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Portions of the Central Nervous System Controlling Reproductive Behavior in *Drosophila melanogaster*

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Drosophila melanogaster *sex mosaics were tested in their courtship interactions with females and then with males. The distribution of genetically male and female tissues in each mosaic was determined with respect to an external cuticle marker and an internal enzyme marker. Performance of malelike courtship was correlated with the genotype of various tissues, with special attention being paid to the genotypes of head and thoracic ganglia. Male tissue in the left or right dorsal brain is necessary and nearly always sufficient to trigger early courtship actions—following of females and wing extension at them—but male tissue in both the dorsal brain and thoracic ganglia is necessary for attempted copulation to occur. Female tissue on or in the abdomen is nearly always necessary and sufficient for a mosaic to be courted by a male.*

KEY WORDS: courtship behavior; *Drosophila* mosaics; nervous system marker.

INTRODUCTION

Drosophila males exhibit a sequence of several specific behaviors in their courtship of females (reviewed by Spieth, 1952, 1974). The mechanisms by which the central nervous system controls these behaviors are largely unknown. Before elucidating the mechanisms, it is necessary to learn what parts of the nervous system exert this control. Ablation and electrical stimulation experiments are one way to find out if there are specific parts of the nervous system involved in male courtship behavior in insects (e.g., as

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reviewed by Huber, 1965, 1967). In *Drosophila,* procedures involving genetic mosaics provide means by which one can study the intact animal to learn which parts of the nervous system have been genetically programmed to control courtship behavior. Unlike stimulation and ablation experiments, the genetic methods of studying courtship control bear on the developing as well as the adult nervous system.

Genetic mosaics in *Drosophila melanogaster* can be generated so that part of the fly has genetically male tissue (with one X chromosome) and the rest has genetically female tissue (with two X chromosomes). The male and female tissues can be scored with respect to genetic markers for external and internal tissues (Kankel and Hall, 1976). These mosaics can be observed to determine which malelike courtship behaviors they might exhibit toward normal females. Also, males can be placed with these mosaics to see which parts of a mosaic must be female to elicit courtship.

In *D. melanogaster,* sporadic observations of the reproductive behavior of sex mosaics have already been made (reviewed by Patterson and Stone, 1938; Manning, 1967). The few data indicate that genetic maleness in the anterior part of a sex mosaic is sufficient to allow some malelike behavior on the part of the mosaic. Thus the genetic constitution of the anterior nervous system might be critical. This preliminary conclusion is not necessarily obvious or expected for an insect. Although control of courtship actions by the head region is the case in wasps (the mosaic experiments of Whiting, 1932, and Clark and Egen, 1975), more posterior segments apparently contain the control center(s) for sex-specific behavior in houseflies and bees (reviewed by Manning, 1967).

Hotta and Benzer (1976) have recently made detailed observations of *D. melanogaster* sex mosaics. The distribution of male and female tissues in the mosaics was scored only with respect to the cuticle. They were able to show that if a mosaic orients toward and follows a female it almost invariably performs wing vibration; the center of control of these behaviors can be localized in the head region by morphogenetic fate mapping (*cf.* Hotta and Benzer, 1972). The fate-mapping parameters are consistent with the assumption that only the left or right side of something in the head need be genetically male to allow following of females and wing extension at them. There are many mosaics which do not perform attempted copulation even though they carry out the early actions, and it appears that genetically male tissue in the thoracic region is required for the control of this behavior. Attempted copulation did not occur in the absence of previous following and wing extension. Finally, genetically female tissue in or on the abdomen is nearly always required for a mosaic to elicit courtship actions as performed by a normal male.

The present investigation confirms and extends the findings of Hotta and Benzer (1976). The analysis here is more direct in that the genotype of internal tissues is examined by histochemical genetic marking. One can thus ask directly if the brain is indeed a control center for courtship behaviors, and, if so, if the center can be localized in only a portion of the head ganglia.

MATERIALS AND METHODS

Production of Sex Mosaics

Sex mosaics were generated for which cuticle mosaicism was scored with the body and bristle color mutation yellow (*y*), and for which internal mosaicism was scored with respect to an acid phosphotase-null mutation (*Acph-1*^{n11b}, Bell and MacIntyre, 1973). *X·Acph-1*$^{+}$*/y*$^{+}$*Y; pal/pal; Acph-1*^{n11b}*/Acph-1*^{n11b} males were crossed to *y/y*; *Acph-1*^{n11b}*/Acph-1*^{n11b} females. As shown by Kankel and Hall (1976), about 0.5% of the initially diplo-X zygotes from this cross are sex mosaics which have undergone somatic loss of the paternally inherited X chromosome. This X chromosome has the normal allele of the *Acph-1* gene translocated onto it, and carries the normal allele of *y*; the somatic loss is caused by the effects of the *pal* (paternal loss) mutation in the father (Baker, 1975). The haplo-X (male) tissues in these mosaics will have a patch of yellow tissue externally, and will have little acid phosphatase activity internally, whereas the diplo-X (female) tissues are not-yellow and have acid phosphatase activity. A total of 395 sex mosaics were collected under light etherization as 0- to 10-hr-old virgins. They had been raised on a cornmeal–agar–molasses medium at 25°C.

Behavior Testing of Sex Mosaics

The sex mosaics were processed as follows. Each mosaic was aged for 3–4 days at 25°C and about 60% relative humidity, in constant light, in 22- by 95-mm shell vials containing the above medium and live yeast. They were then put with female testers, that is, *C(1)DX,y f/Y* (attached-X, females homozygous for the yellow and forked bristle mutations), which had been raised, collected, and aged in the same fashion as the sex mosaics. These genetically marked tester females were used so that mosaics could easily be distinguished from testers during the observations. The observations were carried out in cylindrical plastic mating chambers (diameter 9 mm, height 6 mm): one observation apparatus contained ten such cham-

bers, and ten was the maximum number of sex mosaics observed at any one period. A given chamber contained one sex mosaic and two tester females. The observations were done for 1 hr at 25°C and 60% relative humidity. Flies were moved in and out of the observation chambers without etherization, by means of a rubber and glass tubing aspirator. Each mosaic was observed to see if it would perform malelike orientation at and following of females, left and/or right wing extension at females, attempted copulation (scored positive when there was curling of the mosaic's abdomen, directing the posterior part toward the female's genitals), and copulation itself, *or* only some of the above behaviors. On the following day, the mosaic was tested for elicitation of male courtship by wild-type males (from a Canton-S strain) which had been raised, collected, and aged as described above. There were one sex mosaic and two tester males in a given chamber; again, the observations were carried out for 1 hr. If a sex mosaic copulated, it was placed in a food-containing shell vial to assess its fecundity. The mating behavior observation apparatus was washed and rinsed thoroughly before each use.

Observations of Control Males and Females

The controls for malelike behavior involved observations of 162 y/Y; $Acph\text{-}1^{n11b}/Acph\text{-}1^{n11b}$ males. The control males were raised in the same conditions as described with respect to the sex mosaics, and they were aged and observed for male behavior in the same manner as described above. The male tissues here are relatively isogenic with the male tissues in the sex mosaics. If a very low fraction of control males fail to court normally, then there will be few false negatives in the results from mosaics. In principle, the proper control males would be yellow and acid phosphatase-null, but with no Y chromosome; but it is difficult to construct stocks such that these XO males would be genetically related to the male tissues in the sex mosaics. The Y chromosome is not necessary for male courtship behavior (although it is required for fertility): 32 XO males (generated by crossing Canton-S males to $C(1)M3,y^2/O$ attached-X females) were tested, and all courted in an apparently normal fashion, with 25 of them copulating successfully. Thus the fact that male tissues in the mosaics have no Y chromosome would not appear to affect the experiments.

For controls on the elicitation by sex mosaics of male behavior, 139 virgin females were collected from among progeny of a cross of X $Acph\text{-}1^{+}/y^{+}Y$; $In(2LR)O/pal$; $Acph\text{-}1^{n11b}/Acph\text{-}1^{n11b}$ males to y; $Acph\text{-}1^{n11b}/Acph\text{-}1^{n11b}$ females. Female progeny (heterozygous for y, $Acph\text{-}1^{n11b}$ and pal) were aged and scored in the same manner as for the elicitation tests.

Scoring of Male and Female Tissues in Mosaics

After all behavioral testing of a sex mosaic was completed, the distribution of male and female external tissues was drawn schematically (by observation in a dissecting microscope at 50–80×). Internal scoring was done by sectioning the mosaic for acid phosphatase histochemistry. The method was to cut 10 μm frozen sections (in a S.L.E.E. International cryostat) in the horizontal plane, the entire fly being collected as approximately 80 serial sections. Fixation and staining were carried out as described by Kankel and Hall (1976).

The distribution of male (unstained) and female (stained) tissues in these mosaics was scored in the light microscope (100–430×) and noted on diagrammatic drawings for the head and thoracic ganglia (Kankel and Hall, 1976). Several regions of the cortices of these ganglia were scored for each of several planes of sectioning: three levels in the supraesophageal ganglion and associated optic lobes; one level in the subesophageal ganglion, with associated optic lobes; and five levels in the fused thoracic ganglia. The genotypes of alimentary tissues in each mosaic were noted as well, to assist in morphogenetic fate-mapping analysis (see below).

RESULTS

Behavior of Control Males and Females

Of 162 *y/Y*; *Acph-1*^{n11b}/*Acph-1*^{n11b} males tested, 161 performed following of and wing extension at females, and 158 of these showed both left and right wing extension at different times during courting; three performed right wing extension only. Of 151 which attempted copulation, only 94 were successful. Thus the high frequency of performance of the early stages of courtship validates the observations of sex mosaics, in which the male tissues are expressing the above markers. However, attempted copulation was not so successfully accomplished. This means that a small proportion of false negatives should be expected in the sex mosaic experiment. The copulation focus cannot be mapped in this study because of the low frequency of copulation by control males. In addition, results would be complicated by the fact that many mosaic individuals which attempt copulation are unable to achieve it because the external genitalia are all or part female.

Of 139 control females tested, 128 elicited following and wing extension, 124 elicited the early steps plus attempted copulation, and 91 copulated. The relatively poor performance of the tester males here led to routine retesting of mosaics which did not provoke any male courtship; that

is, in these cases, a second observation was made on them with fresh tester males.

Behavior of Sex Mosaics

Hierarchy of Behaviors Involving Sex Mosaics

Figure 1 shows the gross results from the sex mosaic tests. Of the 395 mosaics, 31% performed at least some male behavior. All began with following of females, except for one which appeared to perform an attempted copulation lunge (once during the hour-long observation). Since the behavior here was dubious, this case is included in the 271 mosaics listed (Fig. 1) as showing no male behavior. 97% of the male-behaving mosaics showed both following and wing extension. Of the four mosaics which followed but showed no other courtship behavior, three followed a female weakly and not in a sustained fashion (less than 30 sec total). One mosaic exhibited several vigorous following bouts in 1 hr, without extending either wing. These data suggest that the foci for following and wing extension are either identical or very close to each other.

Of the mosaics showing following and wing extension, 44% went on to attempt copulation. The separability of wing extension from attempted copulation is far greater than the decrement between these steps in the controls.

In the tests to see which mosaics would provoke courtship, 76% of the mosaics had following and wing extension performed at them and 87% of these elicited attempted copulation (Fig. 1). The proportion of mosaics eliciting courtship was thus substantially lower than the proportion of control females which were courted.

Cuticle Mosaicism and Sex Mosaic Behavior

The sex mosaics were classified with respect to cuticle mosaicism, both for malelike behavior and for elicitation tests (Table I). The focus for following and wing extension can be roughly mapped in the head region: 5% of the mosaics with entirely female or male head were cases where the cuticle genotype did not correlate with the behavior (e.g., female head cuticle, but male behavior); these calculations for the thorax and abdomen are 13% and 64%, respectively, which indicates that the external genotypes of these body regions correlate less with this malelike behavior.

The sex mosaics which did begin courtship of females were divided into different classes with respect to external mosaicism, roughly to localize the attempted copulation focus. Nearly all the external heads for the mosaics perforce have male tissue (see Table IA). The head genotype seems not to

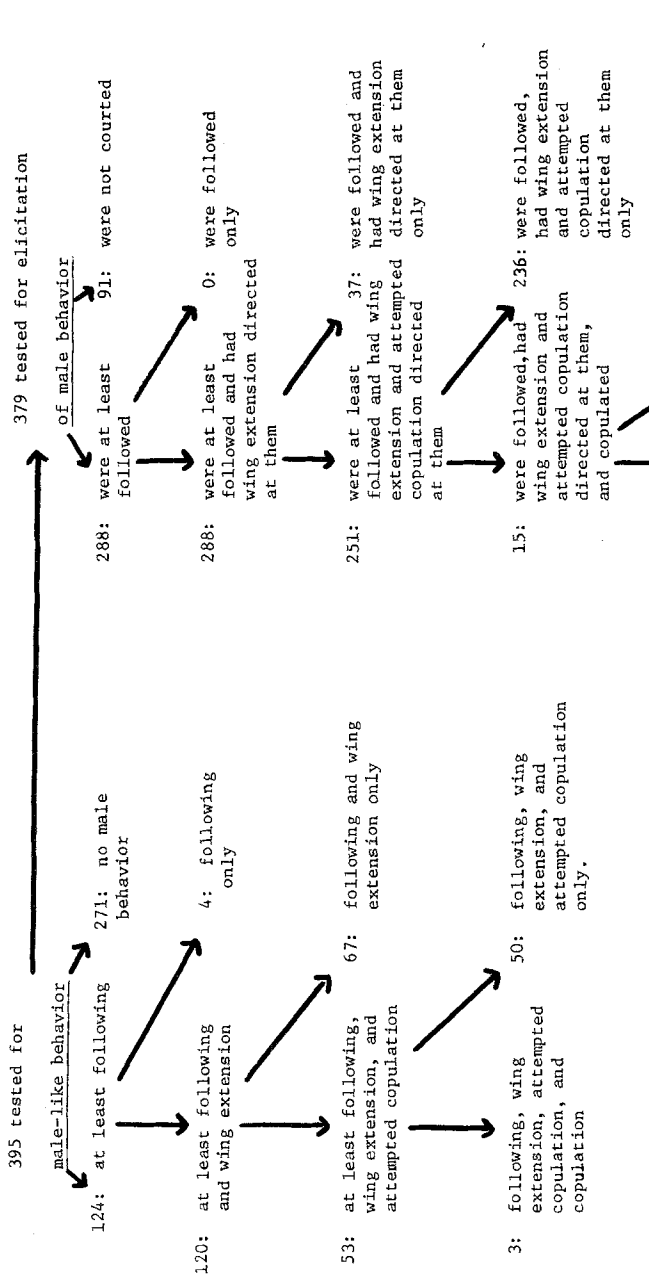

Fig. 1. Behavior of sex mosaics toward females, and behavior of males directed at them.

Table I. Cuticle Mosaicism and Sex Mosaic Behavior[a]

		All male	Mixed	All female
A.	Malelike behavior			
			Head cuticle	
	Male behavior	42	79	3
	No male behavior	7	121	143
			Thorax cuticle	
	Male behavior	9	105	10
	No male behavior	5	177	89
			Abdomen cuticle	
	Male behavior	13	88	23
	No male behavior	23	238	10
B.	Elicitation of male behavior			
			Head cuticle	
	Were courted	33	143	112
	Were not courted	13	47	31
			Thorax cuticle	
	Were courted	5	207	76
	Were not courted	8	65	18
			Abdomen cuticle	
	Were courted	4	253	31
	Were not courted	29	61	1

[a] All mosaics were divided into different categories as to cuticle mosaicism considering the head, the thorax, or the abdomen. A major body region could be all female (y^+), all male (y), or mixed in genotype. For a given subdivision (e.g., heads male), the mosaics were separated into male behaving (at least following) vs. not male behaving (A). The different mosaic categories were also separated into cases which elicited male behavior vs. those which did not (B).

control attempted copulation, since 44% of the mosaics with all male or all female head cuticle had the opposite behavior (e.g., head male, but no copulation attempted). For the posterior body regions, the attempted copulation focus is a bit more closely linked to the thorax than to the abdomen: 21% of mosaics with all male or all female thorax showed the opposite behavior, whereas 28% of mosaics with all male or all female abdomen showed the opposite behavior.

From the tests of mosaics on elicitation of courtship, the cuticle mosaicism indicates that the focus is closest to the abdomen. Table IB shows that in 8% of the mosaics with entirely female or male abdomen the external genotype was not correlated with the elicitation of male behavior (e.g., all female abdomen, but no elicitation). The analogous figures for the thorax and head are 21% and 35%, respectively (Table IB). Some of the cases with entirely male abdominal cuticle which did elicit courtship may be false positives, since males are courted by other males with a probability of about 0.1 (J. Hall, unpublished).

Among mosaics with part male, part female abdomens, those with relatively more female abdominal tissue tend to provoke courtship with a higher probability than mosaics with mostly male abdomens. There was no particular segment of the abdomen for which genetically female cuticle was invariably associated with attempted copulation.

Internal Mosaicism and Sex Mosaic Behavior

Male Behavior and Brain Genotype. Of the 395 mosaics whose behavior was tested, 293 were sectioned and could be reliably scored for internal mosaicism. Of the internally scored mosaics, 32% showed male following (Table II). Substantially higher proportions of the mosaics with male tissue in the left and/or right brain showed male behavior (Table II). For example, nearly all the cases with only left brain or right brain entirely

Table II. Brain Genotype and Male Courtship Behavior of Mosaics[a]

		Total mosaics	Mosaics showing male behavior (proportion)
A.	Left–right brain dividing planes		
	1. L♀, R♀	165	0 (0.0)
	2. L♂, R♀ or L♀, R♂	16	15 (0.9)
	3. L mixed, R♂ or L♂, R mixed	20	18 (0.9)
	4. L mixed, R♀ or L♀, R mixed	39	18 (0.5)
	5. L mixed, R mixed	19	12 (0.6)
	6. L♂, R♂	34	32 (0.9)
B.	Dorsal–ventral brain dividing planes		
	1. sp♀, sb♀	165	0 (0.0)
	2. sp♂, sb♀	1	1 (1.0)
	3. sp♀, sb♂	0	0 (0.0)
	4. sp mixed, sb♂	8	3 (0.4)
	5. sp♂, sb mixed	7	6 (0.9)
	6. sp mixed, sb♀	23	17 (0.7)
	7. sp♀, sb mixed	2	0 (0.0)
	8. sp mixed, sb mixed	53	36 (0.7)
	9. sp♂, sb♂	34	32 (0.9)

[a] Mosaics scored internally (N = 293) were divided into different classes with respect to genotype of (A) left (L) and right (R) supra- and subesophageal ganglion and (B) with respect to supra- (sp) and subesophageal (sb) ganglia taken as units (e.g., considering left and right supraesophageal ganglia together). Mosaics in each class were then separated into cases showing male behavior (i.e., following of females) and those showing none. The optic lobe genotypes are ignored here (but see Fig. 4a). Thirty-two percent of these mosaics showed male behavior. Associated with each number in the tables (e.g., L mixed, R mixed, male behavior) is the proportion of that brain genotype class which it comprises.

male (and the other half female) showed male behavior. Figure 2 shows an example of a split brain, with female tissues on the left side and male tissues on the right side. The left–right dividing line category with the lowest proportion of male-behaving mosaics is that with one-half the brain entirely female and the other half mixed in genotype (Table II). No mosaics with entirely female brain showed male behavior, and thus it seems that male brain tissue in the left or right side is necessary and sufficient to trigger male courtship behavior. However, 10% of mosaics with entirely male brains showed no male behavior; one of the two mosaics in this category appeared rather debilitated in its overall behavior, and so may be a false negative. The other was not noticeably sluggish. Thus the possibility remains open that male brain tissue is necessary but not sufficient for controlling male courtship.

Mosaic divisions with respect to dorsal–ventral planes are rarer than left–right divisions (*cf.* Kankel and Hall, 1976). Yet, from the few relevant cases, an entirely male subesophageal ganglion is not sufficient to trigger male behavior with a much higher probability than the overall proportion of mosaics showing male behavior, i.e., 0.32 (categories 2 and 6 in part B of Table II).

Wing Extension and CNS Genotype. Of the 95 mosaics which followed females, 91 exhibited wing extensions at them (*cf.* Fig. 1). In the one case which showed vigorous and sustained following but no wing extension, the left half of the brain was entirely male, and the right half entirely female. Thus it was the same in brain genotype as many other mosaics which showed both following and wing extension (i.e., all the other male-behaving cases in category 2, part A of Table II).

Of the mosaics showing wing extension, 23% showed only left or right wing extension; this is a substantially higher proportion of such anomalous behavior than is exhibited by control males. An attempt was made to determine if the wing extension is under ipsilateral or contralateral control by the supraesophageal ganglion, the subesophageal ganglion, or the thoracic ganglia, but no consistent association was found. It may be that the mosaics which do show male behavior are—relative to totally male flies with the markers used in this system—defective in their behavior. Indeed, several mosaics showing wing extension did not appear to do it normally in that the behavior was not sustained, and the extension was not complete, as it led to an angle between the extended wing and the long axis of the body which was less than the normal 90°. These bouts of defective wing extension were definitely courtship behaviors in that they were associated with orientation toward or following of females.

A more forceful conclusion from the wing extension–ganglia genotype correlations is that male tissue in only one side of the brain is very

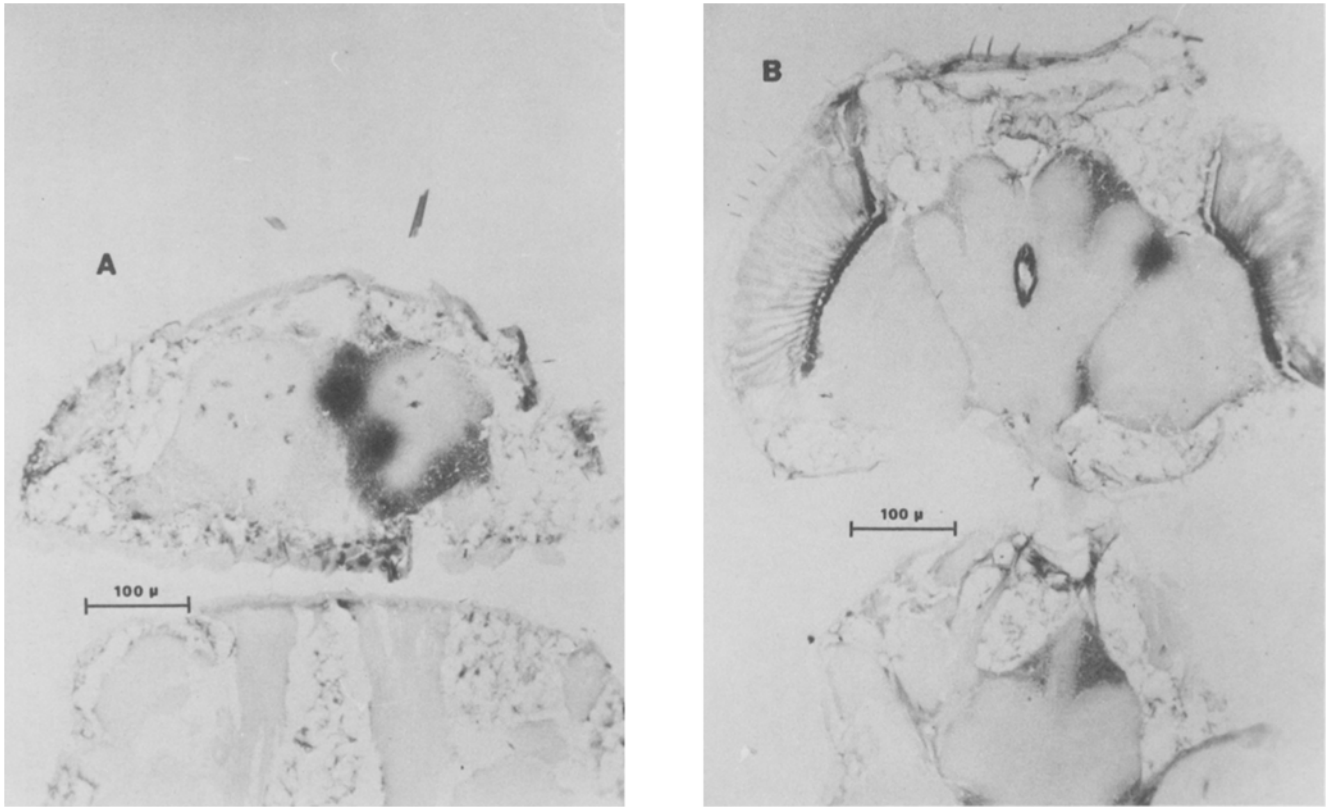

Fig. 2. Split brain mosaic. This sex mosaic followed females, and extended its left and right wings (not simultaneously) at them. Serial sections were cut in the horizontal plane and stained for acid phosphatase activity. Dark staining of nerve cell bodies designates female tissue, while the absence of it designates male tissue. Two of the 10-μm sections are shown, A coming from a more dorsal plane than B. The enzyme activity is present in one side of the brain only. The thoracic ganglia (not shown) were entirely stained, and no copulation was attempted.

Table III. Attempted Copulation and Nervous System Mosaicism[a]

	Total mosaics	Mosaics showing attempted copulation (proportion)
A. Brain mosaicism and attempted copulation		
1. Left (L)–right (R) dividing planes		
a. $L_{♂}$, R♀ or L♀, $R_{♂}$	15	6 (0.4)
b. L mixed, $R_{♂}$ $L_{♂}$, R mixed	18	9 (0.5)
c. L mixed, R♀ or L♀, R mixed	18	4 (0.2)
d. L mixed, R mixed	12	4 (0.3)
e. $L_{♂}$, $R_{♂}$	32	18 (0.6)
2. Dorsal (sp)–ventral (sb) dividing planes		
a. $sp_{♂}$, sb♀	1	0 (0.0)
b. sp♀, $sb_{♂}$	0	0 (0.0)
c. sp mixed, $sb_{♂}$	3	1 (0.3)
d. $sp_{♂}$, sb mixed	6	2 (0.3)
e. sp mixed, sb♀	18	5 (0.3)
f. sp♀, sb mixed	0	0 (0.0)
g. sp mixed, sb mixed	35	15 (0.4)
h. $sp_{♂}$, $sb_{♂}$	32	18 (0.6)
B. Thoracic ganglia mosaicism and attempted copulation		
1. Left (L)–right (R) dividing planes		
a. L♀, R♀	22	0 (0.0)
b. L♀, $R_{♂}$ or $L_{♂}$, R♀	16	10 (0.6)
c. L mixed, $R_{♂}$ or $L_{♂}$, R mixed	9	7 (0.8)
d. L mixed, R♀ or L♀, R mixed	24	10 (0.4)
e. L mixed, R mixed	21	11 (0.5)
f. $L_{♂}$, $R_{♂}$	3	3 (1.0)

frequently sufficient to allow extension of both wings at different times during the period of courtship observation: 66% (21/32) of mosaics which had male tissue in only the left or right dorsal brain showed bilateral wing extension. For the thoracic ganglia, not only is male tissue in one side not correlated with wing extension on that (or the opposite) side, but also 17% (12/70) of the cases showing bilateral wing extension were entirely female in the thoracic ganglia. This is of course consistent with wing extension being closely linked to head but not thoracic maleness (Table IA).

Attempted Copulation and CNS Genotype. Of the mosaics showing male behavior and scored internally, 43% performed attempted copulation (Table III). The brain tissues for the male-behaving mosaics are perforce partially male (Table II). An attempt was made to correlate male tissue in a

Table III. Continued

	Total mosaics	Mosaics showing attempted copulation (proportion)
B. Thoracic ganglia mosaicism and attempted copulation (continued)		
2. Dorsal (d)–ventral (v) dividing planes		
a. d♀, v♀	22	0 (0.0)
b. d♂, v♀	0	0 (0.0)
c. d♀, v♂	0	0 (0.0)
d. d mixed, v♂	5	3 (0.6)
e. d♂, v mixed	1	1 (1.0)
f. d mixed, v♀	4	1 (0.2)
g. d♀, v mixed	0	0 (0.0)
h. d mixed, v mixed	59	33 (0.6)
i. d♂, v♂	3	3 (0.1)
3. Anterior (th)–posterior (ab) dividing lines		
a. th♀, ab♀	22	0 (0.0)
b. th♂, ab♀	2	2 (1.0)
c. th♀, ab♂	1	0 (0.0)
d. th♂, ab mixed	3	3 (1.0)
e. th mixed, ab♂	9	4 (0.4)
f. th♀, ab mixed	4	2 (0.5)
g. th mixed, ab♀	16	5 (0.3)
h. th mixed, ab mixed	35	22 (0.6)
i. th♂, ab♂	3	3 (1.0)

[a] Mosaics showing malelike behavior ($N = 95$) were divided into different genotypic classes with respect to (A1) supra- and subesophageal ganglia left (L)–right (R) dividing planes; (A2) dorsal–ventral brain dividing planes, considering the left and right supraesophageal ganglia dorsal and the left and right subesophageal ganglia ventral; (B1) thoracic ganglia left–right dividing planes; (B2) thoracic ganglia dorsal–ventral dividing planes, considering the first three levels of scoring as dorsal and the last two levels as ventral (see Fig. 3 of Kankel and Hall, 1976); (B3) thoracic ganglia *per se* as anterior, and the abdominal ganglion (fused to the thoracic ganglia) as posterior. These genotypic classes were then further divided into types which attempted copulation or did not. Forty-three percent of these mosaics showed attempted copulation. Associated with each number in the table is the proportion of that genotypic category which it comprises.

particular brain region with performance of attempted copulation (Table IIIA), but there is no category of male–female dividing plane in the head ganglia which is particularly associated with attempted copulation.

Since performance of attempted copulation is more closely linked to maleness in the thorax than to any other region, thoracic ganglia genotypes of male-behaving mosaics were examined (Table IIIB). All of these mosaics with entirely female thoracic ganglia did not attempt copulation; those with entirely male thoracic ganglia did. For the cases with genetically mixed thoracic ganglia, dividing planes with left–right, dorsal–ventral, and anterior–posterior orientation were analyzed (Table IIIB). There is no dra-

matic correlation of attempted copulation performance with male tissue in a particular part of the ganglia. The following can be said:

1. Some male tissue, in any of several different thoracic ganglia regions, appears to be necessary for attempted copulation. Some examples were found with quite small patches of male tissue in a thoracic ganglion, and yet they attempted copulation.
2. Male tissue is often insufficient for triggering this behavior (e.g., Table III, part B1, category b has several mosaics with identical distribution of male tissue in the thoracic ganglia but which showed different behavior). There must be some false negatives among the mosaics with male tissue in the thoracic ganglia which failed to attempt copulation, since only 93% of the control males which followed and showed wing extension attempted copulation. Male thoracic ganglia tissue is certainly insufficient for attempted copulation (i.e., in the absence of male brain tissue) since 56% of mosaics not performing following or wing extension had part male or all male ($N = 2$) thoracic ganglia. Yet only one of these showed attempted copulation; this was the case of the one dubious copulation lunge at a female (see above). The mosaic proved to have entirely female head ganglia, and male tissue was restricted to the thoracic ganglion in the prothorax.
3. More male tissue is associated with a higher probability of performing attempted copulation (e.g., Table III, part B1, category c; or part B3, category h vs. category g). The proportion of male tissue in each of the thoracic ganglia in these mosaics was roughly determined. Here, the histogram for mosaics performing attempted copulation was indeed shifted in the direction of greater degrees of ventral ganglion maleness; yet there were several exceptional cases (e.g., with very little male tissue but performance of this behavior, or *vice versa*).

Fate Mapping of Courtship Behavioral Foci

The parts of the embryonic blastoderm (or "foci" as defined by Hotta and Benzer, 1972) which will develop into tissues that control courtship behaviors were determined. The aims were objectively to confirm the strong suggestions, from the preceding analysis, that the dorsal brain contains a site or sites controlling male following and wing extension, but neither the ventral brain nor the optic lobes possess this focus, and that the following and wing extension focus is "domineering" (as defined by Hotta and Benzer, 1972) in that male tissue in only the left or right side of the dorsal brain is sufficient for male behavior to occur. An alternative is the case where left and right male brain tissue is required for male behavior, i.e., a

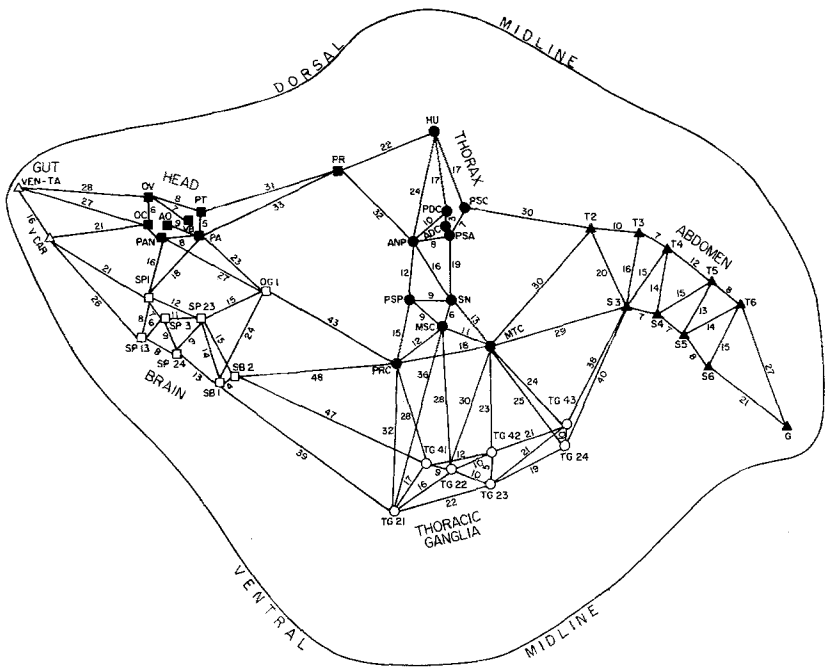

Fig. 3. Fate map of embryonic blastoderm. The map is a formal representation of the sites on the blastoderm (landmarks) which will develop into adult cuticle and internal tissues. Left is anterior and top is dorsal. The length of a line connecting two given landmarks represents the probability that the structures are of different genotype among all the mosaics, calculated according to the formula: (number of mosaic sides with structure *a* of genotype different from structure *b*)/(number of sides with *a* haplo-X + number of sides with *b* haplo-X). The numbers associated with each line are these probabilities times 100. The border surrounding the figure represents the midline of the blastoderm (thus only one side of the fate map is shown here, yet the one for the other side is a mirror image); the distances between various structures and the midline are based on the probabilities that left and homologous right structures are of different genotype. A more complete explanation of these principles is in the review of Hall *et al.* (1976). Head cuticle structures (■): OV, outer vertical bristle; OC, ocellar bristle; PT, postorbital bristle; VB, vibrissae; AO, anterior orbital bristle; PAN, proximal segment (second) of antenna; PA, palp; PR, proboscis. Head ganglia (□): SP1, SP3, SP13, SP23, SP24, supraesophageal ganglion sites (lower numbers are more dorsal); SB1, SB2, subesophageal ganglion sites; OG1, optic ganglia site. These designations are based on the schematic diagram of the nervous system in Kankel and Hall (1976, their Fig. 3). Thoracic cuticle structures (●): HU, humeral bristle; PSC, posterior scutellar bristle; PDC, posterior dorsocentral bristle; ADC, anterior dorsocentral bristle; ANP, anterior notopleural bristle; PSA, posterior supraalar bristle; PSP, posterior sternopleural bristle; SN, notosternal bristle; PRC, MSC, and MTC, bristles on proximal segments of anterior, middle, and posterior legs, respectively. Thoracic ganglia (○): TG21, TG22, TG23, TG24, sites in pro-, meso-, metathoracic, and abdominal ganglia, respectively: TG41, TG42, TG43, sites in meso- and metathoracic ganglia and abdominal ganglia, respectively (more ventral sites than the first four listed); again, see Kankel and Hall (1976, Fig. 3). Abdominal cuticle structures (▲): T2 through T6, dorsal tergites; S3 through S6, ventral sternites; G, external genitalia. Alimentary tissues (△): VEN-TA, anterior portion of thoracic ventriculus (gut), VCAR, ventral portion of cardia (valve in gut).

"submissive" focus. Also, a test was made of the suggestion that the attempted copulation focus is associated with the thoracic ganglia but is diffuse (i.e., not linked to a particular region of the ganglia) and is not strongly domineering or submissive (i.e., cases with a high degree of maleness in left or right thoracic ganglia may or may not attempt copulation, and cases with lower proportions of maleness in left or right thoracic ganglia may or may not attempt copulation).

The fate mapping was accomplished in two steps. First, a general fate map was constructed, showing the blastoderm locations of external and internal landmarks (i.e., parts of the blastoderm which will give rise to these tissues in the adult). The map (Fig. 3) is made by placing two given sites a distance from each other which is based on the probability that the sites are of different genotype. Thus two different head bristles are rarely of different genotype, but any head bristle is frequently of different genotype from any thoracic bristle. The present fate map is consistent with that of Kankel and Hall (1976), with respect to the location of nervous system and alimentary landmarks relative to cuticle landmarks. This consistency extends even to the curious fact that the more dorsal thoracic ganglia sites (*in situ*) are located, on the map, in positions ventral to those for the ventral thoracic ganglia sites. Also, the thoracic ganglia landmarks are farther from thoracic cuticle landmarks than is the case for distances between brain landmarks and head cuticle landmarks. Recall, from the rough mapping of the behavioral foci with respect to cuticle structures, that male following and wing extension are more closely linked to the external head than is attempted copulation to the external thorax.

To map the behavioral foci with respect to the external and internal landmarks, the analytical system of Hotta and Benzer (1972)—as modified by the maximum likelihood method of Merriam and Lange (1974)—is inappropriate because their analysis depends on having mosaics which are, on the average, half male and half female. The *pal*-induced mosaics in the current study have external and internal structures which are XO with a probability of 0.32 ± 0.08 (close to the value of 0.34 ± 0.06 from *pal*-induced mosaics obtained by Kankel and Hall, 1976). When the statistical treatment of Merriam and Lange (1974) was applied to an analysis of male following, neither a domineering nor a submissive focus model was consistent with the data, based on huge χ^2 values obtained for several external and internal landmarks (*cf.* Merriam and Lange's analysis, where the mosaics in different classes—e.g., left brain site male, homologous right brain site female, and behavior male—would be compared to expectations generated from a given model). Most of the contributions to χ^2, for a given pair of landmarks, came from the fact that there are many more mosaics with both homologous landmarks female compared to those with both landmarks

male. These types of mosaics are equivalent, for half male–half female mosaics (Hotta and Benzer, 1972).

A "contour" mapping procedure was applied to the current data. This was developed by Y. Hotta (unpublished) and modified by Feitelson and Hall (1977). A computer selects the mosaics which did not show male behavior (at least following of females), then calculates the probabilities that all scored landmarks are haplo-X. These probabilities (which are different for domineering and submissive models) are fractions computed for each landmark pair as follows: Domineering: for a given mosaic, 2 is added to the numerator if the left or homologous right landmark is XO, and 2 is added to the numerator if both are XO; 2 is added to the denominator for each mosaic. Submissive: 2 is added to the numerator only if both left and right landmarks are XO, and 2 is added to the denominator each time. Contour maps are prepared by connecting landmark positions (Fig. 3) which have roughly the same probability of being XO. The contour lines will converge on the focus such that lines connecting landmarks with the smallest probabilities are nearest the focus.

If the domineering model does not lead to a localization of the focus—where one or a few closely bunched landmarks have a similar and low probability of maleness—then the model is possibly invalid, and the submissive model may yield a convergence. This situation obtains for contour mapping of a mutant which is hypersensitive to mechanical shock (i.e., the domineering model had landmarks with similar probabilities spread all over the fate map, but the submissive model gave a definite convergence: Fei-

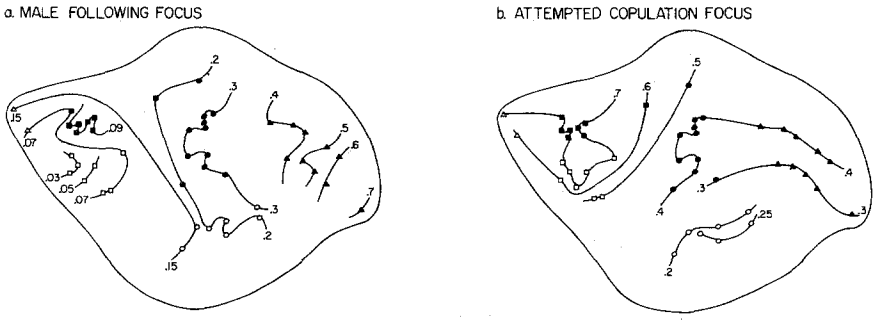

Fig. 4. Contour mapping of male following (a) and attempted copulation (b) foci. These two maps have the external and internal landmarks as shown in Fig. 3 (with the same kind of symbols for different kinds of tissue). Lines are drawn connecting landmarks with similar probabilities of maleness among mosaics which did not show the behavior at issue: the numbers associated with the lines are these probabilities. The line associated with the smallest probabilities is closest to the focus (according to the principle of Y. Hotta, unpublished). The probabilities were calculated based on the "domineering" focus model, which assumes that, for instance, male tissue in only the left or right side is sufficient to allow for the behavior in question. From Feitelson and Hall (1977).

telson and Hall, 1976). If the domineering model gives a convergence, then the submissive model will as well.

The domineering model of contour mapping for male following led to a localization of the focus to dorsal male brain sites (Fig. 4a). No one landmark has a zero probability of maleness, which is a reflection of the fact that a few mosaics with all-male brain tissue, or with all-male brain tissue on left or right side, did not exhibit male behavior (Table II). Subesophageal ganglia, optic ganglia, and head cuticle landmarks are not far from the focus, but the maleness probabilities for these sites are at least twice as great as for supraesophageal ganglia landmarks.

For the attempted copulation focus, the mosaics which had followed females were preselected, and the domineering contour mapping model was applied (Fig. 4b). This led to lines with the lowest probabilities (none of which was very low) which ran through the thoracic ganglia, as if the focus were diffusely located in these ganglia. Submissive models were run on both following and attempted-copulation foci, and these results led to the same localizations as for the domineering models.

DISCUSSION

Performance of Male Courtship by Mosaics

Only about one-third of the mosaics here showed male courtship behavior, compared to the value of 50% found by Hotta and Benzer (1976). The reason for the difference is likely that the mutant *pal* induces sex mosaics, among which the probability of structures (including brain sites) being male is about one-third. The probability for a structure being male in the mosaic-generating system of Hotta and Benzer (1976) is about one-half.

Several of the mosaics showing male behavior performed it more feebly than did control males. For example, wing extension was not always bilateral or a full 90° extension. Clark and Egen (1975) have made the same qualitative findings with respect to wasp mosaics tested for courtship. It is possible that the tissue marker mutations in the system cause these cases of debilitated behavior by mosaics, yet the data from control males argue against this trivial explanation. It may be that parts of the CNS of males and females have different connectivity, which is indeed related to their obvious differences in reproductive behavior. Thus certain dividing planes between male and female tissue in the brain may not only lead to the all-or-none ability vs. inability to perform male courtship, but may disrupt parts of the "male" wiring for mosaics genotypically capable of courting as a male, and so lead to malelike behavior which is defective. Further work on this problem must initially involve the quantification of the amounts of time

spent by mosaics in following, wing extension, and attempted copulation, in addition to testing for possible quantitative abnormalities of courtship wing vibration done by the mosaics. For instance, it could be that mosaics which perform wing extension use an abnormal courtship song, or none at all (see review of Bennet-Clark and Ewing, 1970). Whereas male tissue in the brain controls wing extension, it could be that the focus for a normal song is in the thoracic ganglia.

Brain Control of the Initiation of Male Courtship

The data presented here lead to the conclusion that male brain tissue is required for male courtship behavior since no mosaics with all female brains initiated courtship. The data also suggest that only part of dorsal brain need be of this genotype. The results here are different from those obtained from wasp sex mosaics. Clark and Egen (1975) found that male tissue in the head of *Habrobracon* is sufficient, not only for the initiation of male courtship behavior, but also for the subsequent steps (excluding, of course, successful copulation).

A definitive localization of the control center for the initiation of *Drosophila* male courtship has not yet been obtained, because no exact part of the dorsal brain could be correlated one-to-one with male behavioral control. Also, it is not clear if the foci for following and for wing extension are in one particular portion of the brain or are only close to one another. Finally, since 10% of mosaics with all-male brains did not court females, it is possible that male tissue in the dorsal brain is necessary but not sufficient to allow following of females and wing extension at them.

In spite of the above problems, the dorsal-brain control of male following and wing extension in *Drosophila* is analogous to what is found for the control of courtship song in crickets and grasshoppers (reviewed by Huber, 1965, 1967; Elsner, 1973). Here, stimulation and ablation experiments implicate the corpora pedunculata as sites of control. Moreover, intact corpora pedunculata in only the left or right side of the brain are sufficient to allow a normal song. In *Drosophila,* it may be that the dorsally located left or right corpora pedunculata are the brain regions which must be male in order that courtship be initiated.

Another possibility relating to the domineering following and wing extension focus in the dorsal brain concerns neurosecretory cell clusters there (Köpf, 1957). A diffusible substance, produced by male neurons, on the left or right side may be able to influence the activity on the other side. Thus a male brain might have connectivity identical to that in the female, but the intracellular quality of neurons could be crucial, and this could be determined by male genotype.

Thoracic Ganglia Control of Attempted Copulation

A substantial portion of the mosaics which initiated courtship did not progress to the later stages (*cf.* Clark and Egen, 1975). Those mosaics with genetically male head and thorax tissue tended to show later as well as early courtship actions. Neuronal activity in ventral ganglia of insects can endogenously control copulatory movements of the abdomen (e.g., as reviewed by Roeder, 1967). The data presented here implicate the *Drosophila* thoracic ganglia as such control centers for copulatory movements, although the control is not completely endogenous in that male thoracic ganglia tissue plus male brain tissue is required. The apparent ability of male neurons anywhere in the thoracic ganglia to lead to excitation of abdominal nerves is puzzling. It would appear that hypothetical "male wiring" in a particular thoracic ganglion region is not at issue, but rather that male neurons can operate locally and can influence the activity of other parts of the ganglia by receiving or sending out signals, or possibly through the activity of male-specific substances. Clarification of these results on attempted copulation may come from observations of mosaics where the amount of time spent in attempted copulation is recorded and where attempts are made to break this behavior down into components (e.g., abdomen curling plus genital–genital contact vs. curling only). Also, it could be that male tissue in the brain, in the thoracic ganglia, and in some other unidentified part of the animal is required for attempted copulation. For instance, an analysis of sex comb presence and thoracic ganglia maleness in mosaics may reveal that the attempted copulation focus includes both these tissues. This notion is based on the report of Cook (1975) that surgical removal of sex combs inhibits attempted copulation. Preliminary results (J. Hall, unpublished) are not what one would predict from Cook's findings: 20% of male-behaving mosaics with no sex combs do attempt copulation. Finally, it could be that an abdomen which is all or mostly female may not be able to manifest attempted copulation even if directed to do so by male thoracic ganglia tissue. This could be due to mechanical constraints imposed by gravid ovaries.

Elicitation of Courtship by Mosaics

Female tissue nearly anywhere on the abdomen will usually trigger courtship of a mosaic. This could mean that, when a male taps another fly before beginning to court (e.g., reviewed by Spieth, 1974), he must touch female cuticle and thereby receive information on the sex of the fly through chemoreception of a female-specific surface substance. Another possibility is that female tissues not on but in the abdomen produce the female-specific

courtship-stimulating pheromone (Shorey and Bartell, 1970; Averhoff and Richardson, 1974). Thus the rough mapping of the elicitation focus in the abdomen—but neither in a particular part nor necessary in any part of the cuticle—may lead to an identification of internal tissues whose genotype is relevant for stimulating courtship behavior. Glands which produce sex pheromones in insects are, indeed, frequently located in the abdomen of females (reviewed by Leonard *et al.*, 1974).

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