The *dissonance* Mutant of Courtship Song in *Drosophila melanogaster*: Isolation, Behavior and Cytogenetics

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ABSTRACT

The *dissonance* mutant of courtship song was induced by chemical mutagenesis. This *X*-chromosomal mutation causes the *D. melanogaster* male's acoustical output, resulting from his wing vibrations directed at a female, to include very long and loud tone "pulses." Yet, a given train of pulses starts out as normal, with the signals in all but the shortest singing bouts eventually becoming polycyclic and high-amplitude. The aberrant songs caused by *diss* (map position, *1*-52; cytological interval, 14C1-2 to 14C4-5) were quantitatively compared to those produced by mutant *cacophony* males, whose pulses are much more uniformly polycyclic (due to a mutation mapping elsewhere on the *X* chromosome). Males or females expressing *diss* are normal in several "general" behaviors. Yet *diss* males not only sing abnormally, but they also exhibit longer-than-normal mating latencies in their courtship of females. These decrements seem to be associated, at least in part, with visually aberrant behavior of *diss* flies—measured with regard to male courtships per se, and also in tests of more general visual responses. Such defects were found when testing *diss* males or females, and the genetic etiology of the visual impairments were provisionally mapped to the same locus to which the song abnormality has been localized. Neurogenetic connections between the control of courtship singing behavior and visual system functions are discussed with respect to the new song mutation (*diss*) and the older one (*cac*)—which also turned out to be genetically related to a mutation that causes abnormalities of light-induced behavior and physiology.

THE courtship song of a Drosophila male is produced by his wing vibrations that occur as he orients toward and follows a female (BENNET-CLARK and EWING 1970). These sounds play an important role in the mating success of many species of fruit flies, each of which has its own distinct song (*e.g.*, BENNET-CLARK and EWING 1969; SCHILCHER 1976b; COWLING and BURNET 1981; KYRIACOU and HALL 1986). The male courtship song of *Drosophila melanogaster* consists of tone pulse trains, interspersed with *ca.* 160 Hz hums (reviews: EWING 1977a; HALL 1982). The interpulse interval (IPI; *ca.* 35 ms for *D. melanogaster*) serves as a recognition signal for the conspecific females (BENNET-CLARK and EWING 1969; SCHILCHER 1976a, b; KYRIACOU and HALL 1982). Although a male's mating success is dramatically reduced by the elimination of song through surgical removal of his wings, the courtship song is not a requirement for mating, in that wingless males do mate eventually (*e.g.*, EWING, 1964; ROBERTSON 1982; SCHILCHER 1976b; KYRIACOU and HALL 1982, 1986).

The first single-gene mutation that was found specifically to alter the pulse song is *cacophony* (*cac*, SCHILCHER 1976c, 1977). Individual pulses in the mutant song are polycyclic and larger in amplitude than the normal song. This pulse-song abnormality of *cac* appears to be specific, because courtship hums and flight wing-beats (SCHILCHER 1977), plus flying ability (KULKARNI and HALL 1987), are normal. Recent genetic and behavioral analyses of this mutation (KULKARNI and HALL 1987) showed that it causes only a limited array of well defined defects: longer and louder tone pulses in the song, and marginally depressed locomotor activity. Latencies to initiation of mating with females (measured for *cac* males carrying *X* chromosomes that had undergone recombination with certain marker-bearing *X*'s) were normal, *i.e.*, in spite of the aberrant sounds produced by the mutant.

The neural control of the courtship song has been investigated in mosaics. Normal pulse song was found to be most closely associated with genotypically male tissue in the ventral thoracic ganglia (SCHILCHER and HALL 1979). Mosaic analysis has also suggested a thoracic focus for the *cac* mutant song (HALL *et al.* 1988).

It seemed as if new mutations that might, with a reasonable degree of specificity, perturb the courtship song should be searched for. Thus, we have isolated the *dissonance* (*diss*) mutant, to augment the neurogenetic analysis of this element of reproductive behavior. We present the basic behavioral and genetic characterization of the new mutant. In order to determine if the gene's action could be specific, we

have carried out a detailed analysis of the mutation's effects on singing plus other behaviors, in conjunction with mapping the genetic etiology of these phenotypic changes to a narrowly defined interval of the *X* chromosome, which is separate from the *cac* locus (*cf.* KULKARNI and HALL 1987).

MATERIALS AND METHODS

General: Flies were raised on a cornmeal, agar, molasses, yeast medium, at room temperature (*ca.* 22–23°) and under natural lighting conditions, unless stated otherwise.

Mutagenesis: The preparation of a feeding solution containing *N*-nitroso-*N*-ethyl urea (ENU) and the feeding method have been described by VOGEL and NATARAJAN (1979a, b) and VOGEL and LUERS (1975), respectively. However, we used an unpublished procedure first developed by M. A. CROSSBY and E. B. LEWIS, later modified by H. LIPSHITZ (personal communications). Briefly, 1 g of the mutagen was dissolved by injecting 100 ml of 0.01 M acetic acid into a sealed 150 ml bottle (Isopac) containing the ENU in powdered form. One ml of this solution was mixed thoroughly with 49 ml of 1% (w/v) sucrose in distilled water. This ENU-containing sucrose solution (final concentration of the mutagen: 0.2 mg/ml) was added to mutagenesis "chambers" (½ pint glass bottles), *i.e.*, enough to dampen a cushion made of Kimwipe paper. Then, 200 Canton-S wild-type males, that had just been starved overnight, were gently transferred into mutagenesis chambers (*ca.* 100 males in each bottle) and allowed to feed for 24 hr. Since ENU is extremely carcinogenic, all procedures were carried out inside a large container that had a 1–2-cm layer of 1 M NaOH as a decontaminant. Each mutagenesis bottle was kept in a polypropylene container, modified with inlet and outlet connectors and a silicon-rubber gasket. The air inside the sealed container was continuously aspirated over a 0.1 M ammonium hydroxide decontaminant solution.

Mutagenized males were mass-mated (10 males plus 25 females/bottle) with attached-*X*, *y f* virgin females. These females were allowed to lay eggs for 5 days and later discarded. Approximately 2500 lines were established by mating F_1 males singly to further attached-*X*, *y f* virgin females.

Isolation of putative song mutants: The courtship song produced by the wing vibrations of one F_2 male from each line, in the presence of a virgin female, was monitored through earphones and on an oscilloscope as described previously (KULKARNI and HALL 1987; GORCZYCA and HALL 1988). We looked for any departures from the normal song, in terms of shape and amplitude of pulses, numbers of cycles/pulse (*cf.* typical wild-type numbers: 1–3), or the basic characteristics of sine song (*cf.* SCHILCHER 1976b). If a male from a particular line showed an abnormal song, 5 additional males from it had their songs recorded, to retest the possibly mutant phenotype. Males from such a line were crossed to females homozygous for the *In(1)FM7a* balancer chromosome to establish a balanced stock and, eventually, a true-breeding subline containing hemizygous mutant males plus homozygous mutant females.

From these procedures one new "solid" mutant was isolated (see RESULTS), which was characterized genetically as follows:

Recombination mapping: To determine the approximate location of the new *dissonance* mutation (symbol: *diss*) on the *X* chromosome, males from its balanced stock were crossed to a strain carrying markers *yellow* (*y*, 0.0), *chocolate* (*cho*, 5.5), *crossveinless* (*cv*, 13.7), *vermilion* (*v*, 33.0) and *forked* (*f*, 56.7). F_1 progeny were self crossed, and the songs produced by recombinant F_2 males in the presence of virgin females were observed on the oscilloscope (see above).

Song analysis: Cycles/pulse values were computed as in KULKARNI and HALL (1987) for *ca.* 150 pulses obtained from several complete pulse trains in a given song. A pulse train is a series of characteristic signals, separated from another train by either a sine song bout (courtship hum) or an interval of at least 65 ms of "silence" (*e.g.*, BENNET-CLARK and EWING 1969; KYRIACOU and HALL 1980). Two pulses is the minimum number in a train. Single pulses separated from other signals by ≥65 ms were not analyzed. These "blips" account for less than 5% of the total signals in a song record; more often than not, they are caused by nonspecific wing flicks produced by the male or the female when they are not courting (*cf.* EWING 1977a).

Each cycles/pulse value was determined as in Figure 2A of KULKARNI and HALL (1987) and plotted with respect to its position (pulse number) in the train (see below, Figure 3). A scatter plot was obtained by determining 150–250 cycles/pulse values from several complete trains (usually 15 to 30 per fly), and a regression of pulse number (within a given train) on cycles/pulse values was obtained with the help of a "Statworks" program run on an Apple MacIntosh computer. We found that *ca.* 80% of the trains containing 2–5 pulses/train did not show any aberrant pulses; and only *ca.* 10% of the trains contained more than 20 pulses (see below, Figure 1A). Therefore, the regression analyses were restricted to only those trains which contained 5–20 pulses. Slopes of the regression lines were diagnostic of the variation in cycles/pulse values for *diss* songs, as a function of increasing pulse number in the train (see RESULTS). For each genotype, pulse songs from 3–5 flies were analyzed. Mean slope values for the different mutant *vs.* normal genotypes were compared using the statistics noted in RESULTS.

Amplitudes of pulses were measured directly from the patches of songs displayed on the oscilloscope (*cf.* KULKARNI and HALL 1987). Instrument settings were the same for songs recorded from all different genotypes. The overall amplification (*cf.* GORCZYCA and HALL 1988) was such that pulse amplitudes ranged from 1–4.5 V (on the oscilloscope tracings or on oscillographed hard copy records), depending on the genotype.

Complementation tests: Homozygous g^2 *sd diss f* or *diss f* females were crossed to males carrying a series of translocations (FALK *et al.* 1984), in which deletions had been induced in a segment of the *X* chromosome (13F1-16A2) translocated to the fourth chromosome (see Table 1 and Figure 8). Thus, the "starting" $Dp(1;4)r^+f^+$ covers a region of the *X* corresponding approximately to the *sd f*, *diss*-flanking interval. All deletions induced in the $Dp(1;4)r^+f^+$ were f^+ because the most proximal breaks created by such deletions extended only up to 15B (FALK *et al.* 1984), thus leaving the f^+ containing region (15F1-3) intact. For our complementation tests, the presence of these "deleted duplications" in males marked as specified above could therefore be followed.

We applied several interstitial deletions, involving regions 14 and 15, to *diss* mapping (see Table 1 and Figure 8); they had been induced by B. H. JUDD (unpublished data) and were subsequently characterized (*e.g.*, with regard to breakpoints) by S. BANGA and J. B. BOYD (personal communication). The latter two investigators also have made a slight correction of breakpoint determinations for one of FALK *et al.*'s chromosome aberrations (see above and legend to Table 1).

A semilethal interaction was observed between the 14B-15A deletions and the third chromosomal *tra* mutation of STURTEVANT (1945). That is, when *diss f* males heterozygous for *tra* and a third chromosomal balancer (*In(3LR)TM6B*, dominant marker = *Dichaete*) were crossed to *Df(1)l32; tra/TM6B; T(1;4)r⁺f⁺* (*Df* = 14B17-C1 to 14F4-6) or to *Df(1)E150/FM7; tra/TM6B; T(1;4)r⁺f⁺* (*Df* = 14B3-4 to 14F) females, very few *Df/diss f; tra/tra* pseudomales were obtained (frequency, *ca.* 0.2%). Although these pseudomales seemed weak in their general movements, which precluded their "overall" behavioral testing (*e.g.*, mating success, phototaxis; see below), they nevertheless sang robust songs. More generally, all genotypes involving two *X*-chromosomes and homozygosity for *tra* led to normal courtship songs (in terms of overt appearance of the pulses, cycles/pulse values, and "song-slope" computations, *cf.* Figure 4, below), with the proviso that *diss* was not being expressed. Thus, application of this sex-transforming mutation did not interfere with interpretation of our complementation tests.

Response to mechanical shock: Mechanical shock-induced paralysis of flies was observed as described by GANETZKY and WU (1982). Briefly, 3–5-day-old flies (N = 5–10 of each genotype) were placed in empty culture vials and vibrated on a vortex mixer (Scientific Products, model s8223) at its top speed for 15 s. The length of paralysis was measured as the time required after vortexing until the first fly regained the ability to stand upright. The test was repeated at least 5 times/genotype using different sets of flies each time.

Measurements of male courtship: Before being tested behaviorally, newly emerged flies were collected under ether anesthesia. The flies were then stored for 5 days at room temperature: males 1 per unyeasted food vial, virgin females 5/vial.

Five-day-old flies of each sex were paired singly by introducing them gently into a plastic "mating wheel" with its ten observation chambers (HOTTA and BENZER 1976) at *ca.* 25°. The times elapsing between the moments of pairings and the initiations of copulations were recorded.

The courtship response of the test male was quantified as a courtship index (CI; *e.g.*, SIEGEL and HALL 1979; TOMPKINS, HALL and HALL 1980; TOMPKINS *et al.* 1982), which is the percentage of an observation period during which the male performs any of the courtship behaviors. CIs were measured for 5 min or until initiation of copulation, whichever occurred first.

The ability of a male to "track" the female visually during courtship (*cf.* COOK 1981) was estimated by scoring the number of times the male reoriented towards a female, after breaking away from her for at least 2 s, within a total observation period of 5 min (*cf.* TOMPKINS *et al.* 1982).

Phototaxis: Five-day-old males and females were tested separately in a Y-tube apparatus (QUINN, HARRIS and BENZER 1974), which had been adapted for phototaxis tests as in KULKARNI and HALL (1987). From 15 to 30 flies were placed in a dark "start tube" and allowed to choose between the "light" and "dark" arms of the Y-tube during a 120-s test interval. At the end of this time, the numbers of flies distributed in each arm, and those remaining in the start tube, were counted. Each test of a given genotype was repeated with five or more separate groups of flies. The controls (see Figure 13) included flies blinded by *norpA* mutations (PAK 1979).

Electroretinograms (ERG): These ERGs (*cf.* PAK and GRABOWSKI 1978; HEISENBERG and WOLF 1984) were recorded extracellularly from the adult eyes as follows: all test flies were dark adapted for 2 min. Each fly was immobilized on ice and quickly anchored to a glass coverslip by securing all of its moving body parts with Elmer's glue. The cornea of the illuminated eye was penetrated slightly with a recording electrode (glass, *ca.* 30 megaohms resistance) filled with 0.8% saline. The reference electrode, filled with 3 M KCl, was placed dorsally in the thorax; and light-evoked voltage changes were recorded on a Gould 2400 chart recorder. The illumination at the fly's eye level was 6 foot-candles (f.c.). For each fly, ERGs in response to light pulses of 1, 2, 3, 5, and 10 s durations with 30 s rest in dark before each consecutive stimulus, were obtained. Amplitudes of the "light on" and "light off" transient spikes, plus that of the "maintained component" (PAK and GRABOWSKI 1978), were measured for three flies of each pertinent genotype (see Table 3).

Walking optomotor tests: These tests were conducted based on a method previously described by GREENSPAN, FINN and HALL (1980). Individually stored flies, 3–5 days old, were starved for 3–4 hr in empty vials and tested (at room temperature) for their turning behavior in a visual field. For this, an individual was placed under a 25-mm diameter watch glass in the middle of a plexiglass rotating drum (diameter 15 cm; height 22.5 cm), which had alternating black and white vertical stripes. One black-white pair of stripes subtended a 19° of arc, and the drum was rotated at 12 rpm. White fluorescent light (Sylvania, FC12T10 CW RS) illuminated the center of the drum during the tests. Behavior was scored by counting the number of times the fly ran across a quadrant line in the same direction as the rotating stripes, *vs.* the number of times it ran in the opposite direction (*cf.* GREENSPAN, FINN and HALL 1980). Flies were tested in three successive trials, each consisting of a 1-min clockwise run followed by 1 min of rest, and finally a 1-min run in the counterclockwise direction. Results of all three trials, for a given fly, were pooled separately for the clockwise and counterclockwise runs. Three individuals of each sex were tested for each genotype. The results were expressed as the fraction of total lines which were crossed in the *same* direction as that in which the stripes were moving.

Locomotor activity: These measurements were made on single flies at *ca.* 25° in a cylindrical plastic chamber divided across the diameter by a straight line (*cf.* KULKARNI and HALL 1987). A given individual was transferred to the chamber; after a 5-min "accommodation" period, the number of times the fly crossed the line in the next 5 min was recorded.

Circadian rhythms: Circadian rhythms of locomotor activity were monitored automatically and analyzed as described by HAMBLEN *et al.* (1986).

Flight: A transparent plastic cylinder (BENZER 1973; KULKARNI and HALL 1987) with its insides coated with paraffin oil was placed upright in a petri dish. From 25 to 50 5-day-old flies were gently introduced into the top of the cylinder. The numbers of flies stuck to the sides along the length of the cylinder, at 2.5-cm intervals, were counted. Each test was replicated with five groups of flies of a given genotype.

RESULTS

Isolation of *dissonance* and its basic phenotype: We initially found 8 putative song mutants among the 2500 lines descended from ENU-treated males. Seven of them were immediately noted as involving males with abnormal wing positions, who sang with merely marginal defects in their courtship song pulses. Further, these mild abnormalities did not

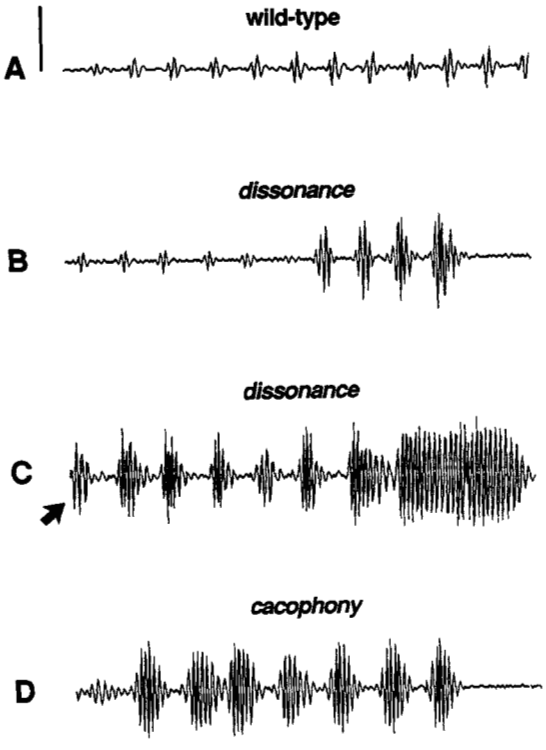

FIGURE 1.—Pulse songs of mutant and normal males. Taped song records (*cf.* KULKARNI and HALL 1987) were "digitized" by a computer and eventually printed from the appropriate files stored on disc. The songs displayed here are plots of voltage (post-amplification, *cf.* GORCZYCA and HALL 1988) *vs.* time. The vertical scale bar shown in A (and referring to B–D as well) thus represents 1200 mV, and the total time for each plot here is *ca.* 400 ms. The entirety of a given train of pulse singing is displayed in each case except for C (see below). (A) A wild-type (Canton-S) male's typical bout of pulse song (*cf.* KYRIACOU and HALL 1980, 1982; SCHILCHER 1977; KULKARNI and HALL 1987; GORCZYCA and HALL 1988). (B) A moderately aberrant bout produced by a *diss* male from the unmarked, true-breeding stock (see MATERIALS and METHODS); note that this train begins with wild-type-like pulses. (C) A "wild-burst" from the same male as in B, though only the latter portion of this pulse train is shown (*i.e.*, the *arrow* points to pulse No. 11; this bout, like that in B, commenced with normal-appearing signals, *i.e.*, 10 wild-type-like pulses). (D) A typical *cac* pulse train (*cf.* SCHILCHER 1977; KULKARNI and HALL 1987).

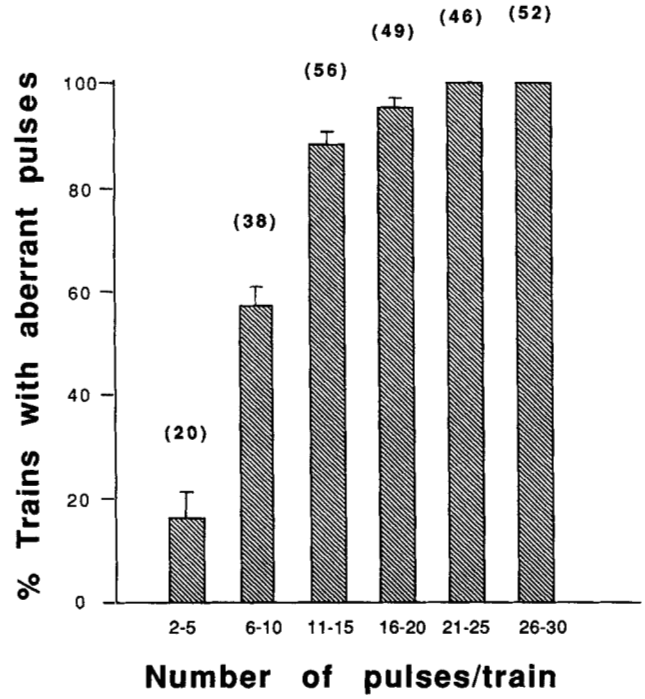

FIGURE 2.—Aberrant pulse song of *diss* as a function of pulse-train length. Bouts of singing—a total of *ca.* 200 pulse trains produced by 10 separate *diss* males (from the unmarked stock)—were grouped into the 7 train-length categories shown. The numbers in parentheses above each bar are overall *percentages* of abnormal (individual) pulses for the trains in that category. [For example, then, longer trains tend each to be half normal (at the beginning) and half abnormal in pulse characteristics; whereas shorter trains are made up of smaller percentages of loud, polycyclic pulses.] The proportions of the 328 trains examined here that fell into the various train-length categories are: 2–5, 28%; 6–10, 23%; 11–15, 17%; 16–20, 23%; 21–25, 5.5%; 26–30, 2.5%.

show up in the F_3 (*re* further crosses to attached-*X* females). Only one isolate, which we named *dissonance* (*diss*), sang in a consistently abnormal manner in the F_2 and beyond.

The pulse song of a wild-type *D. melanogaster* male (Figure 1A) is series of pulse trains, each containing between 2 and *ca.* 35 individual pulses; the median value is *ca.* 7 pulses/train (computed from a total of 100 trains, derived from 5 Canton-S males). The pulse song of a *diss* male is quite dramatically different from that of either wild-type or the *cacophony* mutant (SCHILCHER, 1976c; KULKARNI and HALL 1987). For *diss*, trains containing more than 5 pulses begin with wild-type-like (*i.e.*, mono- to tricyclic) pulses but break into polycyclic, high amplitude pulses (Figure 1B) after a variable number of pulses are produced in a train (Figures 2, 3). In general, short *diss* trains (≤5 pulses) are entirely normal (Figure 2). Two different kinds of aberrant pulse songs, as produced by this mutant, are shown in Figure 1B and C. The "wildly" abnormal train (Figure 1C) began with normal-appearing pulses (see legend to Figure 1), as did the more standardly aberrant *diss* train displayed here (Figure 1B).

The amplitude of mono- to tricyclic pulses in *diss* songs (*i.e.*, early in trains) is similar to the amplitude of wild-type pulses (Figure 1A). In contrast, polycyclic pulses in *diss* songs showed a *cac*-like amplitude (*cf.* SCHILCHER 1977, KULKARNI and HALL 1987), *i.e.*, 50–100% higher than that of wild-type. The amplitude of wildly polycyclic pulses (Figure 1C) was comparable to, or marginally higher than, the *cac*-like pulses in a *diss* song.

The comparison of mean cycles/pulse values of successive pulses from several trains recorded from the songs of wild-type, *diss*, and *cac* males is shown in Figure 3. Data from the *cac* mutant (also see Figure 1D) are included for comparison, because *diss* pulses relatively late in the trains are superficially similar to

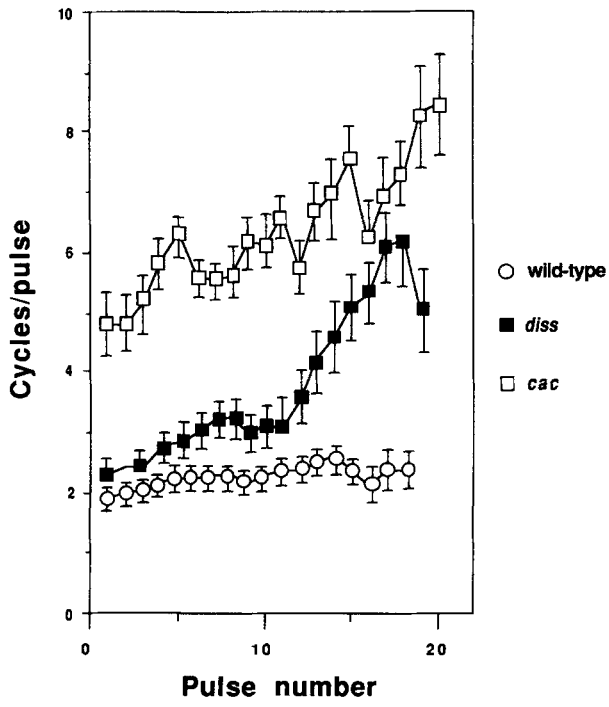

FIGURE 3.—Song characteristics as a function of position within trains. The wild type was a Canton-S male; *diss*, a male from the original (unmarked) strain; and *cac*, a male from the original unmarked stock of this mutant (*cf.* KULKARNI and HALL 1987). Each data point represents a mean cycles/pulse value (±SEM) from *ca.* 35 trains, from each of these three types of male (1 song was analyzed per genotype). The numbers of pulses, contributing to the computations for the various points differ; *e.g.*, there are relatively few trains that are as long as 15–20 pulses (*cf.* Figure 2).

those produced uniformly by *cac* males (*cf.* SCHILCHER 1977; KULKARNI and HALL 1987).

There appears to be, however, a difference in the "*cac*-like" portions of *diss* trains, compared to the pulse singing that is uniformly produced by *cac* males. For the latter, the interpulse intervals, or IPIs (see BENNET-CLARK and EWING 1970; COWLING and BURNET 1981, for definitions), can be thought of as longer than normal (*cf.* SCHILCHER 1977). This would be so if one computes the intervals between pulses with reference to the *peak* amplitudes of any two adjacent pulses. Yet, another way of considering this parameter is to attempt computations of the actual intervals of "silence" that occur between pairs of pulses. In this regard, *cac* IPIs are in reality in the normal range of *ca.* 30–40 ms (*e.g.*, Figure 1D, *cf.* SCHILCHER 1977; KULKARNI and HALL 1987). For *diss*, it is not uncommon for the polycyclic pulses actually to "blend" into one another, such that there is in fact little or no discernible silence interval between two pulses of this kind (see Figure 1C). Yet, it could be that the "peak-to-peak" manner of determining IPIs would show *diss* males to be normal in this character—defined and computed this way. Some of this discussion is confounded as to how one wishes most rationally to specify what is an IPI. Yet, in any event, *diss*'s aberrant singing behavior is once more different in its details from that associated with the other song mutant in this species.

The increase in *diss*'s cycles/pulse values, as a function of pulse number within each train (Figure 3), forms the basis of quantifying the difference between the *diss* and normal songs.

First, complete penetrance of the *diss* song phenotype was observed (on the oscilloscope) in individual song records of 20 *diss* males from the original stock (*i.e.*, whose males are unmarked). In no case did we observe an arbitrary mixture of mono- to tricyclic *vs.* polycyclic pulses. That is, several normal-appearing pulses (all in a row) proceeded into a subsequent series of thoroughly abnormal pulses. Occasionally, entirely polycyclic *cac*-like trains were produced by a *diss* male. These occurred in less than 1% of the *diss* trains (N = 150 trains from 10 males) and always were seen in relatively short trains (5–8 pulses).

Second, we proceeded to quantify the *diss*-induced singing defect more systematically. Since it seemed that the transition of mono/poly-cycles in *diss* is rather abrupt, we initially tried to describe the trains of pulses by fits to polynomial regression curves. However, subsequent applications of linear regressions were found to give better fits (Figure 4). This implies that there is no fixed pulse number, in a given pulse train of *diss*'s song, at which the monocyclic/polycyclic pulse transition occurs. That is, when results from several pulse trains from the same song were pooled, the overall transition appeared to be gradual (hence the superior fits from the linear regressions).

The number of pulses in the individual *diss* trains ranged from 2 to 35 with a median of 6 (N = 200 pulses from 10 songs). The median for wild-type males was 7 pulses/train (see above).

The mean slopes (±SEM) from these regressions, for 10 males of each of the following genotypes, were: wild-type, 0.022 ± 0.008; *cac*, 0.058 ± 0.015; *diss*, 0.200 ± 0.024. The comparison among the distribution of slope values for wild-type, *diss* and *cac* is shown in Figure 5. The difference in slope values between wild-type and *diss* was significant (one-way ANOVA tests, overall $P < 0.001$; $P < 0.01$ for the specific difference between *cac* and *diss*). Except for a small overlap between the higher values of *cac* and lower values of *diss*, the slopes from analyses of *diss* songs are distinctly different from those of either wild type or *cac* males (*cf.* Figure 4). The relatively broad range of slope values obtained for *diss* is due to the variable times (within trains) at which cycles/pulse values begin to increase, as well as variations in degrees of polycyclicity among pulse trains. Although there was a considerable overlap between the slope values of wild type and *cac* (reflecting relatively small variations in their cycle/pulse values within

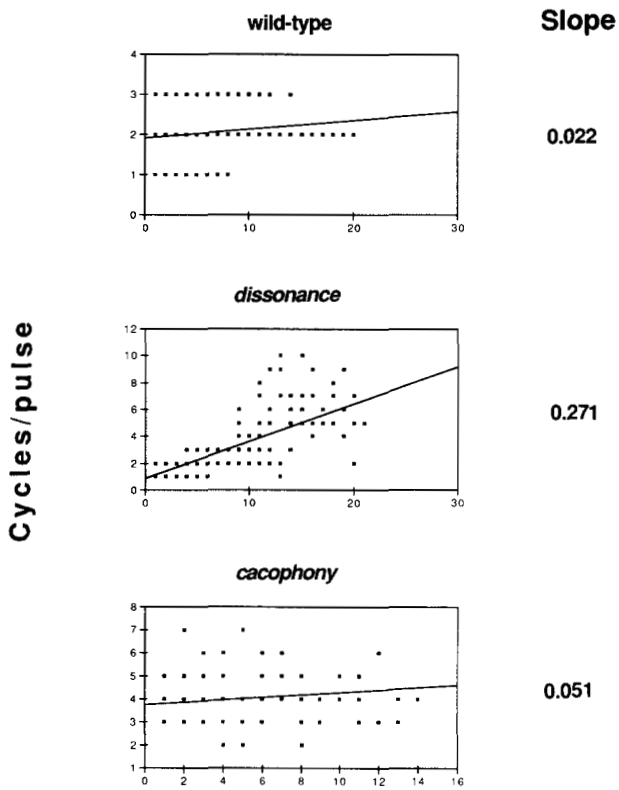

FIGURE 4.—Sample regression analyses of courtship song pulses produced by mutant and normal males. These plots refer to cycles/pulse *vs.* pulse numbers (*e.g.*, "early" *vs.* "late" in a train) for three males: Canton-S wild-type, unmarked *diss*, and unmarked *cac*. Each plot represents *ca.* 150 pulses from *ca.* 20 trains per male. Trains chosen for the analysis contained >5 and <21 pulses/train, thus accounting for *ca.* 70% of pulses in each song (*cf.* Figure 2). The range of regression slopes for the several songs analyzed for each of these genotypes is discussed in the text; also, see Figure 5.

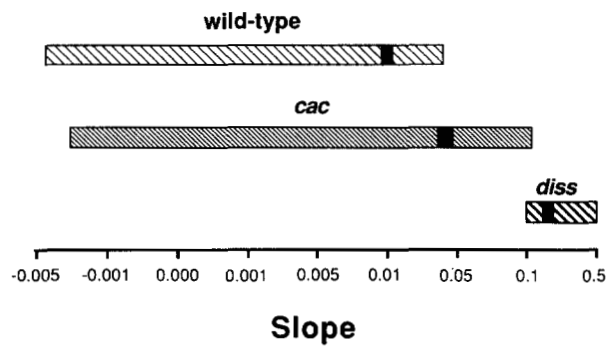

FIGURE 5.—Summary of regression analyses of song pulses. Pulses chosen for the analyses were from trains containing >5 and <21 pulses (*cf.* Figures 2 and 4). The black box within each rectangle denotes the *median* slope for the given genotype (N = 10 flies each). Slopes for the *diss* males' songs differed significantly from those for wild-type (Canton-S) males or *cac* males (see text).

either genotype), the mean cycles/pulse value is higher for *cac* (4.7 ± 0.2; N = 10) than for the wild type (2.0 ± 0.2; N = 10) (*cf.* KULKARNI and HALL 1987).

Therefore, we conclude that *diss* mutation confers distinct pulse song phenotype, which is different qualitatively as well as quantitatively from that of either wild type or the *cac* mutant.

The sine song of wild-type *D. melanogaster* usually occurs in a rather narrow frequency band, *i.e.*, *ca.* 160–180 Hz (SCHILCHER 1976a; COWLING and BURNET 1981; D. A. WHEELER, W. L. FIELDS, and J. C. HALL, in preparation). The sine song spectra for *diss* songs have recently been determined to be similar to those of wild type (major peak, *ca.* 170 Hz; D. A. WHEELER, S. J. KULKARNI, D. A. GAILEY and J. C. HALL, in preparation). Therefore, *diss* does not appear to cause a change in the courtship hums.

Song phenotypes of *diss* males from the true-breeding stock, compared to those in the balanced stock that has been maintained ("over" *In(1)FM7a*) for *ca.* 2 yr, were very similar (slope values, 0.202 ± 0.012 and 0.224 ± 0.031 respectively, N = 3 in each case, *cf.* Figure 4). This suggests that there has been little or no selection of factors that might act to ameliorate this singing defect. (*Note*: The song slope values for the genotypes discussed here, as well as in the following paragraphs and subsequent sections of RESULTS are mean ± SEM for computations obtained from recordings of 3–5 males of each genotype.)

The mutant phenotype was also retained in males of the genotypes *cv v diss*, *diss f*, and g^2 *sd diss f* (slopes: 0.273 ± 0.007, 0.130 ± 0.003, and 0.150 ± 0.015, respectively). These genotypes were obtained by replacing segments of the *X*-chromosome flanking *diss* with material from marker-bearing chromosomes, by recombination (Figure 6). Slope values for controls were: *cv v f*, 0.016 ± 0.001; and g^2 *sd f*, 0.022 ± 0.001. The behavior of these *diss* recombinant males again argues against a contribution of the genetic background (*e.g.*, factors "co-induced" with *diss*) to the mutant song phenotype.

The *diss* mutation appears to be completely recessive. Pseudomales of the genotype *diss/diss; tra/tra* (slope value, 0.145 ± 0.011) produced pulse songs characteristic of *diss*, whereas *diss/+*; *tra/tra* controls generated a song like that of wild type (0.041 ± 0.005). Also, the cycles/pulse computations for these flies yielded normal values (*ca.* 2, *cf.* wild-type result above; and note that the "normal" song slopes, which will frequently be specified below as having resulted from testing certain of the genetic variants located near *diss*, were also accompanied by cycles/pulse values in the normal range).

The *diss* song phenotype is not temperature sensitive, in that mutant song characteristics, as observed on the oscilloscope screen, were neither enhanced nor reduced for *diss* males reared then stored (as adults) at the following temperatures: 18°, 25°, 29°. Also, note that *diss* is not a temperature-sensitive

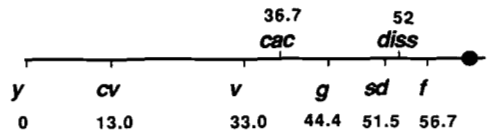

FIGURE 6.—The recombinational map position of *diss*. From a three-point cross between *diss* and flies carrying an *X* chromosome marked with *v* and *f*, 167 recombinants were obtained: 133 crossovers occurred between *v* and *diss*, whereas 34 were between *diss* and *f* (see MATERIALS AND METHODS, and LINDSLEY and GRELL 1968, for descriptions of markers). Therefore, *diss* maps *ca.* 19 map units to the right of *v*, and 5 map units to the left of *f*, *i.e.*, at position 52 on the *X* chromosome. A g^2 *sd diss f* recombinant was recovered from a cross between *diss f* and g^2 *sd*, which essentially led to the same estimate for *diss*'s map position and also flanked the song mutation by *sd* and *f* (these markers being *ca.* 5 map units apart). The map position for the *cac* song mutation is from KULKARNI and HALL (1987).

paralytic or a mechanical-shock-sensitive mutation: mutant adults were quite mobile at 29°, and they were not "stunned" when a container of them was vortexed (see MATERIALS AND METHODS, and below). These tests were performed because of *diss*'s proximity to *para*^{ts}, *easily shocked*, and *bang-senseless* behavioral mutations (GANETZKY and WU 1982; GANETZKY 1984; and see Figure 8, below).

Mapping of *diss*: During the initial mapping of this mutation (using a *y cho cv v f* chromosome, see MATERIALS AND METHODS), the *diss* song phenotype segregated such that recombinants involving all intervals except *v–f* were uniformly normal or mutant in their singing (Figure 6). Thus, the song defect of *diss* was mapped to approximately position 52 on the *X* chromosome (see legend to Figure 6). Finer localization of *diss* was done by using the markers *garnet* (g^2, 44.4), *scalloped* (*sd*, 52.0), and *f* (Figure 6). Thus, *diss* maps within a *ca.* 5-centimorgan interval, flanked by *sd* and *f*.

This region of the *X* chromosome is largely spanned by $Dp(1;4)r^+f^+$. This duplication and a smaller one, $T(1;2)r^{+75c}$ covered the *diss* singing abnormality (Figure 7, Table 1). $Dp(1;3)f^{+71b}$ failed to cover *diss* (Figure 7, Table 1). Males carrying the latter duplication alone (*i.e.*, in a $diss^+$ background) sang a normal song (Table 1). The interstitial deletions *Df(1)l32* (Figure 7) and *Df(1)E150*, when heterozygous with g^2 *sd diss f* in diplo-*X* flies transformed into males by homozygosity for *tra*, led to mutant songs (Table 1). When each of these deletions was heterozygous with $diss^+$ in *tra/tra* pseudomales, the songs were normal (Table 1). When *diss* was heterozygous with the following deletions: *Df(1)D7*, *Df(1)C246*, *Df(1)g-1*, *Df(1)KA9*, and $Df(1)sd^{72b26}$, the songs of the transformed "males" were normal (Table 1).

The singing abnormality of *diss* was not covered by the following "deleted duplications" that are missing certain portions of 14B5-18/distal region 15B:

TABLE 1

Complementation tests involving *diss* and chromosome aberrations

Deletions	*X* chromosome breakpoints	*diss* uncovered?	Song slope (re: (cycles/pulse *vs.* pulse number)
Df(1)E150	14B3-4 to 14E	Yes	0.200 ± 0.007
Df(1)l32	14B17-C1 to 15A2-3	Yes	0.186 ± 0.009
Df(1)C246	11D-E to 12A1-2	No	0.021 ± 0.015
Df(1)g-1	11F10 to 12F1	No	0.028 ± 0.017
Df(1)KA9	12E2-3 to 12F5-13A1	No	0.033 ± 0.009
$Df(1)sd^{72b26}$	13F1 to 14B1	No	0.024 ± 0.012
Df(1)D7	14C7-D1 to 14E3-F1	No	0.047 ± 0.011
Controls:			
$diss^+/Df(1)E150$			0.056 ± 0.001
$diss^+/Df(1)l32$			0.031 ± 0.006

"Deleted duplications"	Deleted segment	*diss* uncovered?	Song slope
T(1;4) + *Df(1)82c3k*	14B3-4 to 14E	Yes	0.189 ± 0.081
T(1;4) + *Df(1)82b6w*	14B5-18 to 14E	Yes	0.246 ± 0.017
T(1;4) + *Df(1)81k19b*	14B5-18 to 15B	Yes	0.229 ± 0.062
T(1;4) + *Df(1)80f18c*	14C4-5 to 15A3-4	No	0.051 ± 0.012
Controls:			
$diss^+/Y$; *T(1;4)* + *Df(1)82c3k*			0.031 ± 0.005
$diss^+/Y$; *T(1;4)* + *Df(1)82b6w*			0.020 ± 0.002
$diss^+/Y$; *T(1;4)* + *Df(1)81k19b*			0.021 ± 0.005

Straight duplications	*X* chromosome breakpoints	*diss* uncovered?	Song slope
In(1)2-4-1-1	13E9-14 to 14C7-8	No	0.022 ± 0.005
In(1)D30	14C6-D1 to 15E-F	No	0.031 ± 0.008
$Dp(1;2)r^{+75c}$	14C1-2 to 15A9	Yes	0.014 ± 0.007
$Dp(1;4)r^+f^+$	13F1 to 16A2	Yes	0.007 ± 0.00
$Dp(1;3)f^{+71b}$	15A4 to 16C2-3	No	0.180 ± 0.026
Control:			
$diss^+/Y;Dp(1;3)f^{+71b}$			0.024 ± 0.009

Deletions = interstitial deletions (for their source, see MATERIALS AND METHODS). These *X*-chromosomal deficiencies were made heterozygous with *diss*, in flies homozygous for *tra*. Deleted duplications = deletions induced by FALK *et al.* (1984) in the *X*-chromosomal segment (13F1-16A2) which had been translocated to the fourth chromosome (re: $Dp(1;4)r^+f^+$). The distal break of *T(1;4)* + *Df(1)80f18c*, previously reported as 14C3-8 (FALK *et al.* 1984), has been revised to 14C4-5 by S. BANGA and J. BOYD (personal communication). These duplications, and the "straight" ones as well, were tested for coverage of *diss* in song recordings of haplo-*X* males. The inversion breakpoints, for *In(1)D30* and *In(1)2-4-1-1*, were determined by GANETZKY (1984) and B. GANETZKY (personal communiation), respectively. These two aberrations were tested for *diss* uncoverage in a *tra* background (see above). Song slope values (means ± SEM for 3–5 flies tested per genotype) distinguish *diss*-like songs (slopes >0.100) from normal songs (slopes <0.100).

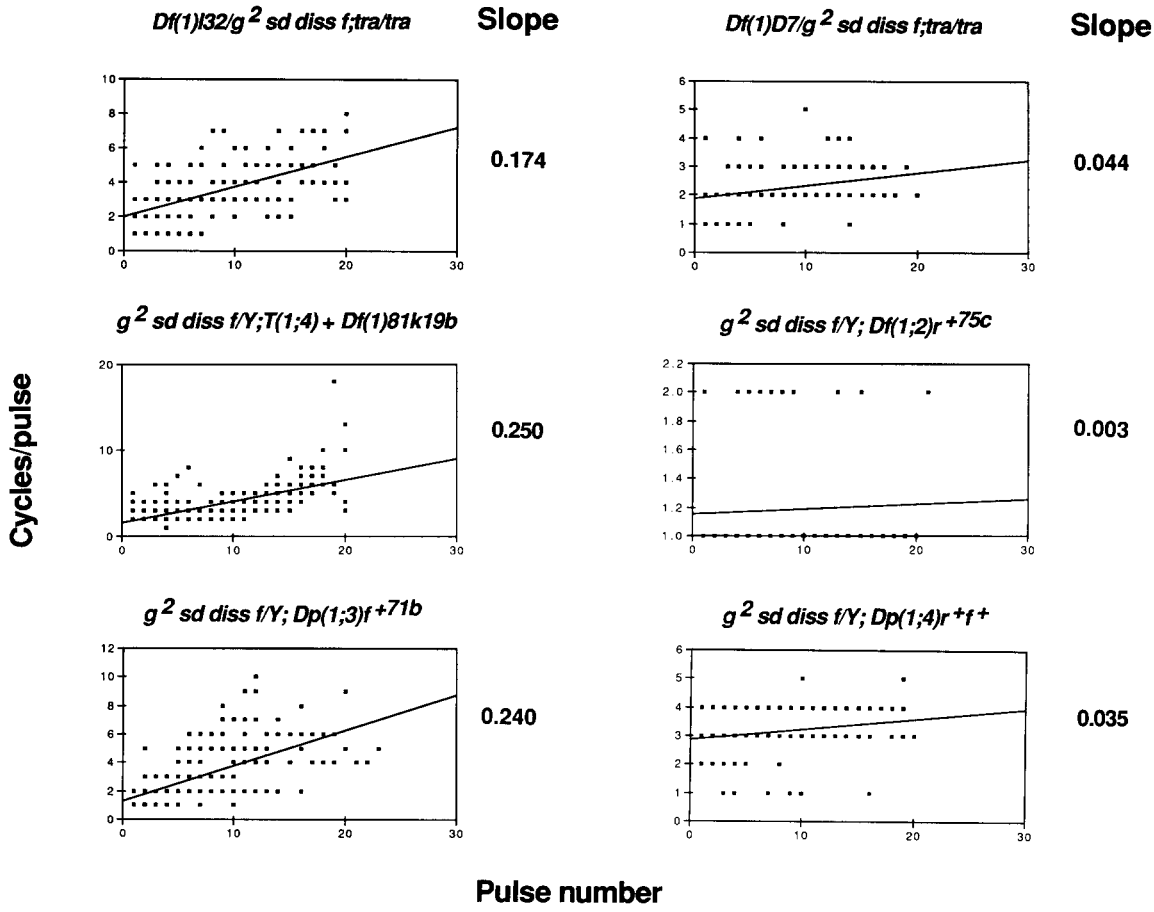

FIGURE 7.—Complementation tests involving a *diss*-bearing *X* chromosome and various chromosome aberrations. The deletions: *Df(1)l32* and *Df(1)D7* (see Figure 8) were tested "over" *diss* in pseudomales of the genotype *Df/diss; tra/tra*; and duplications (see Figure 8) were introduced into a g^2 *sd diss f/Y* genetic background (see MATERIALS AND METHODS). These plots of the song phenotypes associated with such genotypic combinations were prepared as described in the text (also see Figure 5). The "straight" *Dp*'s are $T(1;4)r^+f^+$, $T(1;2)r^{+75c}$ and $T(1;3)f^+71b$, whereas *T(1;4) + Df(1)81k19b* is a "deleted *Dp*" (see MATERIALS AND METHODS). One of the results shown represents a control: the straight duplication $T(1;4)r^+f^+$ in a g^2 *sd diss f/Y* background.

T(1;4) + Df(1)81k19b (Figure 7), *T(1;4) + Df(1)82c3k* and *T(1;4) + Df(1)82b6w*, each in a g^2*sd diss f/Y* background, led to a mutant song (Table 1). When these *T(1;4) + deletions* were present in males hemizygous for $diss^+$, the songs were normal (Table 1). One deleted duplication [*T(1;4) + Df(1)80f18c*], whose missing segment does not totally overlap that defined by the three aberrations just noted above (Figure 8), covered the effects of *diss* (Table 1).

In further complementation tests, *In(1)D30* and *In(1)2-4-1-1* failed to uncover the singing abnormality of *diss* (Table 1). These two breakpoints (which by themselves cause lethality) are non-complementing with *para* mutations (GANETZKY 1984).

Viable mutations not only in the *para* gene, but also at the two closely linked "behavioral loci" *easily shocked* and *bang-senseless* (all mapping within 14B-14C), complemented *diss* in females heterozygous for the respective mutations. Thus, $para^{ts1}/g^2$ *sd diss f* and $para^{ts115}/g^2$ *sd diss f* did not paralyze when placed at 37° for 10 min (N = 20 each). And in tests of mechanical shock-induced paralysis (see MATERIALS AND METHODS), "stun" durations—measured in seconds (± SEM) for 5 groups of 5 flies each—were: eas^{PC80}/g^2 *sd diss f*, 0.4 ± 0.2; eas^{PC80}/eas^{PC80}, 11.4 ± 0.8; eas^{RH11}/g^2 *sd diss f*, 0.6 ± 0.4; eas^{RH11}/eas^{RH11}, *12.2 ± 1.0;* g^2 *sd* bss^{MW1}/g^2 *sd diss f*, 3.4 ± 0.8; g^2 *sd* bss^{MW1}/g^2 *sd* bss^{MW1}, 157.0 ± 17.8; g^2 *sd* $bss^{MW1}/FM7$, 8.4 ± 2.0; bss^{PC75}/g^2 *sd diss f*, 6.4 ± 1.0; bss^{PC75}/bss^{PC75}, 103.0 ± 8.5; $bss^{PC75}/FM7$, 10.0 ± 2.5. Note that bss^{MW1} *and* bss^{PC75} flies are semidominant for the mechanical shock-induced paralysis (GANETZKY and WU 1982, and see above), which accounts for the higher stun values associated with *bss/diss* females.

Two loci affecting visual system function map near *diss*: slow-receptor-potential(*slrp*, *1*-51, PAK 1975) and *no-on-transient-A* (*nonA*, *1*-52.3, PAK 1975, plus W. L. PAK and M. DELAND, unpublished data; also see HEISENBERG and WOLF 1984). Diplo-*X* males heterozygous for *slrp* and *diss* or *nonA* and *diss* produced normal songs: $nonA^{H2}/g^2$ *sd diss f; tra/tra* (slope, 0.029 ± 0.006); $nonA^{P14}/g^2$ *sd diss f; tra/tra* (0.023 ± 0.009); $slrp^{P28}/g^2$ *sd diss f; tra/tra* (0.045 ± 0.028).

Whereas *diss* and *nonA* complement each other with

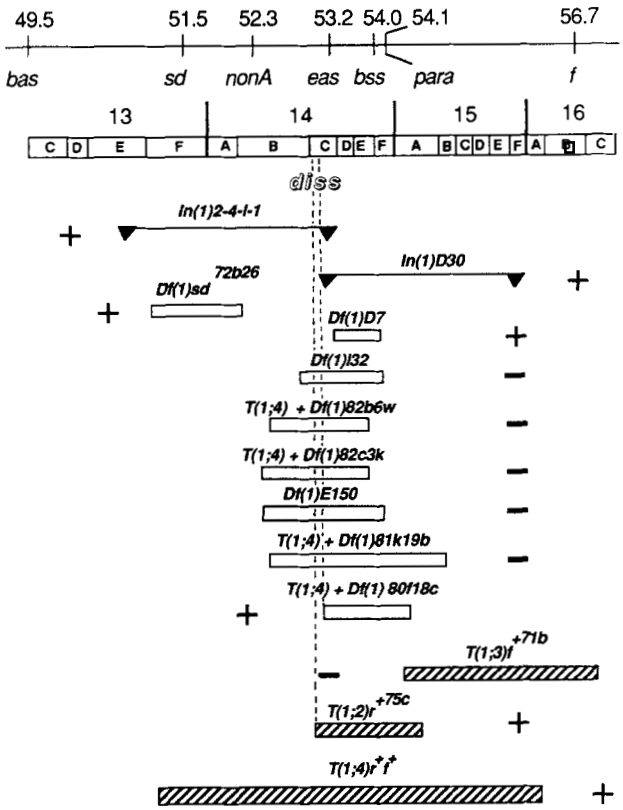

FIGURE 8.—Cytogenetics of *diss*. The top line shows the recombination map of markers and other mutations in the vicinity of *diss* (*cf.* LINDSLEY and GRELL 1968; HALL 1982). Under this line are the numbered and lettered intervals referring to the salivary chromosome map for this relatively proximal portion of the *X* chromosome (*cf.* LINDSLEY and GRELL 1968). Deletions or "deleted duplications" (whereby each empty rectangle designates the *missing* material) were tested for complemention with *diss* in either transformed diplo-*X* pseudomales or in g^2 *sd diss f/Y* males that carried the deleted duplications. The *X* chromosomal breakpoints of these aberrations are indicated, *i.e.*, flanking the horizontal bars. Plus signs denote normal song phenotypes (complementation with or coverage of *diss*), whereas minus signs indicates that the song was *diss*-like when a *Df* was heterozygous with the mutation or when a given *Dp* was present. Certain inversion breakpoints (solid triangles, *cf.* GANETZKY 1984) were tested "over" *diss* (in *tra* "males") and failed to uncover it (hence the two + indications). The one small cytological interval to which the *diss*-induced song defect was localized from these experiments is designated by the vertical dashed lines.

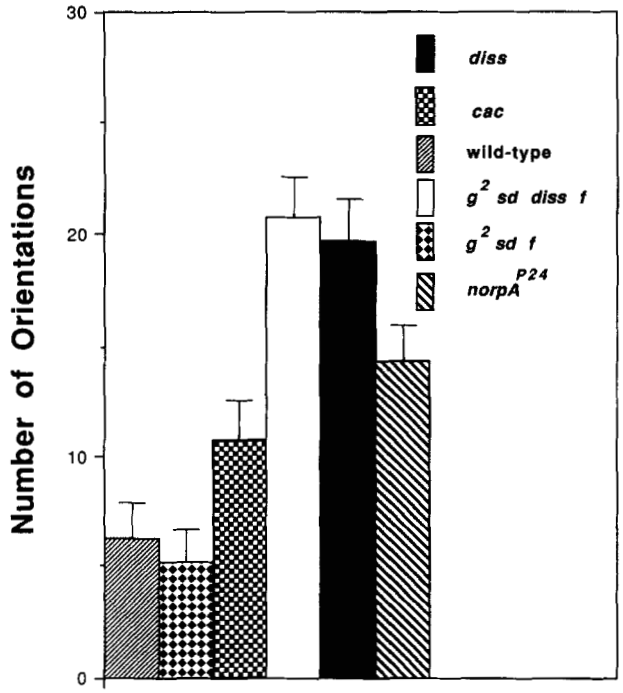

FIGURE 9.—Orientations of males toward females affected by *diss*. Males of the genotypes shown were paired singly with attached-*X* virgin females in courtship chambers at 25° (see MATERIALS AND METHODS). The orientation bouts performed by a given male towards the female—*i.e.*, number of discrete courtship intervals, each separated by 2 or more seconds of noncourtship—were counted for a period of 5 min (see MATERIALS AND METHODS). Twenty males were tested for each genotype, and the results are expressed as the mean number of orientations (±SEM). These scores for both unmarked *diss* and g^2 *sd diss f* recombinants are compared here to those of wild-type males, males with markers alone, *cac* males, and the visually blind males expressing a *norpA* mutation. Males carrying certain "deleted duplications"—*T(1;4) + Df(1)81k19b* and *T(1;4) + Df(1)82c3k* in a g^2 *sd diss f/Y* background—showed a higher number of orientations (18.4 ± 2.5, 14.0 ± 1.4) than g^2 *sd diss f/Y; T* $(1;4)r^+f^+$ and g^2 *sd f/Y; T(1;4) + Df(1)81k19b* controls (4.2 ± 0.4, 5.4 ± 0.7). Similarly, g^2 *sd diss f* males carrying the duplication $T(1;3)f^{+71b}$ reoriented anomalously often (20.1 ± 1.2), whereas g^2 *sd diss f* males carrying the duplication $T(1;2)r^{+75c}$ were nearly normal (6.0 ± 0.8). A one-way ANOVA test, performed with respect to genotype *vs.* number of orientations, sorted the genotypes into three groups: (1) wild type and g^2 *sd f*; (2) *cac* and $norpA^{P24}$; (3) g^2 *sd diss f* and *diss*. Each member of any one of these groups differed significantly from individual members of the remaining two groups ($P <$ at most 0.05, for all such comparisons).

regard to singing behavior, and *nonA* males sing normally (see below), these two kinds of mutations seem to interact in terms of visual responses. We will report elsewhere (S. J. KULKARNI, M. A. VARGO, L. MOROZ and J. C. HALL, in preparation) that *diss/nonA* females exhibit deficits in behavior and physiology similar to those caused by *diss* alone (also see below, Figures 13 and 14) or *nonA* by itself.

Based on the genetic results reported here, the mutation causing the *dissonance* song phenotype defines a genetic locus in the chromosomal interval 14C1-2 to 14C4-5 (Figure 8). Note that *diss* is at a completely separate *X*-chromosomal locus from that of *cac*, which maps in 11A (KULKARNI and HALL 1987). Furthermore, *diss* and *cac* complemented each other in diplo-*X*, homozygous *tra* "males" (slope, 0.040 ± 0.003).

Courtship behavior of *diss*: During visual observations of the courtships performed by individual *diss* males, each with a virgin female in a courtship chamber (see MATERIALS AND METHODS), it seemed as if the mutant males were visually impaired. That is, these males courted females with multiple and abnormally brief orientation bouts. This was subsequently quantified (Figure 9). A *diss* male frequently lost contact with the female in the middle of a wing vibration bout and continued his singing, directed at "nothing." However, *diss* males did not court "air

alone" when they were introduced in a fresh unused courtship chamber, nor did they vibrate their wings in chambers that had been "pretreated" with virgin females for 5 min.

The two song mutants were compared in their responses to moving females. Both *cac* and sn^3 *cac* males performed only half the number of orientations as did *diss* (Figure 9), suggesting further that the *cac* mutant does not suffer from any severe visual impairment (also see KULKARNI and HALL 1987). These *cac* males, however, did reorient themselves significantly moreso than did controls that were free of courtship and/or visual mutations (see legend to Figure 9). This could be caused by the generalized decrement in locomotor activity that is a part of *cac*'s mutant phenotype (KULKARNI and HALL 1987).

Courtship behavioral abnormalities like those measured for the unmarked descendants of the original *diss* isolate were also observed in tests of g^2 *sd diss f* males, and in g^2 *sd diss f* males bearing either of the deleted duplications *T(1;4)* + *Df(1)81k19b* and *T(1;4)* + *Df(1)82c3k* (see legend to Figure 9). In contrast, duplications that cover *diss*'s singing abnormality also complemented the abnormal orientation response (again, see legend to Figure 9). These results indicate that the visual impairment maps at or very close to the song abnormality.

It was thought that a *diss* male might sing with louder and polycyclic pulses in some sort of desperation, *i.e.*, an indirect effect on singing that could be caused by losing contact with the female. We controlled for this by monitoring the courtship behavior of visually blind $norpA^{P24}$ males (PAK 1979). Courtships exhibited by these mutant males were observed while simultaneously observing their pulse songs on the oscilloscope screen (see Figure 10 for an example). Special attention was paid to the portion of a given pulse train produced by a $norpA^{P24}$ male when he strayed from the female and was completing a wing vibration bout. Although $norpA^{P24}$ males showed multiple orientations similar to *diss* (Figure 9), they nevertheless sang a wild-type-like pulse song (slope from the relevant regressions, 0.028 ± 0.006, and see Figure 10)—including the data recorded when these blind males were losing or had already lost contact with females. Males from another visually mutant strain nbA^{EE171} sang normally (*cf.* KULKARNI and HALL 1987); slope, 0.036 ± 0.005. And visually mutant *nonA* males (see above) were also normal in this courtship phenotype: two alleles were tested: $nonA^{P14}$ and $nonA^{H2}$; slopes, 0.028 ± 0.008 and 0.027 ± 0.008 (see Figure 10 for examples, and also note that cycles/pulse values for these visual mutants were in the normal range, *i.e.*, *ca.* 2). These results indicate that the aberrant wing vibration produced by *diss* males are inherent to the "song mutation" per se and are not a secondary consequence of the visual defect that has evidently been coinduced.

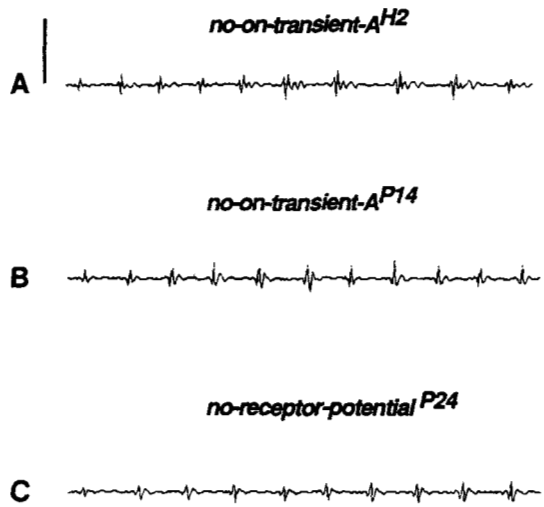

FIGURE 10.—Pulse songs of visual system mutants. Computer printouts of digitized pulse songs were obtained as described in the legend to Figure 1. The vertical scale bar in A represents 1200 mV, and the total time for each song trace is *ca.* 400 ms. Pulse songs of males from the true-breeding stocks expressing the three mutations (two at one locus) indicated here (*cf.* PAK 1975, PAK and GRABOWSKI 1978; HEISENBERG and WOLF 1984) were recorded. All pulse song bouts from these three types of mutants were apparently normal, as exemplified in these traces (*cf.* Figure 1A).

To assess further the mating performance of *diss* males, cumulative percentages of flies initiating copulation during 60-min observation periods were determined (Figures 11, A and B). For all measurements of mating latencies reported, 20 males were tested for each genotype. All of the wild-type control males mated with wild-type females (average time elapsed before mating: 7.2 ± 1.0 min); whereas only 50% of *diss* males did so (average time for those successful: 28.7 ± 4.2 min; Figure 11A). Blind $norpA^{P24}$ males initiated matings faster (latency, 23.8 ± 4.0; 80% matings in 60 min) than did males expressing *diss* (Figure 11B).

To test if song is the only factor governing the abnormally long mating latencies for *diss* males, mating performances of wingless *diss* males were compared to those of wingless wild-type males. Wingless males mate with longer latencies than those measured for intact males (*e.g.*, SCHILCHER 1976b; KYRIACOU and HALL 1982, 1986; KULKARNI and HALL 1987). Wingless *diss* males were still worse than normal (Figure 11A): latency >60, (0% mating in 60 min), *vs.* wingless wild type: 24.4 ± 4.0 min (50% mated). *diss*'s poor performance here is consistent with its song not being the only behavioral abnormality caused by the mutation (see above). Note however, that wingless *diss* males are not sterile. When 10 wingless *diss* males were paired singly with normal females in vials, all of them mated eventually (within 2 days) and produced progeny.

Previously, we showed that the genetic factor(s) associated with the poor mating performance of the *cacophony* song mutant was genetically separable from

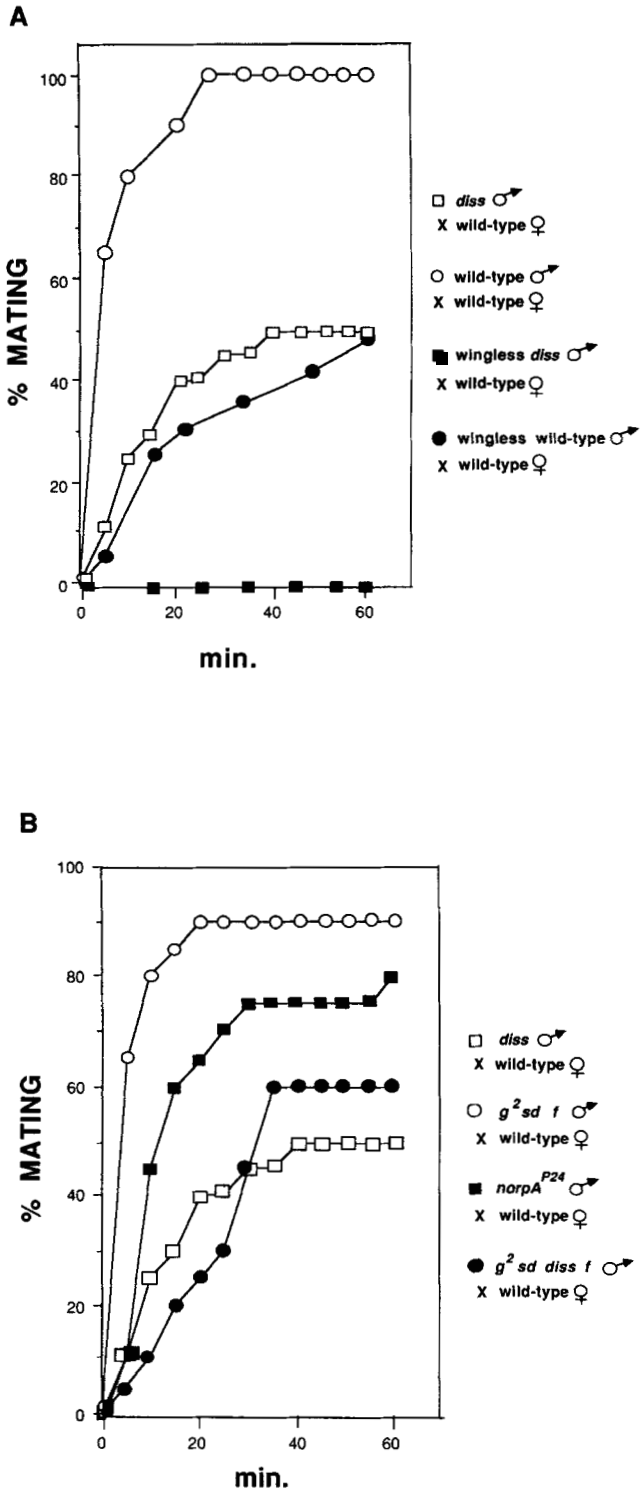

FIGURE 11.—Mating latencies of *diss* and control males. Males were individually paired with females in courtship chambers at 25°, and the time elapsed before initiation of copulation (which = mating latency) was noted in each case. Twenty male/female pairs were tested for each genotypic combination. (A) *Open circles*, wild-type (Canton-S) males and females; *closed circles*, wingless wild-type males with wild-type females; *open squares*, *diss* males with wild-type females; *closed squares*, wingless *diss* males with wild-type females (the former had their wings clipped off with small scissors when they were first collected under anesthesia); (B) *open squares*, *diss* males × wild-type females; *closed squares*, $norpA^{P24}$ males × wild-type females; *closed circles*, g^2 *sd diss f* males × wild-type females; *open circles*, g^2 *sd f* males × wild-type females.

that which causes the song defect (KULKARNI and HALL 1987; *cf.* SCHILCHER 1977). This seems not to be so for *diss*, because the mating performance of g^2 *sd diss f* recombinant males was still poor (latency, 36.9 ± 4.0 min, 60% mating in 60 min) when compared to a g^2 *sd f* control (10.0 ± 2.0 min, 100%, Figure 11B). Also, g^2 *sd diss f* males carrying the "deleted duplications" *T(1;4)* + *Df(1)81k19b* or *T(1;4)* + *Df(1)82c3k* mated with longer than normal latencies (23.4 ± 3.6, 75%; 32.8 ± 3.9, 60%). Control males of the genotypes, g^2 *sd f*; *T(1;4)* + *Df(1)81k19b* and g^2 *sd f*; *T(1;4)* + *Df(1)82c3k* mated rather quickly (5.8 ± 1.5, 90%; 9.1 ± 1.3, 100%). However, g^2 *sd diss f* males carrying the "straight" duplication $T(1;4)r^+f^+$ (cf. Table 1) were rather poor maters (20.8 ± 4.8, 40%). We noticed that this duplication, in a *diss* background, leads to an abnormal wing posture (wings held out); this could have caused these control males (*i.e.*, with *diss* covered, Table 1, Figure 8) to be subnormal in achieving mating success, even though they courted vigorously and without performing an inappropriately high number of orientations (*cf.* Figure 9). The wing posture defect mentioned above seems to be nonspecific because: (1) g^2 *sd f/Y*; $T(1;4)r^+f^+$ males also hold their wings abnormally, and (2) *diss* males bearing $T(1;2)r^{+75c}$—which also covers both the song and visual defects associated with *diss*—hold their wings in a normal position. In conclusion, *diss*'s "courtship visual" defect appears to map at or very near the song defect (also see section below on "vision").

Possible effects of the *diss* mutation on female receptivity to male mating attempts were assessed in tests involving wild-type males paired with *diss* females; and *diss* or *diss* recombinant males paired with *diss* females or *diss* recombinants (N = 20 in each case, unless stated otherwise). Tests of *diss* males × *diss* females from the original stock led to subnormal mating kinetics (16.7 ± 3.3-min latencies, 55% mating within 60 min), as did g^2 *diss f* males × *diss* (original) females (22.0 ± 5.0, 33%). Wild-type male × *diss* female and wild-type male × g^2 *sd diss f* pairings were also deficient (16.7 ± 3.3, 70%; 18.1 ± 4.0, 90%, N = 10 for the latter), although less severely so than for the matings just noted. Therefore, *diss* females might be subnormally receptive to males. This is different from the results obtained from the homozygous *cac* females, for which wild-type males initiated matings with their own or with *cac* females after very similar latencies (KULKARNI and HALL 1987).

From CI determinations of the new song mutant, these measures of overall courtship vigor for *diss* males and g^2 *sd diss f* males were ca. 4× less than controls (Figure 12). However, this could be due, at least in part, to the visual deficit in *diss* males (*cf.* Figure 9). Indeed, blind or mutant *optomotor-blind* males (*cf.* BLONDEAU and HEISENBERG 1982) court with subnormal CIs (review: TOMPKINS 1984). More

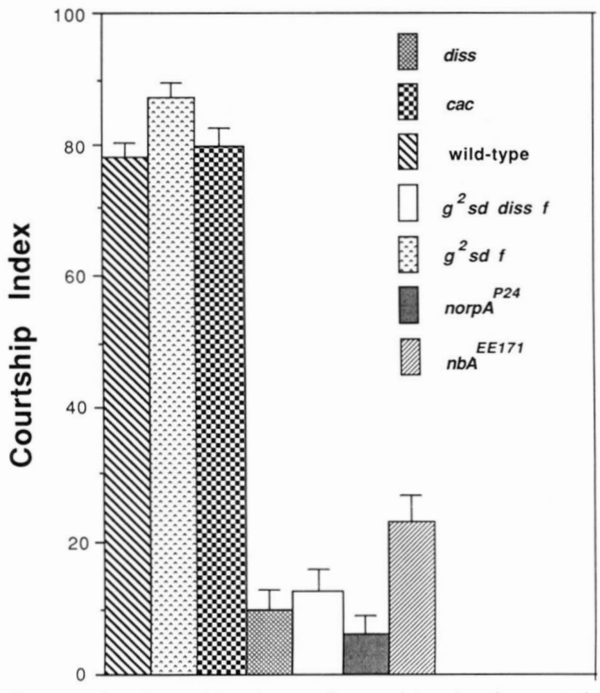

FIGURE 12.—Courtship vigor influenced by the *diss* mutation. Flies were reared at room temperature (*ca.* 22°). Males, which had been stored individually in food vials for 5 days after eclosion, were tested with one virgin female each. Twenty observations for each genotype were made in conditions of incandescent room lighting. Each male was introduced to the female in a courtship chamber at 25°, and his courtship index (CI ± SEM) was determined by observing the pertinent behaviors (see citations, relating to CI, in text) and recording their durations with timers, for 5 min per courtship. Canton-S wild-type along with g^2 *sd f* males served as "positive" controls for *diss*-induced CI decrements, with $norpA^{P24}$ and nbA^{EE171} males being "negative" controls. A one-way ANOVA test, performed with respect to genotype *vs.* CI, indicated that each member of the control group—which included the genotypes +/*Y*, g^2 *sd f*, and *cac*—exhibited CIs that were significantly different from those obtained for each member of the experimental group, consisting of *diss* and g^2 *sd diss f* ($P <$ at most 0.05). However, comparisons among individual members within each group elucidated a significant difference between certain genotypes: for example, +/*Y* differed significantly from g^2 *sd f* within the control group ($P < 0.05$); whereas, within the experimental group, *diss* differed significantly from g^2 *sd diss f* ($P < 0.05$). In addition, both members of the negative control group, $norpA^{P24}$ and nbA^{EE171}, differed significantly from unmarked *diss* ($P < 0.05$). However, only nbA^{EE171} differed from g^2 *sd diss f* ($P < 0.05$).

specifically, in our present experiments, the CIs of *diss* and g^2 *sd diss f* males were comparable to those measured for $norpA^{P24}$ or nbA^{EE171} males (Figure 12). Mutant *cac* males, on the other hand, courted with normal CIs (Figure 12).

Low courtship indices were also exhibited by g^2 *sd diss f* males carrying the deleted duplications *T(1;4)* + *Df(1)81k19b* or *T(1;4)* + *Df(1)82c3k* (CI = 18 ± 3, 13 ± 2, respectively; $N = 10$ in both cases). Control males carrying a "straight" duplication (g^2 *sd diss f*/*Y*; $T(1;4)r^+f^+$) courted vigorously (CI = 86 ± 2, N = 10).

Vision: When a choice between a dark tube and an illuminated tube was presented to *ca.* 150 flies tested in groups of 25–30 in a series of Y-tube tests (see MATERIALS AND METHODS), *diss* flies from the original stock and from one in which markers flanked this mutation (g^2 *sd diss f*) showed subnormal responses to light (Figure 13). No sex-specific difference was found, in that both *diss* and g^2 *sd diss f* males or females were less phototactic than $diss^+$ or *diss*/*FM7* controls. The subnormal phototaxis of *diss* was uncovered (in *Df*/*diss* females, Figure 13) by a deletion *Df(1)l32* that uncovers the song abnormality (*i.e.*, in *tra*/*tra* flies, *cf.* Table 1). Most groups of flies homozygous or hemizygous for *diss* responded more positively to light than did $norpA^{P24}$ adults (Figure 13). This suggests that *diss* flies may be only "partially blind."

A visual mutant, *slow-receptor-potential*, plus two alleles each of *eas* and *bss* (see above), complemented the phototaxis deficit associated with *diss* (data not shown). All of these mutations map near the song mutation (see above and Figure 8). As was previewed above, another mutation in *diss*'s vicinity, *nonA*, fails to complement the song mutant's visual impairments (S. J. KULKARNI, in preparation).

In tests of "walking optomotor" behavior, both males and females—carrying the *diss* mutation in its original (unmarked) form—anomalously moved "against" the rotating stripes (see MATERIALS AND METHODS) with considerably greater frequency than did wild type (Table 2). Similarly, recombinant g^2 *sd diss f* adults of either sex responded abnormally to these moving stimuli. *diss*/ + females tested as somewhat subnormal (Table 2), in contrast to the apparently complete recessivity of the mutation in tests of phototaxis (Figure 13). Females heterozygous for *diss* and the same deletion of the locus as used in the phototaxis tests were like *diss* males or *diss*/*diss* females in their (poor) optomotor responses (Table 2).

Therefore, we tentatively conclude that the factor mapping near (or at) *diss*, which leads to poor "courtship optomotor" responses of mutant males (Figure 9), also causes a more generalized decrement in this kind of visually mediated behavior.

The ERG, a light-induced "gross" electrical response of the visual system, contains "light on" and "light off" transient spikes and a "maintained component" (*e.g.*, PAK and GRABOWSKI 1978). The transients reflect synaptically driven activity in the large monopolar cells, L1 and L2, in the first-order optic ganglion (COOMBE 1986), whereas the main depolarization (corneal-negative, from these extracellular recordings) is the summed generator potentials of the photoreceptor cells (PAK and GRABOWSKI 1978). Both unmarked *diss* and g^2 *sd diss f* flies of either sex yielded ERGs (Figure 14, Table 3) with at least 2× reduced on- and off-transient amplitudes, when compared to signals recorded from wild type, g^2 *sd f*, or

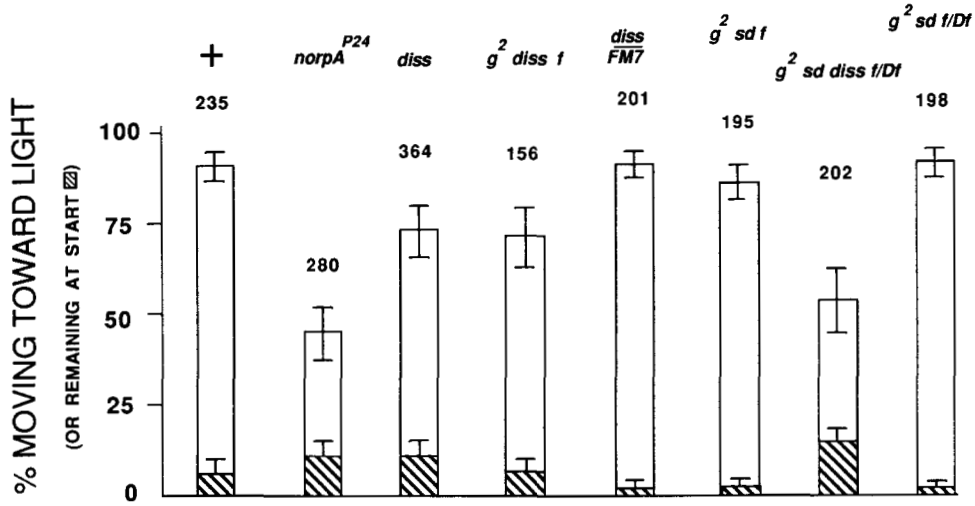

FIGURE 13.—Phototaxis of *diss* and control flies. The preferences of flies for an illuminated *vs.* dark tube of a Y-shaped apparatus (*cf.* KULKARNI and HALL 1987) were assessed in a series of tests involving 25–30 flies/run. The mean percentages (per run) of flies that moved into the "light" tube are indicated by the unshaded bars (±SEM, with total number of flies tested for a genotype noted at the top of each bar). The shaded bars indicate the mean percentages (±SEM) of flies remaining in the start tube of the Y. Males and females were tested separately for each genotype (note: "+" = Canton-S wild-type), except in the cases of heterozygous females (*e.g.*, g^2 *sd diss f/Df*; note: *Df* = *Df(1)l32*; see Figure 8). A two-way ANOVA test indicated that, within a genotype, there was no significant difference between the data from both sexes, so these data were pooled. One-way ANOVA testing, performed with respect to genotype *vs.* positive phototaxis, sorted the genotypes into three groups: (1) wild-type, *diss/FM7*, g^2 *sd f* and g^2 *sd f/Df*; (2) *diss*, g^2 *diss f*; (3) $norpA^{P24}$ and g^2 *sd diss f/Df*. Each member of any one of these groups differed significantly from individual members of the remaining two groups ($P <$ at most 0.05, for all such comparisons).

TABLE 2.

Optomotor responses of *diss* and control flies

Experimental genotype	%CW	%CCW	Control genotype	%CW	%CCW
diss/Y	63 ± 5	58 ± 3	+/*Y*	91 ± 1	95 ± 1
diss/diss	65 ± 2	64 ± 3	*diss/FM7*	60 ± 8	69 ± 6
			+/+	92 ± 1	89 ± 1
g^2 *sd diss f/Y*	69 ± 4	59 ± 5	g^2 *sd f/Y*	80 ± 3	80 ± 4
g^2 *sd diss f/*g^2 *sd diss f*	64 ± 2	62 ± 2	g^2 *sd diss f/FM7*	81 ± 3	92 ± 1
g^2 *sd diss f/Df(1)l32*	43 ± 5	47 ± 8	g^2 *sd f/Df(1)l32*	73 ± 2	69 ± 3
omb^{H31}/*Y*	54 ± 9	55 ± 0			
omb^{H31}/omb^{H31}	55 ± 1	49 ± 4			

Flies were observed individually under a watchglass placed in a cylinder of rotating stripes, as described in MATERIALS AND METHODS. CW designates tests conducted during clockwise rotation of the drum and CCW counterclockwise. The scores in the two columns represent the mean percent of the lines (± SEM) that flies crossed in a given directional test. Three flies of each genotype listed here were tested. "+" denotes *X* chromosomes from a Canton-S wild-type stock. omb^{H31} is the *optomotor-blind* mutant (*e.g.*, BLONDEAU and HEISENBERG 1982, HEISENBERG and WOLF 1984), which served as a "negative" control. Two separate "one way ANOVA tests"—with respect to (1) genotype *vs.* number of clockwise rotations and (2) genotype *vs.* counterclockwise roations—were performed. For both (1) and (2), the experimental genotypes *diss/Y*, *diss/diss*, g^2 *sd diss f/Y*, g^2 *sd diss f/*g^2 *sd diss f*, and *Df(1)l32/*g^2 *sd diss f*—when combined to represent a group—differed significantly from the pooled control group consisting of +/*Y*, +/+, *diss/FM7*, g^2 *sd f/Y*, g^2 *sd diss f/FM7* and g^2 *sd f/Df(1)l32* ($P < 0.05$). However, for both (1) and (2), when individual genotypes in the experimental group (mentioned above) were compared with the control individuals (see above), some overlaps were noticed. For example, in group (1), *diss/FM7* was not significantly different from any of the following genotypes: *diss/Y*, *diss/diss*, g^2 *sd diss f/Y* or g^2 *sd diss f/*g^2 *sd diss f* ($P > 0.05$). In group (2), g^2 *sd f/Df(1)l32* did not differ significantly from *diss/diss* or g^2 *sd diss f/*g^2 *sd diss f* ($P > 0.05$). *diss/FM7* was not significantly different from any of these genotypes: *diss/Y*, *diss/diss*, g^2 *sd diss f/Y* or g^2 *sd diss f/*g^2 *sd diss f* ($P > 0.05$).

cac [*i.e.*, the ERG of the latter song mutant was normal, as predicted from previous behavioral testing (KULKARNI and HALL 1987), and Figure 9 of this report]. The deficits in transients for *diss* were uncovered by *Df(1)l32* (Table 3, *cf.* Figure 8) and by *Df(1)E150* (data not shown, *cf.* Table 1).

In general, the reduction in off-transient amplitudes of *diss* ERGs was more severe than for the on-transient spikes. Occasionally (in <10% of the flies tested), *diss* males or females missing both transient spikes in their ERGs were found. The amplitudes of maintained components in all of the ERGs recorded were rather variable, such that no consistent difference between *diss* and controls could be found (Table 3). One control type, involving $diss^+$ linked to a *garnet* eye color mutation, generally gave relatively high amplitudes of the various ERG components (Table 3), possibly due to depleted levels of screening pigment in its eyes.

Locomotor tests: *diss* males and *diss/diss* females

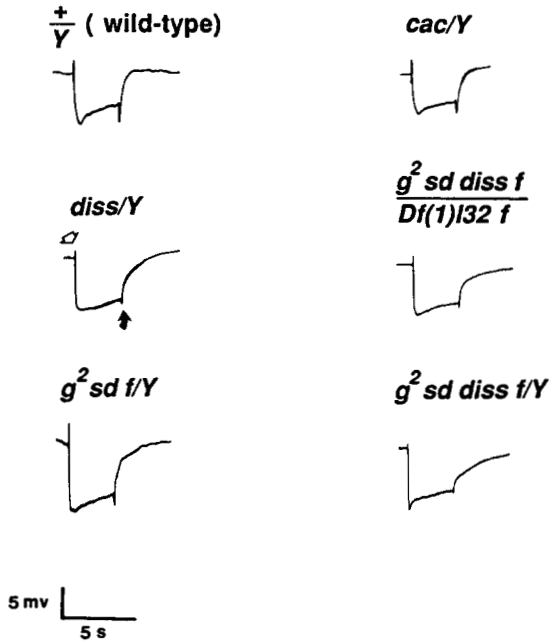

FIGURE 14.—Electroretinograms of *diss* and control flies. Light pulse-induced electrical potentials, at the cornea of the fly's eye, were recorded as a waveform on a chart moving at a speed of 5 mm/s (see MATERIALS AND METHODS for details). Each ERG waveform consisted of a "light on" (*open arrow* accompanying *diss/Y* trace) and a "light off" (*black arrow*) transient spike, plus a maintained component. The light pulse duration for all ERGs shown here was 3 s. A total of three flies (males + females) were tested for each genotype. No sex specific differences within a genotype were found. ERGs of *diss*, g^2 *sd diss f* and *Df(1)l32/diss* are compared to control ERGs of wild-type and g^2 *sd f* males. The ERG for the *cac* song mutant is included for comparison with that of wild-type and *diss*. Flies expressing the *garnet* (*g*) eye color mutation exhibited relatively high amplitude ERG components (see Table 3) hence the inclusion of a g^2 *sd f* control.

TABLE 4.

Locomotor activity of *diss* and control flies

Experimental genotype	Activity	Control genotype	Activity
diss/Y	140 ± 8	*+/Y*	135 ± 8
diss/diss	128 ± 11	*+/+*	121 ± 6
diss/diss; tra/tra	126 ± 13	*diss/+; tra/tra*	147 ± 10
g^2 *sd diss f/Y*	123 ± 9	g^2 *sd f/Y*	144 ± 6
g^2 *diss f/*g^2 *diss f*	116 ± 8	g^2 *sd f/*g^2 *sd f*	123 ± 9
		norpA^{P24}*/Y*	153 ± 15

Flies were reared and tested at room temperature. Activity is expressed as the number of within-chamber lines crossed (± SEM) in a 5-min period (see MATERIALS AND METHODS). Twenty males or females were tested separately for each genotype. "+" is derived from Canton-S wild type.

were as vigorous as either wild-type males and females, or females carrying a normal chromosome heterozygous with *diss* (Table 4). Recombinant g^2 *sd diss f* males or females were as vigorous as g^2 *sd f* controls (Table 4). These results were obtained after rearing the flies at our standard temperature (22–23°) and then testing them at 25°. In addition, locomotor activity results for males and females from both the original and recombinant *diss* stocks—which had been grown at either higher (25° and 29°) or lower (18°) temperatures—were very similar to controls when tested at 25° (data not shown). Thus, *diss* does not seem to affect general motor behavior, whereas *cac* males or females are a bit sluggish in this phenotype (KULKARNI and HALL 1987).

Circadian rhythms: Three males each from *diss* and g^2 *sd diss f* stocks were tested for circadian rhythms

TABLE 3.

Electroretinograms of *diss* and control flies

Experimental genotype	On	Off	Maintained	Control genotype	On	Off	Maintained
diss/Y	0.7 ± 0.0	0.2 ± 0.1	8.6 ± 1.6	*+/Y*	2.6 ± 0.3	4.0 ± 0.6	9.0 ± 0.8
g^2 *ds diss f/Y*	0.8 ± 0.1	0.9 ± 0.1	12.2 ± 0.1	g^2 *sd f/Y*	4.1 ± 0.2	4.7 ± 0.6	12.4 ± 0.8
g^2 *sd diss f/Df(1)l32*	1.1 ± 0.3	0.8 ± 0.0	7.2 ± 0.1	g^2 *sd f/Df(1)l32*	2.7 ± 0.1	4.8 ± 0.1	10.0 ± 2.8
diss/diss	0.5 ± 0.1	0.4 ± 0.1	7.9 ± 1.0	*diss/FM7*	1.4 ± 0.1	2.8 ± 0.5	8.4 ± 1.3
g^2 *sd diss f/*g^2 *sd diss f*	1.8 ± 0.7	1.0 ± 0.1	15.1 ± 0.7	g^2 *sd diss f/FM7*	1.8 ± 0.4	2.7 ± 0.5	8.1 ± 0.8
				cac/Y	2.4 ± 0.1	2.7 ± 0.3	8.0 ± 0.3

Electroretinograms (ERGs) were recorded as decribed in MATERIALS AND METHODS. Amplitudes of light-on ("on") and light-off ("off") transient spikes, as well as of the "maintained" component, were measured in mV. For each fly, several ERGs were recorded, with 30-s dark adaptation periods occurring between any two consecutive light pulses. Light pulse durations were varied from 1 to 10 s. However, such increments in the duration of light pulse did not cause any systematic variation in the amplitudes of transient spikes. Mean amplitude values (*e.g.*, for on-transient amplitudes) were computed from *ca.* 10 individual ERGs recorded from each fly. The approximate standard deviation for a given fly was 20–50% of the mean. Three flies from each of the genotypes listed were tested. The results quoted for a given genotype are means (± SEM) for the three *individuals* (such that these values are means of means; see above). Since amplitudes of the ERG components from *diss*$^+$ flies expressing *garnet* (*g*) were larger than normal, they should be compared to values recorded from *diss* flies carrying this eye-color marker. No sex-specific difference in ERG responses within a genotype was observed. Mutants and controls (*e.g.*, *+/Y*, or a given mutation heterozygous with its normal allele) were always tested in parallel. "+" is derived from Canton-S wild type. A one-way ANOVA test, performed with respect to genotype *vs.* "on" transient amplitude sorted the genotypes into three major groups: (1) wild type, *cac*, and g^2 *sd f/Df(1)l32*, each member of which differed significantly from any in group (2) *diss*, g^2 *sd diss f* and g^2 *sd diss f/Df(1)l32* ($P <$ at most 0.05 for any of these 9 comparisons). g^2 *sd diss f/FM7* did not differ significantly from all genotypes in both (1) and (2), except for *diss* ($P > 0.05$). In addition, *diss/FM7* was not significantly different from the following experimental genotypes: g^2 *sd diss f*, g^2 *sd diss f/Df(1)l32*, and *diss* ($P > 0.05$). One genotype, g^2 *sd f* stood alone ($P < 0.05$). A similar one-way ANOVA test involving genotype *vs.* "off" transient sorted the genotypes in three groups: (1) wild type, g^2 *sd f* and g^2 *sd f/Df(1)l32*; (23) *diss*, *diss/FM7*, g^2 *sd diss f* and g^2 *sd diss f/Df(1)l32*; (3) g^2 *sd diss f/FM7* and *cac*. Each member of any one group differed significantly from every member of the two remaining groups ($P <$ at most 0.05). No differences in the amplitudes of the maintained component, in flies expressing the various *diss*$^-$ or *diss*$^+$-including genotypes, were consistently observed.

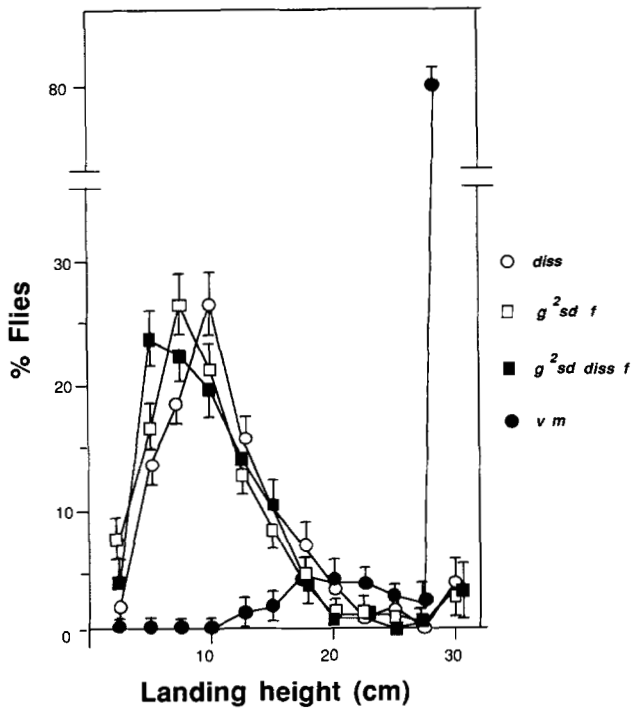

FIGURE 15.—Flight performance of *diss* and control flies. A total of *ca.* 250 flies was tested for each genotype, in a series of runs, each containing 25–50 flies/trial (as described in MATERIALS AND METHODS). Males and females of each of these four genotypes were tested separately; but the results for the two sexes were so similar that these data were pooled within a genotype. The numbers of flies landing horizontally at successive intervals (length of each such landing segment, 2.5 cm) were counted, and the mean values (±SEM) from the separate tests (for a given genotype) were plotted as in BENZER (1973) and KULKARNI and HALL (1987). *Open circles*, unmarked *diss; closed circles*, flightless control flies with mutant *miniature* wings (also carrying the *vermilion* eye color marker); *closed squares*, g^2 *sd diss f; open squares*, g^2 *sd f* controls.

of locomotor activity; all six individuals were rhythmic; average periodicity, 23.8 ± 0.1 hr. This value is in the normal range (*e.g.*, HAMBLEN *et al.* 1986).

Flight: Drosophila flight, as well as the male's wing vibration during courtship, is initiated and powered by several "known" thoracic muscles (EWING 1977b, 1979). To test if the song defect in *diss* could be a secondary consequence of a generalized functional impairment of the thoracic muscles, the flight performance of the mutant was examined.

No differences were found when distributions of *diss* and g^2 *sd diss f* flies along the length of the flight testing cylinder (see MATERIALS AND METHODS) were compared with those for control flies (Figure 15). Flies carrying a *miniature* wing mutation served as a negative control (Figure 15). In addition, the gross morphology of dorsal longitudinal and dorsoventral flight muscles plus the tergotrochanter jump muscle appeared to be normal when frozen sections (10 μm thick) stained with 0.5% Azure C were examined at ×450 magnification using a light microscope. Therefore, it was concluded that the gross flight system is not perturbed by *diss* or other genetic factors present in the stock as originally isolated.

DISCUSSION

Courtship and other behaviors of the *dissonance* mutant: The *diss* mutant of courtship song, which was isolated by directly observing outputs from wing vibrations of males carrying mutagenized *X* chromosomes, turned out to exhibit longer-than-normal mating latencies (also see below). In contrast, the *cacophony* song mutant was initially recovered based on abnormally long mating latencies, which ended up being genetically (KULKARNI and HALL 1987) and "surgically" (*cf.* Figure 11A) separable from the singing defect that was discovered soon after *cac*'s isolation (SCHILCHER 1977).

The pulse song phenotype of *diss* males is distinctly different from that of either wild-type or *cac* pulses: a pulse train in *diss* begins with qualitatively wild-type pulses that have normal amplitudes. Yet most trains—soon or eventually—consist of polycyclic pulses (Figure 1B); and certain *diss* trains become "bursts" of enormously loud, long pulses which merge with one another (Figure 1C). The very next pulse train, however, begins again with normal-looking pulses. The songs of *cac* males, though, are uniformly louder than normal and polycyclic, *i.e.*, in every pulse train. Thus, the wing vibration "machinery" and/or the neural control of it (*cf.* EWING, 1977b, 1979; SCHILDBERGER 1984; HUBER and THORSON 1985) seems to be affected in a different manner in these two mutants. It might be, for example, that the *cac* phenotype has a purely neural etiology and that *diss*'s defect is muscular, *e.g.*, a matter of "fatigue" occurring in the "direct" wing muscles (*cf.* EWING 1979), which could cause pulse trains to degrade into polycyclicity towards the end of singing bouts.

The behavior of *diss*, like that of *cac* (KULKARNI and HALL 1987), seems normal in several nonsexual behaviors. In courtship itself, *diss* males are slow in their mating kinetics. This, however, seems not to be due solely to detrimental effects of the *diss* mutant's abnormal song on female receptivity to the male's mating attempts (*cf.* BENNET-CLARK and EWING 1969; KYRIACOU and HALL 1982). That is, *diss* males exhibit a marked visual impairment as they attempt to orient toward or follow females (Figure 9). In fact, note that *diss* males are slower in their mating latencies than are genetically blind flies (Figure 11B). This could be explained by the singing *plus* visual defects caused by *diss*; whereas *norpA* males sing normally (Figure 10).

The *diss* mutation—or a factor linked very closely to it—has in fact resulted in more generally aberrant responses to visual stimuli (Figures 13 and 14; Tables 2 and 3), though these are not as severe as total blindness. Therefore, *diss* flies could be only partially blind, as reflected in their subnormal (but not usually

absent) ERG transients; or, they might be "optomotor blind" (*cf.* HEISENBERG and WOLF 1984); or both.

The song abnormalities and the visual defects, associated with the original *diss*-bearing *X* chromosome, were also found in recombinant g^2 *sd diss f* flies and in females heterozygous for *diss* and a small deletion that almost certainly removes all of this locus. This provisionally indicates that the genetic factors causing faulty wing vibrations, aberrant visual responses, and the resultant slowdown in mating kinetics are one and the same.

The specific genetic issues concerning two or more behaviors disrupted by the same mutation should also be viewed in the broader context of *diss* and the other song mutant in this species, *cac*, each being connected in some way to visual abnormalities—the former per se, and the latter by its relationship to the *night-blind-A* locus. That is, *cac* fails to complement *L13* lethal mutations, which in turn uncover *nbA*-induced visual defects—though *cac* and *nbA* complement (KULKARNI and HALL 1987).

Another "visual system gene," *no-on-transient-A*, maps on paper quite close to *diss* (W. L. PAK and M. DELAND, unpublished data; Figure 8). Although *diss* and *nonA* are in some sense unconnected, given that two mutations at the latter locus did not cause the courtship song to be abnormal (Figure 10), these mutations seem to interact with respect to visual behaviors (S. J. KULKARNI, in preparation). This suggests that *diss* and *nonA* might be alleles of the same gene, and this is at least a loose analogy to the relationship between *cac* and *nbA* (see above).

It may eventually be possible to map *diss*-associated visual defects plus those caused by *nonA* more finely, *i.e.*, to one tiny interval within—or to two separate but nearby subsegments of—region 14. Furthermore, in the future, it could be that one or more of these genes will prove identifiable molecularly (see below), and this might include cases of simultaneous "transformation rescue" (see, for example, HAMBLEN *et al.* 1986) of all of the relevant phenotypic abnormalities. Thus, a relatively small DNA fragment, cloned from wild type, might turn out to "cover" *diss*'s singing and seeing defects.

General behavioral and neural biology of *diss*: The new song mutant should be tested further in its visual responses (and their precise genetic etiology; see above). For instance, general optomotor testing can be extended (*cf.* BLONDEAU and HEISENBERG 1982), beyond the crude monitoring of "walking" optomotor turning responses so far performed (Table 2). Such further behavioral testing of *diss* could include attempts to "map" tissue "foci" that underly the visual impairments. In this regard, the neural etiology of *nonA*-induced ERG defects (which in reality involve impairments of the light-*off* as well as light-*on* transients; HEISENBERG and WOLF 1984), has been examined in externally marked genetic mosaics: the focus was in photoreceptors (HOTTA and BENZER 1970). But a more *central* cause for the more subtle behavioral problems, seen in a variety of visual testing regimes applied to *nonA*, has been strongly implicated (see HALL 1978, for discussion). If *diss* indeed turns out to be an allele of *nonA*, then the aforementioned central cause could conceivably be "in common" to both *diss*'s song defect and its visual impairments.

This begs the question as to where *diss* foci could be localized in mosaic experiments. Will the song focus be in the thorax (hence in a wholly separate body region from the putative visual focus)—as appears to be so for *cac* (HALL *et al.* 1988), and as has also been determined for the basic sex-specific control of pulse singing (*i.e.*, from courtship behavioral analysis of gynandromorphs; SCHILCHER and HALL 1979)? If *diss* and *cac* can have their respective singing abnomality foci mapped by *internal* markers (cf. HALL 1979; SCHILCHER and HALL 1979), will they be in the same narrowly definable tissue, *e.g.*, in one of the thoracic ganglia? in thoracic muscles? And if a single mutation at the *diss* locus has in fact caused a visual defect to go hand in hand with the aberrant courtship song—will this focus be mappable, say, to a specific optic ganglion (see above, and *cf.* COOMBE 1986)? Finally, is it a coincidence that the two extant song mutants in *D. melanogaster* are each at least indirectly associated with abnormalities of the visual system—and could this lead to the establishment of neurogenetic connections between the control of development and function of the relevant, though seemingly quite separate, portions of the fly's nervous system?

Certain of these intriguing questions can be asked using approaches that should augment the neurogenetic experiments. That is, *diss*, like *cac* (see discussion in KULKARNI and HALL 1987), seems as if it could eventually be isolated molecularly, given the DNA that has been cloned from region 14 of the *X* chromosome (*e.g.*, LOUGHNEY and GANETZKY 1985; FALK and HALLADAY 1986; H. STELLER, K. JONES and G. M. RUBIN, personal communication).

In certain recent examples from Drosophila neurogenetics, molecular experiments on a given gene—if not necessarily augmented by known pleiotropic mutations at the relevant loci—have already suggested that the factor in question may be more "versatile" than would have been thought *a priori*. For example: (1) the *period* "clock gene," at least in embryos, is transcribed in apparently all developing ganglia (JAMES *et al.* 1986), as opposed to what could have obtained—expression in a limited subset of certain portions of the CNS that might house the fly's "central oscillators." In addition, *per* mutants have been reported to be aberrant in phototaxis (PALMER, KENDRICK and HOTCHKISS 1985), as well as in various aspects of rhythmicity (*e.g.*, HAMBLEN *et al.* 1986). Indeed, a *per*-derived protein is detectable not only in the central brain of adults, but in the eye and

optic lobes as well (SIWICKI, ROSBASH and HALL 1987). (2) Learning and memory mutations at the *dunce* locus are more pleiotropic than this: they also cause females to be subfertile or sterile (due to a defect in egg laying, BELLEN *et al.* 1987). This pleiotropy correlates, in a sense, with the broad tissue distribution of *dnc*'s product (SHOTWELL 1983), with the fact that the gene's primary transcript becomes a "family" of alternatively spliced mRNAs (DAVIS and DAVIDSON 1986), and with the sequencing data which reveal that this locus can encode (in a given conceptually translated protein) both a cAMP phosphodiesterase and an "egg-laying-hormone" relative (CHEN, DENOME and DAVIS 1986). (3) The *fushi-tarazu* gene is not only expressed early in embryogenesis (before overt segmentation) in its classically alternating-striped pattern (review: SCOTT and O'FARRELL 1986); but *ftz* expression also reappears at a discretely separate embryonic stage, when it is localized to portions of essentially all of the segmentally arranged neural ganglia (CARROLL and SCOTT 1985; HIROMI, KUROIWA and GEHRING 1985). (4) A "*fasciclin*" gene, isolated via immunological/molecular experiments that began with the specification of this factor as expressed "in" embryonic neural pathways (thus possibly helping to specify them), turned out also to be expressed at an earlier embryonic stage (PATEL, SNOW and GOODMAN, 1987). The latter expression is not solely neural and, moreover, is associated with a different type of spatial pattern from that appearing during times of axonal fasciculation. (5) The *sevenless* gene is one that would appear to act with exquisite temporal and spatial specificity, in terms of effects of *sev* mutations on a particular photoreceptor type (HARRIS, STARK and WALKER 1976; TOMLINSON and READY 1986) plus, at first glance, molecularly monitored expression of the normal allele at the expected developmental stage and in the appropriate imaginal disc; yet, *sev*'s action also *returns* in the adult, in which the transcript from the locus is relatively enriched in the head (HAFEN *et al.* 1987; BANERJEE *et al.* 1987).

It will of course not be a trivial task to move from the cloned, sequences near (possibly including) *diss* to a definitive isolation plus identification of this song gene. But if this can eventually be achieved it will be interesting to ask, at the level of primary and secondary gene expression, where *diss* is expressed—and if a determination of such tissue distribution(s) will be as versatile as one would infer from the different phenotypes influenced by the mutation. It also can be hoped that future studies of the *dissonance* gene at the molecular level will lead to concrete clues as to why and how the information encoded there could be used both in the fly's song and visual systems.

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