and function of the central nervous system* (CNS); (4) *genetic mosaics*, with internal cells and tissues marked and whose nervous systems are mixed in sex genotype, or that express a neurologically defective mutation in a known part of their CNS.

Several of these genetic variants, such as the sex mosaics or mutants isolated on criteria of defective reproduction, appear to involve genotypes that specifically perturb the programming of fixed action patterns, in courtship, that are controlled by particular portions of the fly's genome. Other of the mutants, originally isolated by behavioral geneticists investigating a variety of questions on the development and function of the nervous system, were found later to perturb courtship in informative ways. Surprisingly, perhaps, some of the experiments using these "general behavioral" mutants have undermined the idea that courtship and mating in *Drosophila* involve completely fixed action patterns.

In general, this neurogenetic approach has also shown that mutants and mosaics are excellent tools for obtaining experimental information on the neural control of reproduction. We have been able to manipulate the flies in ways that leave them free of the non-specific debilitative effects associated with other kinds of perturbations, such as surgery or ablation, injection of drugs, and tethering or stimulation with electrodes. In addition to serving as tools, the mode of action of several of the genes whose mutants have been used will hopefully turn out to be intrinsically interesting. Finally, the genetic variants that were discovered on one behavioral criterion and then were shown to affect a very different behavior have revealed some startling connections between courtship and other features of higher behavior in *Drosophila*.

MUTANTS ISOLATED BECAUSE THEY COURT DEFECTIVELY

Drosophila males and females proceed along a courtship pathway in their reproductive behavior (reviewed by SPIETH 1974). This pathway is shown in FIGURE 1. Most of the designated steps refer to overt actions, the most conspicuous of which are wing extension (display and vibration of one wing at a time) and attempted copulation performed by the male. In *D. melanogaster* the female does not exhibit obvious, stereotyped behaviors during at least the early stages of courtship. Indeed, she need perform no behavior in order to stimulate many and sometimes all of the male's actions (discussed in detail later). Several of the male's actions are easily observed with the naked eye, as we routinely do using small plastic courtship chambers (see HALL 1978a, 1979). In some of these tests, more detailed observations are done with a dissecting microscope, in order easily to see relatively subtle behaviors such as tapping (of the female's abdomen by the male's forelegs) and licking (contact of the female's genitalia by the male's extended proboscis). The other salient features of these techniques involve (1) rearing the flies at 25°C in humidified incubators, under a 12 hr:12 hr light:dark (LD) regime; (2) collection of virgin male and female adults under ether anesthesia, followed by 3-5 days of storage in food vials (one adult per vial), under the conditions just noted; and finally (3) behavioral testing in these same conditions (between midmorning to early afternoon of the LD cycle), using the courtship chambers that almost always contained two virgin flies. Additional more quantitative aspects of the behavioral testing are described in subsequent sections.

Figure 1. Behavioral pathway for the courtship of *Drosophila melanogaster*. Mutants noted above the sequence perturb or block the indicated steps.

The sequence of events in courtship can be thought of as a pathway, primarily because they essentially always occur in the indicated order. Thus, males have not been observed to perform licking and attempted copulation without having previously extended their wings to produce the courtship song. It is true, however, that tapping does not always occur, especially in some wild-type strains of this species (BURNET & CONNOLLY 1974). Also, the courting flies can return to an earlier step in courtship. They might break off courtship after an unsuccessful attempt to copulate, and then, when they resume, the male might start in again at the stage of wing extensions.

Five mutants have been isolated that each disrupt a stage of the courtship pathway, or possibly even block that stage. As we will discuss below, each mutant is apparently due to a change in a single gene, and each has ostensibly normal viability and morphology. Morphological observations have been made by care-

ful examination of the external morphology, sometimes using scanning electron microscopy (see below), and by serial sectioning of mutant individuals at 5 μm (after embedding the adults in plastic) or 10 μm (for frozen sections). These sections did not show any obvious defects in internal tissues, when examined at ca.200-400x; but the possibility that there are relatively subtle abnormalities of the internal (or even the external) morphology in these courtship mutants has not been ruled out.

The mutants were each isolated based on abnormal male behavior, and they are listed in the order of steps in courtship that they affect: *cacophony* (defective courtship song); *celibate* and *fruitless* (virtually no attempted copulation); *coitus interruptus* (too-short duration of copulation); and *stuck* (frequent inability to disengage after copulation). These basic defects in the five mutants are summarized both in the diagram of the courtship pathway (FIGURE 1) and in Table 1. The latter lists the results of testing for the performance of each courtship action, ranging from following of females to the end of copulation, either in males expressing an aberrant genotype or in wild-type males (which almost always came from a *Canton-S* strain).

COURTSHIP-SONG MUTANT

The *cacophony* (*cac*) mutant was isolated by SCHILCHER (1976a, 1977), after ethyl-methane-sulfonate (EMS) mutagenesis and a search for *X* chromosomal mutations that caused males to take an abnormally long time to achieve copulation and subsequently proved, as well, to have abnormalities of courtship song. It is thought that courtship wing vibrations from male *Drosophila* are important in stimulating the female to be receptive to copulation, in conjunction with the presumed function of these sounds in species recognition (reviewed by BENNET-CLARK & EWING 1970). Thus, if there is no or reduced song from a male, due to surgery of all or part of his wings, he takes longer than usual to begin copulating (EWING 1964). If the song were qualitatively abnormal (not just less loud), this might also cause the male to be a poor mater.

Males expressing *cac* were found to produce abnormal songs when they sing to females. Instead of the intervals between pulses of tone being ca. 35 msec, as is characteristic of *D. melanogaster*, they are ca. 45 msec in the mutant. The length of each pulse is also increased, from the normal 3 msec to 12 msec; and there are a greater number of cycles of tone per pulse than in wild-type (for which each pulse has one cycle, and sounds like a click). Finally, the amplitude of pulses in *cac* males are increased, so the song is simply louder. All of these observations were made by SCHILCHER (1977). These kinds of data are collected (by him, and in our studies) by placing courting pairs in a small chamber that rests on a ribbon microphone. This feeds into a tape recorder, and then the sound record can be displayed visually on an oscilloscope or onto film (via an oscillograph).

All of the aforementioned abnormalities of courtship song induced by *cac* were mapped near the *vermilion* marker on the *X*

chromosome, i.e. at map position 34.6 (SCHILCHER 1977, and see LINDSLEY & GRELL, 1968, for a description of the *vermilion* and other genetic markers described elsewhere in this paper).

The *cac* mutation does not simply derange all aspects of wing behavior, because the humming noise generated in male courtship (the "sine song," e.g. SCHILCHER 1976b) is normal in *cac* males, as is the wing-beat frequency in flight (SCHILCHER 1977). We have also found recently that both *cac* males and females are normal in their basic ability to fly (using the test described by BENZER 1973). Females homozygous for this mutation are, in addition, ostensibly normal in their overall courtship abilities, but their behavior has not been analyzed thoroughly.

Is the reduced mating success of *cac* males (Table 1) due solely to their altered courtship song? This cannot yet be answered, in that males from this strain are abnormal on other behavioral criteria (SCHILCHER 1976a). They exhibit a reduced number of flight buzzes, are less reactive to a shadow stimulus, and frequently hold their wings in an opened and raised position when in the presence of females. Most damaging to the notion that the aberrant song is solely responsible for lowered mating success is the finding that wingless *cac* males (after surgery) are still at a disadvantage, when compared to wingless wild-type males (SCHILCHER 1976a). However, the general deficits in behavior, some of which are also found in homozygous *cac* females, have not been mapped to the same genetic locus which controls the specific parameters of the courtship song. F.V. SCHILCHER & J.C. HALL are currently analyzing genetic mosaics, each of which expresses *cac* in only a portion of its CNS (using internal cell markers discussed in detail in a later section). These experiments should reveal, not only the part of the CNS in which *cac* expression leads to abnormal song, but also if the other behavioral defects have this same "focus" (as defined by HOTTA & BENZER 1972) or if, for instance, the aberrant shadow response is caused by expression of the mutation at another anatomical site.

NON-MATING MUTANTS

The *celibate* (*cel*) was isolated by D.L. LINDSLEY and colleagues, in a search for EMS-induced, *X*-linked male-sterile mutations affecting spermatogenesis. A small subset of these mutants proved to have ostensibly normal sperm, as shown simply by pulling out the testes with fine forceps, putting the tissues under a cover-slip in buffered saline, then drawing out the fluid with filter paper: the seminal vesicles burst, revealing many motile sperm that can be easily observed with brightfield microscopy. From these observations, it was found that *cel* males have sperm that seem normal in number and appearance. These mutant males, when observed with females in the courtship chambers, court females but do not copulate with them (Table 1). The behavioral sterility was mapped to position 48.5 on the *X* chromosome, using the nearby markers *raspberry*, *dusky*, (*dy*), and *forked* (*f*). In conjunction with this genetic localization, a *dy cel f* chromosome was constructed, followed by removal of these flanking markers, that involved replacing *dy*

and *f* with alleles from our usual wild-type stock. The result was a *cel* strain nearly isogenic with the stock we use in controls (the *cel* stock also carries an *X*-chromosome balancer, *FM7*, in order that the mutation can be maintained in *cel/cel*$^{+}$ females).

Quantification of mutant *cel* behavior showed that, whereas 95% of the tested males performed wing extension, only 16% even attempted to copulate (Table 1). These attempts were never more than a feeble curl of the abdomen, without genital-genital contact (cf. HALL 1979). Courtship behavior was generally vigorous, in that the fraction of observation periods spent by *cel* males in courtship actions directed toward wild-type females was 44 + 3% (mean + SEM). This is near the normal range of "Courtship Indices" or CI's that are recorded routinely for wild-type males (defined as the fraction of an observation period in which the flies actively court, e.g. SIEGEL & HALL 1979, HALL 1979, JALLON & HOTTA 1979; and see below).

The *celibate* males thus seem to be blocked midway in the courtship sequence (FIGURE 1), though they initiate and perform the earlier stages quite well. The interactions of *celibate* males with females are similar to what happens with mutant *fruitless* males, (see below), but *cel* males do not court wild-type or other mutant males in any appreciable frequency, unlike what is the case with *fruitless*.

It can be interesting to ask if a genetic factor affecting male courtship has any influence on females, based on the notion that the male's "signal transmitter" and the female's "signal receiver" are genetically coupled (e.g. HOY 1974). However, the effects of *cel* in females can ostensibly not be tested, because *cel* is *X*-linked; thus a cross of heterozygous females and hemizygous males, necessary to produce *cel/cel* females, is sterile. This was circumvented by the construction of females heterozygous for *cel* and a marked *X*-chromosome inversion, and also homozygous for a meiotic mutant that causes chromosomal nondisjunction at the second meiotic division (DAVIS 1971). When these females are crossed to attached-*XY*/0 males, a fraction of the progeny are females homozygous for *cel*. These individuals were observed to be quite normal in receptivity, in addition to copulation duration and fertility.

Males expressing the *fruitless* (*fru*) mutation discovered by GILL (1963) behave similarly in the presence of males or females. Thus they court other males, whether *fruitless* or wild-type, much more than normal; and they court females in a much reduced fashion (HALL 1978a). The poor courtship of females includes almost no attempts at copulating (Table 1), and no *fru* male copulations have ever been observed (HALL 1978a). (Thus, *fru* is maintained in stocks using males heterozygous for the mutation and a balancer chromosome.) The mutant males would probably be fertile if they copulated, because *fru* males have normally appearing reproductive systems and sperm. Females in the strain exhibit no apparent abnormalities (HALL 1978a).

With respect to aberrant courtship seen when *fru* is expressed in males, it is important to note that wild-type males readily court the mutant males as well as the reverse case (HALL 1978a). The observations on two males courting are made possible by, for example, making a small clip in the wing of the wild-type or the *fru* male, or by introducing a *cuticular* marker into one or the other genotype (e.g. HALL 1978a).

Any of the courtship interactions involving *fruitless* males and other males are sustained, compared to the infrequent and brief courtship bouts recorded between two wild-type males (HALL 1978a, JALLON & HOTTA 1979). For instance, the CI recorded for courtship of wild-type males by *fru* males is 18 $\pm$ 2 (N=43) (HALL 1978a), and for wild-type males courting *fru* males 20 $\pm$ 12 (N=20) (TOMPKINS et al. 1980a). These values are ca. 10-20 fold elevated, compared to CI's routinely recorded from two wild-type males (see below, Table 5).

A commonplace sight in containers of *fru* flies is the formation of chains of courting flies (first noted by GILL 1963), which are not seen among wild-type flies (HALL 1978a). The rules governing stable chain formation are that a female, if present, will occupy the head position, since females never, or almost never (COOK 1975), pursue other flies in active courtship. A normal male in the chain tends to occupy the tail position, since he rejects pursuits by vigorous wing flicking (e.g. HALL 1978a). A *fru* male can occupy any position, since he does not reject pursuit as vigorously (HALL 1978a). It should be noted that the stimulation by *fru* males of courtship performed by wild-type males cannot be explained by an absence of rejection behavior on the part of the mutant males, because their wing flicking, while reduced, is appreciable. There are other defects in wing behavior of *fru* males. When they are following another male or female, they often show a fluttering of both wings simultaneously, with each wing extended less than its usual 90°. At other instances in courtship, the same *fru* males will perform the regular unilateral wing extension. With either kind of wing display, the courtship song (see below) is anomalous as well (F.V. SCHILCHER & C.P. KYRIACOU, unpublished).

All of the abnormalities in the *fruitless* strain just described apparently map to the same genetic locus on chromosome *3*. After sterility was mapped to position 62 on this autosome (HALL 1978a), the nearby flanking markers *Stubble* (*Sb*) and *Delta* (*Dl*) were recombined onto a *fru*-bearing chromosome. *Sb* and *Dl* were then removed, and this resulted in the re-extraction of not only male sterility, but the other characteristics as well, such as enhanced male-male courtship plus aberrant wing behavior. In addition, all the behavioral defects in the strain are uncovered by a small deletion of the appropriate segment of chromosome (Table 1).

Wing defects cannot explain all of the abnormalities induced by *fru*; inadequate stimulation of females and abnormal rejection of males could have such an etiology but apparently not the active courtship by *fru* males of other males. Visual factors too, are apparently not at issue. For instance, in

Table 1. Courtship performance of wild-type and mutant males of *D. melanogaster*. Flies were observed for 30-60 min. Virgin males and virgin females were placed in plastic mating chambers, one pair per chamber (see e.g. HALL, 1978a, 1979). All males that performed a later step in the courtship sequence (e.g. attempted copulation) had performed all of the previous steps (following, wing extension). See the text for explanation of mutant symbols. "*Df*" is a small 3rd-chromosomal deficiency that is missing the *fru* locus (HALL 1978a).

Male genotype	Number tested	% OF MALES: Following females	Wing extending	Attempting copulation	Copulating	Duration of copulation (min ± SEM)
wild-type	180	97	97	88	83	20.1 ± 0.2
cac	150	87	67	54	9	17.2 ± 0.5
cel	56	94	93	15	0	--
fru/fru	209	56	48	1	0	--
fru/Df	53	53	43	2	0	--
coi	159	82	78	53	45	12.0 ± 0.4
X0	93	90	90	87	68	18.1 ± 0.3
sk	213	88	87	83	80	42% not stuck: 19.0 ± 0.9 38% stuck: 841 ± 193

tests using a counter-current device (BENZER 1967), *fru* males have normal phototaxis (though they have to be tested singly, lest they court one another instead of running toward light!). The electroretinogram of the mutant is normal. Moreover, genetically blind males (see later section) court neither each other (N=39 pairs tested)nor wild-type males (N=40). Therefore, a male does not need to see that a potentially courted fly is male in order to ignore it. Blind males court *fru* males quite strongly. Double mutant blind-plus-*fru* males actively court wild-type males and each other.

Anesthetized *fru* males, but not wild-type males so immobilized, are courted quite vigorously by wild-type males (HALL 1978a). This further shows that an absence of male rejection (in *fru* or wild-type males) is not sufficient to cause male-male courtship; and it suggests that *fru* males stimulate other males to court them in a "passive" sense, possibly through the production and release of volatile compounds. These sex pheromones in *Drosophila* are discussed in more detail later, especially with respect to such materials emanating from females. As to the possibility of anomalous pheromones being produced by males, we note here that it is almost certainly the case that some of the aberrant patterns of behavior have an olfactory etiology. Thus, both gas chromatographic analyses and bioassays of volatile compounds from *fru* males show that they make a compound (or compounds) that induces wild-type males to court males (TOMPKINS et al. 1980a). These materials are present in very low or zero amounts in extracts from wild-type males, whose volatile compounds do not induce courtship (TOMPKINS et al. 1980a). It is likely, therefore, that *fru* males stimulate themselves to court other males, through the production of their own pheromone. This material may come from the posterior body segments of the mutant males, since severed abdomens from *fru* (but not from wild-type) males can trigger wing extension as performed by normal males (HALL 1978a).

It must be noted that abnormal production of sex pheromones by *fru* males cannot easily explain all aspects of the mutant syndrome, such as the observations of aberrant wing behavior and the behavioral sterility (which is, after all, the criterion on which *fru* was discovered). Possibly, though, a too-high amount of courtship-stimulating pheromone, present throughout the adult life of *fru* males, causes them to become habituated to these kinds of chemical stimuli; or worse, causes their neural circuitries to become jammed, interfering with their transition to the later steps in courtship.

Thus, it is useful still to think of the effects of this mutation as having one primary defect, and to consider further what such a defect might say about normal courtship. In this light, it must be pointed out that courtship solely involving wild-type males occurs very frequently and in a sustained fashion if the courted male has recently eclosed as an adult (COOK and COOK 1975, JALLON & HOTTA 1979, TOMPKINS et al. 1980a). However, these young flies do flick their wings when courted. Also, it has been known for some time that young adult males of *D. melanogaster* show low levels of courtship directed at fe-

males (for recent data, see JALLON & HOTTA 1979, TOMPKINS et al. 1980a).

Thus, young wild-type males are somewhat similar in their courtship behavior to mature *fru* males (though the former do not actively court other males). Wild-type males, as they mature during the first 1-2 days of adult life, begin to be "able" to court females vigorously and cease being stimulating targets (COOK and COOK 1975, JALLON & HOTTA 1979, TOMPKINS et al. 1980a). Could it be, then, that an important aspect of the defect induced by *fru* is to block this maturation process? If this is so, then young wild-type males could have sex-stimulating pheromone associated with them, and indeed this has been shown to be the case (TOMPKINS et al. 1980a). Also, young *fru* males have the same stimulating effects as young wild-type males (there is no additive effect or synergism--TOMPKINS et al., 1980a), once more suggesting that *fru* may identify a gene that is normally concerned with "turning off" pheromone production in maturing males. However, it is not known if the volatile compounds associated with young wild-type or *fru* males (but not with mature wild-type males) are chemically the same. Nor is it known if any of these compounds are similar or identical to the female-specific volatile compounds which have also been identified in *D. melanogaster*(HEDIN et al. 1972, TOMPKINS et al. 1980a; and see below). Finally, it is not easy to think of the adaptive significance of courtship stimulating materials that are transiently associated with normal males of this species.

Courtship among male flies has been observed in other circumstances, some of which are artificial. For instance, sodium tungstate has been found to induce so-called homosexual behavior (AARON 1977). Other factors having no apparent relationship to chemical stimulation can lead to this behavior, such as playing simulated courtship song to groups of wingless males (SCHILCHER 1976c), or a complex chromosome re-arrangement that is associated with vigorous male-male courtship but requires light in order to have its effects (SHARMA 1977). One of the breaks in this aberrant genotype is on chromosome *3*, but not near the *fru* locus (*fru* was induced by X-rays, yet is not associated with any visible chromosome abnormalities, GILL 1963).

Wild-type males in some *Drosophila* species have been observed routinely to court one another (e.g. WASSERMAN et al. 1971). It is hoped that studies of *fru* in *D. melanogaster* will further reveal important components of wild-type behavior that might not have been uncovered solely through observation of normal courtship in this species.

COPULATION MUTANTS

The final two courtship mutants to be discussed each involve abnormalities of copulation. The *X*-linked *coitus interruptus* (*coi*) mutant was another by-product of D.L. LINDSLEY'S search for EMS-induced mutants defective in spermatogenesis. The *stuck* (*sk*) mutation was found as an apparently spontaneous genetic variant by BECKMAN (1970).

Males expressing *coi* are usually sterile (70-90%), and it appeared as if the reason might be a premature signal to terminate copulation: they copulate for only about 60% of the normal 20-min duration, although having performed the earlier steps of courtship reasonably well (Table 1). The normal copulation span (at 25°C) is quite invariant, and is characteristic of this species (e.g. FOWLER 1973). It is important carefully to control the temperature at which copulation is measured, because copulation is ca. 70% shorter at high temperature (28.5°C) than at low temperature (17°C) (S. BENZER, unpublished). The residual variability of copulation duration (Table 1) could be due to intrinsic differences among individual males. This might explain why selection for separate lines of *D. melanogaster* with male-linked differences in copulation span is successful (MACBEAN & PARSONS 1967). When 34 wild-type males were tested in two successive copulations each, the mean duration for the first trials was 20.6 $\pm$ 0.4 min and for the second was slightly but significantly shorter, 19.2 $\pm$ 0.4 min ($p<0.01$, two-tailed Mann-Whitney test). The durations for the first and second copulations for individual males were not, however, significantly correlated (Spearman's $r_s = 0.09$, $p>0.05$). This suggests that variability among copulation durations is due to stochastic processes.

In copulations of *coi* males, their mean 12-min duration has more variability from individual to individual compared to wild-type; and this may be due to individual differences among mutant males: In double copulation tests of 50 such flies, the first trials had a mean duration of 14.5 $\pm$ 0.5 min, and the second was significantly shorter 12.5 $\pm$ 0.5 min ($p<0.01$), as in wild-type; but different from some other species, e.g. LEOPOLD et al. 1971). Unlike what is found in wild-type the first and second copulations for *coi* individuals are significantly correlated ($r_s = 0.38$, $p<0.01$).

The variability among individual *coi* males is probably not associated with differences in "background" genotype, because the mutants in these tests were relatively isogenic. This had been accomplished in conjunction with genetic mapping of the mutation responsible for aberrant behavior. Sterility was roughly mapped to position 20 on the *X* chromosome. Finer localization was achieved using the markers *carmine*, *cut*, *singed*, and *tan*; these crosses also involved construction of a *coi*-bearing chromosome flanked by nearby markers, followed by removal of them. The re-extracted *coi* factor, mapping at position 22.1, was still associated with poor fertility and dramatically reduced length of copulation time.

Shorter-than-normal copulation could easily lead to poor fertility, including sterile copulations in many cases. This is because sperm do not begin to be transmitted immediately after copulation begins. The kind of interrupted mating experiment (reviewed by FOWLER 1973) that leads to this conclusion is shown in FIGURE 2a, since details of these kinds of data have not been reported previously. Here, the male-female pairs were interrupted various times after the start of copulation. There is an abrupt transition from near-zero fertility to very frequent

fertility, with respect to separations occurring 5-6 min after copulation begins (combined data from J. HALL using pairs separated by vortexing, and S. BENZER, who separated copulating flies with forceps). In addition, counts of the numbers of progeny from such interrupted copulations shows that the sperm are not all transmitted at once, since the fecundity of females rises somewhat gradually to its maximum value (i.e. as seen after uninterrupted copulations), with respect to matings interrupted between 6 and 12 min (S. BENZER and J.C. HALL, unpublished). Further, the kinetics of this rise in fecundity imply that "quanta" of approximately 40 sperm are transferred as separate, sequential units (S. BENZER, unpublished).

Thus, if *coi* males frequently ceased copulating early--and at least one quarter of them terminate between 4-10 minutes--they would frequently have done so before having a chance to transmit appreciable numbers of sperm. One alternative explanation would be that *coi* males are not able to transmit sperm normally, and that this could cause them prematurely to terminate their futile copulations. In attempts to distinguish between these two explanations for the mutant behavior, durations of *coi* copulations were artificially extended. This was accomplished through the use of the *stuck* (*sk*) mutation. This genetic variant, whose properties are described in detail below, causes males frequently not to withdraw from females. If an individual exhibits this mutant behavior he often remains stuck for a long time (Table 1). After such an aberrant copulation, followed by withdrawal by the male on his own or pulling him out with forceps, 90% of the females produce many progeny (with the remainder having no fecundity); thus there are relatively mild effects on fertility in males expressing *stuck* only. Double mutant *coi, sk* males were constructed. Several of the matings involving such males thus lasted beyond the time normally needed for appreciable numbers of sperm to be transmitted to females. (Table 2, cf. FIGURE 2a). However, it was found that even a substantial lengthening of copulation in the presence of *coi* frequently did *not* lead to fertility (Table 2).

These results led to a detailed examination of the reproductive systems in *coi* males (which, near the time of the discovery of the mutant, had shown apparently normal sperm in the few mutant individuals dissected). Recently, 86 mutant males were dissected. Only 54 had motile sperm in their seminal vesicles, and the remainder had either no detectable sperm or sperm that were immobile (not seen in each of 35 dissections of wild-type males). This was found for either the original *coi* stock, or for several of the *coi* stocks which had been reextracted after out-crossing to wild-type. Thus, it appears as if the mutation leads to faulty sperm production, in addition to abnormally short copulation. The former characteristic is probably not due to any residual genetic variability, but instead has a penetrance of less than 100%. Sperm production by the mutants does not eventually cease for all individuals, because the proportion of *coi* males with motile sperm (0.6 ± 0.2) was uncorrelated with their age post eclosion (2-18 days).

It was asked if copulation behavior is correlated with the

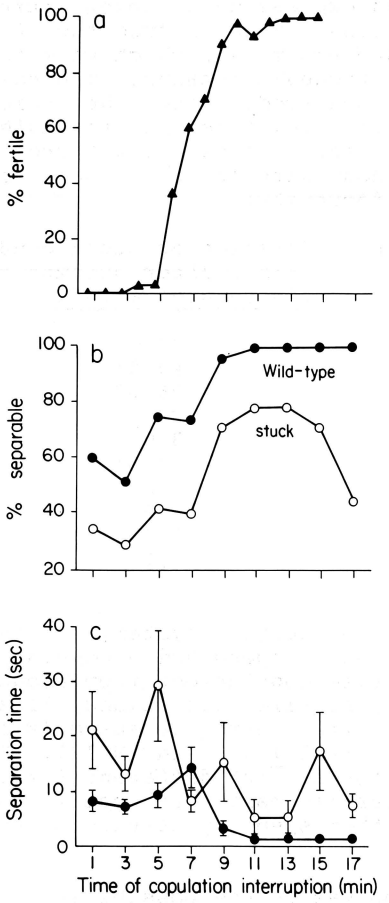

Figure 2. Interrupted copulations of wild-type and *stuck* mutant males.
a. Fecundity of females following artificial separation, by vortexing or use of forceps, of copulating wild-type pairs. There were ca. 40 pairs tested per data point.
b. Ease of separating males after the beginning of copulation, expressed as % of pairs separable by 60 sec of vortexing (Vortex Genie Mixer, Scientific Industries, speed #6). ca. 20 tested pairs per point.
c. Ease of separating males after the beginning of copulation, expressed as mean sec ± SEM (not shown if the error was smaller than the data point) required for separation, for those pairs that did separate (cf. b). For b. and c., data from wild-type males are shown with closed circles, and from *stuck* males with open circles.

Table 2. Simultaneous expression of *coitus interruptus* and *stuck* mutations. Factors from the *sk* strain were introduced into *coi* males, which were tested in copulation with attached-*X* females. Ten cases of stuck behavior were observed. After the indicated durations of copulation (i.e. time when the male first attempted to dismount) the male either withdrew by himself or was pulled away with forceps. Each female was then tested for fecundity.

Duration of copulation (min) before attempts to dismount began	Duration of stuck condition (min), after attempt to dismount (*separated artificially)	Progeny?
7.6	30.0*	no
7.9	45.5*	yes
11.5	26.6*	no
11.6	23.2	no
12.0	3.0	no
13.6	20.5*	yes
14.4	1.0	no
14.7	1.6	yes
15.0	5.0	no
18.6	0.7	yes

abnormalities of the reproductive system, and the results are as follows: males with motile sperm had a copulation span of 12.9 $\pm$ 0.7 min (N=34); fertile copulations from among these males, 14.3 $\pm$ 0.7 min (N=19); sterile copulations, 11.2 $\pm$ 1.0 min (N=15); and the males with non-motile sperm (all of whose copulations were sterile), 12.1 $\pm$ 0.9 min (N=12). The sperm-motile vs. sperm-immotile means are not different ($p=0.4$); but the time spent copulating by fertile males with motile sperm is slightly longer ($p=0.02$) than that spent by sterile males with motile sperm.

A final question was on the possibility that some *coi* males are sterile because their sperm cannot function, even though they are motile and transferred to the female. 45 *coi* copulations were observed, and the females were dissected 3-5 days afterward (during which time fecundity could be assessed). 28 of the copulations (duration, 12.2 $\pm$ 0.8 min) did not result in sperm transfer, though 13 of the males proved to have motile sperm; all females here were of course sterile. Of 17 copulations (duration, 13.3 $\pm$ 0.9 min) that led to motile sperm in the ventral receptacle or spermathecae, 4 were nonetheless sterile.

It is therefore concluded that there is a wide range of sperm abnormalities among *coi* males, and that these abnormalities can be present even if the sperm are motile and are transmissible to the female. Copulation behavior is routinely too short in the mutant, and this likely explains why many copulations do not result in sperm being transferred. In this light, the longer copulations tend to be those that resulted in sperm transmission and (usually) fertility. However, copulations

with no sperm transfer are not due entirely to their shorter duration. This is partly because of the obvious lack of motile sperm in some of these males, whose copulations are not appreciably shorter than for males with motile sperm. And even the males with motile sperm may not always be able to transmit them, in spite of the fact that they may have copulated for what would appear to be a long enough time to do so.

In the initial tests of the *coi* mutant, it seemed as if the abnormal gene might lead to a defect in the "copulation program." However, there are many abnormalities in reproduction caused by the mutation. And the abnormalities of sperm production or transmission cannot thoroughly explain the behavioral effect on copulation span. This is because other genetically defective males with abnormal sperm copulate much longer than do *coi* males. The former are males expressing no mutations, but missing their *Y* chromosome. They were constructed by crossing wild-type males to attached-*X*/*O* females (using several different attached-*X* strains). The resulting *XO* males in the F_1 have no motile sperm (indeed no sperm could be seen at all when these males were dissected). Their general courtship behavior is ostensibly normal (Table 1, and cf. HALL 1977, 1979; vs. one report of abnormal behavior of *XO* males, ASLUND et al. 1978). When they copulate, the mean duration is certainly in the normal range (Table 1), though it is actually significantly lower than the 20-min value from the *XY* males (carrying the same *X* chromosomes used to generate the *XO* individuals). Obviously, though, the absence of motile sperm is insufficient to "feed back" on the control of copulation time and reduce it substantially.

The *stuck* mutation was not only a useful tool for studying *coi*, but is also of intrinsic behavioral interest. These *sk* males behave normally until the end of the courtship pathway, but then about half of the copulations are not terminated normally (Table 1). In these cases, the male is apparently trying to dismount but cannot withdraw. Thus he and the female tug at each other, frequently facing in opposite directions. The duration of this state is highly variable, in that about 70% of the pairs can separate within 4×10^2 min; 25% were stuck for $1\text{-}3 \times 10^3$ min; and the remaining 5% were stuck for ca. $4\text{-}6 \times 10^3$ min. For roughly half the cases in the range of $1\text{-}6 \times 10^3$ min there was no withdrawal at all. Here, both the male and the female died, thus at an age much shorter than the normal lifespan of *Drosophila* (e.g. HALL 1969). The low penetrance (ca. 0.5) of the stuck phenotype may be underestimated, because in tests of mutant males that copulated twice, about one third of the individuals that did not get stuck on the first trial did on the second (Table 3).

Residual genetic variability may, however, be in part responsible for variable behavior on the part of *sk* males. This is because the major genetic factor responsible, when re-extracted after outcrossing, results in lower than usual penetrance (which otherwise has remained invariant, during the course of mass transfer of the *sk* stock during the several years subsequent to its discovery). These genetic mapping

experiments first showed that *sk* is a semi-dominant, since 4% of F_1 males (N=474) from a cross of mutants to wild-types exhibited stuck behavior. This is in contrast to tests of 1155 males from the wild-type stock: only 2 showed any sign of difficulty in dismounting after the normal copulation period. One pair remained stuck, apparently trying to pull away, and succeeded in doing so after 5 min; in the other case this required one hr. On inbreeding progeny from these two matings, no males with stuck behavior were found in subsequent generation.

Table 3. Double copulations by *stuck* mutant males. Males expressing *sk* were tested in copulation with wild-type virgin females. If they copulated and then withdrew immediately on dismounting or by finally pulling away after being stuck, they were tested again with fresh virgin females.

Males copulating twice	Males showing stuck behavior twice	Males getting stuck after 1st copulation, not 2nd	Males getting stuck after 2nd copulation, not 1st	Males showing no stuck behavior
39	4	2	14	19

More detailed genetic tests showed that, when the *X*, 2nd, and 3rd chromosomes from the *sk* strain were totally replaced with dominantly marked, inverted balancer chromosomes, ca. 10% of the resulting males showed the stuck phenotype. This implies that the major genetic factor is on chromosome *4*. Thus males from a *sk* stock were crossed to eyeless-dominant females (which carry the 4th chromosome marker, ey^D); heterozygous F_1 females were taken only from crosses in which the parental males had been stuck for at least 1 hr. These F_1 heterozygotes were backcrossed to *stuck* males, and the following F_2 males (again, only from crosses in which there was strong stuck behavior) were tested: not-ey^D, 12% stuck (N=85); ey^D, none stuck (N=33). In further tests, when the 4th chromosomes from the *stuck* strain were totally replaced, none of the resulting males (N=83) were able to get stuck. Finally, *stuck* males were crossed to attached-*4/0* females, and the F_1 males that were *4/0* (with their 4th chromosomes from the *stuck* strain) were tested: 7/20 copulations were of the stuck type (vs. 0/30 such cases from *4/0* males with their 4th chromosome from a normal strain). The genetics of this behavioral mutant are discussed in some detail, because it is important to realize that it was not possible to extract the original frequency of stuck behavior among the progeny of backcrosses (see the ca. 10% values noted above, vs. usual penetrance of *sk*, Table 1). Thus, other factors may be segregating in the original *sk* strain, several of which must be present for a relatively high-probability stuck phenotype. In this light, *stuck* males were inbred to their sisters for 15 generations, in matings chosen because they always involved stuck behavior. The fraction of tested males that showed mutant be-

havior during the course of this attempted selection fluctuated from 30-60% during the course of this attempted selection, without a continual increase or decrease. It should be noted in this context that sisters of mutant males have no influence on the behavioral abnormality, in that a *sk* male can get stuck with an equal chance in females from the *sk* strain or those from any other source. Also, wild-type males (N=23) did not get stuck in mutant females. The females from this strain do not transfer the abnormality to their brothers (via copulation): 5 *sk* males, collected as very young virgins, were able to become stuck when they matured and were eventually tested with normal females; and 7 wild-type males, after copulating with one mutant female each, showed no abnormal behavior on subsequent testing.

The biological, if not the genetic, etiology of stuck behavior might have been rather simple. For example, *sk* could be thought of as leading to a defect in the cuticle of the male genitalia, causing the males to have "merely" mechanical difficulties in separating from females. This would be analogous to the stuck behavior observed in mating between *D. subpalustris* males and *D. palustris* females, thought to be due to a difference in the morphology of the spines on the penis between inter- and con-specific males (GROSSFIELD 1972a). But this hypothesis to explain *sk* in *D. melanogaster* fails in its simplest version, because scanning electron micrographs at ca. 1000x showed that for 20 virgin males from the *stuck* stock, or 10 that had copulated and withdrawn normally, all were normal in external morphology (compared to 20 virgin and 13 mated wild-type males): with respect to the appearance of the penis, its associated spines, or nearby bristles and appendages (Figure 3a). Data from males that had become stuck after copulating, and then were either pulled out or eventually withdrew on their own, showed a dramatic difference from wild-type: 30 of 82 such males had their penis greatly extended and the clasper appendages had nearly disappeared (FIGURE 3b). This change could also be seen under light microscopy at 160x, so that affected males could be chosen and re-tested behaviorally. The abnormal positions of the appendages were maintained for several days. Eight such males were re-mated, and 5 became stuck again while 3 terminated copulation normally. Of the 3 males that had normal copulations, the penises of 2 had returned to a normal position, and the claspers had re-emerged. The abnormal positioning of claspers was not found in normal males with penis extended due to anesthesia (as sometimes happens with ether and is especially pronounced with n-pentane or n-hexane). Wild-type males separated from females by vortexing (cf. FIGURE 2) before the end of copulation did not show extended penises or retracted claspers. Thus, *sk* leads to a rather unique phenotype, which, as far as can now be determined, is related to the manipulation of the external genitalia, instead of their morphology per se. The mutation may even lead to a sex-specific change in neural or muscular physiology.

That *sk* should be viewed as having a "dynamic" abnormality is further suggested by tests on vortexing copulating pairs. In the interrupted mating tests performed to assess the time of initial sperm entry, it was noticed that wild-type males are

quite difficult to separate early in copulation, and much easier to vortex apart during the second half (FIGURES 2b & 2c). *Stuck* males, on the other hand, show only a slight decrease in coupling strength; and they may become even more strongly bound as the normal time for disengagement approaches (FIGURES 2b & 2c) totally unlike wild-type males. For copulations involving mutant *stuck* males and vortexed during the second half of copulation, those that did *not* separate nearly all became stuck at the end; whereas those vortexed during the first 1-9 min that did not separate showed the more usual frequencies of eventually getting stuck (10-40%). Finally, when 8 cases of stuck males were vortexed for 2-5 minutes, *after* the apparent end of ca. 20-min copulations, they were impossible to separate.

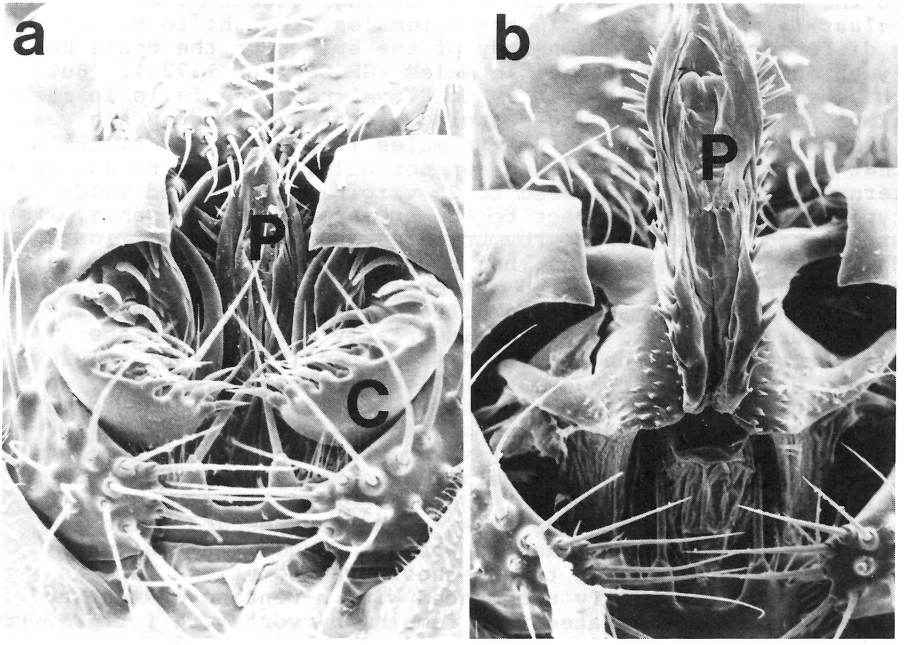

Figure 3. Genitalia of mutant *stuck* males in scanning electron micrographs. Ventral views; caudal end toward top. a. virgin *stuck* male, with apparently normal penis (P) and claspers (C). b. *stuck* male, after copulating, becoming stuck, and eventually pulling away from the female. The penis is extended and the markedly retracted claspers are not visible. The scale bar at the bottom of each micrograph represents 10 µm.

These experiments show that *sk* affects essentially the entire process of copulation, not just its termination. And it seems even more likely that stuck is a true behavioral mutant, not a "structural" one.

MUTANTS WITH ABNORMAL SIGHT AND SMELL THAT COURT DEFECTIVELY

From the foregoing discussion of "courtship-specific" mutants, it was clear that no mutations yet discovered affect the earliest stage of the pathway. Such mutations would, for instance, cause males not to initiate courtship. This begs the question as to what information passing between females and males is responsible for triggering courtship. Visual cues and chemical ones might be expected to be important, since *Drosophila* see and respond to moving objects (e.g. KALMUS 1948), including, it seems, moving females (COOK 1979). The importance of chemical communication might be invoked, due to observations that males tap female abdomens at the beginning of the pathway (FIGURE 1), and that something on or in the fly's abdomen must be female in order that this individual trigger courtship. The latter conclusion comes from experiments on gynandromorphs, the results of which have shown that the behavioral focus for female sex appeal is most closely associated with abdominal tissue (reviewed by HALL 1978b, GREENSPAN & HALL 1979). Thus, when the male taps the abdomen, he may be stimulated by contact chemoreception from female-specific surface substances. These stimuli would enter through the tarsus of the foreleg, which is rich in chemoreceptors (e.g. FALK & ATIDIA 1975). Alternatively, or additionally, the mapping of female sex appeal to the abdomen could mean that volatile compounds are produced under the sex-specific control of these body segments, and possibly released from such posterior tissues as well. The suggestions of a role for sex pheromones in *Drosophila* came initially from behavioral experiments, some of which have tested the possibility that females can influence male behavior when individuals of the two sexes are physically separate (e.g. SHOREY & BARTELL 1970, AVERHOFF & RICHARDSON 1974, JALLON & HOTTA 1979).

If sight and smell are important in at least the initial stages of courtship, then mutations that interfere with either sensory modality might block the pathway at the beginning, causing, for example, male sterility. Here we discuss results showing that visually or olfactorily defective mutants are able to court and mate; this would explain why no male-sterile mutants have been found which then turned out simply to be blind or unable to sense odors. However, males expressing these kinds of mutations have been found to have abnormalities in their courtship.

VISUAL MUTANTS

Of the many blind mutants isolated in *D. melanogaster* (reviewed by PAK 1975, HALL & GREENSPAN 1979), all are able to mate. Even the most severe mutants, expressing alleles of the *no-receptor-potential* (*norpA*) gene, court females with a high proba-

bility; and they achieve copulations with only a 2-3 fold lower frequency than that recorded for wild-type males in 10-min observation periods (SIEGEL & HALL 1979, cf. MARKOW & MANNING 1980). These findings are not surprising, because it has been known for some time that *D. melanogaster* can mate in total darkness (for the most recent reports, see HARDELAND 1972 and GROSSFIELD 1972b). Other *Drosophila* species, however, require light to mate (see below).

The observations on genetically blind flies, however, provide a bit more detail on the role of vision, because it is easy to observe that *norpA* males are badly misdirected in many of their courtship actions, and that their Courtship Index (CI, as defined above) is significantly reduced (see below, Table 5).

Since the absence of vision does cause defective mating behavior, is it most important that a normal male simply see the object of his courtship, or is it more important that he detect her movement? This question has been addressed by testing mutant males with more subtle visual defects than total blindness. The courtship performance of *optomotor-blind* (omb^{H31}) males was measured. Flies expressing this mutation are able to see, but are very defective in their turning responses to movements in their environment (HEISENBERG et al. 1978). This behavioral defect is almost certainly related to the fact that omb^{H31} flies have severe reductions in giant fibers normally present in one of the optic lobes. More peripheral genetic defects in the eye or the optic lobe just proximal to it (which is apparently not affected by omb^{H31}) lead to marked abnormalities of the electroretinogram (ERG). For instance, many *norpA* alleles abolish the ERG. The omb^{H31} mutation, however, has a normal ERG (HEISENBERG & GÖTZ 1975).

When observed in courtship, omb^{H31} males show the same two-fold decrement in CI as found for *norpA* males (TOMPKINS et al. 1980b, and see below, Table 5). Moreover, the turning responses toward moving females are severely depressed: when two lines interecting at 90° were placed beneath the courtship chamber (dividing it into quadrants), wild-type males crossed 41 ± 2 lines per min (these males were from a *Berlin* strain, unlike all other control flies we have used, because omb^{H31} was isolated on that genetic background). The omb^{H31} males crossed only 2 ± 0 lines per min (TOMPKINS et al. 1980b). These mutant males therefore perform essentially no orientation toward moving females, which apparently is necessary for initiating sustained courtship bouts between normal flies. The number of courtship bouts is increased 3-fold in tests of omb^{H31} males, compared to wild-type; the bout lengths, however, are much shorter, accounting for the reduction in CI.

Thus the observations of this mutant suggest that the detection, using vision, of female movement (not merely her presence) is an important feature of the input from female to male in the courtship of *D. melanogaster*. The adaptive significance of optomotor responses to female movement is further emphasized by the results of mating tests involving the omb^{H31} males: they take an abnormally long time to achieve copu-

lation. Whereas wild-type males took only about 2 hrs (at most) for 80-100% of the individuals to copulate, the mutant males required approximately 2 days to reach this same level of success (TOMPKINS et al. 1980b). This re-enforces the original suggestion, from the measurements of defective courtship actions in short-term tests of *norpA* and omb^{H31} males, that visual cues are far more important than would be concluded merely from the fact that light is not required for mating.

However, though it is helpful for the *D. melanogaster* male to see a moving female, female actions in the courtship of other species seem to be more critical. For instance, if a *D. subobscura* or *D. auraria* female is immobilized by decapitation, she is courted poorly or almost not at all by a male of her species (SPIETH 1966). This is consistent with the crude results on the absolute requirement for light, if mating is to occur in these species (reviewed by GROSSFIELD 1966). And the notion that the male and female must see each other performing fixed action patterns is strongly suggested by observations on the complex movements performed separately by individuals of each sex in *D. subobscura* (BROWN 1965, PINSKER and DOSCHEK 1979). On the contrary, *D. melanogaster* females appear as if they are only "required" to move as such, during at least the early stages of courtship, instead of exhibiting ritualized postures and other positive actions. Indeed, when *D. melanogaster* females are immobilized by decapitation (SPIETH 1966), or anesthesia (e.g. HALL 1978a), courtship is sub-normal but still occurs at a reasonably high frequency; and copulation involving immobile females is possible.

We have recently examined the role of female movement in *D. melanogaster* courtship in more depth (also see COOK 1979). Our tests first involved males present with females that expressed *shibire-temperature-sensitive* (shi^{ts}) mutations (cf. GRIGLIATTI et al. 1973). Such females can be almost completely immobilized, due to paralysis caused by shi^{ts} at 27°C (or higher). At this temperature, courtship involving a wild-type male and a wild-type female is normal, or, if anything, slightly enhanced (SIEGEL & HALL 1979). However, when wild-type males were put with shi^{ts} females and the temperature raised to 27°C, the female soon became paralyzed, and the CI values abruptly plummeted 50-fold. In the reverse experiment with a separate group of flies, the temperature was lowered from 27° to 20° (a permissive temperature for the mutant); and when the females recovered from paralysis, CI's increased almost 4-fold (TOMPKINS et al. 1980b). Thus, we can modulate courtship up and down by causing the female to start moving or to stop, respectively. But there is not, for example, a complete turn-off in these kinds of experiments, because females are able to stimulate courtship levels that are significantly above zero, even when they are not actively involved in interactions with the courting males.

Additional experiments on female movement led eventually to a connection between visual behavior and olfactory responses in the courtship of *D. melanogaster*. First, we note that in the tests involving genetically blind males, a change in the

patterns of female activity was found to be correlated with the longer-than-normal times required for copulation to begin. When it takes a male "too long" to initiate copulation, the female slows her general movements dramatically. These data were collected in a manner similar to those relating to turning responses of *optomotor-blind* males; that is, a line was placed through the test chambers, and the numbers of *female* crossings were noted during successive time intervals after a male had been introduced into the chamber. For wild-type males in the light, there was no substantial decrease in female activity, apparently because these normal courting pairs copulate so soon after the first male wing extension occurs: an average of 0.5 min to the first wing extension, and an additional 1.9 min required for copulation to begin (Table 4). In only 3 of these 24 tests was the female "brought to a stop" (defined as 0-5 line crossings per min) for a full minute or more (Table 4). The females generally crossed ca. 20 lines per min, during the 2-3 min required for the average copulation to begin.

Visually defective flies were tested in a similar manner, using wild-type pairs in the dark (observed under red light, in which conditions *D. melanogaster* cannot see, HAMILTON 1922) or near-blind males expressing the tan mutation (e.g. HOTTA & BENZER 1969). The times to the first wing display were an average of 1.0 and 2.2 min, in 24 tests each of normal flies in the dark or tan males, respectively. Copulations were initiated a relatively long time later (compared to normal pairs in the light) in these two aberrant situations (Table 4). During the course of these relatively long time intervals, the females were often brought to a stop: in 12 of 24 tests for wild-type pairs in darkness, and in 19 of 24 tests with the mutant males. But they did not "stop" until the last 1-2 min before copulation, and continued to cross ca. 20 lines per min during the preceding 4-9 min.

This kind of female behavior apparently is a positive response on her part to relatively sustained courtship by a male that nonetheless takes "too long" to be fulfilled. This is because in tests of females with very young males [which court little if at all, and do not copulate (e.g. JALLON & HOTTA 1979)], we found that the female is rarely brought to a stop (2 of 24 tests), even after long periods of time during which these pairs are left together.

COOK (1973, 1977, 1979) has also concluded that the females of *D. melanogaster* must slow their general movements if copulation is to occur. His and our results would suggest that she must be a reasonably immobile target if the male is to initiate copulation successfully. Her near cessation of movement is only momentary when she is courted by a wild-type male under normal conditions; but she will respond to abnormally long courtship in an exaggerated way, by markedly slowing down. Recall, though, that if a female is absolutely immobile and is not even standing up (due to anesthesia or effects of the shi^{ts} mutation) she mates very poorly. Successful courtship, then, appears to require an optimal "medium" between the extremes of zero activity from the female and "too much" movement.

Table 4. Influence of visual and olfactory behavior on female behavior and mating success. Male-female pairs of the indicated genotypes were observed for 30 minutes, in the plastic mating chambers described by HALL (1977, 1979), which were divided in half by a diagonal line. The conditions involved either the use of fluorescent white light or dim red light ("dark" for the flies). The "% of females stopping" refers to cases in which these females eventually ceased significant movement throughout a one-minute period (i.e. crossed the line only 0-5 times during that minute). The "mean time to start of copulation" was calculated for those pairs which did begin copulating within 30 minutes.

male genotype	female genotype	conditions	number of pairs tested	% of females stopping for 1 min	% of pairs copulating	mean time to start of copulation (min)
wild-type	*sbl*$^+$	light	24	13	100	2.4
wild-type	*sbl*$^+$	dark	24	50	67	5.1
tan	*sbl*$^+$	light	24	79	96	11.1
wild-type	*sbl*	light	24	0	63	7.4
wild-type	*sbl*	dark	25	0	24	14.2
tan	*sbl*	light	29	0	10	16.8

OLFACTORY MUTANTS

What does the female detect, in order to respond by slowing down when a male is taking a long time to achieve copulation? We suggest that the male may generate an odor that the female senses, and whose effects eventually accumulate, resulting in her reduced activity. This conclusion comes from tests of the effects of a mutation, *smell-blind* (*sbl*) that was recovered by ACEVES-PIÑA & QUINN (1979) on the basis of an inability to sense certain artificial odorants. Perhaps such flies cannot smell any airborne chemical stimuli, or cannot process such sensory information because of a chemical defect. It is the case that *sbl* females in the presence of defectively courting *tan* males do *not* respond by coming to a near halt in the courtship chambers. None of the females stopped (re the criterion stated above) in 30-min tests (Table 4). They continued to make at least 20 line crossings per minute, for many minutes. Analogous tests were also conducted using wild-type males and females in the dark; none of these females stopped either (Table 4). Some previous experiments using olfactometers have given precedence to the idea that females in this species can respond to volatile compounds emanating from males (VENARD 1980).

Correlated with the apparent inability on the part of *sbl* females to sense the lengthy presence of a defectively courting tan male, and hence for the females to slow their move-

ments, is the fact that copulation was even more inefficient in these tests: there was no copulation at all in 26/29 cases vs. only 1/24 such failures in analogous tests of tan males with normal females (Table 4).

Mutant *sbl* females are defective in their stopping and copulation behavior, even under more optimal conditions involving wild-type males observed in the light. In these tests, all the females eventually stopped, but only momentarily, not by the standard criterion (Table 4). Correlated with this relatively poor "stoppage" was a mediocre copulation frequency: 9/24 pairs failed to initiate copulation in 30 min, vs. 0/24 such failures in tests of wild-type males plus wild-type females in the light (Table 4).

It has been found that males as well as females of *D. melanogaster* apparently respond to courtship pheromones from other flies. These relatively recent studies have substantially augmented the earlier suggestions of the relevance of sex-stimulating volatile compounds in these flies (e.g. SHOREY & BARTELL 1970). The current work has made important use of the *sbl* mutant. It was found first that females produce volatile compounds that (1) are different from male materials on gas chromatographic criteria (HEDIN et al. 1972, TOMPKINS et al. 1980a); (2) stimulate *two males* to court each other at greatly accentuated levels--compared to such male-male interactions in the presence of nothing else or volatile compounds from mature male flies (TOMPKINS et al. 1980a, VENARD 1980); (3) can stimulate males to court one another when the pheromones are in the chamber with the males or are as much as 7-8 mm removed (TOMPKINS et al. 1980a); (4) have an optimum concentration for courtship stimulation above and below which they are less effective. (TOMPKINS et al. 1980a, VENARD 1980); and (5) will stimulate males of a closely related species to court, but have no effect on males of a distant species (TOMPKINS et al. 1980a).

These volatile compounds from virgin females are thus similar in their effects to those from the fruitless mutant males discussed earlier. The experiments on substances from females again show that the usual near-zero level of male-male courtship in *D. melanogaster* can be dramatically enhanced by chemical stimuli. In male-female interactions, the chemical stimuli seem to be important, because mutant *sbl* males show substantially reduced courtship activity in the presence of virgin females--about the same 2-fold reduction in CI as effected by mutations affecting vision, and associated with similar abnormalities of male orientation toward the female (Table 5). In addition, tests of pairs of *sbl* males in the presence of volatile compounds from females show no response of these mutant individuals, no matter what concentration of the pheromones was used (TOMPKINS et al. 1980a).

Table 5. Courtship by *D. melanogaster* males with visual and olfactory defects. Blind $norpA^{EE5}$ or gl^3 males, olfaction-minus *sbl* males, and *sbl*, gl^3 males, which are both blind and cannot smell, were tested in courtship chambers, with either two males of the same genotype per chamber, or with one male plus one virgin female per chamber. See TOMPKINS et al. (1980a) for explanation of virgin female extract, how it is used in these courtship tests, and the Courtship Index (CI) that results.

Number of males in each test	Stimulus	Number of tests	% of tested males or pairs performing wing extension	CI ± SEM
1 wild-type	virgin female	20	100	68 ± 2
1 $norpA^{EE5}$	virgin female	10	100	37 ± 4
1 gl^3	virgin female	10	90	27 ± 4
1 *sbl*	virgin female	25	88	31 ± 3
1 *sbl*, gl^3	virgin female	12	17	5 ± 1
2 wild-type	none	112	6	1 ± 1
2 $norpA^{EE5}$	none	10	0	2 ± 1
2 *sbl*	none	10	0	2 ± 1
2 wild-type	virgin female extract	10	40	16 ± 2
2 $norpA^{EE5}$	virgin female extract	10	40	9 ± 1
2 *sbl*	virgin female extract	10	0	2 ± 0

Therefore, it appears as if one can eliminate either sight or smell in tests of the roles that these sensory modalities play in courtship. These tests of the individual mutants suggested that a double mutant might court essentially not at all, since either a blind mutant or an olfactory-deficient mutant has about one-half of its courtship "removed" (Table 5). This prediction was partially borne out: *sbl* was combined with a blind, *glass-eye* (gl^3) mutation. (Males expressing these mutations separately gave CI's that are 46% and 40%, respectively, of the wild-type value; Table 5). When the double-mutant males were tested with virgin females, they courted with the extremely low CI of 5 (TOMPKINS et al. 1980a, Table 5). This courtship performance is barely greater than the "background" level seen between two mature males that are together in a chamber with no other stimuli (Table 5), or, for example, volatile compounds from mature males (TOMPKINS et al. 1980a).

In spite of the low level of courtship activity recorded in short-term observations of *sbl*, gl^3 males, when double-mutant individuals were put with females for several days (one male plus five females per food vial), most of these males inseminated at least one female (TOMPKINS et al. 1980a). It would seem, therefore, that other sensory modalities are sufficient for mating, if the two "major" ones are removed by mutations. For instance, contact-chemoreception or even mechanoreception that is hypothetically associated with male tapping may eventually provide enough male-female "communication" for copulation to occur. In addition, or instead, sound communication of some kind from female to male may tell the sensorily deprived *sbl*, gl^3 male of her presence, stimulate the low-level courtship that is observed, and finally overcome the absence of vision and olfaction. The "important" sound stimuli in *Drosophila* courtship are usually thought to travel from male to female (see above), but auditory stimulation of the male's own reproductive activity has been reported (SCHILCHER 1976c).

MUTATIONS AND MOSAICS AFFECTING THE CNS'S CONTROL OF COURTSHIP

GYNANDROMORPHS AND PHYSIOLOGICAL MUTANTS

Genetic variants affecting the central nervous system and higher behavior in *Drosophila* have begun to be used intensively in studies of courtship. Sex mosaics (gynandromorphs) have different nervous systems from normal flies. Through the use of a cell marker involving acid phosphatase activity, it was found that the brain must have haplo-*X* male tissue in order that a gynandromorph court a female (HALL 1977, 1979). This confirmed the suggestion from earlier work that involved cuticular markers only (reviewed by MANNING 1967a, and studied more quantitatively by HOTTA & BENZER 1976). In addition, studies of marked nervous systems showed that male tissues in only a portion of the brain, in dorsal posterior regions in the left or right side of the head (or both), are sufficient to control qualitatively normal behavior at least at the beginning of the pathway (cf. FIGURE 1). However, subsequent stages of courtship are under the sex-specific control of the ventral ganglia in the thorax. Thus the courtship song is aberrant or absent, and there is no attempted copulation, unless both the brain and the thoracic ganglia have male neurons (SCHILCHER & HALL 1979, HALL 1979).

It has been suggested that these courtship foci relate to male-specific intracellular aspects of neurons in certain portions of the CNS, and/or sex-specific connectivity among neurons (e.g. HALL 1979). The latter possibility would be analogous to sex specificity of morphology seen in parts of mammalian brains whose structure and function is related to reproduction (e.g. RAISMAN & FIELD 1973, GORSKI et al. 1978). There is recent evidence along these lines from diptera: the lobula optic lobe in the visual system of larger flies has sex-specific patterns of fibers (STRAUSFELD 1980); and a thoracic ganglion in *Drosophila* has extra or more extensive fibers in males (STRAUSFELD & SINGH 1980). In the first case, at least some of the cell bodies of neurons whose axons innervate this

optic lobe are in the dorsal posterior brain (STRAUSFELD 1980), to which a courtship focus in *D. melanogaster* has been mapped. However, it is unlikely that sex-specific input through the visual system is solely responsible for male courtship in *Drosophila*, because male flies that have *no* such input still court females (see above).

It has been suggested that the male courtship focus in the brain could relate to male-specific structure or function of the dorsal mushroom bodies; these are bundles of axons, some of whose cell bodies lie at or near the focus in the posterior cortex of the fly brain (cf. STRAUSFELD 1976, and Figure 4, below). Such axonal bundles, forming various lobes, have been postulated to control many (perhaps too many) features of higher behavior in insects (reviewed by HOWSE 1975, and see recent evidence from WADEPUHL & HUBER 1979). Genetic evidence on the possible role of the mushroom bodies in courtship comes from HEISENBERG (1980), who has isolated a *mushroom-bodies-deranged* (*mbd*) mutant in *D. melanogaster*. Axons in at least one major lobe of the mushroom bodies appear to be "rolled up" near their cell bodies in the dorsal, posterior cortex; few fibers, then, run through this lobe in the normal locations. Yet, when males expressing *mbd* are tested in courtship, they are strikingly normal (HEISENBERG 1980)--even in the dark; and even in subtle features of male-female interactions, such as those involving experience-dependent actions (discussed below, cf. SIEGEL & HALL 1979). However, the one mutant allele of *mbd* that is extant does not allow a definitive test of the function of the mushroom bodies in controlling various behaviors, because even the most severely affected individuals in the strain still have ca. 20% of the normal numbers of fibers present in the relevant lobe of the dorsal brain. An *mbd* allele leading to no fibers--if it would still allow survival--is needed if one is to rule out the importance of the mushroom bodies in courtship.

Even if the male courtship focus in the brain of *Drosophila* is dramatically abnormal, male behavior can still be mediated. This conclusion comes from studies of gynandromorphs expressing the neurochemically defective acetylcholinesterase (AChE) mutations (*Ace*) in the male parts of their central nervous systems. The mutations cause embryonic lethality if the whole animal is mutant (HALL & KANKEL 1976); but many of the mosaics expressing an Ace^- allele in only part of the fly survive to adulthood. Several anatomical and functional abnormalities have been seen in these mosaics, including defects in higher behavior (HALL et al. 1979, GREENSPAN et al. 1980). The fact that the Ace^- male tissues in the dorsal brain of such mosaics have aberrant metabolism of the neurotransmitter acetylcholine and are associated with condensation, apparent disorganization and even degeneration of the axonal neuropile, *but* can still mediate male courtship suggests the following possibility: What if male-specific structure and function per se were irrelevant and it is only necessary to have *non-female* tissues at the courtship focus? This requirement would be met if the relevant brain tissues were male and normal or male and defective (FIGURE 4). Thus, female tissues, if present in both left and right brain, might actively inhibit courtship. This

inhibition would be released--and thus visual, olfactory, and other sexual stimuli would be processed--if the focus in one side of the brain had its female-specific function "destroyed."

This hypothesis could be tested, perhaps, through brain ablation experiments on adult flies; but an attractive alternative, still in the realm of known neurochemical dis-

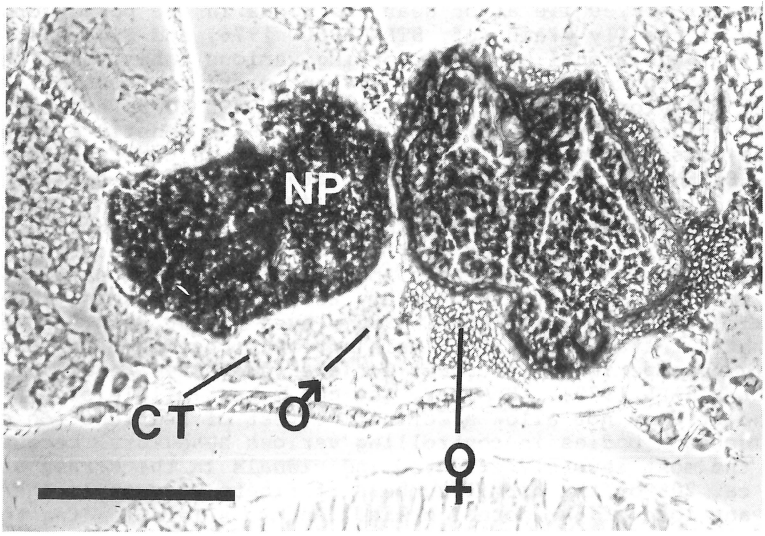

Figure 4. Mosaicism for acetylcholinesterase in *Drosophila* gynandromorph that courted. This horizontal frozen section (10 µm thick) is from the dorsal brain of a sex mosaic constructed so that haplo-*X* male tissues (left side) would be mutant (Ace^-) for the gene that codes for acetylcholinesterase in the CNS. The diplo-*X* female tissues (right side) are Ace^+. This mosaic exhibited courtship, including wing display, when placed with a female. After subsequent sectioning and histochemical staining, essentially the only Ace^-, male tissue proved to be in the dorsal, posterior brain, as shown by the absence of AChE staining in the cellular cortex (CT); the neuropile (NP) adjacent to this mutant cortex shows differences from normal morphology that are characteristic of Ace^- clones in the CNS (see GREENSPAN et al. 1980, who reported behavioral results from tests of this and other acetylcholinesterase mosaics). The scale bar represents 100 µm.

ruptions, would be to construct mosaics for AChE activity that are not gynandromorphs. Instead, these flies would be female throughout but part Ace^+, part Ace^- (all cells would be diplo-*X*,

but the Ace^- tissues would have undergone somatic loss of a small genetic duplication that has been constructed to carry the normal allele of a cuticular marker along with Ace^+--J.C. HALL & A. GROSS, unpublished). If the Ace^- tissues are in the dorsal posterior brain (cf. FIGURE 4), such *a female might court other females*. Such aberrant behavior has been observed in some *Drosophila* strains (e.g. COOK 1975).

The thoracic courtship foci have also been studied in AChE mosaics that are not gynandromorphs; diplo-*X* tissues were turned into male ones through the use of the transformer mutation of STURTEVANT (1945). These thoroughly male mosaics were tested in their courtship song, which is at least in part under thoracic control (SCHILCHER & HALL 1979). In spite of the usual morphological defects seen in association with the Ace^- neural tissues in the thorax, rather subtle defects have been observed. These relate to the regular fluctuations of intervals between pulses of tone that have recently been discovered to be a feature of normal courtship wing vibration in *Drosophila* (KYRIACOU & HALL 1980). Such inter-pulse intervals (IPI's) increase and decrease in a sinusoidal fashion (FIGURE 5). A full cycle of this variation lasts ca. 1 min in the song of wild-type *D. melanogaster* males (but only ca. 60% of that value in *D. simulans*, FIGURE 5). In the male *Ace* mosaics, if one side of the thoracic ganglion is AChE-negative, then the corresponding wing has been observed to show an absence of the oscillations of IPI (HALL et al. 1980). This is perhaps remarkable, because (1) the courtship song produced by this wing does not merely degenerate to random noise or silence, in spite of the severe morphological defects in the underlying ganglion; and (2) the opposite wing, corresponding to Ace^+ tissue, shows the usual sinusoidal pattern of fluctuation, during moments of courtship when that wing is used; it, and the abnormally singing one, were extended normally at various moments. Finally (3) the independent control of one wing or the other, by the respective halves of the thoracic ganglia, is not what might have been expected from studies of gynandromorphs. These courting mosaics (with male tissues labelled by the benign acid phosphatase marker) showed that thoracic maleness in only the left *or* right part of the ventral ganglia is sufficient to control normal song from either wing (SCHILCHER & HALL 1979).

The brains were thoroughly Ace^+ in most of the male mosaics we have analyzed, and showed abnormal song (C.P. KYRIACOU, J.C. HALL and A. GROSS, unpublished). Thus, thoracic control of the IPI fluctuations is strongly implicated. However, the Ace^- clones analyzed so far have not been localized enough to determine if only a particular small portion of the ventral nervous system is relevant.

Oscillating aspects of courtship song have been analyzed preliminarily with another kind of neurophysiologically defective mutation. The no-action-potential (nap^{ts}) mutant is a temperature-sensitive paralytic whose electrical impulses vanish when larval nerves are treated with high temperature (WU et al.

1978). We have assumed that such action potentials would be absent during high temperature treatments of nap^{ts} adults as well. In this light, it was interesting to find out that a nap^{ts} male, which courts normally under our standard conditions of (e.g.) 25°C, can have his reproductive behavior interrupted by heat-induced paralysis, but then will resume courtship almost immediately if the temperature is lowered. When he resumes singing, his fluctuations of IPI begin to occur again in the regular manner (cf. FIGURE 5). Two such mutant males have had their song patterns analyzed in detail, before and after nap^{ts}-induced paralysis. It has been found that the sinusoidal oscillations are not resumed "in phase" (cf. below), in that the IPI produced is out of phase by the same amount of time that the male was paralyzed (KYRIACOU & HALL, unpublished). Thus, the mechanism controlling these oscillations has not "kept running" during the time when the mutant males were paralyzed.

In striking contrast are the results from normal males: either those tested under our usual conditions (N=32 males tested, cf. FIGURE 5) or those treated transiently with high temperatures (N=2). In these cases, it appears as if the males are "singing to themselves" during intervals when they break off courtship (as happens routinely, since Courtship Indices are never 100%, cf. Table 5). This means that, when a male resumes his following of and wing display at a female, it is often seen that the courtship song is resumed with an IPI that would be predicted if the curves drawn to define the oscillations were simply extrapolated onward (FIGURE 5). Whatever the mechanism is that keeps running during non-singing intervals, the results from the nap^{ts} males suggest that it requires normal nerve impulses to be maintained.

Other attempts to disrupt the normal patterns of IPI oscillations have involved the application of different temperatures or light-dark regimes to wild-type males during developing and/or adult stages. These treatments have been found not to affect these highly regular, built-in periods of fluctuating acoustical behavior (KYRIACOU & HALL 1980).

CIRCADIAN-RHYTHM MUTANTS

The fluctuations of IPI in courtship song are so regular that it seemed as if it would be valuable to test the effects on this behavior of other mutations, particularly those in *Drosophila* that were isolated on the basis of abnormal "timing". These are the period (*per*) mutants of KONOPKA & BENZER (1971) which express aberrant circadian rhythms. Three different EMS-induced alleles of this *X*-chromosomal gene alter periodicity of diurnal rhythms in different ways: either shortening them ca. 20% (from the usual 24 hours), lengthening them ca. 20%, or abolishing them altogether. Rhythms of both eclosion and general locomotor activity are affected in the same way in each mutant. But it was not known whether these relatively long-term rhythms--in *Drosophila* or in other organisms--have a relationship to mechanisms controlling short-term oscillations (e.g. PYE 1969, 1971).

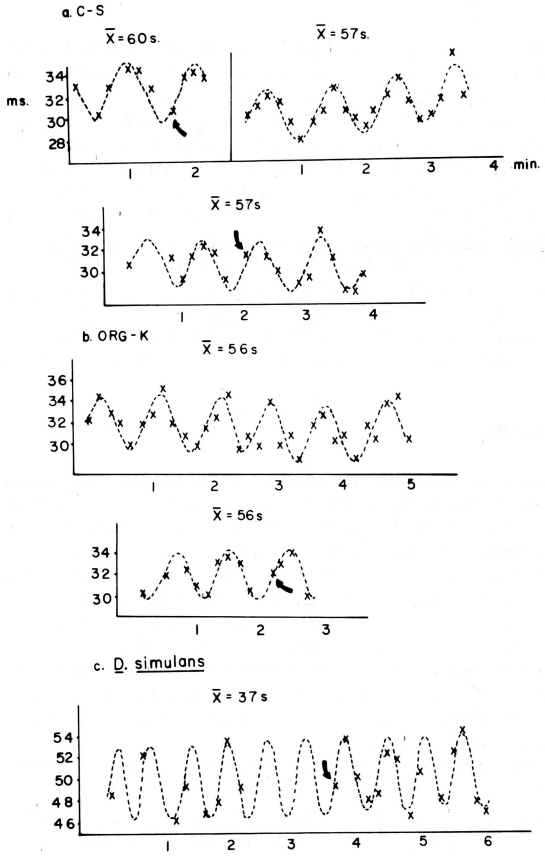

Figure 5. Variation in inter-pulse-interval (IPI) for courtship songs of wild-type *Drosophila* males. These males had the wing vibrations they directed at females recorded. Mean intervals between pulses of tone (IPI's in millisec = ms) were calculated for successive 10-sec (=s) fractions of the 3-6 min observation periods. Each point represents such a mean IPI (the standard errors range from the same size to 2-fold the height of the symbols). Symbols (x) do not appear for all time frames monitored, because the males often broke off their courtship during some of the 10-sec intervals. When they resume singing, their mean IPI for that 10-sec interval tends to be what would be predicted from the functions that are expressed as dashed lines (see arrows for examples). These sinusoidal curves were fitted by eye to have a near-constant period length ($\bar{X}$) of ca. 1 min from (a) 3 *Canton-S* wild-type males of *D. melanogaster* and (b) 2 *Oregon-K* wild-type males from this species; the *D. simulans* male (c) had a much shorter period length. All of these IPI's do fluctuate in a non-arbitrary sinusoidal fashion, as shown by the statistical analysis presented in KYRIACOU & HALL (1980).

When the three *per* mutations were tested (N=at least 12 for each mutant) in males for effects on courtship song, it was found, strikingly, that males expressing the *per*-short allele have period-lengths of IPI fluctuations of only 40-45 sec; *per*-long males have 80-90 sec periods; and the arrhythmic per^{o} males sing with different IPI's during different moments of courtship, but there was neither a sinusoidal nor any other regular pattern of fluctuation (KYRIACOU & HALL 1980). All of these mutant males, like wild-type, had IPI's that were in the range of 30-38 msec. Males expressing per^{o} showed more variability of IPI per se, in addition to no regular periodicity of fluctuation. Yet neither the two mutants with regular oscillations, nor the wild-type males with their ca. 1-min periods have anything approaching invariant lengths of time between pulses.

LEARNING MUTANTS

The final kinds of higher behavioral variants to be discussed involve learning and memory. It would seem that conditioning is irrelevant to courtship and mating in *Drosophila*, because of anecdotal reports showing that, for instance, male flies if isolated from eclosion, then put with females, will nonetheless court them. No previous "experience" would apparently be required. However, detailed measurements of this kind of courtship have not been made. What if, for example, the fluctuating features of courtship song were not performed, unless the male had previously been exposed to all the details of normal auditory behavior? It is conceivable that this is the reason for the newly emerged male to release pheromones that cause him to be courted so vigorously (see above).

The fact that conditioned behavior is sometimes relevant to *Drosophila* courtship comes from studies involving olfactory information passing from female to male. It is known that mated females of *D. melanogaster* stimulate relatively little courtship, compared to virgin females (e.g. SIEGEL & HALL 1979), addition to the well-known fact that the former kind of females do not readily re-mate until a few days after their initial copulation (e.g. MANNING 1967b, BURNET et al. 1973). Further examination of this phenomenon showed that the mated females are almost certainly producing an aversive pheromone, along with the stimulating substances made by virgins (TOMPKINS & HALL 1980).

The possible relationship of learning to the effects on males of mated females came from experiments showing that there is an after-effect on male behavior: that is, a male put with a mated female for an hour or more shows poor courtship when *subsequently* put with a virgin female (SIEGEL & HALL 1979). This after-effect lasts at least 2 hours. It was first thought that the male is merely debilitated, because he was exposed to "noxious" materials associated with mated females, and thus needs many minutes to recover. However, these males do not appear to suffer decrements in their general behavior after being exposed to mated females (SIEGEL & HALL 1979). More interesting is the fact that a bona fide memory mutation, amnesiac (*amn*--

QUINN et al. 1979) results in a much shorter-than-normal after-effect, when mutant males are tested with mated females and then virgins (SIEGEL & HALL 1979). The memory mutant was isolated on criteria unrelated to courtship, and, in addition, appears to have normal olfaction (QUINN et al. 1979); moreover, *amn* males definitely sense that females have mated, when they are in the presence of such females (SIEGEL & HALL 1979). They simply "forget" rather quickly that they were with the flies that had given them negative cues. If males were generally debilitated by exposure to mated females, or, more subtly, had poor function of olfactory receptors induced, then both wild-type and *amn* males should show similar after-effects. The results from the mutant, then, suggest that wild-type males show poor courtship of potential partners for some time after having been with mated females, in order to "avoid" such aversive stimulation.

It was predicted that another mutation affecting conditioned behavior, *dunce* (DUDAI et al. 1976), would also show abnormal responses to "treatment" by mated females. Flies expressing this mutation essentially do not learn, let alone remember. Thus, *dunce* males were examined with mated females (and found to court such females poorly, as usual), with the idea that the males would show no after-effect when subsequently placed with virgins. However, these mutant males do show the normal decrease in courtship in the second test. This negative result does not necessarily undermine the idea that learning is involved in male-female interactions. This is because *dunce*, after being isolated on the basis of defective learning in "shock-odor" experiments (DUDAI et al. 1976), was later found to be able to learn in other kinds of tests (DUDAI & BICKER 1978, DUDAI 1979).

Other non-learning mutants were induced after *dunce* was found (QUINN et al. 1979, W.G. QUINN, unpublished). Two of these *X*-chromosomal mutations, *turnip* and *rutabaga*, have been found in preliminary tests to result in males being poorly trained, if at all, by exposure to mated females (R.W. SIEGEL & D. GAILEY, unpublished). Additional learning mutants should be tested, to ask further if *dunce* is an exceptional case in the mated-female experiments. It will also be important to analyze the involvement of all of these genes in courtship situations that do not involve mated females, in which the effects of previous experience are suspected (O'HARA et al. 1976, PRUZAN 1976, PRUZAN et al. 1977, SCHILCHER 1976b).

CONCLUSIONS

It is obvious that courtship in *Drosophila* is complex, involving many sensory modalities and also the function of, no doubt, many portions of the central nervous system in order that the sophisticated actions performed by males and females take place. However, we believe that it has been possible to dissect this complexity, by interfering with different aspects of the fly's excitable tissues, one at a time. The mutant genes have, we hope, allowed relatively specific perturbations to be made so that only sight, only smell, or only learning are affected in

the courting flies expressing the relevant genetic variants.

One problem with our approach is that we cannot prove that a given mutation, with hopefully one specific defect in neural structure or function, is devoid of other non-specific defects in its behavior. Decrements in courtship could thus have unknown causes. Therefore, it may be better to use non-genetic intervention for some courtship experiments. For instance, it is easy to cut off all or part of a male wing, and test the effects of defective courtship song on female receptivity. A wingless mutant with defective courtship might have other abnormalities besides the overtly observable ones. For visual defects, which we have induced genetically in order to ask if blind flies do not court properly, it might be best to turn off the lights and monitor behavior. However, some of the aspects of vision we have tested involved more subtle genetic defects, that is, normal sight per se but defective detection of movement. Such an abnormality might be impossible to induce by surgically manipulating the fly or changing its environment. The flies with defective olfaction were rather easily obtained genetically; and there is no alternative method known to remove all sensory input of this kind. When disruptions of the central nervous system were used to see what aspects of higher function might be related to courtship, it was perhaps essential to use circadian-rhythm mutants, learning mutants, and genetic variants of neurotransmitter metabolism. For these aberrant genotypes, we again do not claim to know that the various mutations are entirely specific in their action. But drug treatments aimed at blocking learning or various features of acetylcholine function would very likely also cause the fly to be generally debilitated. With mutants, though, even lethal variants (such as those eliminating acetylcholinesterase) are available for use in courtship experiments: because the expression of such mutations can be readily removed in a particular portion of the nervous system. This kind of genetic mosaicism of enzyme expression was important in other experiments on courting flies, and it is important to stress the desirability of directly identifying the genotype of various parts of the CNS, after behavioral testing and sectioning of the genetically mixed individuals.

All of these genetically abnormal flies--whether expressing simple genotypes such as an individual sensory mutation, or a complex one, such as sex-chromosomal mosaicism in conjunction with a neurotransmitter mutation--were readily testable in courtship. The altered fly was usually "healthy" in general, and thus could simply be put with a potential courting partner. Since a male and a female must move about, use their appendages, and achieve various postures as they court, it is very desirable to allow them to do all of their actions except for those one would like to perturb selectively. To probe the neurobiology underlying ostensibly simple reflex behaviors may be possible using tethered animals or physiological "preparations." To study complex action patterns, though, often requires observation and measurements of the whole, freely moving organism.

What have we found to be important for the initiation and successful conclusion of reproduction in this organism? Without re-iterating the details of the results, it is important to recall our findings that the flies can still court even in the absence of visual behavior, auditory behavior, olfaction, and even in the presence of rather severe abnormalities of the CNS. Thus none of these features of neural structure or function are necessary. Are they important at all? They seem to be, because significant quantitative decrements are found if only one factor is perturbed, and times required for actual mating to occur can be substantially lengthened. Furthermore, if two known factors are perturbed simultaneously--such as sight and smell--then mating ability nearly vanishes, similar to what is found for mutants actually blocked at an intermediate stage of the courtship pathway. These mutants, though they were found with respect to defects in courtship, are not yet known to have specific defects in the nervous system's morphology, physiology, or neurochemistry. But the genes defined by such variants will hopefully prove to be interesting etiologies. It will be important to keep these mutants in mind, because the wild-type function of genes such as *celibate* and *fruitless* can be absolutely required for successful reproduction. Even the genes whose functions are not absolutely required may be more important than could be suggested from our laboratory studies. Mutants such as *cacophony*, *no-receptor-potential*, *optomotor-blind*, *smell-blind*, or *per*^{0} might have little or no chance of mating in the wild. Thus, the decrements in courtship that we have shown to be associated with such variants are possibly of considerable significance.

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Dr. Jeffrey C. Hall demonstrating the courtship behavior of *Drosophila*. Dr. Maurice Green is shown on the right.