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Evolution

Behavioral and sensory basis of courtship success in *Drosophila melanogaster*

(sexual selection/mating/fruit fly)

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ABSTRACT **In *Drosophila* some individuals are more successful at mating than others. Reproductive fitness is strongly dependent upon the ability to recognize and compete for members of the opposite sex. Experiments were designed to answer two questions. (*i*) What behavioral components are characteristic or predictive of successful courtship? and (*ii*) How important is the information transmitted in the different sensory channels for courtship success in each sex? These questions were approached by two experimental procedures. Flies having a sensory deficiency (olfactory, auditory, or visual) competed with wild-type flies of the same sex for mates. Males were found to rely upon sensory channels different from those used by females in order to court successfully. In addition, the courtships of pairs of various genotypes were recorded and subjected to multivariate analysis. The multivariate courtship profiles deviated most widely from those of successful wild-type pairs when the male or female was unable to receive information in the sensory channel most important for successful mating by that sex. Both sequential and quantitative courtship properties were altered when one participant was deficient in ability to receive certain sensory information.**

Drosophila has long provided a popular model system for studying the evolutionary aspects of variable mating success among and between populations. However, the behavioral basis underlying differential courtship success of males and females has not been completely defined. During courtship, information is exchanged through a finite number of sensory modes. The work of Ewing and Bennet-Clark (1), Averhoff and Richardson (2), Tompkins *et al.* (3), and Markow (4) shows that males and females are influenced by different kinds of sensory input during courtship. For example, in *D. melanogaster*, females obtain auditory cues from males, but males obtain no auditory cues from females. Precisely how any given type of sensory information influences the behavior of the receiving sex and the subsequent outcome of courtship is unknown. It should be possible, however, to approach this question given the ability to control sensory input and a means of describing courtship in comprehensive and quantitative terms. A number of mutants are available that selectively interfere with particular sensory channels. Markow and Hanson (5) devised a multivariate courtship profile (MCP) for use in comparative studies designed to examine the behavioral basis of courtship success and showed that successful courtships are characterized by a complex constellation of behaviors having qualitative, quantitative, and sequential properties. In the work to be described, the MCP was used in conjunction with sensory-deficit mutants to analyze the behavioral basis of courtship success. A series of experiments was conducted to ask the following questions: (*i*) Are there particular MCP components that are good indicators of courtship success? and (*ii*) How does inability to receive information via a particular sensory channel affect courtship success of the sensory-deficient fly and of wild-type flies paired with sensory-deficient flies?

MATERIALS AND METHODS

Fly Strains. The wild-type strain TM3 came from ≈20 pairs of wild flies trapped in an orchard in Tempe in May 1980. The sex-linked *norp A* (no receptor potential) gene controls a step in the phototransduction process in the compound eye (6); flies of the *norp A* strain are blind. The olfactory blind mutant *sbl* is also sex-linked (7) and was obtained from J. Hall (Brandeis University). The auditory pathway may be disrupted by making flies homozygous for two autosomal recessive genes, aristaless (*al*) and thread (*th*) (8). The *al th* strain came from the California Institute of Technology Stock Center (Pasadena, CA). Genetically marked multiple inversions were used to insert the mutant genes into the TM3 wild-type backgrounds to eliminate potential behavioral effects from variations at other loci. For *norp A* and *sbl* only the autosomal genetic background could be controlled.

Sexual Behavior of Sensory-Deficient Males. Two experiments were done to evaluate the sexual behavior of mutant males. In the first experiment, a wild-type TM3 female was placed with two males, one wild-type TM3 and one mutant, *norp A*, *sbl*, or *al th*. Fifty pairs of courting males were observed for each combination. The genotype of the mating male was recorded. In the second experiment a comprehensive multivariate analysis of courtship behavior was done on single males of each mutant type paired with a wild-type TM3 female. In all these experiments, a single 4-day-old virgin female was aspirated into a 1-inch-diameter (2.5 cm) round observation chamber of plexiglass and filter paper construction containing one 4-day-old virgin male. The complete courtship was videotaped through a Wild dissecting microscope at ×120 by a JVC (Elmwood Park, NJ) color video camera and JVC HR 3600 videorecorder using a light intensity of 210 footcandles (2260 lux). Courtships not resulting in copulation within 10 min were excluded.

Sexual Behavior of Sensory-Deficient Females. Ranking of the mating success of mutant females was obtained by placing a wild-type TM3 male with a wild-type TM3 female and a sensory-deficient female ($n = 50$) and recording which female mated first. In another series of experiments mutant females were placed with wild-type males, and the courtships were videotaped for multivariate analysis. The MCPs of both sexes were analyzed.

Multivariate Analysis of Courtship. A 19-in Sanyo color television monitor was used for playback of the courtship records. Two observers logged nine male and seven female behaviors simultaneously on a TRS80 level II computer

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Abbreviation: MCP, multivariate courtship profile.

(Tandy Radio Shack, Ft. Worth, TX) programmed to serially record events and store all this temporal and sequential information on diskettes. Recorded male behaviors are listed in Table 3; most of these behaviors have been defined (9). The term orient is used in a modified sense from that originally employed by Bastock (10). During both orient-front and orient-back, the male's head is directed toward the front or back half of a stationary female. This is a useful distinction because orient-front may influence females in the visual mode. Female behaviors are also listed in Table 3. Multivariate analyses were done as reported by Markow and Hanson (5). All analyses were performed on observations for 10 pairs of flies per category except for the sequential and stationary probabilities tests; these latter two parameters were compared on equal numbers of transitions.

RESULTS

Sexual Behavior of Sensory-Deficient Males. When sensory-deficient males were competing with wild-type males for a wild-type female (Table 1), the following ranking for successful courtships was obtained: wild type > *al th* (auditory deficiency) > *sbl* (olfactory deficiency) > *norp A* (visual deficiency).

The next question asked was whether there are features of the MCP that serve as predictors of courtship success. Wild-type pairs of flies were placed in one of two groups before analysis—pairs in which a mating took place during the observation period and pairs in which no mating was observed. Observation records were compared to detect MCP differences between successful and unsuccessful wild-type pairs. In addition, the courtships between males from each of the mutant strains paired with wild-type females were recorded and analyzed. With the exception of *norp A* males, courtships involving mutant males and wild-type females were also successful, although sometimes they took longer to achieve copulation.

Differences in the sequential properties of the male behaviors displayed by the various genotypes during courtship are depicted in Fig. 1. Only transitions that have a significant ($P < 0.05$) frequency of occurrence are shown. Several major differences are seen between the diagrams generated by successful and unsuccessful wild-type pairs. In addition to a minor alteration in the spatial relationships in the multidimensional scaling, several of the sequential dependencies present in one pair do not occur in the other. In successful courtship the only behavior that follows a copulation attempt is a chase, whereas in unsuccessful pairs males performed orient-back and vibrate behaviors or a chase after a copulation attempt (Fig. 1*B*). Males from unsuccessful pairings go from chase and vibrate to chase, whereas males in successful pairs show the opposite transition. Successful males also show a locomotion to orient-front transition that is absent in unsuccessful pairs. The stationary probabilities, i.e., the probability of a given behavior occurring (5), of orient-front behaviors are greatest in unsuccessful wild-type pairs.

Table 1. Mating success of sensory-deficient flies in competition with wild-type flies for wild-type mates

	Successful mating, no.		
	Wild-type	Mutant	χ^2 (1:1)
Mutant male[*]			
al th	28	22	0.72
sbl	35	15	8.00[†]
norp A^{p24}	50	0	—
Mutant female[‡]			
al th	39	11	15.68[†]
sbl	34	16	6.48[§]
norp A^{p24}	31	19	2.88

[*] A single mutant male and a single wild-type male competing for a single wild-type female.

[†] $P < 0.01$.

[‡] A single mutant female competing with a single wild-type female for a wild-type male.

[§] $P < 0.025$.

By far the greatest deviation from the pattern seen for successful wild-type pairs is that of *norp A* males (Fig. 1*C*). None of these pairing resulted in a mating during the observation period. Besides a complete rearrangement of the multidimensional scaling relationship only two significant transitions appear: locomotion to chase and locomotion to chase and vibrate. Although males having *sbl* are not as aberrant as *norp A* males, their courtship picture does differ in several respects from that of wild-type (Fig. 1*D*). They show a transition from orient-back and vibrate to orient-front not seen in wild-type pairs. Many transitions that occur in only one direction in wild-type flies are bidirectional when males are *sbl*. The picture for *al th* males shows more vibration behaviors than that of wild-type (Fig. 1*E*).

Average bout lengths for male behaviors are seen in Table 2. Lick and copulation attempt appear as interbout intervals, or rates, because both are short pulse-like behaviors. There is much variability in bout lengths for many of the behaviors between groups. Two male behaviors, copulation attempt rate and, to a lesser extent, lick rate, are predictive of time until mating—being lowest in *norp A* males, *sbl* males, and unsuccessful wild-type males. Although different types of males varied in bout lengths of other behaviors, these differences were insignificant except in the case of *norp A* males for chase and for chase and vibrate, and, in the case of unsuccessful wild-type males for orient-front and vibrate.

Another quantitative aspect of courtship that revealed interesting differences was the time distribution for each behavior over the course of courtship (Table 3). In the courtships of successful wild-type pairs, chase and chase and vibrate decrease during the second half of courtship. This decrease is not observed for unsuccessful wild-type males or *norp A* males. Unsuccessful wild-type, *smb*, and *al th* males also increase their orient-front behavior during the second half of courtships.

In addition to comparing behavioral profiles of different types of males, the behaviors of females paired with them were also analyzed. Wild-type females had a significantly higher probability of decamping (jumping or flying away from a male) if the male was *sbl* or *norp A* (Table 4), a factor associated with the reduced copulatory success of these males. It is also worth noting that in successful wild-type pairs, as well as in other pairs where matings occur, females decrease locomotion and increase the amount of time spent standing still in the second half of courtship (Table 3).

Sexual Behavior of Sensory-Deficient Females. Females of each sensory-deficient genotype competed with wild-type females for wild-type males (Table 1). The following ranking of female success was obtained: wild type = *norp A* (visual deficiency) > *sbl* (olfactory deficiency) > *al th* (auditory deficiency). Because female behaviors consist of only terminal double transitions, diagrams of female behaviors are omitted; instead, stationary probabilities appear in Table 4. Although females of different genotypes vary in the stationary probabilities for any given behavior, the only significant differences involve the increased probability of a double wing flick in *sbl* and *norp A* females.

Male behavior differs when the female is mutant compared to wild-type. Males that court *norp A* females do not decrease the amount of chase and chase and vibrate behaviors (Table 3). Also males that court mutant females show longer locomotion bout lengths and orient-front bout lengths than males courting wild-type females (Table 2).

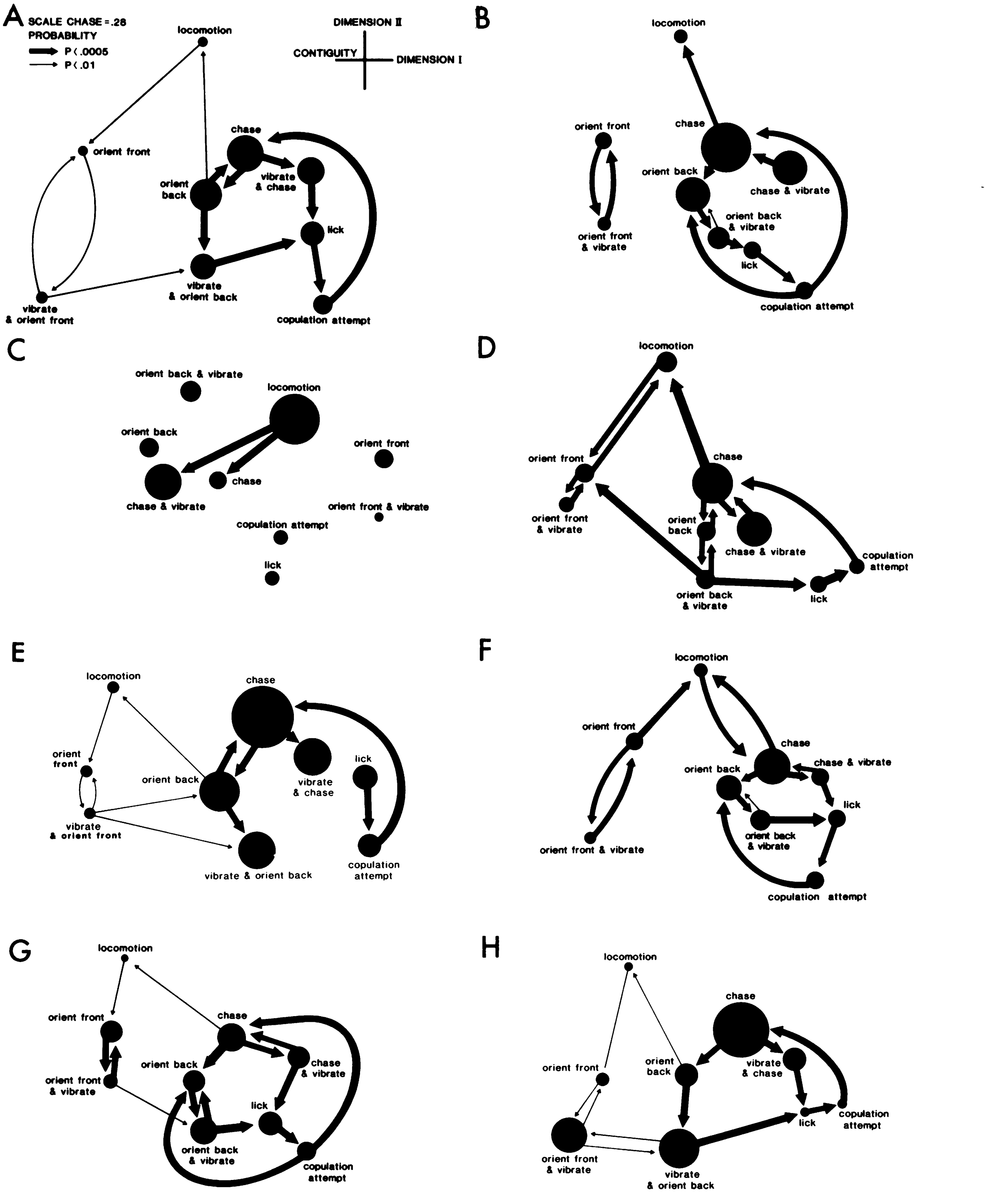

FIG. 1. Probabilities and sequential dependencies in *D. melanogaster* male courtship. Solid circles represent each behavior; the stationary probability of each is proportional to the circle diameter. Coordinate positions of each circle were derived by multidimensional scaling solutions that place each behavior in the two-dimensional contiguity space of all behaviors. Multiple (2×) sequential dependencies are represented by arrows between circles; the arrow width designates level of significance (from a binomial test) of the frequency of each transition (thick arrows, $P < 0.005$; thin arrows, $P < 0.01$). (*A*) Successful courtship between wild-type males and females; (*B*) unsuccessful courtship between wild-type males and females; (*C*) *norp A* male, wild-type female; (*D*) *sbl* male, wild-type female; (*E*) *al th* male, wild-type female; (*F*) wild-type male, *norp A* female; (*G*) wild-type male, *sbl* female; and (*H*) wild-type male, *al th* female.

DISCUSSION

Competitive mating experiments between wild-type and sensory-deficient flies revealed contrasting orders of importance for sensory channels in each sex. The orders reported here are probably highly specific for *D. melanogaster* because other *Drosophila* species differ in requirements for visual cues during courtship (11) and in the use of female songs.

An intact visual system appears more important to a

Table 2. Average bout lengths for various male behaviors (in sec) for wild-type successful pairs, wild-type unsuccessful pairs, and pairs in which either the male or female had a sensory deficiency and the other pair member was wild-type

	WT-S	WT-U	*smb*♀	*norp A*♀	*al th*♀	*smb*♂	*norp A*♂	*al th*♂
Locomotion	9.44	8.43	21.28*	25.52*	30.4*	5.51*	19.06	15.61
Lick (IBI)[†]	20.79	32.31	14.93	13.85	20.53	44.39*	29.51	13.14
Orient back	1.65	3.08	3.36	2.32	3.55	1.54	2.68	2.22
Orient back, vibrate	1.35	1.72	0.91	1.55	2.37	1.19	0.70	1.51
Orient front	1.37	2.07	1.15	3.41*	5.17*	1.20	1.03	1.71
Orient front, vibrate	1.0	3.32*	1.14	1.17	2.85*	0.84	1.30	1.15
Chase	3.61	4.73	3.83	2.08	4.77	3.74	0.0*	0.71
Chase and vibrate	1.01	1.13	0.62	1.19	0.76	0.72	0.05*	0.71
Copulation attempt (IBI)[†]	26.07	34.07*	30.11	17.24*	31.97	48.19*	55.34*	15.68*

WT-S, wild-type successful pairs; WT-U, wild-type unsuccessful pairs.

*$P < 0.05$ difference from WT-S pairs.

[†] Interbout interval = rate.

courting male than its olfactory capabilities in order to begin courting and to maintain contact with a female. *Norp A* males exhibit what Connolly *et al.* (12) have labeled inappropriate courtship; that is, they performed vibration not oriented toward a female, but to a position where a female had been moments earlier. Possibly the female presence leaves a residual airborne stimulus to which the blind males react. Vision is also important in enabling a male to properly execute certain courtship behaviors when near a female. For example, wild-type males always vibrate the wing closest to the female's head (Fig. 1*A*). *Norp A* males fail to do this (Fig. 1*C*), suggesting that visual cues alone dictate the orientation of wing vibration and its song. Olfactory cues are also important for courtship initiation and proper execution of courtship behaviors. Males with *sbl* exhibit delayed courtship initiation and misdirected behavioral components. Wild-type males always lick the female's posterior abdomen and direct copulation attempts to the same place (Fig. 1*A*). *Sbl* males, conversely, were frequently seen licking and attempting to copulate with the female's head. If quantified information on inappropriately directed courtship interests an investigator, such could be designated as a separate behavior on the event recorder and analysed.

The order of importance of each sensory channel differs for females. Blind females are not at all disadvantaged compared with wild-type, but clearly females must hear courtship songs and, to a lesser degree, perceive olfactory stimuli to become receptive.

Unsuccessful wild-type pairs and pairs with a mutant male showed MCPs that differed from wild-type successful pairs. Because each type of pair varied in a different way, it is difficult to label any one behavioral deviation as an absolute indicator of reduced success; nevertheless, some generalization is possible. In wild-type successful pairs females reduce their locomotion, and males in turn reduce their chasing. This may be the primary means by which females of this species indicate their receptivity (3). In unsuccessful pairs (wild-type and *norp A*) no such "slowing down" was detected. Females may display signs of a lack of receptivity other than slowing down, such as decamping or wing flicking.

Another possible generalization is that the greatest reductions in male success appear associated with the largest aberrations in the MCP. Those males, such as *norp A* and *sbl*, that were least successful in mating were the slowest to begin courting and, once courting, showed the most deviant behaviors. It is impossible to assign a relative importance to courtship latency compared to the quality of the courtship once initiated. However, if two wild-type males exhibited similar MCPs, but one of them began courting earlier, the advantage to the male who courts first can easily be envisioned. Usually the early male also is more persistent, and unless the female rejects his signals in favor of signals from another male the early male is likely to be more successful.

Table 3. Time spent at each behavior in the first half vs. the second half of courtship among wild-type successful pairs, unsuccessful wild-type pairs, and pairs in which either the female or male was mutant and the partner was wild-type

	WT-S	WT-U	*norp A*♀	*smb*♀	*al th*♀	*norp A*♂	*smb*♂	*al th*♂
Male behavior								
Locomotion	+/−	+/−	+/−	+/−	+/−	=	+/−	+/−
Lick	=	=	=	=	=	=	=	=
Orient back, vib	−/+	−/+	−/+	−/+	−/+	−/+	=	−/+
Orient front, vib	−/+	−/+	−/+	−/+	−/+	=	−/+	−/+
Chase	+/−	=*	=*	+/−	+/−	=	+/−	+/−
Chase and vibrate	+/−	=*	=*	+/−	+/−	=	+/−	+/−
Orient back	−/+	−/+	−/+	−/+	−/+	=	−/+	−/+
Copulatory attempt[†]	=	=	=	=	=	=	=	=
Orient front	=	−/+*	−/+	−/+	−/+	=	−/+	−/+*
Female behaviors								
Locomotion	+/−	=	+/−	+/−	+/−	=	+/−	+/−
Stand still	−/+	=	−/+	−/+	−/+	=	−/+	−/+
Preen	−/+	=	−/+	−/+	−/+	=	−/+	−/+
Kick	=	=	=	=	=	=	=	=
Double flick	=	=	=	=	=	=	=	=
Single flick	=	=	=	=	=	=	=	=
Decamp	=	=	=	=	=	=	=	=

WT-S, wild-type successful pairs; WT-U, wild-type unsuccessful pairs; vib, vibrate. Ratios are time in first half of courtship/time in second half of courtship.

*$P < 0.05$ different from WT-S pairs.

Table 4. Stationary probabilities for female behaviors among successful wild-type pairs, unsuccessful wild-type pairs, and pairs having a mutant female or male

	WT-S	WT-U	*smb*♀	*norp A*♀	*al th*♀	*smb*♂	*norp A*♂	*al th*♂
Locomotion	46.9	44.9	41.9	44.7	43.8	45.2	41.2	44.7
Stand still	24.4	28.5	29.5*	18.6*	22.4	20.1	21.6	18.3*
Preen	15.8	13.6	15.3	15.7	14.9	12.6	12.8	14.9
Kick	0.9	0.0	0.4	0.1	0.2	0.0	0.1	1.1
Single flick	1.2	0.9	1.0	2.6	1.1	3.6*	2.9	3.5*
Double flick	3.0	5.7	8.9	11.1*	3.6	0.5	2.9	3.5
Decamp	8.8	6.6	2.8	7.2	5.4	13.9*	20.1*	5.3

WT-S, wild-type successful pairs; WT-U, wild-type unsuccessful pairs.
*$P < 0.05$ difference from WT-S pairs.

In addition to association with reduced success, an aberrant behavioral profile results in other visible changes. Often the wild-type partner of a mutant showed behavioral features not seen in pure wild-type/wild-type pairings, supporting the idea of Markow and Hanson (5) that information is exchanged between the sexes. This also suggests that a male or a female can change its behavior in response to information received from the opposite sex.

Some caution is necessary in interpreting the above findings. Although it is tempting to conclude that the reduced success of mutant males is a function of their measurable behavioral differences, these differences may not, in fact, be the primary behavioral lesions caused by the mutant alleles. Even though licks and copulation attempts do not follow any particular female behavior, females may be controlling in some yet undetected way those male behaviors; thus females may somehow elicit these behaviors after perceiving other qualities of courting males. What can be concluded, then, is that a relationship exists between courtship success and the MCP—the greater the aberration in the MCP, the less successful the male under competitive conditions. Furthermore, certain perturbations of the MCP, such as continuing female locomotion or increased frontally oriented behaviors by males, are more useful predictors of courtship success.

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