CONTROL OF MALE REPRODUCTIVE BEHAVIOR BY THE CENTRAL NERVOUS SYSTEM OF DROSOPHILA: DISSECTION OF A COURTSHIP PATHWAY BY GENETIC MOSAICS[1]

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ABSTRACT

In gynandromorphs of Drosophila, a detailed examination was made of the association between male courtship behavior and the chromosomal genotype of various parts of the central nervous system. Mosaic flies that behave as males repeatedly show a shorter courtship than normal males. If there is to be male behavior, the posterior dorsal brain must be haplo-*X* on at least one side for occurrence of the early courtship events. tapping, following of females and wing extension. Licking (proboscis extension) has nearly the same focus but is submissive; that is, male tissue must be present in both left and right dorsal brain. The next courtship step, attempted copulation, has a focus (especially for actual genital contact) located in the thoracic ganglia, though apparently not in a discrete region. Attempted copulation, which can occur even in mosaics with a gravid abdomen, may be correlated with the presence of sex combs. The role of courtship foci are interpreted in terms of known sensory inputs to and functions of the major insect ganglia.

CERTAIN portions of the central nervous system (CNS) of *Drosophila melanogaster* control different features of male reproductive behavior. This conclusion comes from investigations of genetic mosaics that are part male, part female (HOTTA and BENZER 1976; HALL 1977; NISSANI 1977; COOK 1978; and earlier work reviewed by MANNING 1967a). Courtship behavior of these gynandromorphs was observed, and their distributions of male and female tissue in the CNS were determined by use of a cell marker in one of these studies (HALL 1977). It was found that male-specific behavior was correlated with a haplo-*X* genotype in the left or right side of the brain. Such courtship nearly always included orientation toward and following of the female, along with unilateral wing extension directed at her. However, these courting mosaics frequently did not attempt copulation; if they did, there was always some male tissue in the ventral thoracic ganglia (HALL 1977). More recently, SCHILCHER and HALL (1979) have mapped the actual wing vibration in courtship (which produces the species-specific song) to the thoracic ganglia. Only the left or right ventral nervous system needs to be male for normal sonic output from both wings.

The current paper extends the foregoing studies by (1) mapping the foci for

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additional male behaviors (which were impossible to observe by the earlier, cruder techniques) (2) asking if mosaics are quantitatively defective in courtship, in addition to being qualitatively blocked in many cases (for example, a mosaic may follow a female and perform its wing display to her, but not show any attempted copulation), and (3) attempting to rule out false-negative mosaics —the few cases with male tissue in the brain, but no male behavior (HALL 1977)—through more rigorous behavioral testing.

As a result of these further experiments, finer localization of the courtship foci has been achieved. The purpose was not to repeat the fate mapping of male courtship foci by an analysis of all gynandromorphs that could be produced from a given cross, but was instead to analyze in detail the internal tissue of selected mosaics, especially those behaving as males. The overall findings allow extensions of the earlier suggestion (HALL 1977) that the "mushroom bodies" in the fly's dorsal brain control the initiation of male courtship.

MATERIALS AND METHODS

Sex mosaics were produced by induced somatic loss of an *X* chromosome from initially *diplo-X* zygotes. Males that were *X·Acph-1+/y+Y; pal/pal; Acph-1*^{n11}*/Acph-1*^{n11} were crossed to *y/y; Acph-1*^{n11}*/Acph-1*^{n11} females (*cf.*, HALL 1977). Males tissues, resulting from *pal*-induced loss of the *Acph-1+*-bearing *X* chromosome in a small fraction of the progeny that are mosaics, are marked externally by the yellow (*y*) bristle mutant and internally by the acid-phosphate-null (*Acph-1*^{n11}) mutant. After behavioral scoring, the external distribution of male and female tissues was drawn. Internal mosaicism was noted on score sheets after serial sectioning and histochemical staining for acid phosphate, as described in KANKEL and HALL (1976).

The flies were raised, collected and stored as described by HALL (1977). Mosaics, control males, and females used to assess male behavior were raised and stored in the same manner. After storage, behavioral testing was done more extensively and in more detail than previously. Each mosaic was first observed for 20 min with the naked eye in the presence of a female (wild type, or attached-*X*, *y f*) in the relatively large plastic chambers used before (volume *ca.* 0.4 cm^3, HOTTA and BENZER 1976; HALL 1977). For this test (Test 1), following of females, wing extension at them (left and/or right) and gross attempted copulation were readily scorable (*cf.*, HALL 1977). In addition, the fraction of time spent by a mosaic in total courtship (including any and all courtship actions) was recorded as described by HALL (1978). Each mosaic was tested a second time the next day, again for 20 min; however, a different virgin female was used, as well as a smaller chamber (volume *ca.* 0.2 cm^3, HALL 1978) in which courtship interactions could be scored at 10× magnification. For this observation (Test 2) tapping, licking, and components of attempted copulation were readily observable, in addition to the behaviors recorded in Test 1. For attempted copulation, the separate components recorded were abdominal curling only (by the mosaic) or abdominal curling that included contact of mosaic-with-female genital regions. The time spent in courtship was also recorded in Test 2.

The usual control males were *XY* brothers of the mosaics; these males expressed the same genetic markers as in the male tissues of the mosaics. In addition, a few *XO* control males were produced that carried the same markers as the *XY* control males. The *XO* males were a small fraction of the progeny from the cross used to generate mosaics (see above and BAKER 1975). Both kinds of control males had their behavior recorded in the two separate tests described above.

RESULTS

Focus for male behavior in general

Most of the mosaics chosen for the current analysis of sex behavior and CNS

genotype had male tissue externally on the head. The great majority of such mosaics were expected to court as males (HOTTA and BENZER 1976; HALL 1977).

Mosaics not behaving as males: Several mosaics did not behave as males. Table 1 shows the genotype of different parts of the ganglia in the head for these 58 mosaics. For each pair of sites in the anterior CNS, the majority of these non-courting mosaics had a female genotype in both the left and right side of the brain (a "site" is an arbitrarily labelled region of the cortex of the ganglion in the head, from a particular plane of section; see KANKEL and HALL 1976). The tissues least frequently male are in the dorsal brain, *i.e.*, for the sites labelled SP 1, 2, 3, and 13. The other tissues in more ventral portions of the supra-oesophageal ganglion, in the sub-oesophageal ganglion (SB), and in the optic lobes (OG) are male on the left and/or right side, with substantially higher probabilities (20 to 40%). The sites with the lowest maleness probabilities within the dorsal supra-oesophageal ganglion tend to be in the more posterior tissues (Table 1).

TABLE 1

Genotype of sites in the anterior CNS, for mosaics not behaving as males

Sites in the brain	♂/♂	♂/♀	♀/♀	Domineering contour value for focus
Dorsal brain				
SP1	2	2	54	0.07
SP2	0	5	52	0.09
SP3	0	3	54	0.05
SP11	9	13	36	0.38
SP12	4	15	37	0.34
SP13	0	1	56	0.04
SP21	7	15	36	0.38
SP22	2	20	35	0.39
SP23	1	8	48	0.16
SP24	4	6	48	0.17
Ventral brain				
SB1	4	14	37	0.35
SB2	5	15	36	0.37
Optic lobes				
OG1	8	2	47	0.18
OG11	9	2	47	0.19
OG23	9	2	46	0.19

Fifty-eight mosaics were analyzed. The sites in the supra-oesophageal ganglion are designated SP: the lower numbers are most dorsal, and SP2, 3, 13, and 24 are in the posterior cortex. SB and OG represent sites in the sub-oesophageal and optic ganglia, respectively. All of these designations are from KANKEL and HALL (1976, their Figure 3). "♂/♂" means that, for a given part of the brain, both the left site and the homologous site in the right brain were male; "♂/♀" means that the left site was male, the right female, or *vice versa*; "♀/♀" refers to sites that were female in both the left and right brain. A very few pairs of sites among the mosaics were: one side male (*e.g.*, left), the other side of mixed genotype; one mixed, one female; both mixed; or too difficult to score. These rare cases are not listed (thus the totals are not always 58). The "domineering contour value" is the fraction of mosaics with respect to a given pair of sites that are both male plus those that are one male, other female. The *lowest* values, are nearest the focus controlling male courtship, since these mosaics did not behave like males (*cf.*, HALL 1977).

However, these maleness values are not zero: 5% of the noncourters had male brain tissue at site SP3 in at least the left or right side of the CNS. These mosaics did not court even though given two trials to do so. Yet, even the all-male controls failed to show females in a similarly small fraction of their tests (see below).

The notion was entertained that the abdomen, as well as the head, should usually be male for vigorous male behavior since the abdomen contains the focus for female sex appeal (NISSANI 1975, 1977; HOTTA and BENZER 1976; HALL 1977), perhaps related to a sex pheromone produced by these posterior female tissues. A mosaic with a male brain, but all or part of the abdomen female, could become habituated to the putative pheromone and thus not court when eventually put with a regular female in the current experiments. However, there was no tendency for greater-than-normal amounts of female tissue to be found in the abdomens of the male-brain cases that showed no male behavior. For example, among the noncourting mosaics with abdomens that were either uniformly male or uniformly female, 44% had all-female abdomens; for the male-behaving mosaics with abdomens of uniform genotype, a higher proportion, 73%, had all-female abdomens.

Mosaics showing male behavior: The courtship behavior of control males was quantified for subsequent comparison with mosaics that behaved as males. Of 68 such XY males observed in chambers, 66 followed, and performed wing extension at females. Of the 66 courting males, 64 had tapped before following, and 59 licked a female (extension of the male proboscis toward the female's genitalia); 60 males attempted copulation (one of these had not licked), but only 41 achieved genital contact; of these, 37 were able to copulate. All the above numbers refer to males that performed the above steps at least once during the two successive 20-min observation periods. It should be noted, however, that tapping, licking and genital contact are observable only during the one trial that involved magnification; thus, while the fraction of these control males that followed females is maximal, from the aggregate behavior in two trials, the fraction that licked might have been higher had both trials been observed at 10×. The mean fraction of time spent courting by the *XY* control males in Test 1 was .79 ± 0.12 (± standard deviation); and this value for Test 2 was 0.82 ± 0.20. The variation in performances of individual males, from trial to trial, appeared not to be due to individual differences since the courtship times of individual males were not positively correlated: Spearman's correlation coefficient (r_s) was in fact −0.09, but was not significantly negative ($p > 0.05$). A control was performed with *XO* males (see MATERIALS AND METHODS) and gave similar results. Of 20 such males tested, all tapped, followed, performed wing extension, licked and curled their abdomens in attempted copulation. Fourteen of the males achieved genital contact, and none copulated. The mean fraction of courtship time, for Test 1 was 0.72 ± 0.16, and for Test 2 0.76 ± 0.21. These values were not significantly different from those obtained with males carrying a *Y* chromosome ($p > 0.05$) Mann-Whitney test, two-tailed); also, as in the *XY* male control, there was no positive correlation in courtship times from Tests 1 and 2 ($r_s = -0.20, p > 0.05$).

For marked *XY* control males observed with females in vials, the courtship behavior was qualitatively no different from that seen in the small plastic chambers, although the fraction of time spent courting was less: 0.34 ± 0.19. This appeared to be due to the fact that the male-female pairs in the relatively large vials (*ca.* 18 cm^3) were frequently able to break contact as they moved about. Thus, timing data from control males and mosaics tested in chambers may have inflated values, if the behavior in vials can be considered to approximate natural conditions more closely.

The male-behaving mosaics tested in chambers behaved much differently from the control males tested the same way. Of 180 mosaics showing some male behavior, seven performed only tapping and following; 170 exhibited tapping, following, and wing extension; and three followed and extended wings in the absence of previous tapping. Only the wing-extending cases (N = 173) proceeded to later steps in courtship: 33 ceased courtship at this step (on both trials); 38 went as far as licking (on at least one trial) but no further, 101 went on to attempt copulation, but only 33 of these achieved genital contract. Finally, there was one mosaic that, after tapping, following and wing extension, did attempt copulation without any previous licking. This anomalous behavior is not necessarily interesting in this mosaic, because it is occasionally seen in a control male (see above).

In the two different trials for each mosaic, the results were qualitatively very similar. Occasionally, males were observed that went one step further in the courtship sequence during one of the trials: *e.g.*, through licking in one trial and through attempted copulation in another. However, no cases of grossly different behavior were noted: *e.g.*, tapping and following only in one trial, but a progression through the behavioral sequence to attempted copulation in the other. For the seven anomalous cases(not seen in the controls) of tapping and following but no wing extension, all exhibited this behavior in both trials. These mosaics were not vigorous courters (mean courting fraction 0.03 ± 0.02). For the analysis of mosaicism in the CNS, as correlated with the various steps in courtship, the mosiacs were put into different behavioral categories (*e.g.*, through attempted copulation *vs.* through licking only, see below), based on the maximum behavior of each individual, that is, the farthest step in the courtship sequence achieved in either trial.

The quantitative courtship performances of individual mosaics were also very similar, in spite of the different observation chambers used in the separate tests. For Test 1, the mean fraction of time spent courting was 0.36 ± 0.32; for Test 2 this was 0.32 ± 0.31. For individual mosaics, the quantitative vigor of courtship was highly correlated from trial to trial: $r_s = 0.82$, $p < 0.01$. This result is very different from the "random" variation seen in the values for Tests 1 and 2 of control males. The mosaics obviously differ from the controls in other ways: (1) The overall courtship times are less for the male-behaving mosaics than for males; and this is not due trivially to the fact that many mosaics fail to proceed to later steps in courtship, because the mosaics that attempted copulation still had significantly lower values than the controls (mean fraction of time spent court-

ing for such mosaics is 0.49 ± 0.24). (2) There is more variation among the mosaics, as reflected in the higher standard deviations, and the fact that 8% of the mosaics showed no courtship at all during one trial; by definition, these cases courted during their other trial, but the mean fraction of courtship time for these individuals was low, 0.04 ± 0.04 (as might be expected from the high correlation of courtship times for the different trials of the mosiacs in general).

Internal mosaic analysis of male-behaving mosaics: The data from histochemical scoring of gynadoromorphs that did show male behavior are listed in Table 2. Sites in the dorsal brain are more frequently male than sites in more ventral regions of the brain (including the sub-oesophageal ganglion) or in the optic lobes. Only one brain region was never female on both sides of the brain. This is in the dorsal, posterior cortex of the supraoesophageal ganglion, site SP3. In spite of the absence of bilateral femaleness in this site, SP3's (and all other brain sites as well) were frequently male on one side and female on the other.

SP1, another site in the dorsal brain near but anterior to SP3, was bilaterally female with as high a frequency as ventral brain sites (Table 2). The other nearby dorsal site, SP2, is a posterior one, and this region was very rarely bilaterally female. Figure 1 shows a section from the brain of a male-behaving gynandromorph in which the anterior dorsal brain is bilaterally female, but the posterior tissues are male on one side.

TABLE 2

Genotype of sites in the anterior CNS, for mosaics behaving as males

Sites in the brain	♂/♂	♂/♀	♀/♀	Frequency both sites female
Dorsal brain				
SP1	91	63	19	0.11
SP2	115	54	4	0.02
SP3	127	47	0	0
SP11	104	65	9	0.05
SP12	85	87	6	0.03
SP13	83	80	11	0.06
SP21	99	68	7	0.04
SP22	80	88	7	0.04
SP23	85	78	16	0.09
SP24	98	67	7	0.04
Ventral brain				
SB1	66	85	22	0.13
SB2	59	91	21	0.12
Optic lobes				
OG1	117	44	19	0.11
OG11	118	44	18	0.11
OG23	116	44	17	0.10

One-hundred and eighty mosaics were analyzed. The sites in the supra-oesophageal, sub-oesophageal, and optic ganglia are designated as in Table 1. The very few cases for which individual sites on one or both sides were of mixed genotype are again not listed. For the values under "frequency both sites female," the lowest values are nearest the focus controlling male behavior, and indeed the dorsal, posterior site SP3 is never bilaterally female.

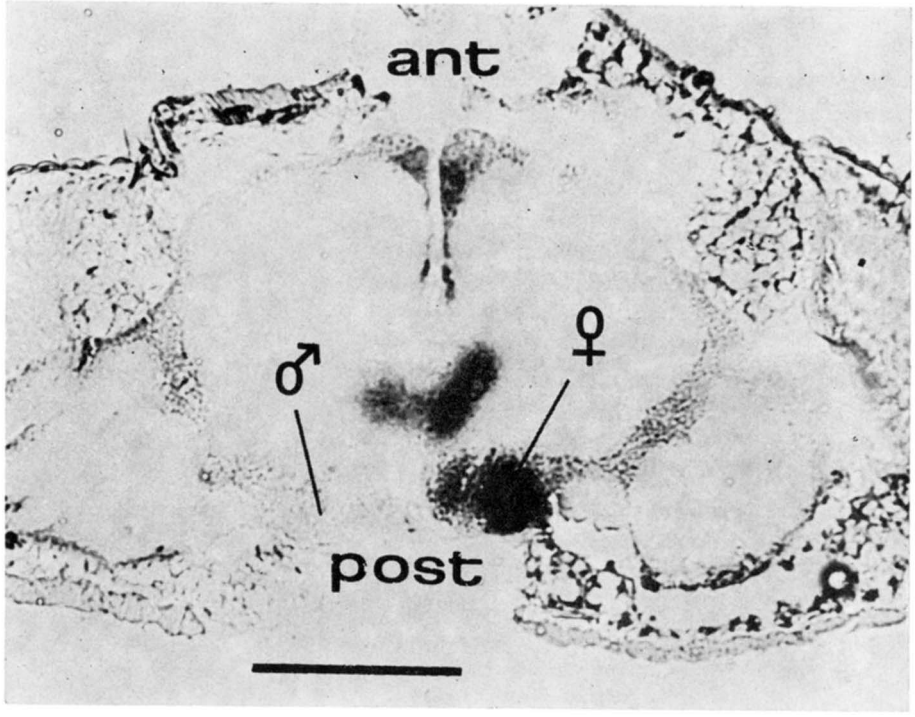

FIGURE 1.—Male-female mosaicism in the brain of a male-behaving Drosophila gynandromorph. This is a relatively dorsal, horizontal section (at 10 μm) through the brain of a mosaic that tapped, followed, and extended both wings at a female (there was neither licking behavior nor attempted copulation). The unstained cells in the cortex are male (♂), the stained tissues female (♀). The anterior parts of the brain (ant) were bilaterally female in this and other dorsal sections. The posterior sites (post) were female on the right side, male on the left. The bar represents 100 μm.

These findings put the focus for male behavior in the dorsal posterior brain. They also indicate, in conjunction with the data on nonmale-behaving mosaics (Table 1 and HALL 1977), that part of the dorsal brain must be male on only one side for male behavior to occur; yet maleness in this region (SP3) is not always sufficient for male behavior.

The focusing results just described are based on the qualitative considerations of mosaic behavior: did a gynandromorph show any male actions, no matter how brief, or none at all? The quantitative variability among male-behaving mosaics was also considered in terms of internal mosaicism in the brain. For all mosaics with qualitatively similar behavior (which did not progress as far as attempted copulation), there is a weak correlation between the overall degree of maleness in the dorsal brain (excluding the scored sites in the optic ganglia and suboesophageal ganglion) with the fraction of time spent courting: Spearman's $r_s = 0.22$ $p \simeq 0.05$. Consistent with this correlation is the fact that those mosaics with male tissue on both left and right sides of the brain tended to court more vigorously. But there were exceptions to this overall pattern: a case with

an entirely male brain whose courting fraction was 0.12; a case with one entire side of the brain male, the other side female and a 0.36 courtship value; finally, a case for which both left and right sides of the brain were mixed male and female with 0.40 courtship. All of these cases had male tissue in the critical region of the dorsal posterior brain on at least one side.

The poor qualitative performance of many of the courting mosaics would not seem to be due to the fact that the male tissues are *XO* and express the cuticular and internal markers (y and $Acph\text{-}1^n$), because the control males described earlier were not very defective at initiating courtship. Yet, the mosaics have another, possibly trivial, source of behavioral problems: the female tissues are slightly aneuploid, since one of the *X* chromosomes in all female cells has an $Acph\text{-}1^+$ duplication (KANKEL and HALL 1976) that causes a 25% decrease in viability of flies that carry it. Even though these aneuploid tissues are female, they could lead to nonspecific debilitation of the mosaics' behavior. This possibility seems to be ruled out by the following. Transformed males (diplo-*X*, but expressing the *tra* mutation, STURTEVANT 1945) were generated. Some carried the $Acph\text{-}1^+$ duplication on one of their *X* chromosomes; brothers of these males had two completely normal *X*'s. All of these transformed males were homozygous for the $Acph\text{-}1^n$ mutation, so that the genotype to be tested behaviorally was essentially the same as the female tissues in the mosaics. The males carrying the duplication had courtship value of 0.59 ± 0.27 ($N = 14$). Their brothers without the duplication had a value of 0.57 ± 0.27 ($N = 15$), which is statistically the same as the first value (Mann-Whitney test). Thus, aneuploidy in the courting mosaics is apparently not responsible for the behavioral deficits.

Focus for proboscis-extension behavior

A courtship action occurring after wing display, not previously analyzed with internally marked gynandromorphs, is proboscis-extension, or "licking." While a significant proportion of the male-behaving mosaics ceased their sex-specific actions at the stage of wing extension, another substantial group of mosaics progressed one further step, showing proboscis extension, but did not attempt to copulate. These findings could mean that the licking focus is in a part of the CNS separate from the foci for the preceding courtship steps. The hypothesized separability of the licking focus from the other behavioral foci is analogous to the separation, in mosaics, of the foci for wing extension and attempted copulation (*cf.*, HOTTA and BENZER 1976; HALL 1977).

A focus in the head for the control of licking would seem more likely than one in the thoracic nervous system (if only that the mouthparts are appendages of the head.) Indeed, a thoracic focus for licking could be quickly eliminated, because an appreciable proportion of each pair of neuromeres in the ventral ganglia were bilaterally female among gyndromorphs that did lick (as well as those that did not). In addition, 9% of the licking mosaics had entirely female tissue in their thoracic ganglia. The brain genotypes for the mosaics that did or did not lick are listed in Table 3. In general, there is much more male tissue in the brains of the mosaics that licked. More specifically, a given brain for the

TABLE 3

Licking behavior in male-behaving mosaics

Sites in the brain	No licking behavior ♂/♂	♂/♀	♀/♀	Submissive contour value for focus
Dorsal brain				
SP1	9	11	12	0.28
SP2	7	25	2	0.21
SP3	3	31	0	0.09
SP11	12	21	1	0.35
SP13	6	20	2	0.18
SP21	12	20	2	0.35
SP22	8	24	2	0.24
SP23	9	20	5	0.26
SP24	13	17	4	0.38
Ventral brain				
SB1	7	20	5	0.22
SB2	7	20	7	0.21
Optic lobes				
OG1	15	12	7	0.44
OG11	15	12	7	0.44
OG23	15	12	7	0.44
Dorsal brain				
SP1	82	49	7	0.59
SP2	108	26	2	0.79
SP3	124	12	0	0.91
SP11	91	39	7	0.66
SP12	79	56	4	0.57
SP13	77	59	3	0.55
SP21	87	46	5	0.63
SP22	71	63	4	0.51
SP23	73	57	9	0.53
SP24	84	50	3	0.61
Ventral brain				
SB1	58	63	16	0.42
SB2	50	71	14	0.37
Optic lobes				
OG1	100	27	12	0.72
OG11	102	27	10	0.73
OG23	100	28	9	0.73

For the mosaics that did initiate male behavior, the genotypes of brain sites are listed for the mosaics that did not lick (N=33) *vs.* those that did (N=139). As in the previous two tables, the sites in these ganglia are designated as in KANKEL and HALL (1976), and the rare sites for which one or both members of the pair were of mixed genotype or unscorable are not listed. The "submissive contour values" are merely the probabilities of bilateral maleness for these brain sites; for the nonlicking mosaics, the lowest values are nearest the focus, and for the licking mosaics, the highest values are nearest the focus (*cf.*, HALL 1977).

licking cases tends to be male in both left and right sides of the head (especially for the more dorsal sites, *i.e.*, approximately SP1 through SP13); but, for the nonlickers, there is an enrichment for sites that are male on one side, female on the other, or even female on both sides. The dorsal posterior site, SP3, could not

have been female on both sides of the brains in lickers and nonlickers since these mosaics showed some male behavior (*cf.*, Table 2). But it is in fact this site (SP3), and those nearby, that have the least likely chance of being even bilaterally split (one side male, one side female) among the mosaics that extended their probosces.

Thus, the licking focus is in or near the dorsal posterior brain. It is largely submissive, since licking usually requires that male tissue be present in both homologous parts of the brain cortex. The focus, though, is not submissive in an absolute sense, because 9% of the SP3 sites were bilaterally of opposite genotype in the mosaics that licked. It could be, however, that the individual flies with one of the SP3 sites female have a nearby site male, and thus did have male tissue in the dorsal brain on the left and right sides. This possibly was ruled out by expressing the mosaic data in a different way from Table 3. That is, about 8% of the mosaic individuals that licked had an all-female supra-oesophageal ganglion on one side (Figure 2). Obviously, they did not require dorsal male tissue on both sides for this behavior. Nevertheless, the rest of this analysis shows that the nonlicking individuals are much more likely to have split brains, or even brains that are all-female on one side and mixed genotype on the other (Figure 2). This pattern is consistent with the data in Table 3, pointing toward

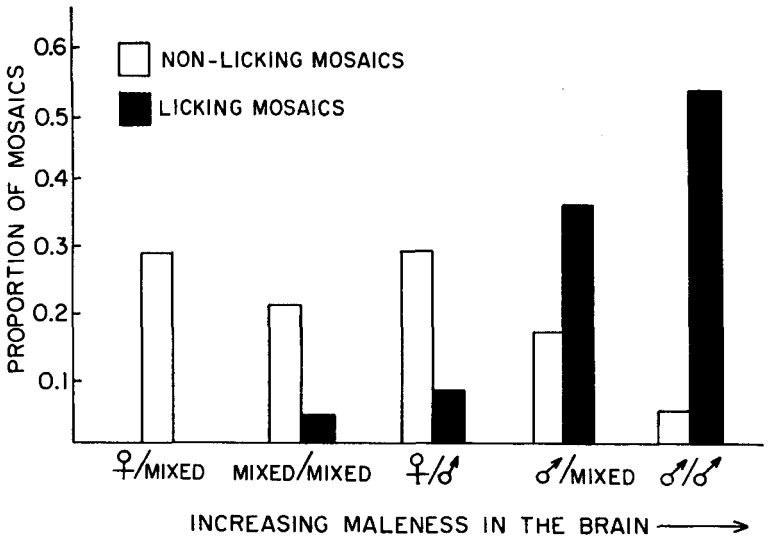

FIGURE 2.—Proboscis extension (licking) by Drosophila mosaics, as a function of left-right genotype in the dorsal brain. The male-behaving mosaics of two behavioral categories—licking (solid bars) *vs.* nonlicking (open bars)—were separated into five rough categories of brain genotype. Thus, the overall pattern of mosaicism was considered for these gynandromorphs, for which the more detailed data on licking are in Table 3. Here, the abcissa shows the five overall genotypic categories, for the supra-oesophageal ganglion, for which "Mixed/♀" (for example) means left side of the brain of mixed genotype, right side female; or left side female, right side mixed. For each of the two behavioral categories here, the bars express the proportion of mosaics distributed among the five genotypic classes; thus for a given behavioral category, the proportions sum to 1.0.

a partially submissive focus. A fly with male tissue in both sides of the brain will not always lick (*cf.*, results from control males), but the positive occurrence of licking in a few of the mosaics with bilaterally split brains may be more significant than the rare negative cases.

Focus for attempted copulation

The late stage of male courtship, involving a curl of his abdomen toward the female's genitalia, was investigated in more detail than previously. The current study was designed to clarify some of the issues having to do with the connection between thoracic maleness and attempted copulation. For example, virtually all mosaics that attempted copulation had licked previously, suggesting that licking is a prerequisite for the next stage. In the previous study (HALL 1977), there were very likely false negatives: *i.e.*, mosaics that should have been able to attempt copulation (given their thoracic maleness), but did not, because they lacked male tissue in both sides of the brain and thus had not licked. Since the current mosaics were observed microscopically, the nonlicking cases were excluded from further analysis of their thoracic genotypes. For instance, 13% of the nonlicking mosaics had all-male thoracic ganglia (a category otherwise associated with vigorous attempted copulation).

The data in Table 4 show that there is much more male tissue in the thoracic ganglia among the licking mosaics that did attempt copulation than among those that did not. From this general picture, one can note further that (1) the pair of abdominal ganglia are most frequently female in both the left and right sides of the posterior thorax among mosaics that did attempt to copulate; these sites (TG1, 13, 24, 32, and 43) are in the ganglion that innervates the abdomen, though the sites are designated "TG" since this posterior-most ganglion is fused to the other ganglia in the thorax (*cf.*, KANKEL and HALL 1976); these posterior tissues can almost certainly be excluded as possible sites for the focus, (2) certain sites in the thoracic ganglia *per se* (especially TG11 and TG21—prothoracic; TG22—mesothoracic; TG23 and TG42—metathoracic) are very frequently male on both sides of the thorax, and these are the sites that are most rarely female on both sides, and (3) many more of the sites in the thoracic ganglia for mosaics that attempted to copulate are male on both sides, compared to the pattern of mosaicism in the mosaics that ceased male behavior at the licking stage. Many sites, however, for the copulation attempters are bilaterally split in genotype, and these left-right male-female dividing lines are frequently observed among the mosaics that did not attempt to copulate.

In summary, the abdominal ganglion is almost certainly not involved in the male focus for attempted copulation, but there is no clear-cut single focus for this behavior elsewhere in the ventral nervous system. However, the notion of "diffuse" focusing suggests that there are a relatively small number of sites in the thoracic ganglia whose male genotype may control this behavior. These sites run from anterior to posterior, and from dorsal to ventral regions. Male tissue on both sides of the thoracic ganglia is infrequently associated with a failure to attempt copulation, but male tissue on only one side seems sufficient to allow

TABLE 4

Attempted copulation in male-behaving mosaics

Sites in the thoracic ganglia	No attempted copulation ♂/♂	♂/♀	♀/♀	Frequency both sites female
Dorsal sites				
TG1	1	13	24	0.63
TG11	1	28	9	0.24
TG12	6	20	10	0.28
TG13	1	14	23	0.61
Mid-ganglia sites				
TG21	1	29	8	0.21
TG22	4	22	11	0.30
TG23	4	25	9	0.24
TG24	0	14	23	0.62
TG31	4	22	11	0.30
TG32	0	14	24	0.63
Ventral sites				
TG41	0	25	11	0.31
TG42	4	24	9	0.24
TG43	0	15	23	0.61
Dorsal sites				
TG1	10	48	40	0.41
TG11	28	61	8	0.08
TG12	23	59	12	0.13
TG13	10	47	40	0.41
Mid-ganglia sites				
TG21	30	59	9	0.09
TG22	25	64	4	0.04
TG23	24	63	4	0.04
TG24	8	55	33	0.34
TG31	31	48	15	0.16
TG32	6	57	35	0.36
Ventral sites				
TG41	25	50	20	0.21
TG42	28	54	9	0.10
TG43	6	54	38	0.38

For the mosaics that did proceed to the licking stage of courtship, the genotypes of thoracic ganglia sites are listed for the mosaics that did not attempt copulation (N=38), and those that did (N=101). As for the sites in the brain, the designation of these locations in the ventral nervous system are defined in KANKEL and HALL (1976): TG11, TG21 are in the pro-thoracic ganglion; TG12, 22, 31, 41, are in the meso-thoracic ganglion; TC23, 42 are in the meta-thoracic ganglion; and TG1, 13, 24, 32, 43 are in the abdominal ganglion (fused to the other three more anterior ganglia). The lower numbers within a ganglion are more ventral. The other features of the data in this table are as explained in the legends to Tables 2 and 3.

attempted copulation to occur in some cases and not in others. The focus, then, is not clearly domineering or submissive, which is correlated with its general diffuse nature. The diffuse character of the focus implies both that any of several portions of the thoracic ganglia, if male, will allow attempted copulation; and that if large portions of the thoracic ganglia happen to be male, there is a greater chance of attempted copulation occurring (see below).

Additional information on bilateral mosaicism and attempted copulation is derived from a comparison of mosaics that only curled their abdomen toward the female, and those individuals that were able in addition to achieve genital contact. All thoracic sites in the gynandromorphs whose attempted copulation was limited to an abdominal curl have some probability of being bilaterally female: here, the lowest probabilities are associated with sites TG22 and TG23 (Table 4). These two sites were never bilaterally female in the mosaics that both curled their abdomens and contacted females. Moreover, the highest frequency of mosaics with bilateral maleness in the thoracic ganglia is in the category that achieved genital contact (Figure 3). These kinds of mosaics are absent in the category that attempted no copulation at all. Indeed, 23% of these mosaics in that category had completely female tissue in both sides of their thoracic ganglia (Figure 3).

The internal mosaicism in the mosaics studied intensively in regard to the late

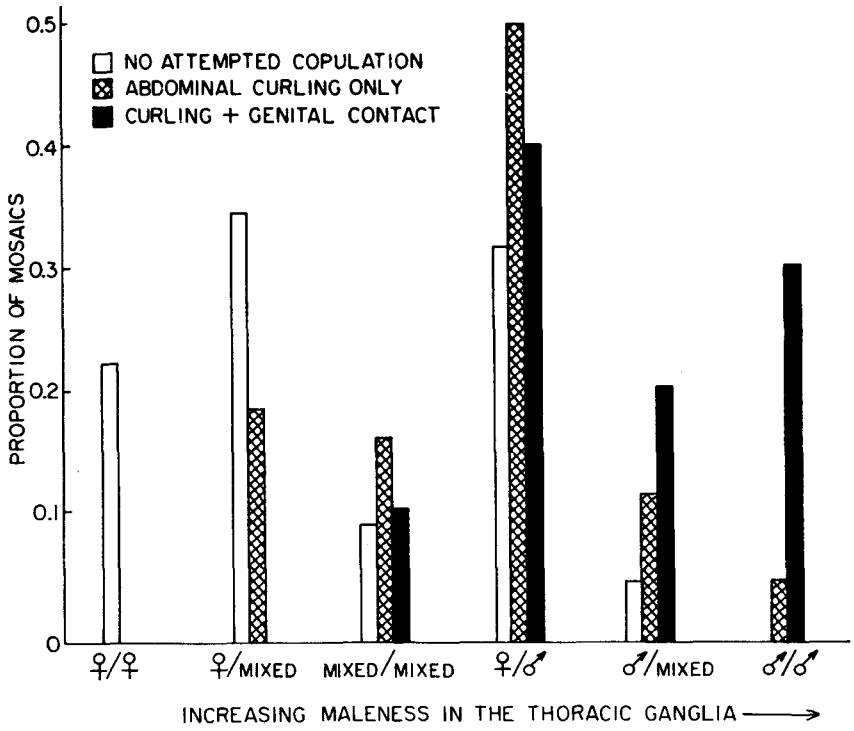

FIGURE 3.—Attempted copulation by Drosophila mosaics, as a function of left-right genotype in the thoracic ganglia. The mosaics that did progress to the licking stage were further broken down into cases that showed no attempted copulation (open bars), abdominal curling only in their attempts to copulate (cross-hatched bars), and those that showed curling plus genital contact (solid bars). The overall patterns of mosaicism in the thoracic ganglia as a whole were then separated into genotypic classes analogous to those in Figure 2. For each of the three behavioral categories, the bars express the proportion of mosaics distributed among the six genotypic categories.

stage of courtship have not resolved all the difficulties raised earlier. Thus, the existence of a "point" focus is unlikely. However, it is the case that increasing degrees of male tissue are very highly correlated with increasing levels of performance of attempted copulation.

Other peripheral issues having to do with maleness associated with the ability to attempt copulation were dealt with as follows. COOK (1977) suggested that sex combs are important for actual genital contact in conjunction with attempted copulation, based on surgical experiments with nonmosaic males. In the current study, the copulation attempters that curled averaged 0.9 sex combs per individual, including 15% with two sex combs and 22% with no sex combs. Clearly, these male parts are not required for the initial stages of attempted copulation (*cf.*, HALL 1977). The curling mosaics that also achieved genital contact had 1.7 sex combs per individual: none lacked sex combs entirely, and 71% had two sex combs. It would seem, then, that either one or two sex combs favors the chance that a mosaic will progress to genital contact (confirming COOK 1977); or else that the presence of sex combs is simply correlated with a high degree of thoracic maleness in general (which is associated with vigorous attempted copulation). The latter notion would mean that sex combs on the vigorous copulation attempters is coincidental. If attempted copulation-with-contact requires one or two sex combs, then some mosiacs in the noncontacting category could be false negatives, in that maleness in their thoracic ganglia would otherwise have been sufficient for genital contact. Thus, excluding the sex comb-less cases from the curling-only category might cause the proportion of maleness in the thoracic ganglia of these mosaics to drop significantly (*cf.*, Table 4) and be more divergent in thoracic genotype than the mosaics showing genital contact. Yet, when this analysis was performed, no significant decrease in maleness for the curling-only class resulted.

Another feature of sexual dimorphism that might interfere with a straightforward interpretation of the attempted copulation results is the presence of many eggs in some of the mosiacs. Thus, a mosaic that perhaps should have attempted copulation (given its thoracic maleness) might not have been able to because of trivial mechanical constraints imposed by gravid ovaries. The presence or absence of eggs was noted in the scoring of internally marked mosaics; it was found that 52% of the mosaics with curling-only had substantial numbers of eggs on both sides of their abdomens. For the curling-plus-contact cases, 30% had many eggs on both sides of their abdomens. Clearly, gravid ovaries do not pose much of an impediment to high-level attempted copulation.

DISCUSSION

Defective behavior of gynandromorphs

Most sex mosiacs in Drosophila are defective in male courtship. The obvious defects have to do with the fact that a given gynandromorph has a high probability of simply ceasing its male behavior at some particular intermediate stage of the courtship pathway (Figure 4). Also, many gynandromorphs are defective in the individual male actions that they are able to perform. Recordings of these

actions showed that mosaics in general carry out these behaviors in a significantly more feeble fashion than regular males do. Quantitative differences in repeated tests of individual males show random variations; but, for the mosaics, there are differences among the individuals, whose measured performances are highly correlated from test to test.

The possibility was considered that quantitative defects in courtship are trivial results of (1) the genetic variants (yellow, acid phosphatase) used to mark male tissues in the mosaics, or (2) the genetic aneuploidy (duplication) present in about two thirds of the tissues in the average mosaic. The quantitatively vigorous courtship by nonmosaic males, with or without the small *Acph-1*$^+$ duplication, argues against these trivial explanations for the quantitative defects in the behavior of mosaics.

The sex mosaics may be defective in their overall courtship behavior for more interesting reasons than those related to the technology of generating and marking the gynandromorphs. For proper behavior, it may be important to have more than male neurons in a particular portion of the CNS. The connectivity among neurons may also be important. In the rat, there are sex-specific differences in the numbers of synapses of a particular kind in a part of the brain believed to be related to functional sex differences (RAISMAN and FIELD 1973). If there are male-specific (and female-specific) neuronal connections in Drosophila, then defects in the CNS could be present at interfaces between male and female axons. The neurons of different genotype might not simply develop independently of each other, but could interact as connections are being formed. Under this hypothesis there would be three general types of cell interactions in the developing Drosophila brain, depending on the sex of presumptive neurons: normal interactions involving only male cells or only female cells, and a third, novel interaction involving the simultaneous presence of male and female cells in mosiacs. Different mosaics, even those in the same category of genotype (*e.g.*, bilaterally split brain) could have different defects at male-female interfaces, and this could be related to the wide variation in quantitatively defective courtship seen among mosaics.

The implications of the above suggestions are related, first, to defective courtship reported in previous studies of mosaics. Some individual wasp gynandromorphs show male courtship behavior at prey (which are usually stung only by females) and stinging behavior at female wasps instead of prey (CLARK and EGEN 1975). Drosophila mosaics, in an unexpectedly high frequency, show extension of only one wing at a female (never using the other one in their courtship bouts (HALL 1977; SCHILCHER and HALL 1979). Worse, mosaics of this species (even if they extend both wings at a female) frequently have no courtship song associated with wing behavior; or, they may produce apparently random, "noisy" wing vibrations, bearing no resemblance to the normal pattern of sound pulses. These qualitative defects in courting mosaics may, too, be related to defective "wiring" in the CNS (such as an absence of certain necessary axon-axon connections, or "crossed wires" in the anomalous wasp cases). These results suggest that there is male-specific neuronal connectivity in sexually important

parts of the CNS. The axons forming hypothetical "male connections" may originate from the posterior cortex of the dorsal brain, since that is currently the best location for the focus that must be haplo-*X* if a mosaic is to show any male behavior. Parenthetically, it should be noted that cells in anterior dorsal brain (near brain site SP1) are not prime candidates for further analysis on possible sex differences related to courtship. This cluster of presumed neurosecretory cells has been found to be involved in pre-conditioning or controlling certain features of male sex behavior in insects (PENER, GIRARDIE and JOLY 1972; PENER 1974); but this involvement apparently does not extend to a requirement that the genotype of these cells in Drosophila be correlated with sex-specific behavior (Tables 1, 2).

For the posterior cells, on the other hand, the possibility should be raised that the dorsally located mushroom bodies have male-specific connections. In Diptera, many of the axons in the mushroom bodies originate from the dorsal, posterior cortex (STRAUSFELD 1976). This "association center" in the brain is also known to receive a great deal of olfactory input in a variety of insects (reviewed by HOWSE 1975, and also reported for a fly by STRAUSFELD 1976). Thus, the Drosophila male may need to receive pheromonal input from a female of his species, and may need to process this information in terms of connectivity from olfactory pathways to axons in his sex-specific mushroom bodies.

Drosophila sex pheromones have in fact been implicated in previous studies of courtship (SHOREY and BARTELL 1970; AVERHOFF and RICHARDSON 1974, 1976). More recently, volatile compounds, distilled and extracted from females, have been shown to stimulate courtship among males in the presence of the isolated compounds only, whereas a similar extract from males has no such stimulatory effects (L. TOMKINS, J. HALL and L. HALL, in preparation).

Other possibilities for important input into the male brain could originate from two other sources: (1) contact chemosensory, having to do with information received by a male from a female when he taps her with his tarsi, which possess contact chemoreceptors (*e.g.*, FALK and ATIDIA 1975), and (2) auditory, related to the possibility that the male may stimulate himself (as well as the female) with certain components of his courtship song (SCHILCHER 1976a). Tarsal chemoreceptors synapse with first-order interneurons in the thoracic ganglia, and some of these interneurons send axons anteriorly, where they eventually enter "antenno-glomerular tracts" and then project to the mushroom bodies (STRAUSFELD 1976). As for auditory input into the brain, these pathways apparently end up in dorsal regions, after the initial mechano-reception (*e.g.*, from sound-induced movement of part of the antenna) is sent to more ventral interneurons in the "deutero-cerebrum" (STRAUSFELD 1976).

The auditory, contact-chemosensory, and pheromonal information from the female's presence and the male's behavior could be integrated in the dorsal brain where signals from these three sensory pathways seem to converge (Figure 4). An argument against this possibility is, for instance, the recent finding of BURNET, EASTWOOD and CONNOLLY (1977) showing no apparent effect of removing the male's antenna on his courtship. That sensory appendage (and the Johnston

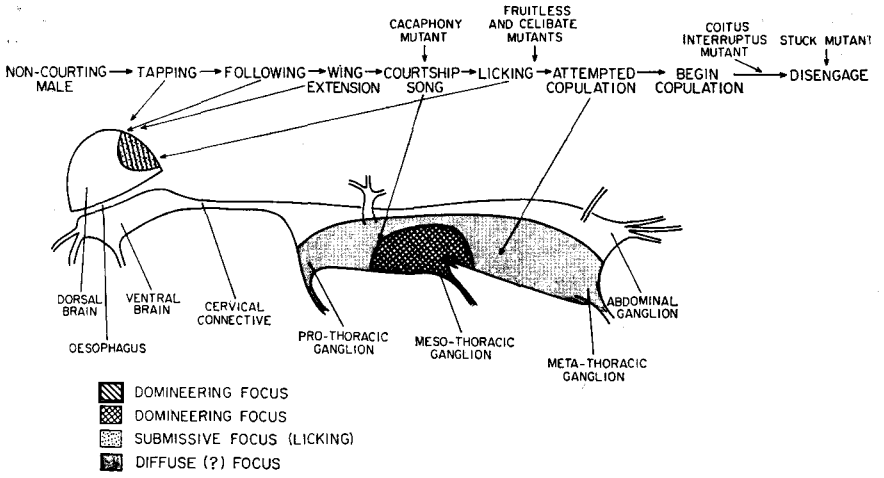

FIGURE 4.—Schematic representation of the courtship pathway in *Drosophila melanogaster*, analyzed with genetic variants. The parts of the pathway "controlled" by different parts of the central nervous system are shown by arrows from the behavioral step to a part of the CNS shown here in sagittal section (and the various portions of this section through an adult are shown across the bottom of the figure). These aspects of the breakdown of the pathway are from the analysis of courting mosaics in the present paper, as well as that of SCHILCHER and HALL (1979). For the foci in the head that are shown in approximately the same portion of the brain, the control of tapping, following, and wing extension are all domineering, while the control of licking is essentially submissive. The thoracic song focus is domineering. The "diffuse" focus for attempted copulation is discussed in the text. The pathway can also be broken down somewhat by courtship mutants which affect various steps of the sequence: the cacophony mutant (SCHILCHER 1976b, 1977), with its altered courtship song; the fruitless mutant (GILL 1963; HALL 1978) ceases courtship of females at the attempted copulation stage (and also will stimulate courtship among males); the celibate mutant (HALL and BENZER in preparation), which, too, stops courting at attempted copulation (but does not lead to male-male courtship); and the two mutants affecting copulation: coitus interruptus (HALL and BENZER in preparation), which copulates for only about 60% of the normal copulation span (which is *ca.* 20 min.) and has abnormalities of sperm and/or sperm transmission; and the stuck mutation which causes males frequently to be unable to disengage at the end of copulation (BECKMAN 1970; HALL and BENZER in preparation).

organ within it) presumably receives the input from courtship wing vibration (MANNING 1967b).

Neural foci for male-specific behavior

The information in this paper on parts of the CNS correlation with male courtship confirms and extends earlier results (HOTTA and BENZER 1976; HALL 1977). Here, though, there is an emphasis on more detailed dissection of the individual parts of the animal whose genotype is related to the performance of the various steps in courtship. The use of "sturt" values for fate mapping of courtship foci by triangulation (*cf.*, HOTTA and BENZER 1976) would be inappropriate because the population of mosaics is a selected subset (enriched for cases that are candidates for male behavior) and because the gynandromorphs

in the current study are not half-male half-female. If one tries to circumvent these biological and computational problems by applying more complicated mapping techniques (*e.g.*, FLANAGAN 1977), one still has to consider the possibility of special, quantitative neural defects in the current sex mosaics (discussed above). These would not be likely to interfere with the conventional mapping of behaviors unrelated to sexual dimorphism. But "distances" of particular tissues from particular sex behavioral foci could be distorted, in the face of many mosiacs that did have the focus of appropriate genotype but failed to perform because of non-trivial defects in neural organization or function.

From the more qualitative mapping in the current gynandromorphs, there is a domineering focus for following the wing extension in the dorsal brain (*cf.*, HALL 1977). The results that most forcefully lead to this conclusion are from the gynandromorphs that did show male behavior (Table 2). A new finding is that the focus for male tapping behavior is also a domineering one in the dorsal brain, essentially inseparable from the other two actions involved in the beginning of courtship. It should be mentioned that NISSANI (1977) concludes that the early male actions have submissive focus in the head (contrary to the present and other earlier papers). However, NISSANI performed no real mapping of the male courtship in his gynandromorphs; he merely noted that 28% of his all-male-head mosaics, determined solely from cuticular scoring, failed to court.

The nonperformance by some male-brain mosaics could be explained by (1) the equivalent proportion of control males that do not court, (2) the possibility that a tissue (not necessarily neural) near the dorsal brain must also be male in order that male courtship occur, or (3) the general debilitation found for some sex mosaics. The last possibility is intriguing in view of the reproducibly poor quantitative performance of mosaics that is documented in these experiments.

The focus for licking is in the brain, and is essentially submissive, unlike the focus for earlier male behaviors. COOK (1978) also came to the conclusion that the focus for licking is submissive in the anterior part of the fate map, from experiments on gynandromorphs whose male-female distributions were marked on external tissues only. In the present work, the licking focus was found to be "incompletely submissive," because some mosaics with male tissue in only one side of the brain were able to lick (Figure 3). This introduces the possibility that, in an analysis of mosaics by determination of genotypic distributions in internal tissues, the finding of an absolutely domineering or submissive focus may be unobtainable. That is, one does not have the luxury of a formal analysis of the focus, based on external scoring only, followed by triangulations on the fate map (*cf.*, HOTTA and BENZER 1976). The analysis of licking was completely consistent with a submissive focus, when the algebraic and statistical procedures of noninternal fate mapping were applied (*cf.*, HOTTA and BENZER 1972; MERRIAM and LANGE 1974); but this result does not reveal the complete picture.

The essentially submissive focus for licking emphasizes the principle that two behaviors that are separable from one another among the mosaics (*e.g.*, many mosaics extend their wings, but not their probosces) will not necessarily map to entirely different tissues: instead, the focus for one behavior may be domi-

neering (wing extension), but the other submissive (licking), and both behaviors could be under the genotypic control of approximately the same part of the CNS.

The other behavior late in the courtship sequence analyzed here was attempted copulation. This behavior is dramatically separable from the previous step in the pathway, in that many of the lickers do not attempt to copulate. The attempted copulation "focus," apparently in the thorax, also has a very different anatomical location from the focus for the previous step of the behavioral sequence. In totally male flies, attempted copulation not only follows, but even seems to be "triggered" by licking. This is based on the impression one gets from observing the close temporal association of licking and attempted copulation (first noted by BASTOCK and MANNING 1955). Many mosaics, though, are qualitatively blocked before the stage of attempted copulation. Among the gynandromorphs that are not so blocked, it is important to emphasize the neural results from the positive behavioral cases: all mosaics that attempted to copulate had male tissue somewhere in their thoracic nervous system (*cf.*, HALL 1977). Quantitatively, though, the thoracic focus for attempted copulation remains without a single sharp focus. However, the more vigorous and complete attempts to copulate were correlated with higher proportions of male tissue in the ventral nervous system (Figure 3).

If the focus for attempted copulation is within the CNS, then it cannot easily be defined as submissive or domineering. Thus, while more than two-thirds of the licking mosaics attempted copulation (implying a domineering focus, Table 4), many mosaics with a bilaterally split ventral nervous system failed to attempt copulation, or they performed feebly with no genital contact (but only about 90% of male-behaving controls attempted copulation and only about 60% achieved genital contact). Three types of behavior are common for this one category of genotypically split thoracic ganglia: no attempted copulation, an intermediate level, and intensive copulatory attempts (Figure 3).

When the "sturt" algorithm for behavioral mapping was applied to the current data on attempted copulation, a rough focusing (without statistical treatment, cf., MERRIAM and LANGE 1974) yielded a domineering focus in a part of the blastoderm that is quite posterior to the posterior-most sites scored in the CNS. Possibly, then, the male focus for attempted copulation is not even neural. Yet, the problems inherent in the algebraic mapping approach and the key fact that no mosaics analyzed to date (also see HALL 1977) have attempted copulation and then proved to have all-female ventral ganglia finally force one back to the predilection in favor of a focus in the CNS. If the focus for this behavior were exclusively nonneural and far from the CNS, then copulation attempters possessing all-female tissues in their ventral nervous system should have been routinely observed. As it is, though, the supposedly long sturt distances from the sites in the thoracic ganglia are largely due to cases that did not attempt copulation, but had male tissue in their ventral nervous system. For these cases it can once more be hypothesized that different kinds of male-female interactions in an apparently uniform class of mosaic (such as the bilaterally split thoracic category) are associated with the three different behavioral results (Figure 3).

The problems arising from these putative defects in some of the mosaics, due to sexually dimorphic ventral ganglia, are, again, unlikely to be dispensed with merely by highly sophisticated data processing (*e.g.*, MERRIAM and LANGE 1974; FLANAGAN 1977).

The analysis of courtship by mosaics may have proceeded to the point where the pathway of actions has been sufficiently dissected in terms of the overall patterns of both the behavioral and mosaic data. Figure 4 summarizes this pathway, showing the neural tissues whose sexual genotype is important, and including for reference steps of the courtship pathway interrupted by behavioral mutants (HALL and BENZER in preparation). Further work should involve detailed anatomical studies on possible sex differences in the specific and in some cases limited portions of the central nervous system that are associated with male courtship foci. This dissection of this behavioral pathway by internally marked mosaics permits such a manageable histological analysis.

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