CONTROL OF FEMALE REPRODUCTION IN DROSOPHILA: GENETIC DISSECTION USING GYNANDROMORPHS

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Manuscript received April 24, 1981
Revised copy accepted September 25, 1981

ABSTRACT

The sexual behavior of *Drosophila melanogaster* gynandromorphs was studied to analyze the relationship between different steps in the female reproductive pathway. It was assumed that, in some gynandromorphs, certain female functions are missing because the corresponding control sites (foci) are either composed of male tissue or did not develop. A given gynandromorph can show elements of both male and female reproductive pathways. None of the steps of the female reproductive pathway appeared to be dependent on any other, in contrast to male behavior where, for example, following of females is a prerequisite for attempted copulation. By correlating each of the behaviors with the genotype of the cuticle, we confirmed previous findings that the focus for the female sex appeal is located in the abdomen, but receptivity to copulation is controlled by a site in the head. Many of the gynandromorphs did not lay eggs, presumably because either the focus controlling egg transfer from the ovaries to the uterus or the one controlling egg deposition was composed of male tissue. Many of the nonovipositing gynandromorphs laid eggs while dying or could be induced to deposit eggs after implantation of hormone-producing glands or topical application of a juvenile hormone analog. Some of the noninseminated gynandromorphs laid eggs at the rate characteristic for inseminated females, suggesting that an oviposition focus (mapping in the head region) suppresses oviposition in virgin females, but not in gynandromorphs whose focus is composed of male tissue. Some of the inseminated gynandromorphs oviposited eggs at a low rate, possibly because the focus responsible for detection of insemination could not function properly. Some of the inseminated gynandromorphs laid unfertilized eggs, revealing the importance of the focus controlling sperm release from the seminal receptacle. Foci controlling egg transfer, egg deposition and sperm release are located in the thorax, according to mosaic fate mapping results and studies on the reproductive behavior of decapitated females. The location of egg deposition in the culture vial seems to be controlled by a brain site. Sexual behavior in Drosophila does not depend on the presence (or absence) of the ovary or germ line.

GYNANDROMORPHS of *Drosophila melanogaster* have been effectively used to dissect the various components of sexual behavior, especially male courtship behavior. Male tissue in the posterior dorsal brain is necessary for the male behaviors of *following* the females and *wing display* (HALL 1977, 1979). An anatomical site that controls a single step in the sexual behavior is called a "focus."

The focus for *licking* of female genitalia is located close to that for following and wing display (COOK 1978; HALL 1979). For *courtship song* (SCHILCHER and HALL 1979) and *attempting copulation* (HOTTA and BENZER 1976; HALL 1979), male tissue must be present in both the brain and the thoracic ganglia.

Less is known about female reproductive behavior. Elicitation of male courtship or *sex appeal*, an indication of sexual attractiveness of the females, is controlled by a posterior focus (HOTTA and BENZER 1976; HALL 1977; NISSANI 1977; JALLON and HOTTA 1979). *Receptivity* to male copulation and oviposition seem to be controlled by an anterior focus near the head (HOTTA and BENZER 1976; NISSANI 1977; COOK 1978). In addition, a female needs to perform other sex-specific actions to be normal in reproductive behavior, such as control of oviposition, sperm release, the location of oviposition, etc. In some of the gynandromorphs, the corresponding control sites may not function properly, causing a defect that may bring about abnormal behavior.

In addition to their arrangement in the adult fly, the origin and development of foci controlling male and female reproductive behavior are also important. There are indications that elements of male- and female-specific behavior patterns can coexist in gynandromorphs, in that some of these mosaics show both male and female components of the reproductive pathways (COOK 1978). Male and female genital structures can also be found simultaneously in gyandromorphs, demonstrating that they are derived from separate primordia (NÖTHIGER, DÜBENDORFER and EPPER 1977).

The aims of the work reported in this paper were: (1) to dissect major steps of the female reproductive pathway, (2) to localize the anatomical sites that control these steps, and (3) to determine the relationship between foci governing male- and female-specific elements of the reproductive pathways.

MATERIALS AND METHODS

Gynandromorphs were recovered from a cross between *y v f mal* (or *y w* f^{36a} *mal*) homozygous virgin females and males carrying the unstable ring-*X* (X_R) chromosome *R(1)2,In(1)*w^{vC}. Loss of X_R in the *y v f mal*/X_R zygote results in the formation of XX_R//*XO* female//male mosaic or gynandromorph (for reviews see HALL, GELBART and KANKEL 1976; JANNING 1978). Male (*XO*) parts of the gynandromorphs show the phenotype of the recessive marker mutations (*y v f mal*), which are linked to the rod-*X* chromosome, whereas the female (XX_R) regions are phenotypically wild type. (For a detailed description of the mutations, see LINDSLEY and GRELL 1968). The marker mutation *mal* allows identification of some internal organs. The *mal* hemizygous male tissues do not possess aldehyde oxidase activity and remain unstained, while the female tissues stain blue in a histochemical assay (JANNING 1976). The marker mutations were *y v f mal* for 333 (81.4%) and *y w* f^{36a} *mal* for 76 gynandromorphs.

Gynandromorphs were isolated every second day so that some of them could have mated by the time of isolation. We analyzed only those gynandromorphs that had female terminalia and could therefore conceivably deposit eggs. Individuals were transferred into glass vials with 2–3 Oregon-R wild-type males each. The vials contained standard Drosophila food with live yeast and were kept at 25°. Flies were transferred into new vials every 3–5 days. The number of eggs, their location (on the food, the glass wall, or both) and the presence of larvae were recorded. The egg production of each gynandromorph was monitored for at least 10 days.

Five to seven days after their isolation, the sexual behavior of the gynandromorphs was tested. First, they were put with two to three virgin wild-type females beneath a watch glass

on a white ceramic plate. Courtship was observed under a binocular microscope at 25–27°. Gynandromorphs that did not court the females in the first test (within at least 20 minutes) were retested two more times. Five to 10 minutes after the first test, each gynandromorph was put with two 5–7 day old wild-type males and tested to determine whether it elicited male courtship. This test was not performed for gynandromorphs that yielded larvae by the fifth to seventh day after their isolation. While transferring flies for the sex behavior test, they were narcotized briefly with CO_2.

At least 10 days after isolation, we tested to see whether the gynandromorphs laid eggs while dying. When a normal female is first placed in 96% ethanol for about 1 sec and then transferred into Ringer's solution, she squeezes out the egg that happens to be in her uterus. If there is no egg in the uterus, the female exhibits a characteristic sequence of events including contraction of abdominal muscles and extrusion of the ovipositor.

For dissection, gynandromorphs were decapitated with fine scissors and the abdomen was separated from the thorax to expose the corpus allatum and the corpus cardiacum. The abdomen was then cut open to analyze the reproductive organs. The seminal receptacle was isolated and examined by phase contrast microscopy for the presence of sperm. Gynandromorphs were considered to be *receptive* when they had sperm stored in the seminal receptacle. Internal structures were stained for aldehyde oxidase activity and mounted in Faure's solution, along with the adult cuticle. The phenotype (sex) of both the cuticular and the internal structures was scored with the compound microscope (100–400×). Various organs (brain, seminal receptacle, ovary with oviduct isolated from young wild-type females, corpus allatum and corpus cardiacum complex of 5-day-old wild-type females, and the ring gland of late third-instar larvae) were implanted into gynandromorphs that did not deposit eggs for at least 10 days. In the case of some of the nonovipositing gynandromorphs, 0.15 μg of the juvenile hormone analog ZR-515 (isopropyl 11-methoxy-3, 7, 11-trimethyl dodeca-2-,4-dienoate) was topically applied to the abdomen (HANDLER and POSTLETHWAIT 1977). The egg production of the host and the ZR-515-treated gynandromorphs was monitored for at least five more days. Some flies (both Oregon-R wild-type females and gynandromorphs) were decapitated with fine scissors and kept in 100% humidity to study their egg production, receptivity and capacity to elicit male courtship.

RESULTS

A total of 409 gynandromorphs with female terminalia were analyzed. An additional 158 flies were isolated from the cross yielding gynandromorphs that appeared to be female on the basis of their cuticular features. These females were also tested for female-type reproductive behavior. All of them elicited male courtship, were receptive and deposited eggs from which larvae eclosed.

The relationship between different components of the reproductive pathways in gynandromorphs is summarized in Table 1.

The role of germ line and ovary: Of the 409 gynandromorphs studied, 365 had at least one ovary with egg chambers, although an ovary was sometimes composed of only 2–8 ovarioles. Forty-three (11%) of the gynandromorphs were agametic: either the ovaries were missing (36 cases) or they were represented only by the mesodermal component of the ovaries (7 cases). In a number of gynandromorphs, undifferentiated testes or only the testicular coat could be identified. One of the gynandromorphs laid one egg although the ovaries appeared to be agametic at the time of dissection.

There was no difference between the sexual behavior of the gametic and agametic gynandromorphs. Equal fractions of the two types elicited male courtship, were receptive and courted virgin wild-type females ($P > 5\%$, chi-square test). Of the courting gametic and agametic gynandromorphs, equal fractions attempted

TABLE 1

Relationship between female and male components of the reproductive pathways in Drosophila gynandromorphs

		Flies tested	Reaction	Germ line Yes	Germ line No	Female behavior: Sex appeal Yes	Sex appeal No	Receptivity Yes	Receptivity No	Oviposition Yes	Oviposition No	Location of egg deposition sites: Food	Food & wall	Male behavior: Following Yes	Following No	Wing display Yes	Wing display No	Licking Yes	Licking No	Attempted copulation Yes	Attempted copulation No
	Germ line	408	Yes	365	0	267	33	202	152	285	80	166	106	119	186	116	189	110	195	58	247
			No	0	43	29	5	17	25	0	43	0	0	16	11	16	11	16	11	10	17
Female behavior	Sex appeal	335	Yes			297	0	219	73	229	68	160	61	86	189	83	192	79	196	36	239
			No			0	38	0	36	22	16	1	19	32	4	32	4	30	6	19	17
	Receptivity	397	Yes					219	0	183	36	156	22	37	165	36	166	36	166	12	190
			No					0	178	98	80	10	79	92	29	90	31	84	37	53	68
	Oviposition	409	Yes							286	0	166	106	86	169	83	172	78	177	38	217
			No							0	123	0	0	50	28	50	28	49	29	30	48
	Location of egg laying sites	272	Food									166	0	14	151	14	151	11	154	1	164
			Food & wall									0	106	64	18	62	20	59	23	30	52
Male behavior	Following	333	Yes											136	0	133	3	127	9	68	68
			No											0	197	0	197	0	197	0	197
	Wing display	333	Yes													133	0	125	8	68	65
			No													0	200	2	198	0	200
	Licking	333	Yes															127	0	68	59
			No															0	206	0	206
	Attempted copulation	333	Yes																	68	0
			No																	0	265

copulation. These results indicate that the presence or absence of the female germ line (or ovary) is not a prerequisite for female sex appeal, receptivity and courting behavior. These findings also suggest that the foci controlling female sex appeal, receptivity and courtship derive from sites on the blastoderm that are distant from the pole cells and the region that gives rise to the mesodermal component of the ovaries (GEHRING, WIESCHAUS and HOLLIGER 1976).

Sex appeal and receptivity: Most of the gynandromorphs studied (88%, Table 1) elicited male courtship, which is to be expected since only gynandromorphs with female terminalia were studied. Such gynandromorphs tend to have female or mosaic abdomens. The focus for female sex appeal may be located inside the abdomen (JALLON and HOTTA 1979, and Table 3). Among the 335 gynandromorphs analyzed for female sex appeal, three had completely male abdomens and thoraces although they elicited male courtship. The focus responsible for their sex appeal, seemingly an internal one, might have comprised female tissue. Four gynandromorphs did not elicit male courtship even though they had fully female abdomens.

Of the 297 gynandromorphs that possessed female sex appeal, 292 could be tested for receptivity. Seventy-three of them (25%, Table 1) elicited male courtship, but did not store sperm in the seminal receptacle and, hence, were not receptive. This observation confirms the reults of HOTTA and BENZER (1976) and COOK (1978) that sex appeal and receptivity have separate control sites and that these foci are distant from one another. There were 11 gynandromorphs (among 219) that stored sperm in the seminal receptacle, but did not elicit male courtship during the test periods. These flies were considered to have female sex appeal. Some of them might have been previously fertilized (see MATERIALS AND METHODS) and therefore possessed a lower degree of sex appeal (COOK 1978; SIEGEL and HALL 1979).

In principle, some of the gynandromorphs could have been nonreceptive because they did not notice courting males; however, most of the nonreceptive gynandromorphs repelled males by wing flicking and extrusion of their ovipositor (see COOK 1978; BASTOCK and MANNING 1955).

Oviposition: Of the 365 gynandromorphs in which mature eggs were present, 80 (22%) did not lay eggs during the test period (Table 1). Several of the nonovipositing gynandromorphs had two ovaries, often with more than three mature eggs per ovariole. Retained eggs, especially those at the distal end of the ovarioles, often had a rounded shape and disorganized egg cytoplasm. Five of the nonovipositing gynandromorphs had blocked ovipositors, accounting for the lack of oviposition. Most of the nonovipositing gynandromorphs attempted to squeeze out an egg while dying and, indeed, 19 of them (25%, Figure 1) each laid one egg. This result suggests that the lack of oviposition in the nonovipositing mosaics is usually not due to physical blockage of the genital tract; rather, the focus controlling egg deposition is probably composed of male tissue and consequently cannot induce egg deposition. Many (57 out of 76) of the nonovipositing gynandromorphs extruded their ovipositor and contracted the abdominal musculature while dying, typical actions during oviposition, but did not lay eggs. There were

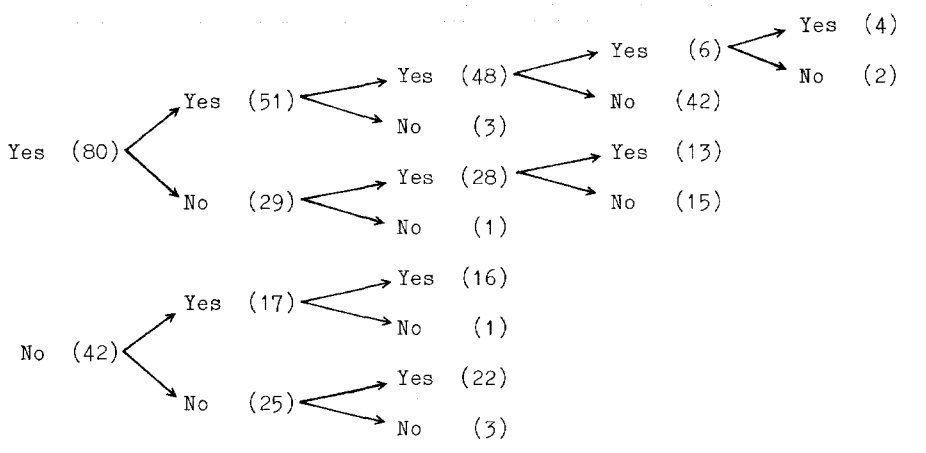

FIGURE 1.—Distribution of nonovipositing gynandromorphs according to receptivity, egg deposition while dying, and yield of larvae. Figures in parentheses show the number of gynandromorphs in each group.

no eggs observed in the uteri of these gynandromorphs at the time of dissection. Only those gynandromorphs that stored an egg in the uterus squeezed out eggs while dying (COOK 1978). This result suggests that transfer of eggs from the ovaries to the uterus is also controlled by a female-specific focus, so that, if the focus is composed of male tissue, egg transfer cannot be induced. Thus, for oviposition to occur, the proper function of two foci is evidently needed—one controlling egg transfer and the other controlling egg deposition.

There were larvae in four of the eggs deposited just prior to death, an observation showing that eggs can become fertilized at the time of or immediately after reaching the uterus. In two other eggs, however, no larvae developed even though the gynandromorph had been inseminated. This failure of fertilization is presumably due to lack of sperm release to the uterus.

Gynandromorphs could be classified into three categories according to the rate of oviposition (Figure 2). Most of the receptive flies (164, 75%) laid eggs at the high rate characteristic of normal inseminated females (10–30 eggs per day), and most of these yielded larvae (158 out of 164, Figure 2). However, six (3.7%) of the gynandromorphs were receptive and deposited eggs at a high rate, and none of their eggs was fertilized. This latter result indicates that release of sperm from the seminal receptacle is under control of a female-specific focus. We propose that, when this focus is made of male tissue, no sperm are released from the seminal receptacle.

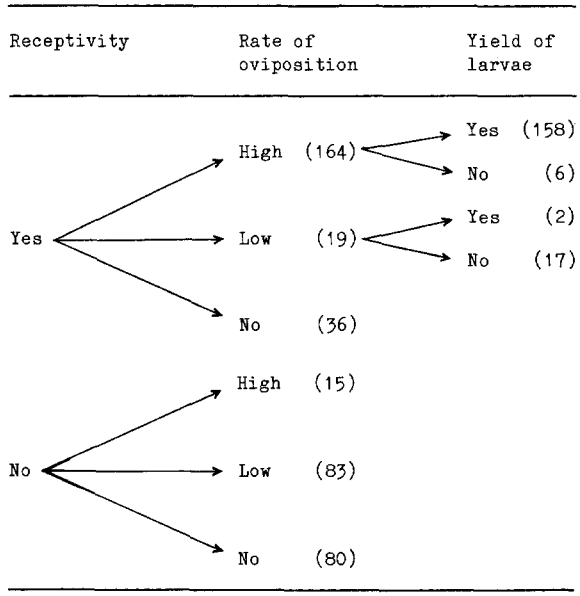

FIGURE 2.—Distribution of gynandromorphs according to receptivity, rate of oviposition, and yield of larvae. Figures in parentheses show the number of gynandromorphs in each group.

It is surprising that 19 (8.7%) of the receptive gynandromorphs laid eggs at the low rate characteristic of the virgin females (1–3 eggs per day). This group of the gynandromorphs probably consists of cases where insemination was not detected, possibly because the anatomical site responsible for detection of insemination was made of male tissue. This group of the gynandromorphs was divided into two subgroups: one that yielded larvae, another that did not (Figure 2).

About half (83, 47%) of the nonreceptive gynandromorphs deposited eggs at the low rate characteristic of virgin females. However, 15 (8.4%) of the nonreceptive gynandromorphs deposited eggs at as high a rate as is seen in inseminated females (Figure 2).

Location of egg laying sites: A relatively high fraction (39%, 106 of 272; Table 1) of the ovipositing gynandromorphs deposited eggs both on the food and on the glass wall of the culture vial, whether it was standing or lying on its side. All but two of the 158 control females deposited eggs only on the food. In two cases, however, 2.3% of the eggs (3 and 7 eggs) were deposited on the glass wall. The glass wall covered 88% (28 cm^2) of the surface where eggs were found; thus, if gynandromorphs laid eggs in arbitrary locations, about 88% of the eggs would be found on the glass wall. Of the gynandromorphs that deposited eggs on the glass wall, 43 laid enough eggs to determine the percentage of eggs on the wall. Among those flies, 26 laid eggs in arbitrary locations (Figure 3). The results suggest that there is a female-specific focus that controls the location of egg-laying sites, and, when this focus does not function properly (as in some gynandromorphs), eggs are deposited arbitrarily.

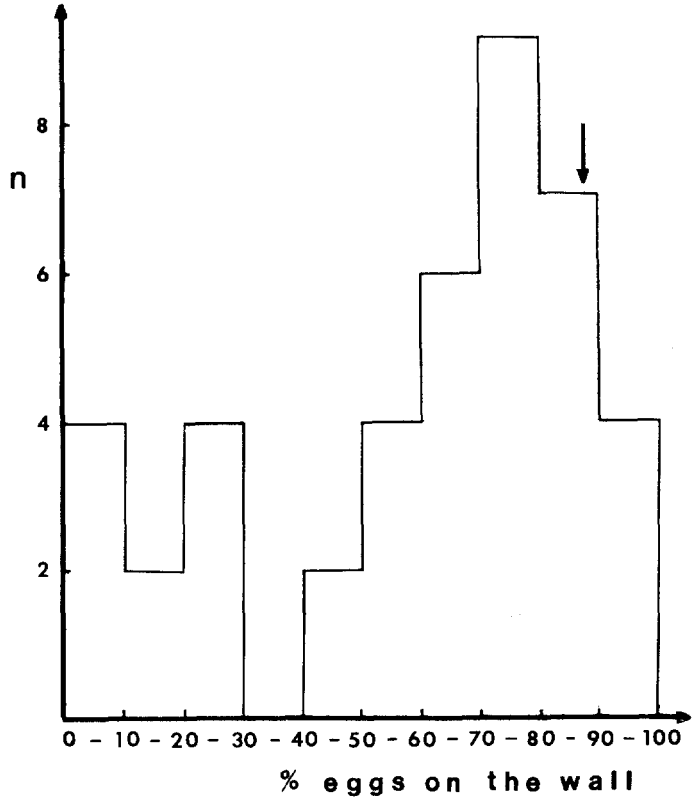

FIGURE 3.—The distribution of gynandromorphs as a function of the percent of their eggs laid on the glass wall of the culture vial. The arrow points to the value corresponding to the surface ratio of the glass compared to the total. No eggs were laid on the cotton stoppers.

Results considering the male type of behavior of the gynandromorphs are in agreement with those published earlier and show the hierarchical constitution of the male courtship pathway. Gynandromorphs did not attempt copulation unless they also followed virgin females and showed wing display. However, only 50% (68 out of 136) of the courting gynandromorphs attempted copulation (Table 1).

Seven of 136 courting gynandromorphs began to lay eggs when they started to court wild-type females, and four of them did lay eggs. All of them had male head cuticle and did not deposit eggs solely onto the food.

Implantation experiments and treatment with juvenile hormone analog: To determine whether the nonovipositing gynandromorphs could be induced to lay eggs, different organs of wild-type females were implanted into those mosaics that contained eggs but did not deposit any for at least ten days. Implantation of most of the organs (brain, seminal receptacle and ovary with oviducts) of the wild-type females did not bring about egg deposition. However, after implantation of hormone-producing glands (for review, see GILBERT *et al.* 1980) egg deposition was achieved. For example, when the corpus allatum–corpus cardiacum complexes were implanted (two pairs of glands per host), 12 of 17 host gynandro-

morphs started to lay eggs (Table 2). Similarly, when ring glands of wild-type female larvae were implanted into the nonovipositing gynandromorphs (two glands per host), all of the six hosts deposited eggs. Topical application of 0.15 μg ZR-515, a juvenile hormone analog, also brought about egg deposition in four of the five treated nonovipositing gynandromorphs. Those gynandromorphs that laid eggs after implantation of the endocrine glands or treatment with ZR-515 deposited an average of 1.2 eggs per day. Seven each laid one egg and six flies laid 15–37 eggs in 10 days. Neither implantation of the hormone-producing glands nor treatment with ZR-515 induced other female-specific characteristics of the host gynandromorphs; for example, none of them became receptive and, except for two, they failed to deposit eggs only on the food.

To ascertain the sex of the corpus allatum and corpus cardiacum is important with respect to oviposition, we determined whether these glands were composed of female or male tissue in those gynandromorphs that showed reproductive behavior characteristic of normal females. Among such gynandromorphs 49% had *mal* hemizygous male glands. Hence, the sex of the corpus allatum and corpus cardiacum seems to have little if any regulatory function in oviposition. Rather, the sex of that part of the central nervous system that controls the activity of the hormone-producing glands seems to play a key role in the regulation of oviposition. By implantation of the hormone-producing wild-type glands or treatment with the juvenile hormone analog, the hormone titer was elevated to a level that induced activity of the foci controlling oviposition.

Decapitation experiments: The failure of some of the gynandromorphs to oviposit might have been due to inhibition by the central nervous system. It is known that decapitated females can show some components of reproductive behavior, suggesting that the corresponding control sites are located outside the head (GROSSFIELD and SAKRI 1972). In our experiments none of the 12 previously nonovipositing gynandromorphs that were decapitated laid eggs. The ovipositing gynandromorphs behaved as did wild-type females after decapitation: two of five laid a few eggs, all of which were fertilized. Of the 35 inseminated and ovipositing wild-type females, ten deposited eggs after decapitation. They laid on the average four eggs within five days and larvae hatched from all the eggs. The be-

TABLE 2

Egg deposition by previously nonovipositing gynandromorphs after implantation of organs from wild-type females and topical application of the juvenile hormone analog ZR-515

Organs implanted	Host*	Ovipositing*	Eggs laid
Brain	7	2	3
Seminal receptacle	11	0	0
Ovary with oviducts	11	0	0
Corpus allatum and cardiacum	17	12	102
Ring-gland	6	6	52
Juvenile hormone alalog	5	4	47

* Number of gynandromorphs.

havior of virgin wild-type females decapitated within two hours after eclosion was also tested. They were brought together with males on the fifth day after decapitation, and ten (out of 27) were found able to elicit male courtship. These results suggest that foci controlling sex appeal, transfer of eggs from the ovaries to the uterus, sperm release from the seminal receptacle, and egg deposition are located outside the head.

Mapping of behavioral foci: The mosaic fate-mapping technique makes use of gynandromorphs and establishes the relative positions of the blastoderm regions from which larval and imaginal structures are derived (for reviews see HALL, GELBART and KANKEL 1976; JANNING 1978). If the precursor cells for a focus controlling a given behavior step are in close proximity on the blastoderm to the precursor cells for an imaginal structure, the two sets of precursor cells will commonly have the same genotype in gynandromorphs (GARCIA-BELLIDO and MERRIAM 1969; HOTTA and BENZER 1972). We established the correlation between the sex of the imaginal cuticle and the presence or absence of female and male specific steps of the reproductive pathways (Table 3). Based on the data listed in Table 1, we also calculated the distance between foci controlling different steps of the reproductive pathways (Table 4). The distance between the blastoderm regions giving rise to the foci is given in *sturts*, a unit that denotes the percentage of gynandromorphs in which the sex of two sites are different (HOTTA and BENZER 1972). A word of caution is that departures from a random gynandromorph population will bring about distortion in sturt values in comparison with their proper usage involving no preselection of certain classes of gynandromorphs.

Because gynandromorphs with female terminalia were selected, 25% (104) of them had entirely female and only 1.9% (8) had entirely male abdominal cuticle. A predominant fraction (45% or 184) had male and a smaller fraction (29%, or 117) had female head cuticle, demonstrating the deviation from the usual 1/1 ratio (Table 3).

Receptivity to male courtship seems to be controlled by a focus that maps close to the head cuticle. That the focus is probably located in the brain is suggested by two observations. Most of the receptive gynandromorphs (82%, 165 of 202, Table 1) did not court virgin females, while of the nonreceptive ones 76% (92 of 121) did. Based on these frequencies, we estimate the distance between foci controlling receptivity and early courtship behavior at about 20 sturts.

It was shown above that proper functioning of at least two foci is needed for oviposition to occur. One focus controls egg transfer from the ovaries to the uterus, the other controls egg deposition. The data in Table 3 suggest that the egg transfer focus is located in the thorax: egg transfer took place in the 62 gynandromorphs with female thoraces, but the gynandromorphs that failed to transfer eggs had entirely male thoracic cuticle. The observation that each decapitated female can deposit more than one egg also argues that egg transfer is controlled by a focus outside the head. The focus controlling egg deposition is probably also located in the thorax, as indicated by the observation that 97% (62 of 63, Table 3) of the gynandromorphs with a female thorax laid eggs, and 94% of the nonovipositing

TABLE 3

Correlation between sex of cuticle and behavior. Matrices for gross mapping of some sex-related characteristics

					Female behavior																		Male behavior			
Region	Sex of cuticle*	Cuticle	Germ line Yes	No	Sex appeal Yes	No	Receptivity Yes	No	Oviposition† Yes	No	Egg transfer Yes	No	Egg deposition Yes	No	Sperm release Yes	No	Detection of insemination Yes	No	Rate of oviposition Nor-/Abnormal		Location of egg laying sites Food	Food & wall	Following Yes	No	Attempted copulation‡ Yes	No
Head	Male	184	160	23	96	31	37	135	101	59	101	19	101	18	84	17	8	14	74	22	7	86	103	32	59	45
	Female	117	111	6	113	1	107	6	103	8	103	2	103	0	103	0	97	0	97	1	100	2	1	108	1	0
Thorax	Male	91	74	17	45	19	16	70	43	29	43	10	43	12	36	7	5	4	35	8	4	33	53	14	34	20
	Female	68	64	4	63	0	57	8	62	2	62	0	62	1	1	1	47	1	53	3	54	6	3	57	0	3
Abdomen	Male	8	6	2	3	4	2	6	2	4	2	2	2	2	2	0	0	0	2	0	0	1	4	1	4	0
	Female	104	98	6	87	2	78	25	87	11	87	1	87	3	1	6	65	5	84	10	63	21	14	72	1	13

* Gynandromorphs with mosaic cuticle were not included.
† For egg producing gynandromorphs only.
‡ For gynandromorphs that followed females.

TABLE 4

Distance, in sturts, between foci controlling sex-specific steps of the courtship pathways

	Female behavior				Male behavior	
	Sex appeal	Receptivity	Oviposition	Location of egg-laying sites	Following	Attempted copulation
Germ line	19	43	28*	64	39	21
Sex appeal		22	27	26	35	42
Receptivity			34	12	20	40
Oviposition				64	34	43
Location of egg laying sites					13	45
Following						50

* For egg producing gynandromorphs only.

ones (29 of 31) had male thoracic cuticle. The latter deduction is also supported by the fact that decapitated females can deposit eggs, but eggs are never laid by isolated abdomens (A. M. HANDLER, personal communication). There is some uncertainty in locating the focus for egg deposition because in some of the gynandromorphs the function of the oviposition focus could not be determined as no eggs were transferred to their uteri. The focus regulating sperm release from the seminal receptacle could not be located because only 23 of the recovered gynandromorphs were receptive but had no larvae hatching from their eggs (Figure 2). That the focus is not located in the head is indicated by the finding that larvae hatched from all the eggs deposited by inseminated and decapitated females and gynandromorphs.

The rate of oviposition appears controlled by a focus located in the head. Thirty-four gynandromorphs had an abnormal rate of oviposition. Nineteen of these were inseminated but laid only a few eggs, while 15 were not inseminated but laid as many eggs as do normal inseminated females (Figure 2). The rate of oviposition is strongly correlated with the sex of the head cuticle: most of those gynandromorphs that deposited eggs at an abnormal rate had male head cuticle (22 of the 23 with either male or female head cuticle), while 97 of 98 with female heads oviposited at the expected rate (Table 3).

Insemination apparently stimulates oviposition only if it is "detected" by the female. Nineteen gynandromorphs (of 219) were inseminated but laid eggs at the rate characteristic of virgin females (Figure 2). Seventeen laid unfertilized eggs. Of the 19 gynandromorphs, 14 had male and 5 had mosaic head cuticle (Table 3), suggesting that insemination is detected by a focus in the head.

The location at which eggs are deposited is controlled by a focus in the head. This conclusion is supported by the finding that almost all the gynandromorphs with female head cuticle (100 of 102, Table 3) deposited eggs on the food surface exclusively. Those with male head cuticle tended to lay eggs both on the food and on the wall of the test vial (86 of 93, Table 3). That the focus may be part of the brain is suggested by the strong correlation between subgroups of the gynandromorphs: 92% of the flies that laid all of their eggs on the food did not court virgin

females and 78% of those that deposited eggs on the wall of the vials also courted (Tables 1 and 4). The early steps of the courtship pathway are controlled by brain sites (HALL 1977, 1979).

Our results with respect to male-specific behavior patterns in the gynandromorphs are in agreement with previous findings (HOTTA and BENZER 1976; HALL 1977, 1979). There is a strong correlation between the sex of the head cuticle and the courtship behavior, *i.e.*, most of the gynandromorphs that followed virgin females (103 of 104, Table 3) had male head cuticle. With one exception, gynandromorphs with female heads did not court virgin females. This result indicates that progenitor cells of the head cuticle and the foci controlling early steps of the courtship pathway are close together on the blastoderm.

DISCUSSION

In this study we analyzed the sexual behavior of gynandromorphs having female terminalia. More than half of them had abnormal behavior which resulted in sterility in most cases. We assumed that foci that control female-specific behaviors do not function or may not develop in gynandromorphs when the foci or their primordia are composed of male tissue. If the part of the blastoderm from which behavior-controlling foci develop comprises male tissue, male types of behavior can develop. Female and male behaviors are evidently not controlled by the same set of foci since about one tenth of the gynandromorphs studied concomitantly demonstrated both male and female types of sexual activities (see also COOK 1978). A few of the gynandromorphs showed almost complete female and male behaviors, whereas others lacked any sexual activity. NÖTHIGER, DÜBENDORFER and EPPER (1977) reported a similar coexistence of male and female genital structures in the same gynandromorph. Apart from the genitalia all the other structures of the adult cuticle can only be present in either male or female form. The authors cited above proposed that male and female genitalia are nonhomologous in origin, and are derived from separate segments during early embryogenesis. In gynandromorphs both or neither primordia can develop, depending on the position of the line separating male and female parts of the embryo (NÖTHIGER, DÜBENDORFER and EPPER 1977). The control sites of the male and female reproductive pathways seem to be organized in a similar fashion at least formally analogous to that for the genitalia.

Hierarchical organization is a well-known feature of the courtship pathway; manifestation of the first step (following of the females) allows performance of subsequent steps (BENZER 1973; HOTTA and BENZER 1976; HALL 1977, 1979; NISSANI 1977; COOK 1978). Our findings agree with those of previous investigations in that only those gynandromorphs which followed virgin females engaged in wing display. Moreover, no gynandromorphs attempted copulation without first following the females and dispalying their wings. In contrast to the courtship pathway, we could not find evidence for hierarchy among elements of the female reproductive pathway. The data in Table 1 suggest that receptivity may depend on sex appeal since no gynandromorph was receptive that did not elicit

male courtship. However, a few of the gynandromorphs seemed to have no sex appeal (they did not elicit courtship), yet had sperm stored in their seminal receptacles. These gynandromorphs were considered to possess female sex appeal in this study. They might have copulated though not via the usual route of first stimulating males to court and later allowing them to mate, but rather by forced type of copulation (SPIETH 1966). Aside from this example, we observed gynandromorphs demonstrating a wide variety of different combinations of components of the female reproductive pathway. Elimination of any particular step does not imply absence of any other particular steps.

About ten percent of the gynandromorphs were sterile either because they had no ovaries or because no germ line cells were present in the ovaries. The ovaries develop from both gonadal mesoderm and germ line cells (KING 1970) and the progenitors of these cells come from different regions of the blastoderm (GEHRING, WIESCHAUS and HOLLIGER 1976). Agametic gynandromorphs result whenever the female/male borderline runs between the above groups of progenitor cells bringing together cells of the opposite sex, a combination which does not allow the formation of functional gonads (VAN DEUSEN 1976; MARSH and WIESCHAUS 1978). The sexual behavior of the agametic gynandromorphs did not differ from that of the gametic ones, indicating that for normal reproductive behavior neither the germ line nor the mesodermal components of the ovaries are needed.

Some of the gynandromorphs were sterile due to nonreceptivity. One quarter of these gynandromorphs that elicited male courtship did not store sperm in the seminal receptacle, indicating that they were not receptive. This finding confirms the observation of HOTTA and BENZER (1976), NISSANI (1977) and COOK (1978) and indicates that female sex appeal and receptivity to copulation are controlled by different foci. MANNING (1967) suggested that the corpus allatum releases juvenile hormone into the hemolymph and that the increase of the hormone titer is responsible for the "switch-on" of receptivity. It has been reported recently that the juvenile hormone titer is twenty-fold higher in newly eclosed females than in males (STRAMBI *et al.* 1981). The lack of receptivity of the gynandromorphs is apparently not due to a defect in juvenile hormone production since 1) many of the nonreceptive gynandromorphs contained several mature eggs in their ovaries and it is known that vitellogenesis and uptake of yolk proteins require juvenile hormone release from the corpus allatum (HANDLER and POSTLETHWAIT 1977; POSTLETHWAIT and HANDLER 1978); and 2) none of the nonreceptive gynandromorphs became receptive after implantation of juvenile hormone-producing glands into their abdomens or after treatment with a juvenile hormone analog. The nonreceptivity of these gynandromorphs may be explained by assuming that the focus controlling their receptivity was composed of male tissue.

One-fourth of the gynandromorphs was sterile because they did not lay eggs even though there were many mature eggs in their ovaries. Another one-quarter of them laid eggs while dying, an observation first reported by COOK (1978). The above finding allows two conclusions: (1) The failure of egg deposition is not due to blockage of the reproductive tracts but rather there is a focus which controls

egg deposition. (2) the ovipositor functions autonomously when "triggered," but no trigger signal arrives when the *egg deposition focus* consists of male tissue. The other three quarters of the gametic but nonovipositing gynandromorphs did not deposit eggs while dying, and upon dissection no eggs were found in their uteri (see also COOK 1978). These gynandromorphs reveal the importance of a focus that controls egg transfer from the ovaries to the uterus. We propose that when the *egg transfer focus* consists of male tissue no eggs are deposited. This defect cannot be related to the ovaries since they consisted of female tissue in these animals.

Oviposition also seems to be controlled by the juvenile hormone titer. When juvenile hormone-producing glands of wild-type larvae or females were implanted into nonovipositing gynandromorphs oviposition was achieved. Topical application of a juvenile hormone analog also induced egg deposition. Implantation of brain, seminal receptacle, or ovary with oviducts did not induce oviposition. We do not know the mechanism by which juvenile hormone induced oviposition of the previously nonovipositing gynandromorphs. The induction may have occurred via simultaneous activation of both the egg transfer and the egg deposition foci, or perhaps through an "oviposition focus" which subsequently activated the other two foci. It is also unknown why these gynandromorphs laid only few eggs after elevation of the juvenile hormone titer. They could have laid many more eggs since there was an excess of mature eggs in their ovaries.

The corpus allatum has a passive role in induction of vitellogenesis, uptake of yolk proteins and initiation of receptivity and oviposition; the gland was made of male tissue in about half of the receptive and ovipositing gynandromorphs. While releasing juvenile hormone into the hemolymph the corpus allatum simply executes a message from the central nervous system (for a review see GILBERT *et al.* 1980) and its hormone-producing capacity does not depend on its being composed of female or male tissue. When the message-releasing part of the brain (the *pars intercerebralis*) is destroyed, females remain unreceptive and do not lay eggs (BOULÉTREAU-MERLE 1976).

The rate of oviposition must also be controlled in Drosophila females; virgin females lay very few eggs, and insemination is followed by a remarkable increase in the rate of oviposition (MANNING 1967; LEAHY and LOWE 1967). Several of the gynandromorphs deposited eggs at an abnormal rate. About ten percent laid very few eggs even though they were inseminated. On the basis of this observation, we suggest that insemination is detected by a female-specific focus and when this focus is composed of male tissue insemination is not detected and eggs are deposited with a rate characteristic of virgin females. About ten percent of the noninseminated gynandromorphs behaved as if they had been inseminated and laid eggs at a rate characteristic for inseminated females. We explain this result by invoking a focus that, when female, prevents a high rate of oviposition in virgin females. When it is male, as expected in some gynandromorphs, oviposition proceeds with a high rate.

A few of the gynandromorphs were sterile because none of the eggs they deposited were fertilized. Drosophila females are extremely economical in using the stored sperm received at copulation: most of the time they appear to introduce

only one sperm to each of the eggs (HILDRETH and LUCCHESI 1963). We explain the lack of fertilization of the deposited eggs as a failure of sperm release from the seminal receptacle, a sign of male function of the "sperm release focus."

Another unusual feature of the reproductive behavior of the gynandromorphs was the locations where they deposited eggs. Normal females deposit all their eggs on the food, a site which can support development of the subsequent generation. However, almost 40% of the gynandromorphs deposited eggs not only on the food but also on the glass wall of the culture vial. These findings show that there is a female-specific focus which controls the location of egg deposition. It appears that when this focus is composed of male tissue the location of egg deposition is not controlled and consequently eggs are deposited at arbitrary sites.

By correlating the sex of the cuticle with various behaviors the foci that control the behaviors can be located (HOTTA and BENZER 1972; for reviews see HALL, GELBART and KANKEL 1976; JANNING 1978). Our data on sexual attractiveness of the gynandromorphs are consistent with previous findings and show that the stimulus that induces male courting behavior is produced by a focus inside the abdomen (HALL 1977; NISSANI 1977; JALLON and HOTTA 1979). Based on the correlation between receptivity and the sex of the cuticle, the focus controlling receptivity was located in the head; it may even be part of the brain, since there is a highly positive correlation between receptivity and male courting behavior, which has been shown to be controlled by brain sites (HALL 1977, 1979). That the focus controlling receptivity (along with some other foci) comprise part of the nervous system could be determined by using marker mutations that allow histochemical identification of the sex of this tissue (KANKEL and HALL 1976). Both the egg transfer and the egg deposition foci are located in the thorax, as suggested by the strong correlation between the sex of the thoracic cuticle and egg transfer and deposition; almost all the gynandromorphs with female thoraces laid eggs while most of those with male thoracic cuticle did not. That the egg transfer and the egg deposition foci are located in the thorax is also supported by the observation that decapitated females can deposit more than one egg each (also reported by GROSSFIELD and SAKRI 1972) and all but one of these must have been transferred after decapitation since the uterus holds no more than one egg at a time (see also HOLZWORTH, GOTTLIEB and SPECTOR 1974). The foci do not seem to be located in the abdomen since eggs are not laid by isolated abdomens (A. M. HANDLER, personal communication). The "sperm release focus" also appears to be located outside the head because larvae hatched from all the eggs deposited by inseminated and decapitated females. It is possible, however, that the above mentioned foci are activated at or shortly after eclosion, as was found for the initiation of vitellogenesis (HANDLER and POSTLETHWAIT 1977).

Foci that control the detection of insemination, the rate of oviposition and the location of oviposition seem to be located in the central nervous system, as suggested by the following correlation: in those gynandromorphs that either failed to detect insemination, oviposited at an abnormal rate or oviposited at arbitrary locations, the head cuticle was either entirely or mostly composed of male tissue. The correlation was weaker for the thoracic and even weaker for the abdominal cuticle. Also, most of the above gynandromorphs courted virgin females and early

courtship actions are known to be controlled by brain sites (HALL 1977, 1979). Females with partially destroyed *pars intercerebralis* could not detect insemination and laid eggs with a rate characteristic for virgin females (BOULÉTREAU-MERLE 1976).

Inseminated Drosophila females laid several eggs a day under normal conditions leaving very few if any mature eggs in their ovaries. However, in many of the gynandromorphs studied, the rate of oviposition was much lower than that of egg maturation, leading to accumulation of matured eggs (see also COOK 1978). Accumulation of mature eggs in the ovarioles has also been observed in virgin females and several female sterile mutants; sealing of the vaginal opening brings about the same effect (BAKKEN 1973). The egg accumulation effect clearly shows that the control of production of new egg chambers and maturation of eggs has little to do with the control of reproductivity but is a rather autonomous process (HANDLER and POSTLETHWAIT 1977).

The valuable comments of P. J. BRYANT, F. EPPER, J. C. HALL, A. M. HANDLER, W. HANRATTY, L. I. HELD, JR. and B. J. SEDLAK are greatly appreciated.

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Corresponding editor: T. C. KAUFMAN