The Mating of a Fly

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Courtship in *Drosophila* is influenced by a wide variety of genes, in that many different kinds of pleiotropic mutations lead to defective courtship. This may seem to be a truism, but the broad temporal and spatial expression of most of the fly's "neuro genes" makes it difficult to exclude elements of such genes' actions as materially underlying reproductive behavior. "Courtship genes" that seem to play more particular roles were originally identified as sensory, learning, or rhythm mutations; their reproductive abnormalities have been especially informative for revealing components of male or female actions that might otherwise have gone unnoticed. Further behavioral mutations seemed originally to be courtship-specific, turned out not to have that property, and have led to a broadened perspective on the nature and action of *Drosophila's* sex-determination genes.

"A fly got into the transmitter pod with me that first time . . .
My teleporter turned into a gene splicer, a very good one . . .
You look so pretty . . . Help me, please help me."

—BRUNDLEFLY (*1*)

The connections among flies, genes, and romance, implied by the opening remarks, will be discussed in this review from the perspective of *Drosophila* genetics. Courtship and mating behavior of the fruit fly is influenced by several different categories of mutants and other kinds of genetic variants (Table 1). One such category includes strains isolated on the basis of apparent "courtship-specific" abnormalities. A second, which does not materially include mutations, involves gynandromorphs, flies that are chromosomally part male and part female; these have been analyzed by correlating the performance of sex-specific behaviors with the presence of chromosomally female (XX) versus male (XO) tissues in different body parts [reviewed in (*2*)]. The chromosomal genotypes just noted are the primary genetic signals that send a *Drosophila* embryo down one of two separate, sex-specific developmental paths [reviewed in (*3*)]. Thus, the double-X and single-X types form a bridge to the third genetic category within this behavioral subject, which involves genes acting downstream of these primary sex-determining signals (*3*).

At present, relations among (i) mutants originally found and studied from a solely behavioral perspective, (ii) others initially identified in the context of sex-determination, and (iii) sexually dimorphic structures and substances have finally intersected at the place where males meet females. Before considering how these topics have become interrelated, it is useful to introduce the biological characters and the pertinent behavioral phenotypes. These reproductively related matters include a bonus: They make some general points about how genes act to build and operate a nervous system.

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How Flies Mate

Courtship in *Drosophila melanogaster* involves a series of behaviors, most of which were caught on film during the production of a certain blue movie (Fig. 1). Once the male and the female have come into some reasonable proximity (perhaps on a food source or when experimentally put together in a mating cell), they quickly sense each other. Primarily, this seems to be the male detecting the female by using more than one sensory modality. Soon after the male reveals that he has noticed the female [which one infers by observing the orientation of his body toward hers (Fig. 1A)], he taps the female's abdomen (Fig. 1B). If she is walking about, he follows her (Fig. 1C) during most of the time that she is moving in this manner [no courtship occurs in flight, unlike the capabilities of some other dipterans (*4*)]. As the male orients to a stationary female—including circling her (*5*)—or follows a mobile one, he frequently sticks out one wing or the other (Fig. 1D). This extension of the wing is accompanied by its vibration (Fig. 1E), which produces a "love song" that can be recorded with specialized microphones (*6,7*); the measurable components of these sounds are among the more salient species-specific elements of fruit-fly courtship. Several seconds to a few minutes after the two flies have begun to interact, the male extends his proboscis and licks the female's genitalia (Fig. 1F). Licking is almost immediately followed by the male's first copulation attempt (not shown in Fig. 1), which involves an abdominal bending by the male; this can be viewed in more contorted form by looking at the posture accompanying copulation per se (Fig. 1G).

If an attempted copulation fails, the male may cease courting for some moments. Thus, overt courtship interactions occur only about 60 to 80% of the time when the male and female are together (called the Courtship Index) [reviewed in (*8*)]. When the male resumes courting, he almost always drops back to the orientation and following or singing stages (that is, not to tapping or licking) and continues through the rest of the sequence. This series of actions and inter-fly interactions is successful in more than 90% of short-term laboratory observations of wild-type pairs. "Success" means copulation, which has a species-specific duration (about 20 min in *D. melanogaster*) (*9*).

Female courtship actions in this species are not very apparent to a human observer. In fact, the female need not "do anything" to elicit all elements of male courtship [paralyzed *D. melanogaster* females are still courted (*10,11*)]. Yet the female is not thoroughly passive. When she is in principle "receptive" to the male's advances (see below), she nevertheless performs some rather subtle (and mild) rejection behaviors (*12*) (Fig. 1C), which are considered to represent "female coyness" (*13*). Eventually, she indicates receptivity by slowing down her general locomotor activity, apparently to make her a better target for the male's copulation attempts. For an attempt to succeed, it appears as if the female must assume a particular posture and manipulate her external genitalia appropriately (*13*).

If a female of this species is not inherently receptive to courtship because she had mated recently, then she produces a more overt rejection response to male courtship: A fertilized female extrudes her ovipositor in the face of a male who tries to mate with her (*12*); this blocks most copulation attempts. The chemical profile presented by the female is also altered as a result of mating (*8,14,15*). This pheromonal change, along with copulation-blocking extrusion, influences the male's courtship in a manner that will bring us to the question of just how "fixed" is the "action pattern" constituting courtship in this fruit-fly species.

Courtship and Significant-Other Mutants

There are almost no *Drosophila* mutants that specifically exhibit courtship defects, possibly because few dedicated screens for such variants have been carried out. Thus, the *D. melanogaster* genome is far from being "saturated" with identification (by mutagenesis)

of reproductive-behavioral genes, whether or not the loci would somehow be dedicated to courtship control. Indeed, no saturations have been documented in the area of neural function in *Drosophila* (*16*), as opposed to what seems to have been accomplished with regard to certain categories of developmental mutants (*17*).

Courtship mutants are pleiotropic in two ways. On the one hand, generally enfeebled genetic variants will be defective in their reproductive (and possibly all other) behaviors; thus, the demanding motor patterns that make up courtship may be especially sensitive to things like general metabolic defects. On the other hand, there are some more informative cases of *Drosophila* mutants—usually isolated by criteria unrelated to courtship—that also exhibit well-defined abnormalities in their reproductive activities (Table 1).

Generally defective mutants. Almost any mutant with an extremely visible abnormality might be revealed as defective in courtship if scrutinized with enough care (*13*, *18*). Some potentially more interesting cases include body-color mutants (notably *yellow* or *ebony*), the males of which have been observed to court abnormally; this may be because such pigment defects often go hand-in-hand with neurochemical problems (*18*).

Several of the large number of "general locomotor" mutants of Homyk and colleagues (*19*) were tested in courtship and found wanting. Many of these mutants were by definition sluggish (the *hypoactives*), so it is not surprising that their courtship vigor was subnormal. The same is true for the *inactive* (*iav*) mutant (*19*), which has been subjected to detailed courtship observations; *iav* also seems to be a neurotransmitter variant, although this does not also reveal itself as an off-colored fly.

More and more *Drosophila* mutants identified in studies of the nervous system have come from reverse genetics, and what I call quasi-reverse genetics. Take *couch-potato* (please); the mutant male exhibits the *Drosophila* equivalent of preferring a stupor to a potential mate (*20*). The locus (*cpo*) was first found by "enhancer trapping": Expression of a reporter gene carried in transposons that became integrated near *cpo* resulted in a staining pattern that promoted further study of this strain. When certain of the *cpo*-locus transposons were made homozygous, a sluggish behavioral phenotype resulted (*20*). Other screens of this general sort, which have not yet involved assessment of reporter expression in the mobilized transposons, led to courtship mutants at some new loci and an old one: One was dubbed *cuckold*, owing to the mutant males' frequent failure to mate; the two allelic *cuc* mutants also exhibited decreased longevity (*21*). Another recently "tagged" locus (*21*) had previously been identified by a most intriguing courtship mutation, known as *fruitless* (see below). An independent search for transposon-tagged reproductive variants led to what seems to be the only female-specific behavioral mutant known: *spinster*, which is subnormal in mating receptivity and performs accentuated repelling behaviors (*22*).

A thoroughgoing reverse genetic approach (*23*) begins with a search for the normal gene or some version of its expression, as in the case of *cpo*. The quasi-reverse strategy examines an aspect of the fly's neurochemistry or neuroanatomy, finds the normal gene that controls these features of the fly's biology, then tries to find mutations at that locus and tests them for behavioral effects (*23*). Courtship studies in the area of quasi-reverse genetics include tests of brain-damaged mutants, whose males exhibit generally subnormal courtship vigor: *minibrain* and the "central complex" mutant *no-bridge* (*23*). Separate kinds of screens have been carried out in which anomalous profiles of adult pheromones were sought; one mutant that resulted is *nerd*, which is another poor male courter (*24*); how this seems not to be related to the pheromonal phenotype will be discussed below.

Should any of these mutants be dignified with the adjective "courtship," and will an intensive molecular analysis of the relevant genes (so far performed only in the case of *cpo*) reveal anything interesting about how the fly is genetically programmed to court? The easy answer is "no." Yet, many *Drosophila* genes that are legitimately considered interesting from the standpoint of neural function are expressed very broadly (in tissues, as well as during the life-cycle) and are able to mutate to lethality—even for cases in which the original (and necessarily viable) mutant exhibited behavioral defects of a particular kind not accompanied by

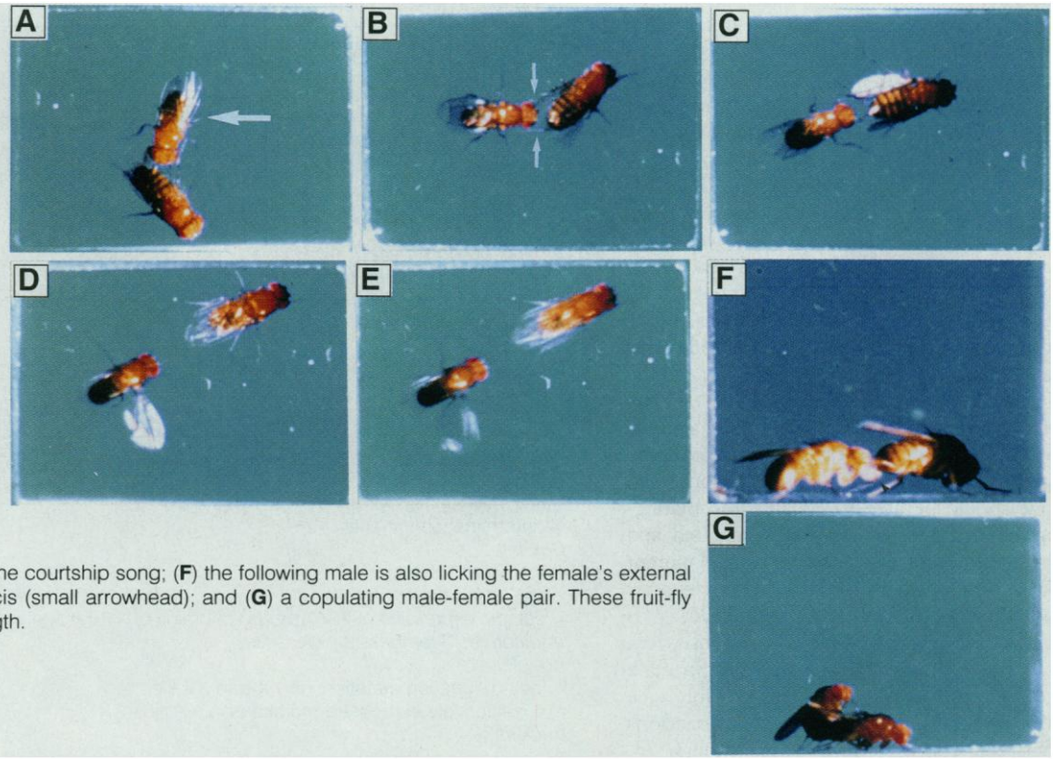

Fig. 1. Courtship of *D. melanogaster*. A virgin male-female pair was placed in a plastic mating cell, as implied by the border of each of these seven images. The courtship sequence proceeded in the order indicated by (A) to (G) (see text for further details). In (**A**), the male (large arrow) is orienting toward the female; (**B**) he taps the female by reaching out toward her abdomen with both of his forelegs (small arrows); (**C**) the male follows the female, who has almost finished flicking her wings at him, this being one of the mild rejection responses exhibited by virgin females (*12*); (**D**) the following male is also extending one of his wings, in preparation for (**E**) vibrating the wing to produce the courtship song; (**F**) the following male is also licking the female's external genitalia by extending his proboscis (small arrowhead); and (**G**) a copulating male-female pair. These fruit-fly adults are about 2 to 3 mm in length.

generalized hypoactivity. Thus, even some of these "sick" mutants (*iav, cpo, cuc*) could define factors that are materially involved in building and operating portions of the nervous system that control courtship. Alleles of such a gene—subtle variants, or forms of the gene that vary in different species—could therefore help to "program" a part of the nervous system that is relatively dedicated to courtship control. Other, more severely mutated variants at such a locus, which result in an overall decrement in the adult fly's health, or death during development, would thus mask the fact that the gene really is in part a behavioral one.

Mutants with more particular defects. This category includes genes that can more comfortably be viewed as real behavioral factors, even though the effects of the mutations at these loci are once again pleiotropic. Such phenotypic defects, however, (i) tend not to be global (as opposed to some of the cases noted above, in which "any" kind of behavior is poorly performed); (ii) are therefore bi- or tri-modal in their features (a small number of particular, although ostensibly unrelated, behaviors are defective); and (iii) frequently involve genetic loci that cannot mutate to developmentally lethal forms.

1) Visual mutants. Courtship studies in *Drosophila* began (near the dawn of this organism's genetics) with experiments on sensory mutants. Thus, *white* (*w*) eye mutants were shown to court in a mediocre manner, largely the result of their poor visual acuity (*25*): A *w* male's optomotor responses to moving females are impaired, and he "tracks" the female in a particularly anomalous manner (*25*). That optomotor behavior is important in courtship was emphasized by the demonstration that a mutant, *optomotor-blind* (*omb*) male courts subnormally. In fact, *omb* males were found (*26*) to be just as courtship-defective as are thoroughly blind males, expressing a *no-receptor-potential-A* (*norpA*) mutation (*16, 26*). The pleiotropy issues belabored above are relevant to these studies: Are *w* or *omb* males courting subnormally for reasons other than their visual defects? [Indeed, *omb* turned out to define a developmentally vital locus, whose various allelic forms lead to a fairly wide array of neural and other tissue defects (*27*); and the *norpA*-encoded product is expressed well beyond the eye (*17*)]. If not, and thus if a purely visual etiology for these mutants' courtship problems is the correct interpretation, then one should be able to equalize the mating-initiation latencies of visually mutated and wild-type males by performing parallel mutant and control tests in the dark. This is exactly what happened for *w* and *omb* (*25, 26*).

2) Olfactory mutants. It might seem obvious that the manner in which a male sees and tracks a female (and possibly vice versa) would promote sexual interactions between the two flies [even though *D. melanogaster* readily mates in total darkness, as do flies of several but not all *Drosophila* species (*28*)]. Odor control of male-female recognition could also be judiciously guessed to play a role in the reproduction of these insects. Olfactory influences on courtship have been investigated (*8, 29, 30*) by bioassaying the effects of compounds extracted from females (virgin or mated) or males (several-day-old versus very young). Males can smell females (*31, 32*), but only over a distance so short that the distinction between smelling and sensing by contact chemoreception is obscured (recall that the male taps the female early in the courtship sequence).

In these experiments, male courtship could be influenced by placing nearby either a virgin female or compounds extracted from her (along with a chemically neutral courtship object); mutant males expressing *smellblind* or *olfactory-D* mutations were unresponsive to either odor source (*31, 32*). These two mutations—which cause the flies to be insensitive to a variety of odorants—turned out to be allelic to each other; moreover, each is also mutated at a Na^+ channel–encoding locus called *paralytic* (*para*) (*33*).

The application of $para^{sbl}$ and $para^{olfD}$ in courtship experiments showed that these mutant males are, perhaps surprisingly, not all that bad at courting and initiating cop-

Table 1. Mutants, manipulated genes, and *Drosophila* courtship.

Mutant	References
General decrements in courtship vigor and male mating ability	
yellow and *ebony* body-color mutants	18
inactive	19
couch potato	20
cuckold	21
minibrain and no-bridge brain-damaged mutants	23
nerd	24
Visual mutants	
white eye mutants, depleted of screening pigment	25
optomotor-blind	26, 27
no-receptor-potential-A blind mutants	16, 26
Olfactory mutants	
smellblind (*sbl*) and *olfactory-D* (*olfD*) alleles of *paralytic* gene	31–33
Abnormalities of female receptivity	
spinster	22
sbl and *olfD* olfactorily defective mutants	31, 32
sex-peptide gene ectopically expressed in female transgenics	112
Rhythm variants	
period (*per*) mutants	7, 37, 43, 44
per gene from *D. simulans*	60, 61
Learning and memory variants	
dunce	2, 48, 50, 52
rutabaga	2, 48, 50
amnesiac	2, 47, 48, 50
Shaker	57
ether-à-go-go	49, 57
CAM-kinase–depleted transgenic	49
Courtship song mutants	
cacophony	62, 63, 78, 85
dissonance allele of *no-on-transient-A* gene	67–69, 78, 85
croaker	22
fruitless	78, 85
Behavioral male sterility and bisexual orientation	
fruitless	11, 22, 72–74
Sex-determination variants	
Sex-lethal	84
transformer	
tra^- in XX flies	37, 63, 67, 68, 83
ectopic expression of TRA^F protein in brains of XY transgenics	89
transformer-2-temperature-sensitive	86
doublesex	
loss-of-function mutations in XX and XY flies	83, 79
constitutively expressed mutations in XX flies	79
fruitless?	75, 79, 81

ulation with females (*31, 32*) (they are less defective than the aforementioned visual mutants). However, a female homozygous for either of these two *para* alleles courts abnormally in that she tends to keep moving about the mating cell and for this reason, it seems, takes longer than normal to be receptive to mating attempts (*25, 32*). The male-dominated view of *Drosophila* courtship seemed to assume that the main odor cue would be a female aphrodisiac, acting on the male (but not summoning him from a long distance). In addition, the genetic dissection of these behaviors indicated that male-to-female chemical communication plays a role.

None of these sensory cues is by itself essential in laboratory courtship settings. Are they therefore "redundant" stimuli? No; in laboratory courtship tests of these mutants, all of the visual and olfactory variants exhibit measurable if subtle decrements. Moreover, as the usual wheeze goes: "In nature, any such mutant would be behaviorally sterile." In an artificial setting, however, virtual sterility could only be achieved by rendering both males and females blind as well as unable to smell and, in the case of the male, mute (*32*).

3) Rhythm mutants. A silent courting male is created by removal of his wings, which leaves him willing to court vigorously and able to copulate (*34, 35*), but this mute male's mating-initiation latency is lengthened, and the female seems to move too much and for too long when an acoustically challenged male is after her (*35*). One can ameliorate this problem by playing electronically mimicked song to the courting pairs (*35, 36*). The key components of these acoustical signals—the "pulses" of tone generated by wing-vibrations—are depicted in Fig. 2.

This playback-elicited enhancement of courtship success worked best if an intriguing feature of the song was programmed into the electronic song-simulator (*36*). This is a song rhythm in which the intervals between pulses gradually lengthen, shorten, then lengthen again over the course of a given courtship minute (*36, 37*). The song-rhythm story became an extensively genetic one after it was found that circadian rhythm mutations also affect the 55- to 60-s periodicity that accompanies the songs of *D. melanogaster* males (*7, 37*). The principal rhythm variants applied in these courtship experiments are *period* (*per*) "clock" mutations (*38*). *per* is a key factor in the operation of the fly's circadian pacemaker, but in the current mania legitimately swirling around the expression and action of this gene (*39, 40*), it has conveniently been forgotten that *per* is not only an ultradian (<<24 hours) clock-controlling factor, but also influences an infradian (>1 day) cycle (that is, the total time from fertilized egg to newly emerged adult) (*41*). The molecular models for *per* function (*39*), and that of its fungal soul-mate *frequency* (*40*), do not permit a facile explanation of *per*'s pleiotropic timing effects.

Such pleiotropy seemed as if it might extend to an effect on female receptivity, that is, to the male's oscillating song signals. Would the "sender" and "receiver" be genetically "coupled?" (*42*). Thus, a *per* mutant male, singing with a long-period rhythm (about 80 s), could be received best by a female carrying that same (per^L) mutation; the same sort of sender-receiving matching could have been shown with regard to short-period (40 s) per^S male-female pairs. But the per^L and per^S females responded best to normal (1 min) song rhythms (*43*), so that *per*'s pleiotropy does not extend to an effect on the reception or processing of cyclically varying song signals.

4) Learning mutants. Reproductive behavior in *D. melanogaster* is not hard-wired. The ability of males to discriminate between females that have recently mated (largely as a result of the different pheromonal profile a mated female presents, as compared to a virgin female) and to "learn" to diminish the amount of courtship they direct at fertilized females helped to reveal this fact (*2, 30*). The male also remembers that he should not court a recently mated female (*44*), because she can block most copulation attempts. The female becomes receptive again once all the sperm from the first mating are used up (*45*). It makes sense for the male who mates early to "turn off" the female, in that, if a second-mating occurs, the sperm from that "β-male" tends to be used first (*46*).

Decrements in male-with-mated-female courtship, and their afteraffects (*30*), are not only useful to the flies but also involve information storage and retrieval: (i) The male must associate negatively acting cues (the natural one being the anti-aphrodisiacs presented by fertilized females) with the presence of a courtship object, such as the mated female herself, who continues to generate her usual aphrodisiac (*13, 47*). (ii) Learning and memory mutations, isolated by associative learning and memory criteria unrelated to courtship, cause abnormalities in what can be called conditioned courtship (*30*). (iii) These defects involve inappropriately vigorous male courtship, exhibited by the classical *dunce* (*dnc*), *rutabaga* (*rut*), and *amnesiac* (*amn*) mutants (after they were trained in the presence of mated females); thus, these mutant males are not defective in conditioned courtship because of some generalized enfeeblement, which might have been the interpretation (*48*). (iv) Certain neurochemical variants, originally induced or molecularly engineered without any connection (then) to learning and memory, were shown to affect experience-dependent behavior in part through the mated-female inhibition system (*49*). In one of these variants, a dominant-negative calcium-calmodulin (CAM)–kinase transgenic that had been manipulated further to

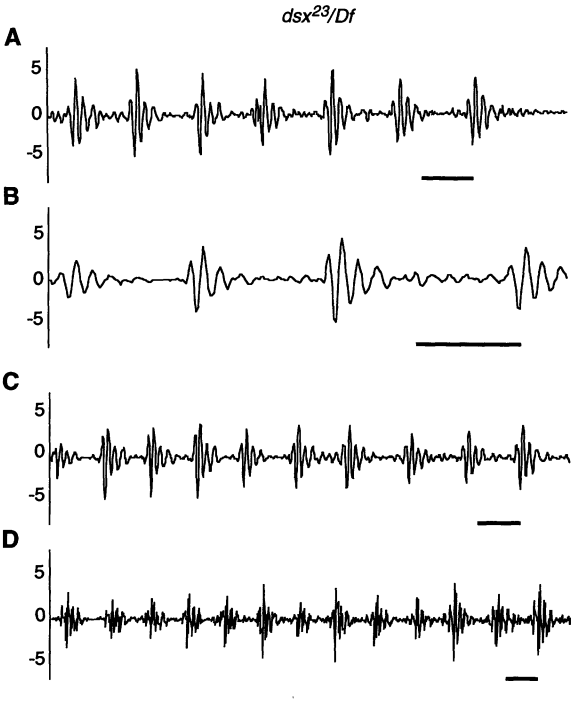

Fig. 2. The love song of *D. melanogaster*. These song traces resulted from recording of the wing vibrations (Fig. 1E) generated by two separate courting males. (**A** and **B**) A male heterozygous for a *doublesex* loss-of-function mutation (dsx^{23}) and a deletion (*Df*) of this autosomal locus [compare (*3, 79*)]. The trains of pulses shown came from two separate singing bouts (A and B), which were recorded with an electret microphone (*6, 90*); the taped record was digitized and converted to these visual images (*67, 83, 90*); in (B), a higher resolution trace was printed to better reveal the cycle substructure of the four pulses shown. These pulses look essentially identical to those produced by courting wild-type males of this species, and the various song parameters (extracted from analyses of the digitized records) revealed that this mutant type sings in a largely normal manner (*79*); for example, wild-type *D. melanogaster* males generate about 25 to 30 pulses per second. All scale bars, 30 ms. (**C** and **D**) Two separate pulse trains generated by another male of the same *dsx*-mutant genotype as in (A) and (B); (C) is indistinguishable from a wild-type train; in (D), the pulses are somewhat more raggedy, but within the normal range.

effect a more severe decrement in the enzyme, males acquired the effects of training in a mediocre manner, let alone exhibited diminished memory of training (*49*).

The engineered CAM-kinase variant was also subnormal (*49*) in a nonassociative component of courtship that is once again based on the flies' previous experience. Naïve, virgin females subjected to song prestimulation exhibit enhanced receptivity (as compared to nonserenaded controls) when males are subsequently introduced to these females; to be effective, this prestimulation (by machine-simulated pulse-song) must include the appropriate rhythmic component (*50*). The CAM-kinase–depleted females were subnormal in the extent to which they could be acoustically primed (*49*). The earlier experiments of this kind had provided one of the first demonstrations of connections between the genetic control of [and cyclic adenosine monophosphate (cAMP) involvement in] associative and nonassociative learning, in that *dnc*, *rut*, and *amn* females exhibited minimal or aberrantly short-lived effects of song pre- stimulations (*50*). But what if these pleiotropic mutants (*48*) were merely deaf? Not so for the three mutants just noted: In experiments based on the fact that song stimuli in *Drosophila* are input through the antenna (*35*), the learning mutants were shown to hear quite nicely (*50*).

There is one other kind of courtship conditioning that also seems to be a nonassociative phenomenon: Mature males court immature (<1-day-old) males very vigorously (*51*). This is a waste of the courter's time, if not the courtee's (see below); but the former does "learn" to cut it out, and subsequently he courts a young male hardly at all (*2, 30, 44*). All the usual learning mutants are aberrant in this regard (they keep courting vigorously) (*2, 30*). The adaptive significance of this element of conditioned courtship was demonstrated in experiments testing for short-term evolutionary fitness (*52*).

The young males elicit so much courtship because they possess a special stimulating pheromone (*29, 32, 51*), which is not the same as the female-produced aphrodisiac (*54, 56*). Unlike what (and how) a mature male learns from an experience with a mated female, he exhibits courtship decrements in the presence of an immature male after exposure to the sex-stimulating substances only, which can be extracted from that very young fly (*32, 54*). This seems to be habituation, the converse of the sensitization-like phenomenon described above in regard to song prestimulation of females.

The courtships that males experience with mated females or immature males provided a way to test another part of the "molecular learning machinery" that had been uncovered in experiments on molluscs (*55*). Thus, in addition to cAMP and phosphorylation reactions being involved in both organisms, potassium channels would seem to be as well. For flies, the *Shaker* (*Sh*) and *ether-à-go-go* (*eag*) mutants (*56*) have been revealed as defective in the acquisition and retention of learning, principally by testing with the aforementioned kinds of unreceptive courtship objects (*49, 57*). Previously, no phenotypic features of these genetic variants permitted them to be called behavioral mutants. Their phenotypic defects (shaking, which is most pronounced under anesthesia, as the name for *eag* implies) did not refer to any specific or meaningful features of wild-type function—mere well-being (in Sh^+ or eag^+) not being a behavior. In contrast, conditioned responses to stimuli are bona fide behaviors, which are readily quantifiable in dedicated testing regimes applied to genetically normal flies. This permits comparison of mutant "learning indices" to values measured for the wild type, whether they are obtained in contrived situations involving electric shocks and artificial odorants (*47, 58*) or more "natural" ones involving courtship.

Courtship song. This feature of the male's reproductive actions also involves real behaviors, which can be delved into with precision and quantitative detail, permitting extensive comparisons between the courtship behavior of wild-type males and those expressing a variety of mutants. Other song variants, which are just as important to analyze genetically whenever possible, are the different acoustical signals generated by males of different *Drosophila* species (*6, 34, 59*). Descriptions of this species-specificity, augmented by electronic simulator experiments, have revealed that this element of courtship communication is adaptively significant and almost certainly plays a role in species recognition (*34–36, 63*).

One of the more advanced interspecific song experiments involved mapping the genetic etiology of a rhythm difference between *D. melanogaster* and *D. simulans* to the X chromosome (*60*). This is where the *per* locus resides (*38*), which prompted the creation (by DNA mediated transformation) of flies whose only functional *per* gene was from *D. simulans*. Song analysis of such males showed that this gene is solely responsible for the 55- to 60-s periodicities of *D. melanogaster*, which is distinct from the 35- to 40-s cycle durations in the sibling species (*61*). The *per* gene from these two species is indeed molecularly different, and chimeric "constructs" showed that the control of a faster as compared with a more leisurely rate of pulse-rate change maps within a subset of this gene that seems to be the most divergent (*61*).

1) The *cacophony* mutant. Most of the other song mutants were isolated in screens that specifically searched for them (after chemical or transposon-mobilization mutagenesis). The first one found was *cacophony* (*cac*) (*62*): A given bout of *cac* singing usually results in polycyclic pulses, each containing more than the typical two to three cycles (Fig. 2). The rationale for finding this mutant provides an object lesson: As was implied in the foregoing discussions, one idea about why males sing is to tell the female that they are of the same species; a corollary is that the female's "final" receptivity is influenced by these sounds (after being sung to for a while, she slows down; thus, auditory as well as olfactory inputs to the female are relevant at this stage of the courtship sequence). *cac* was found among the subset of the approximately 2500 screened strains that included males who took too long to mate (*63*). In the same spirit as the experiments that investigated whether visually defective males court poorly for that reason alone (*25, 26*), *cac* males were de-winged, and their mating-initiation kinetics were found to be still worse than those of wingless wild-type (*62, 63*). Thus, *cac* was provisionally inferred to be pleiotropic (affecting more than the male's song); it is, but this is not the reason. The slowness to begin mating turned out to be genetically separable from the mutation causing the song defect (*63*). Thus, *cac* was isolated as a double mutant and indeed would not have been found had the two courtship-related mutations not been present on the relevant (X) chromosome. This is far from being the only coincidence of this kind (*64*).

To find out "from where" *cac* causes its song defect an analysis of the courtship behaviors exhibited by sex-chromosomal mosaics was performed. Such gynandromorphs, which were part XX and part XO but otherwise carried no behavioral mutations, were used to show that certain portions of the brain must be XO for the fly to "think that it is a male" and hence to follow, wing-extend, and (if there is enough male brain tissue) lick the female (*2*). If the gynandromoph is to sing like a male, at least a portion of its thoracic nervous system must also be XO (*65*); when these ventral ganglia were entirely XX, a wing-extending mosaic generated either no sounds or acoustical gibberish. *cac* was subsequently analyzed with chromosomal gynandromorphs whose XO tissues expressed the recessive song mutation. The behavior of such sexually mixed animals would not have been interpretable (see above) if these chromosomal gynandromorphs had not been turned into all-male flies by making the flies homozygous for the *transformer* (*tra*) mutation (see below). They were, and the tissue etiology of *cac*'s polycyclic song was "mosaically mapped" to the same body region (*63*) where basic song control had

been determined to be, using straight (cac^+, tra^+) gynandromorphs (65).

Thus, one might think that *cacophony* is a "thoracic gene," in that the normal allele would be expressed only at this site in order to control the development or operation of the fly's song circuitry (if such a thing exists). But *cac*, and other genetic factors with which it interacts, are more pleiotropic than this. This locus influences non-courtship phenotypes, and *cac* is allelic to lethal mutations that had been mapped nearby; those lethals are in turn allelic to *night-blind-A* (*nbA*) visual mutations (63). The original genetic study of these relationships showed that *cac* exhibits no obvious visual defect, nor do *nbA* mutant males seem to sing abnormally (63). Moreover, *cac/nbA* XX flies (turned into males with *tra*) exhibit complementation for both the song and visual phenotypes. Subsequently, however, it has been revealed that *cac* has a subtle abnormality in its electroretinogram (ERG), and certain *nbA* mutations cause mild song defects; moreover, an *nbA* mutant sometimes exhibits cyclical fluctuations in ERG potentials in the absence of any light input, and *cac* males have been observed (during courtship tests) to go into momentary spastic fits; some, or all, of these phenotypes could fit with the emerging possibility that this locus encodes a calcium-channel α subunit (66).

2) The *dissonance* mutant. The second song mutation found by mutagenesis and screening was isolated by looking (on an oscilloscope screen) at the acoustical signals per se (67). This procedure resulted in the identification of *dissonance* (*diss*), once more among approximately 2500 candidate strains. *diss* could have been more specific than *cac*, given the search strategy. But it turned out also to be a visual mutant, behaviorally as well as in terms of lacking light-on plus light-off transient spikes in the ERG. These phenotypes, and *diss*'s X-chromosomal map position, suggested that the song mutation had occurred in a previously known visual gene. Indeed, *diss* turned out to be an allele of the *no-on-transient-A* (*nonA*) locus, as was shown genetically and molecularly (67, 68). A *nonA* clone was also found by starting with antigens extracted from chromatin; this (among other studies) led to the prediction that the gene may encode an RNA binding protein (68); in fact, it may be a member of a distinct interspecific family of such factors (68). In *Drosophila*, the *nonA* gene products are essentially ubiquitous [egg-to-adult, and probably all tissues therein (68,69)], suggesting that it could be a vital gene, but this is not the case. Creation of a *nonA*-null mutant led to viable adults that exhibit *dissonance*-like visual and song defects (69); this implies that the *diss* allele, which results in normal levels of NONA protein and is mutated in a featureless region of the putative RNA binding polypeptide (68), is effectively a near-null itself. It is worth speculating on a possible interaction between *nonA* and *cac*. Since alternative splicing of transcripts encoded by calcium-channel genes is such a common phenomenon (70), could *nonA* participate in the regulation of *cac*?

3) The *croaker* mutant. The newest song mutant, *cro*, was also isolated by direct observation of song traces (22). Like the two genes just discussed, *cro* is pleiotropic, its song defect (as yet ill-defined) and decrement in male mating-success being accompanied by subnormal flight (22). In contrast, *cac* and $nonA^{diss}$ flies are normal in this aspect of their wing usage (63, 67), so they are not wildly pleiotropic.

4) Male recognition of females. If the courtship song emanating from a male's unilateral wing vibrations provides a key cue for the female, how does a male tell whether a female is a valid object of his attention? It is likely that chemical stimuli provide the main component of this recognition event, at least in part because certain odor molecules are species-specific (see below). An additional chemical cue could come from the tapping behavior performed by the male as an early courtship step (Fig. 1B). However, it is not known whether there are species-specific components to the contact-chemosensory input, which one infers to pass from female to male at this stage of the behavioral sequence, though removal of the distal forelegs (the appendage used in tapping) causes a breakdown in male discrimination between females of his species and those of a closely related one (71). Licking (Fig. 1F) also smacks of contact chemoreception and could, like tapping, involve elements of sexually dimorphic anatomy (see below); but licking occurs so late in the sequence that it might be unrelated to recognition by the male of the female's characteristics, such as the species to which she belongs.

5) The *fruitless* mutant. Matters of "whom shall I court?", as well as chemosensory and auditory courtship cues, are associated with the last courtship mutant to be discussed, *fru*. Although *fru* has long seemed to be specific in terms of its reproductive behavioral defects, this turned out not to be the case, providing a further entry in the long list of behavioral pleiotropies. The original fruitless mutant (fru^1) was isolated as an autosomal male-sterile by Gill (72). Only a small proportion of the (many) male "sterility loci" identified in screens of this kind are associated with behavior—that is, as opposed to spermatogenesis (22, 73).

fru^1 males court females vigorously (although somewhat subnormally) and yet do not even to attempt to copulate with them (11): A fru^1 male never bends his abdomen toward the female (11, 74). The most dramatic reproductive anomaly associated with *fruitless* is that the fru^1 mutant courts another male just as vigorously as he does a female. Moreover, groups of fru^1 males will snake around a chamber, forming "courtship chains" in which most individuals are simultaneously courters and courtees (72, 74) (Fig. 3). Chaining is also exhibited by another *fruitless* variant (fru^2), which was originally known by virtue of a transposon inserted, as it turned out, at the *fru* locus (74, 75). In spite of fru^2's intermale courtships, separate tests revealed these mutant males to be quite willing and able to mate; they court females vigorously and in fact prefer them to males in direct observations (76).

fru^1 is a mild song mutant. Its wing vibrations were recorded because these males sometimes perform anomalous bilateral wing displays when they court another fly (Fig. 3A) and also are subnormal in their performance of the wing-flick rejection responses (11) that normally occur when a male is courted by another one [for example (77)]. Yet this mediocre flicking does not explain the chaining behavior: Wingless fru^1 males do not exhibit accentuated chaining, and wingless (let alone intact) wild-type males never form such conga lines (76). The slightly abnormal sounds emanating from fru^1 males involve interpulse intervals that are longer than normal, on average (78).

If *fruitless* were not already complex enough, consider that the original *fru* strain turned out to be yet another instance of a behavioral mutant isolated as a double mutation, with both such factors (once again) being defective in related features of the fly's biology (64). Thus, fru^1 males elicit courtship from normal ones, as well as courting other *fruitless* males (11, 79). However, the elicitation phenotype has nothing to do with the chromosome lesion (in an autosomal region called 91B) that results in behavioral sterility, courtship directed at males, and the defective song (74, 78). A nearby chromosomal breakpoint (in region 90C) is the cause of elicitation; indeed, fru^1 is a short inversion, with one breakpoint at each of the autosomal sites implied in this discussion (74). fru^2 has a transposon-insert only in 91B, and this *fruitless* mutant does not elicit anomalously high levels of courtship (74). The aforementioned fru^3 and fru^4 alleles contain inserts in region 91B (22), and one therefore predicts that these mutants also will not stimulate other males to court them. The 90C lesion could have something to do with pheromonal abnormalities (31), because males homozygous for fru^1, or heterozygous for that mutation and a 90C-deletion, can elicit high levels of courtship even when paralyzed or cut into pieces (11, 74).

Yet it does not appear as if *fruitless* simply causes the mutant male, when it is a mature adult (≥2 days old), to be pheromonally like an immature wild-type male (*31, 53, 54*). Recall that such normal males are courted vigorously because of a special pheromone (*51*).

Behavioral and Neurogenetics of Sex Determination

The *fruitless* mutation may define a sex-determination factor as well as a courtship gene. This hypothesis has emerged in several separate ways and locations (investigatorily and geographically) (*22, 79, 117*).

In the course of looking for a reason why *fruitless* does not bend its abdomen in the presence of the fly it is courting, it was discovered that flies expressing the most severely mutated forms of the locus are missing a male-specific muscle (*75*) (Fig. 4), named the Muscle of Lawrence (MOL) after its discoverer (*80*). This was the first association of *fru*'s action to a physically tangible aspect of sex specificity. Preliminary evidence suggests further that appropriately located terminal arborizations of motor neurons innervating developing abdominal muscles are morphologically aberrant in *fru* mutants during metamorphosis (*81*). All of these defective anatomical phenotypes map to the same fru^1 lesion (91B) that results in the mutant male's active (as opposed to elicitation-related) behavioral abnormalities (*74, 75, 78*).

The fru^2 mutant was used to pose a question about the function of this male-specific muscle and hence to reveal that *fruitless* may be involved in more than behavior and reproduction. When fru^2 was placed over more severe genetic variants at the locus, the MOL was usually absent from a given individual, yet such males could readily bend their abdomen toward the female and copulate (*74,75*). Thus, this structure may be devoid of behavioral significance, but—as an element of *Drosophila*'s sexually dimorphic anatomy—it provides a segue into the broader discussion of sex differentiation. Here I will concentrate on behavior and the nervous system because general aspects of this topic have been extensively reviewed (*3*). In addition, several genetic associations have emerged in the last few years between the control of that system's development and the development of maleness as compared with femaleness (*82*). It is not clear whether this is merely more "promiscuity" of gene actions (*82*) or whether the fact that sex-determination mutants turn out not infrequently to be neural-developmental mutants (or vice versa) is revealing a more special relation.

In the sex-determination hierarchy (Fig. 5), "upstream" mutations seem fully to sex-transform not only the fly's external appearance but also its behavior (*83, 84*) and certain chemical features of maleness or femaleness (see below). Thus, viable *Sex-lethal* (*Sxl*) genotypes (involving hypomorphic mutations) and *tra* or *transformer-2* (*tra-2*) null mutations cause XX adults to behave as males (*83, 84*); they can even copulate, but are sterile because their internal abdominal anatomy is far from fully male. These upstream mutants (when chromosomally female) elicit very little courtship, just as (mature) wild-type males do not (*83, 84*).

A chromosomal male carrying such a gain-of-function *Sex-lethal* mutation (leading to ectopic expression of female-specific *Sxl* functions) performs less courtship than does a wild-type male and elicits anomalously high levels of interest from the latter (normal) type (*84*); thus, it could be inferred that some kind of "internal intersexuality" in this mutant goes hand in hand with its externally intersexual appearance.

The behavioral maleness of the *transformer* mutants has been more extensively tested and manipulated than in the case of *Sxl* mutants. Thus, XX flies homozygous for (the classical) *tra* mutation not only court females vigorously, but also sing to them in an essentially normal manner (*37, 67, 68, 85*). There is a conditional mutation of the *tra-2* gene; this permits the investigator to rear an XX animal as essentially a female at low temperature, then "turn off" the gene by heating the animals at a relatively late stage of the life cycle. With this technique, it was shown that putting $tra\text{-}2^{ts}$ mutants in a genotypically male state as pupae and even young adults could still cause these chromosomal, externally appearing females to court as males (*86*). Implications of this late-stage plasticity will be discussed in the next section. For now, it should be realized that "females courting females" is not, in general, a genetic miracle; but the earlier example of this so-called *Lesbian* behavior (*87*) was so complicated genetically that possible tie-ins to sex-transforming factors (or any identifiable genes) were never elucidated.

Another manipulation of *tra* involved

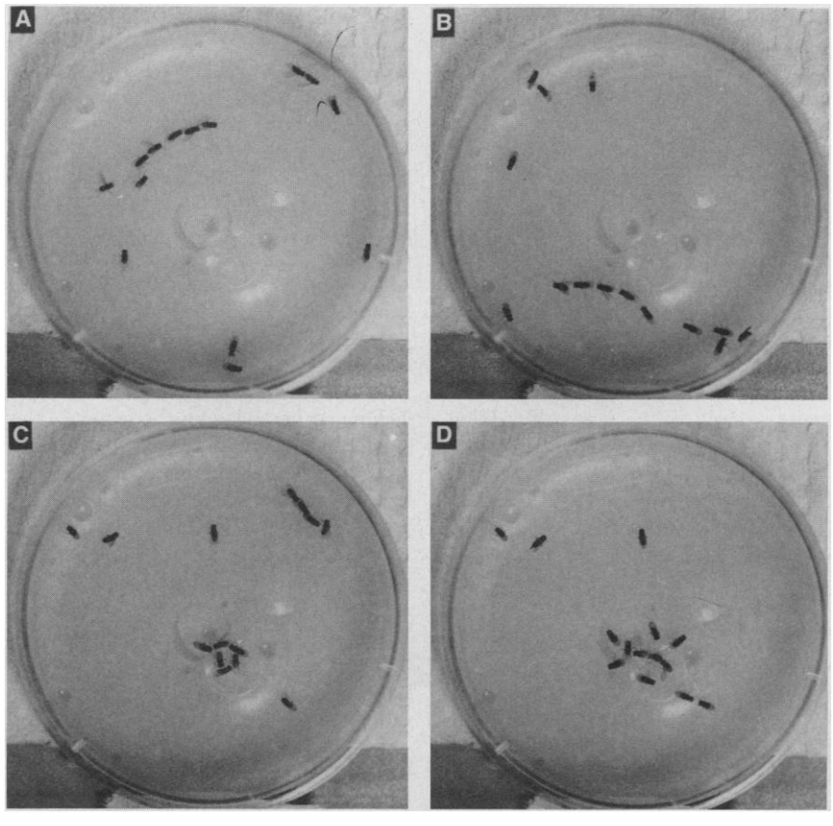

Fig. 3. Intermale courtships performed by *fruitless* males. All flies in each of these chambers were heterozygous for the original *fru* mutation and an autosomal deletion [*Df(3R)P14*]; one of the third-chromosomal breakpoints of this *Df* is within region 91B, where the key genetic lesion is located in the fru^1 mutant (*74,75*). In (**A**), a chain of six or seven courting males is shown, along with a chainlet of two to three males; note that four or five of the courters are exhibiting unilateral wing extension; (**B**) exemplifies similar behavior to that shown in (A); (**C**) depicts a small circle of courters, along with a short chain in the upper right; in (**D**), a geometrically more complex group of intercourting fru^1/Df males is shown.

engineering the normal female-specific form of that gene's function (TRA^F) (Fig. 5), so that the gene was present in only certain portions of the brains of XY adults. For this, the GAL4(trans-acter)/UAS(cis-acter) system, which has been co-opted from yeast and transgenically introduced into *Drosophila*, was used. This strategy is aimed at spatially manipulating fly genes by enhancer trapping of transposons that encode GAL4 (*88*). For the *tra*-related behavioral study, several lines were created with the GAL4 transgene scattered in a variety of genomic locations; these are in effect a series of mosaic strains, in which all intra-strain animals are identical to their siblings (*89*). Certain of these lines included anomalously behaving flies, in that the male with a "partially feminized" brain courted females and other males indiscriminantly. This is *fru*-like behavior. The transgenic types behaving in this manner expressed TRA^F in brain regions that are targets of antennal inputs—a sensory system that does have sex-specific features (see below).

The downstream genes in the sex-determination hierarchy that have been considered from a behavioral standpoint are *doublesex* (*dsx*) and to some extent *intersex* (*ix*) (Fig. 5). The former, whose loss-of-function mutations result in external intersexuality of both XX and XY flies, is regulated by the *transformer* genes: dsx^+ makes female- and male-specific transcripts (hence DSX^F and DSX^M proteins) by alternative splicing (*3*). Superficially a *dsx* null mutation seems to cause XY animals to be intersexual behaviorally: As in the case of the *Sxl* gain-of-function mutant, $XY;dsx^-$ flies courted females poorly and elicited abnormally high levels of (male-performed) courtship (*83*). Yet this and other *dsx*-mutant effects have been recently reexamined (*79*), resulting in a revision of the nature of *dsx*-related behaviors: (i) XY flies expressing other *dsx*-null alleles courted females at subnormal levels, but much more vigorously than in the original tests of one homozygous mutant allele (*83*). (ii) That these $XY;dsx^-$ flies are largely males behaviorally was emphasized by analysis of the sounds emanating from their reasonably vigorous wing extensions, which produced essentially wild-type courtship songs (Fig. 2) [if these flies were intersexual in this region of the nervous system, they should have produced semigibberish or have been unable to sing (*69*)]. (iii) XX flies, in which *dsx*'s expression and function was jammed into the male mode—by applying dominant, constitutive *dsx* mutations that cause a thoroughgoing transformation into external maleness (*3*)—resulted in no male-like courtship behavior whatsoever (*79*).

The anomalously high level of courtship elicitation exhibited by an $XY;dsx^-$ mutant (in the original study) need not necessarily be viewed as partial femaleness. Recall that young wild-type males elicit such behavior, and thus it could be that this (partially) sex-transformed mutant is developmentally delayed in a non-specific manner, so that it was in effect an immature male when tested with mature wild types. It is important to keep in mind that *dsx* (and *ix*) are the two genes considered to be downstream of the *transformers* (*3*) (Fig. 5). The behavioral findings just discussed make it seem as if the part of the hierarchy branching off to *dsx* and *ix* may have little to do with courtship behavior and perhaps not much to do with elements of *Drosophila*'s internal sexuality, at least the part of it that underlies behavior. The following discussion of sex-specific internal anatomy, in the context of both courtship control and sex-determination genes, may reinforce this suspicion.

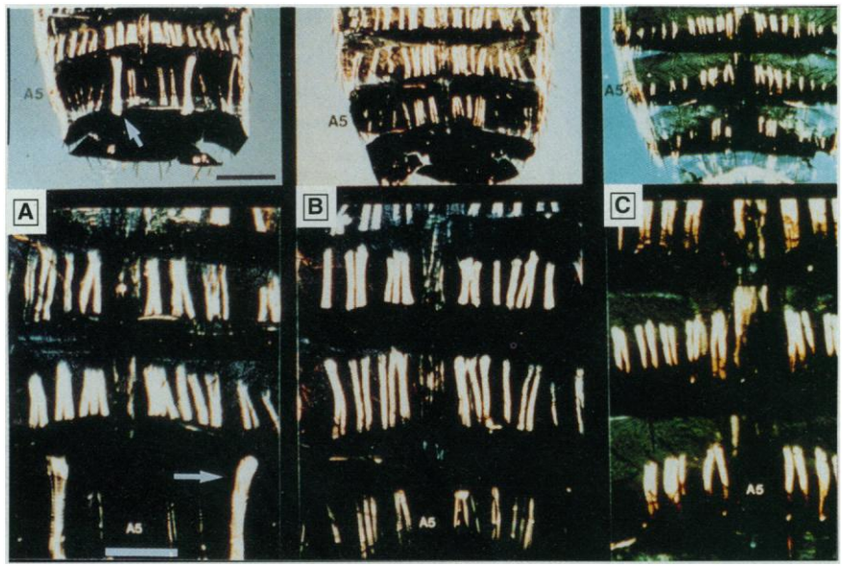

Fig. 4. The Muscle of Lawrence (MOL) as affected by the *fruitless* gene. (**A**) The posterior abdomen of a wild-type adult male, dissected open from the ventral side; after removal of the internal organs, dorsal abdominal muscles were visualized by their birefringence in polarized light (*75*); the bilaterally symmetrical Muscles of Lawrence (arrows) are attached to the cuticle in the anterior region of the fifth abdominal segment (A5) and in a similar part of the adjacent (more posterior) segment, as is visible in the bottom part of this panel. (**B**) The same abdominal view of a male heterozygous for two separate autosomal deletions [*Df(3R)Cha*^{M5} and the *P14(3R)Df* in Fig. 3]; each has a breakpoint in common at the *fru* locus in region 91B of the third chromosome; the MOLs are absent; this nominally fru^- male exhibits a more severely mutant MOL phenotype than does a male expressing the fru^1 mutation, which causes this muscle's absence in some males and results in a rudimentary form of it in others (*75*). (**C**) The same abdominal view of an adult female; as in (B), all the muscle structures are relatively small longitudinal ones (*75, 80*). Scale bar for the lower-magnification images (upper left), 200 μm; and for the higher-magnification ones (lower left), 100 μm.

Sex-Specificities of Internal Phenotypes

Sexually dimorphic anatomy. The male- and female-specific structures and substances discussed below will be considered for their possible relevance to sexual behavior as well as for being under the genetic control of factors that may be downstream of the core components of the sex-determination hierarchy. Consider once again the Muscle of Lawrence (Fig. 4). Despite its unknown function, viewing the MOL from a developmental perspective is useful, because it places the control of this muscle's differentiation somewhere within that hierarchy of gene action. The appearance of this structure during metamorphosis is more than a matter of muscle differentiation: Mosaic analysis revealed that a chromosomally male (XO) genotype of the nerves innervating the MOL is necessary for its formation (*80*). This result is almost certainly going to fit with results that are emerging from studies of genotypically controlled differences in these nerves' anatomy (*81*).

Studying the male muscle's development in conjunction with sex-determination mutations provided an early clue that the corresponding genes are not all that involved with neuronal and behavioral phenotypes. Thus, this muscle's appearance and differentiation are unaffected in *dsx* mutants that are either intersexual externally ($XY;dsx^-/dsx^-$) or fully male externally (XX;*dsx*-dominant/dsx^-); that is, the full presence or complete absence of the MOL goes with chromosomal sex (*90*). Therefore, *dsx* does not seem to be

the factor that is downstream of the *transformer* genes insofar as this aspect of neuromuscular development is concerned. What could be at this position, relatively low in the hierarchy? The only tangible candidate so far is *fruitless*, given its anatomical effects on the relevant neural and muscle morphologies (*75, 81*). Yet *fru* might not be located directly downstream of the *transformer* genes' actions. This has been argued by invoking the hypothetical *geneZ*, which may or may not be equivalent to *fru* (Fig. 5). *geneZ*'s place in the sex-determination hierarchy is imagined to be essentially parallel to that of *dsx*, immediately downstream of *tra*'s, but in this separate branch that is dedicated to the development of the nervous system and the control of sex-specific behavior (*79*).

However, *dsx* does influence at least one aspect of central nervous system (CNS) development (during the larval stage and after); the effects of *dsx* mutations involve certain sex-specific neuroblasts in the abdominal ganglia (*91*) whose function is unknown.

A few other bits of *Drosophila* morphology, which are different in males and females, can be listed, although there tends to be little understanding of their reproductive functions. Consider the mushroom bodies, dorsal brain structures that are different in males and females (*92, 93*). Mushroom-body development resumes after the animal has become an adult, provided that such young flies are exposed to environmental inputs (*92*). Could one of the relevant stimuli be courtship song, of which an immature male indeed hears a great deal (see above)? This might exert a salutary influence on the young male's ability to court, a couple of days later; there is some support for this idea (*94*). One wonders, furthermore, whether the late-in-the-life-cycle manipulations of *tra-2*'s action [and how this influences behavioral maleness (*86*)] might be connected with postnatal brain development that occurs in the mushroom bodies. The problem with these notions is that males made mushroom-bodiless by anatomical-brain mutations or by a certain chemical treatment (*95*) are still able to court in what seems to be a normal manner. These brain structures are only known to be involved in learning [as mediated by association of electrical and olfactory stimuli (*48, 95*)], although not in conditioned courtship (*95*).

The visual system is different in the males and females of other dipteran species (*96*), and this almost certainly has to do with males tracking females in courtship flights (*4*). It is only very recently that a sexual dimorphism in one of *Drosophila*'s optic ganglia has been uncovered (*93*), and hence it is too early to tell if this structural difference may bear a relation to visually mediated tracking of fruit-fly females by courting males (*25, 26*). That ganglion (the lobula) is a deeply located optic lobe but is in a sense part of the peripheral nervous system (PNS).

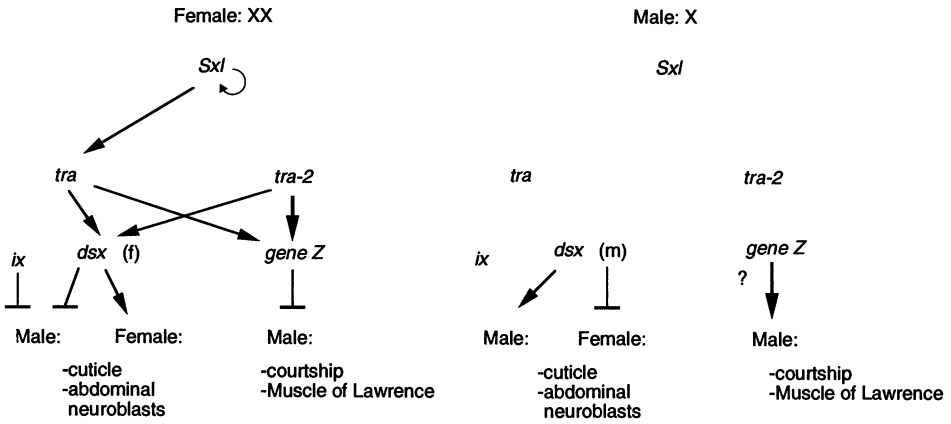

Fig. 5. The genetics and molecular biology of sex determination in *Drosophila*. This hierarchy of gene actions and interactions has been elucidated by a long and extensive series of investigations (reviewed in *3, 82,* and *111*). This depiction of the sex-determination hierarchy (there are many) is based on one that was recently developed by Taylor *et al.* (*79*). Briefly, several dosage-sensitive "early" factors (*82*) activate *Sxl* in chromosomal females (XX) but not in chromosomal males (XY or XO). The protein encoded by this gene (SXL) acts autocatalytically (circular arrow) by influencing the splicing of *Sxl*'s primary transcript such that a functional SXL protein is continually produced in developing (and even adult) XX animals. This female-specific SXL also regulates splicing of the pre-mRNA transcribed from *tra*; the resulting female-specific transcript encodes the only functional form of the TRA protein (see below). In conjunction with the action of TRA-2, female-specific TRA (TRA^F) regulates splicing of the *dsx*-encoded pre-mRNA into a female-specific transcript (DSX^F). Working with the product of the *ix* gene, DSX^F suppresses male types of differentiation (lines ending in "T-bars") in obvious tissues (such as the external genitalia). DSX^F also plays an active role in that it enhances *yolk-protein* gene transcription (*111*) and also helps control the initiation and maintenance of cell divisions by certain female-specific neuroblasts in the abdominal CNS (*91*). Another internal phenotype—development of the male-specific MOL—forms independently of *dsx* action (*90*). Thus, a hypothetical factor *geneZ* is postulated to be interposed between the actions of *tra* and *tra-2* in terms of suppressing MOL development in, and active courtship performed by, females. *geneZ* has also been called *ambisex* (*3, 79, 90*)—an as-yet unidentified factor. In single-X animals, no functional SXL is translatable, and so the only type of *tra* mRNA is one that generates nonfunctional TRA; this results in default-splicing of *dsx* pre-mRNA, leading to male-specific DSX^M. This protein suppresses differentiation of female characteristics, such as external ones and yolk-protein (YP) production (*3, 111*). DSX^M plays at least one positive role: initiation and maintenance of divisions by male-specific abdominal neuroblasts in haplo-X animals (*91*). One way to view *geneZ* = *ambisex* is that it has a positive action (downward pointing arrow), which controls the development of masculinity insofar as male-specific behavior and a muscle are concerned—in a manner analogous to dsx^+ function in males being necessary for the abdominal neural divisions. If this were the case, then the role of the TRA and TRA-2 proteins (in diplo-X animals) would be to cause *geneZ*'s primary transcript to be spliced into a nonfunctional form. One candidate for *geneZ* is *fru*. Under this view, the MOL's absence and an inability to consummate courtship with females in *fru* mutant males can be viewed as resulting from a decrement in or absence of the pertinent "positive gene-action arrow" (accompanied by a "?" in the right-hand section of the scheme). However, it is not at all clear how a loss of *fru* function would lead to intermale courtships. Among the nonovert attributes of maleness and femaleness are reproductively related pheromones. The influence of sex-determination genes on pheromone production is illustrative of the hierarchy's operation and also results in some additional puzzles (analogous to those just noted in terms of *fruitless*). Thus, the effects of *Sxl* mutations and a *transformer* mutation on the production of male- versus female-specific pheromones, by flies of a given sex-chromosomal genotype, make sense (*85, 115*). However, the case of *doublesex*'s involvement in pheromone control seems odd. The simplistic prediction for *dsx* effects on pheromones would go as follows: In chromosomal females, the action of DSX^F (acting with the ix^+ product) represses male functions (see above), so that XX;*dsx*-null mutant adults could be predicted to make male pheromones and yet still product female ones—by analogy to the manufacture of YPs by XX;dsx^- flies; such proteins are also made by XY flies expressing a dsx^- mutation (*3, 111*). Therefore, provided that the relevant pheromone-producing tissues (which in general are unknown) are present in an XY;dsx^- mutant, this fly could be predicted to make the female aphrodisiac, which it does not, along with male-specific odors, which it does (*115*). Another prediction along these lines might suggest that XX;dsx^- adults would still produce female aphrodisiac, as well as having the repression of male-odor production relieved; the latter result was obtained, but the former was not (*115*).

Other PNS dimorphisms have been described in *Drosophila*: The antennae are different in males and females (*97*), and a certain antenna-expressed enhancer-trap line is sexually dimorphic in the staining pattern of the reporter enzyme (*98*). These anatomical descriptions could, in terms of courtship behavior, be related to olfactory inputs that in general terms enter the fly through that structure (*99*); the antenna is also the receptor for the anti-aphrodisiacs emanating from mated females (*100*). This result suggests that a "feminized" antennal system may not correctly process inhibitory odor cues that come from mature males, which in turn could be connected with the intermale courtships exhibited by the aforementioned GAL4/UAS-TRA^F transgenics (*89*).

The *Drosophila* antenna is also the receptor for the love song (*35, 50, 101*), so this appendage's structure could permit the specialized reception and processing of acoustical as well as olfactory cues (*102*). Lest one get carried away by the antennal's significance for (at least) the male's courtship, an object lesson about the pitfalls of "surgery" effected by genetic laziness should be registered (*102*).

The adult fly's legs are sexually dimorphic at their distal periphery, as is the morphology of sensory-nerve endings that project from these chemosensory structures into the thoracic nervous system (*103*). The anatomical differences could underlie the male's ability to sense something about the female's external chemistry as he taps her early in the courtship sequence (Fig. 1B). The male could also receive chemosensory information from his mouthparts during the licking stage (Fig. 1F), and an anatomical substrate for this could involve sexually dimorphic features of the maxillary palps (*104*). However, it is unclear whether these mouthparts, in addition to the actually extended proboscis (Fig. 1F), come into contact with this part of the female.

Continuing to move down the fly, we come to these so-called "terminalia." Here, sexual differences in the sensory neurons elaborated from the genital disk have been well described and nicely manipulated by the application of sex-determination mutations (*105*). Such dimorphisms can be regarded as expected (those imaginal disks also produce male- and female-specific mechanosensory bristles), although the functional significance of, for example, a male pattern of posterior input to the CNS is unknown. *Drosophila* also remains unexamined for sex-specific morphology motor neurons within the abdomen [as have been found in other insects (*106*)]; these parts of the abdominal anatomy could be involved in the control of the penultimate and final stages of the courtship sequence (Fig. 1G).

Sexually dimorphic biochemistry. Some of the PNS elements just noted would seem to develop in a female or male manner, in part to mediate the effects of reproductively related chemicals whose inputs into *Drosophila* can thereby trigger, sustain, or modulate the flies' courtship.

1) Pheromones. The aforementioned influence of pheromones on the fly's reproductive behavior involve cuticular hydrocarbons that are male- and female-specific (*29,107*); further, the overall pheromonal profiles of different *Drosophila* are species-specific as well (*108*). Some intraspecific pheromonal differences are being encountered as natural variants or have been deliberately induced with mutagens (*15, 29, 109*). They are interesting from two perspectives: (i) Insofar as a mutation of this type may diminish the levels of, say, a certain male-specific substance—and cause a decrement in the mating-receptivity of a (wild-type) female being courted by that mutant allele—this very likely ties in with the similar effects on such receptivity of olfactory mutations expressed in females (*26, 32*). In this regard, observations of male-female interactions involving the pheromonally depleted *nerd* male implied that this mutant does not induce a virgin female to be as receptive as she would normally be (*24*), although this does not readily explain the fact that this male's courtship actions per se are also sluggish. (ii) The pheromone-controlling genes implied by mutations such as *nerd* (*24*) could be among the factors that mediate elements of observable male- and female-specificity in a proximate manner [see (*109*) for additional candidates]; these would be very far downstream in the sex-determination regulatory hierarchy (Fig. 5). Perhaps the genetic loci implied by the aforementioned enhancer-trap strain, and by sexual dimorphisms of antennal proteins (*98*), are additional such genes.

Drosophila pheromones include not only courtship-stimulating cues but also anti-aphrodisiacs. How the latter substances come to be associated with fertilized females has been a complex and sometimes controversial story; this is in part because one of the chemicals that received a good deal of attention in this regard (*107*) seems now to be less related to courtship than to generalized "aggregation" behavior, which is not materially connected to male-female interactions (*110*).

2) Small peptides. In addition to olfactory stimuli, other categories of sex-specific substances are involved in the fly's reproduction. These are the precious bodily fluids in abdominal glands of the male; he transfers these molecules to the female during copulation along with his sperm (*111*). Such substances seem entirely under the control of the sex-determination hierarchy as it can be conventionally viewed, before invoking the newly appreciated involvement of factors such as *geneZ* (if there is such a thing) and *fruitless*. A corollary is that the female-expressed *yolk-protein* genes are controlled in the same manner (*111*).

Some interesting manipulations of the sex-specific tissues and substances involved in these elements of chemical communication have been performed: A particular male substance known as "the" Sex-Peptide (S-P) was produced in unmated females who had been engineered to carry the *s-p* gene under the control of an inducible promoter (*112*). This caused such females to act as if they had mated, in terms of nonreceptivity to copulation attempts and the performance of correlated rejection behaviors (*12*). However, the sex-ectopic expression of S-P was able to sustain these behavioral changes for only 1 day, which is much less time than what happens after an actual mating, in part because there is a sperm effect on the female's receptivity as well as a substance-related one (*45*). Nevertheless, S-P alone was able to cause a more sustained activation of female rejection behaviors in another transgenic type that added a *yolk-protein* gene's enhancer to the S-P–encoding construct (*112*).

Use of the same heat-shock promoter that was applied in these molecular experiments on S-P has effected ectopic expressions of many genes cloned from *Drosophila* in developmental studies carried out over the past several years (*17*). Another kind of promoter–fusion-gene innovation involves engineering the flies in order to ablate specific structures with an expressed toxin, so that the biological consequences of eliminating certain cell types or tissues can be investigated (*113*). One of the first applications of this transgenically based technology was to eliminate most of the seminal fluid–producing cells from the male's abdominal accessory gland (*114*). Females that had copulated with such males, who would have lost their S-P and other accessory-gland products, exhibited excellent receptivity to subsequent mating attempts by males. Thus, fluids known to be secreted from other tissues in the male's genital tract (*111*) could not be solely responsible for the chemically mediated effects on mated female behavior. These results were confounded by the unexpected elimination of sperm by the toxin gene carried in these transgenic males; controls (using fluid-rich but spermless mutant males) showed that the accessory-gland substances are partly responsible for a short-term inhibition of remating (*114*).

Prospects and Some Puzzles

Courtship genetics is deepening, as studies of the relevant genes increasingly involve molecular and neurobiological phenomena. This subject has also become quite extensive genetically, which some wags will infer to

mean that "everything is expressed everywhere and affects everything." So, why wouldn't courtship, too, go down the drain (or at least circle it) in a mutant involving such a promiscuous gene? In the first place, one's sense that courtship is bound to be a complicated sensory-motor phenomenon needs nevertheless to be backed up by actually demonstrating something about the cues and actions that are involved or required—even if it turned out to be "too easy" to disrupt part of the sequence with a mutation that has broad phenotypic effects and defines a widely expressed gene (for example, see *21, 27, 68,* and 69). Second, the view that "any genetic variant will court poorly, in general" is mitigated by the examples of modal pleiotropies. The discussion of these phenomena involved genes whose mutations do not have global effects and that established particular connections between events such as learning and the non–fixed action pattern that comprises reproductive behavior in these flies (*30, 49*). Other mutations with nicely specifiable effects on neural structures and functions have been informative in terms of the surprisingly normal (or minimally disrupted) courtships of which certain brain-damaged and olfactorily challenged mutants are capable (*32, 95*).

Studies of courtship behavior and reproductively related substances have helped the subject of sex determination become a neurogenetic one, as well as a success story with respect to the basics of how a *Drosophila* embryo becomes a male or a female (*3*). That certain sex-determination mutations do not disrupt the developmental genetics of internal phenotypes connected (at least in part) with courtship control (*79*) have suggested that the hierarchy of actions of and interactions between factors within this genetic pathway are due for an expansion. The caveat is that some of the effects and inferred noneffects of these mutations are not very clean. For example, even the revisionist view of *dsx* leaves this gene with effects on certain neural and behavioral phenotypes (*79, 91*); and the anomalous courtship-elicitation phenotype reported for an XX;dsx^{-} mutant (*83*) does not seem in accord with pheromonal findings on (chromosomal) males of this same genotype; moreover, these biochemical results themselves are ostensibly puzzling (see legend to Fig. 5).

Nevertheless, why not close with a recapitulation of some of the issues revolving around sex-determination factors—specifically, how the *fruitless* gene seems to be sneaking into this hierarchy of gene actions. The following considerations may stimulate a recall of several themes that ran through this review:

fru is a versatile gene. It causes several behavioral abnormalities when mutated and has neuromuscular consequences for (at least) abdominal development as well (*74, 75, 78, 79*). If any mutant type in *Drosophila* is a true behavioral one, then this is it. In this respect, the equal preference of a *fru* male for courting a female or another male (*11, 75, 76*) may address how the fly senses and interprets the presence of potential courtship objects. The *fru* gene could therefore act in part within the PNS to influence the development or function of sensory structures that are known or suspected to be involved in initiating and sustaining courtship (*71, 97, 98, 100, 101, 103, 104*). Inasmuch as one *fru* variant is a double mutant that is likely to include a pheromone mutation, this autosomal region points to the question of what actual dimorphisms underlie the sex-specific production of these courtship-modulating substances: Is the origin of something like the aphrodisiac pheromone a matter of female-specific chemistry or could it also involve an as yet unknown element of sexually dimorphic anatomy? *Drosophila* is underanalyzed with respect to the tissues sources of its pheromones and in terms of the male and female sensory structures responsible for inputting each of the relevant chemical stimuli (*116*).

That a *fru* male so avidly courts other males and seems so utterly unwilling to mate with a female may imply a central defect in the mutant (whether or not there is also a peripheral one). For this to occur, an involvement of the gene in the differentiation of ganglia within the head can be tentatively predicted. As for the courtship-song defect exhibited by *fru* males (*78*), this strongly suggests that the gene also influences the structure or function of the thoracic nervous system. *fru*'s male-muscle defect (Fig. 4) and its neural etiology (*80, 81*) imply that the gene is expressed in neurons within the abdominal ganglion as well as in the more anterior CNS regions just noted. These suppositions are based in part on the behaviors and MOL phenotypes exhibited by gynandromorphs, whereby the "foci" responsible for a fly's thinking it is a male, singing like one, and developing the male-specific muscle are located in the brain, thoracic ganglia, and abdominal ganglia, respectively (*2, 65, 80*).

Thus, *fruitless* is predicted to be neurally broad in its expression pattern. If this turns out to be so—that *fru* products are found in these various ganglia and possibly in the PNS as well—the results will not be interpreted as strange, owing to the large number of genes whose products are widely expressed in this manner despite the behaviorally specific effects of certain mutant alleles that may have defined these factors in the first place. The fact that three of its mutant alleles are transposon-tagged (*22, 74, 75*) should facilitate determination of *fru*'s pattern of gene-product expressions. A substantial bonus will be that the material to be cloned from this locus can also be assessed as to how it may fit into the sex-determination hierarchy (Fig. 5): Will upstream factors specifically interact with *fru* to control its expression?

Finally, how would the *fruitless* product function? Perhaps it helps control downstream genes involved in effecting particular features of sexual differentiation. These could involve things like male- and female-specific connectivities in certain portions of the nervous system—such as those listed above in terms of *fruitless*-mutant phenotypes. In this regard, singing behavior has long seemed a matter of differential wiring, at least in regard to the varying "song circuitries" that are hypothesized to have evolved in different species (compare *6, 34, 60,* and *85*). As for the brains in males and females, perhaps the fact that one can turn off the *tra-2* gene at a such a late stage, and in so doing turn on male courtship (*86*), would suggest that sex-specific chemistries are involved in these differential behaviors, but the postnatal and neuroanatomical plasticity related to the development of at least one brain region (*92,93*) allows the alternative (but not mutually exclusive) view that sex-specific brain wirings tell the male to interpret and process cues coming from another fly, then act accordingly.

In any case, molecular studies of *fru*'s expression and action could help to reveal if there are sex-specific patterns of neuronal connectivity in *Drosophila,* in which ganglia they are located, and how they may mediate the behavioral differences controlled by varying genotypes. Thus, this so-called courtship gene may be nicely situated between the generally acting regulatory factors—which determine the initial and basic aspects of sex—and other genes whose jobs are more in the realm of manufacturing the details of sexually dimorphic phenotypes. In this sense, *fruitless,* more than many of the other genes mentioned in this review, may be poised to be especially revealing of how courtship in *Drosophila* is controlled.

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