

Genetic and Behavioral Studies of Female Sex Appeal in *Drosophila*

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*The sex appeal of a *Drosophila melanogaster* female is defined here as the stimulus (or set of stimuli) which induces wing vibration in courting males. A quantitative measure of sex appeal is the cumulative duration of wing vibration induced by a given female averaged over several consecutive test intervals using different standardized male testers (sex appeal parameter, SAP). By use of SAP, both males and females are found to have the same amount of sex appeal on the first day after eclosion. However, males rapidly lose it by the next day, so that mature males become distinct from females. We report the ontogeny of the male's response to sex appeal. By the SAP method, we also demonstrate that the male's response is dependent on his previous encounter with females. The sex appeal of 287 gynandromorphs was examined in order to localize the sex appeal focus by means of blastoderm fate mapping. Most mosaic flies were classified as either positive (femalelike, with high SAPs) or negative (malelike, with SAPs of zero). Sixteen percent of the gynandromorphs had intermediate levels of SAP, inducing only short vibrations, a response which males rarely give to normal females. Assuming that the gynanders with such intermediate sex appeal must have both female and male foci, distances to the foci from external landmarks were calculated. The center of the focus seems to be an internal structure mapping to the ventroposterior region of the blastoderm fate map, close to the primordia of the anterior sternites. The focus might include a large mesodermal area, but only part of it must have a female genotype for the sex appeal to be expressed. A possible involvement of the fat bodies in*

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production of the sex appeal stimulus is discussed in relation to these findings. Consistent with this conclusion is the fact that females whose abdomens were amputated still retain enough sex appeal to induce male wing vibrations.

KEY WORDS: sexual behavior; *Drosophila melanogaster*; genetic mosaics; ontogeny of behavior; wing vibration.

INTRODUCTION

Many authors have described and analyzed in detail the sequential behavioral patterns displayed by both sexes of *Drosophila melanogaster* during courtship (Sturtevant, 1915; Spieth, 1952, 1974; Bastock and Manning, 1955). When a mature male detects presence of a female, he orients toward her and vibrates one of his wings, which is extended at a right angle to his body axis. He continues courting by following her, until he licks her genitalia and attempts copulation.

This behavioral pattern has recently received renewed interest, since it was shown that such a complex behavior can be dissected genetically, both by mutations affecting a specific aspect of the pattern (Burnet and Connolly, 1974) and by the use of sexual mosaicism (Hotta and Benzer, 1976). Hotta and Benzer (1976) used Sturtevant's (1929) fate map technique to analyze the embryonic origin of structures affecting courtship behavior. A fate map, or two-dimensional representation of blastoderm sites fated to become adult tissues, is constructed using gynandromorphs. Hotta and Benzer (1976) demonstrated that one side of a male brain is sufficient for the execution of male courtship behavior up to wing vibrations, while the sex of other structures such as the surface sense organs including antennae, the wings used for the behavioral display, and the thoracic ganglion which coordinates movement does not need to be male.

However, the female's role in courtship is understood less well. She produces stimuli which increase the propensity of males to court. She may terminate courtship by accepting or rejecting the male; this latter behavior has been shown to be under neuroendocrine control (Manning, 1967). This article is concerned with the initiation stimuli. Several possibilities have been postulated; among them are visual, tactile, and olfactory cues (Spieth, 1974). The existence of female sex pheromones has been claimed (Sturtevant, 1915; Shorey and Bartell, 1970; Averhoff and Richardson, 1974). As it was not clear whether a single stimulus releases the male's entire courtship, we first concentrated on the stimulus (or set of stimuli) releasing a very conspicuous male courtship behavior, wing vibration. We call the stimulus that releases wing vibration "sex appeal." As to the physiological and biological nature of sex appeal, several fundamental questions may be

raised: (1) Is it sex specific, and, if so, is this true for all developmental stages? (2) Which structure of the body needs to be genotypically female in order for the individual to possess it? Such a structure will be called "sex appeal focus." (3) Which structure of a female produces, stores, and releases it? Such a structure will be called the "sex appeal bearing structure." In the present study we have tried to answer these questions, by combining behavioral, physiological, and genetic methods. Two articles also dealing with sex appeal appeared during completion of the manuscript of this article (Nissani, 1977; Hall, 1977); they will be commented on in the Discussion section.

MATERIALS AND METHODS

Strains

All strains and the mutant genes used were originally derived from stocks at the California Institute of Technology (for details, see Lindsley and Grell, 1968). The wild-type used was a Canton-S strain fed on the usual yeast-cornmeal-agar medium and raised at 25°C.

Courtship Chamber

The courtship chamber used was a shallow watchglass of 30 mm diameter and 2 mm internal height, placed on a glass plate. This chamber was carefully washed with detergent before use. For an examination of courtship behavior, a pair of flies were introduced into the courtship chamber and observed for 5 min under a binocular microscope. Their encounter could never culminate to copulation because of the limited height of the courtship chamber.

Measurement of Sex Appeal

We define sex appeal as a stimulus emitted by a female which induces male's wing vibration. To measure it, we let each tested female meet with a tester male in the courtship chamber and observed their behavior for 5 min. We chose the male response of wing vibration as the most conspicuous signal of early male's excitation and measured the cumulative time during which a tester male showed it against the tested female. The cumulative wing vibration time reflects the quantity of female excitatory stimuli. It may also be dependent on inhibitory stimuli and the physiological state of the courter affecting responses to the female's stimuli. To reduce variability from tester males, we used an average value obtained with several male testers whose environment was standardized in the following way. Wild-type

males, without any apparent anomaly, were collected within 12 hr after emergence, isolated in separate vials, and kept on fresh food. When these flies were 3 days old, they were introduced into the courtship chamber individually with a wild-type female whose sex appeal had to be measured. The tester male's behavior was observed for 5 min, and the time he spent vibrating wings was measured cumulatively. The same female was then retested successively with five other standard males. The values were averaged and defined as the sex appeal parameter (SAP) of a given female.

Sex appeal of body parts was also measured by introducing a freshly dissected body part into the courtship chamber with a tester male.

Production of Mosaics

Gynandromorphs (gynanders), genetic mosaics with respect to sex, were produced by mating males with recessive sex-linked alleles of morphological marker genes such as *y* (yellow cuticle and bristles) and *w* (white eyes) to *In(1) w^{vc}* [Inversion (1) white-variegated Catcheside] heterozygote females (Lindsley and Grell, 1968). F₁ daughters with the genotype of *In(1) w^{vc}/y w* tend to lose the ring-shaped *In(1)w^{vc}* chromosome from one of the early cleavage nuclei to become gynanders. In these mosaics, female parts [*In(1)w^{vc}/y w*] have a wild-type phenotype, since the recessive marker genes are hidden by normal alleles present on the *In(1)w^{vc}* chromosome. On the other hand, male parts (*y w/0*) can be readily recognized by the hemizygous recessive *y* and *w* marker genes, as far as surface genotype is concerned.

Scoring Mosaics

Surface genotype pattern of each gynander was scored for 40 pairs of surface landmarks. For names and location of the landmarks, see Fig. 2 of Hotta and Benzer (1972).

After gynanders were 4 days old, their sex appeal was measured. Correlations of surface genotype patterns with presence or absence of sex appeal were analyzed by the blastoderm fate mapping method. The basic concept and procedures of the mapping are described in Hotta and Benzer (1972). Modifications of the method will be given in the text.

RESULTS

Experimental Measurement of Sex Appeal

SAP of Normal, Mature Individuals

We measured SAP of 71 Canton-S wild-type females aged 4–7 days using the method described above (Fig. 1). There was a maximum between

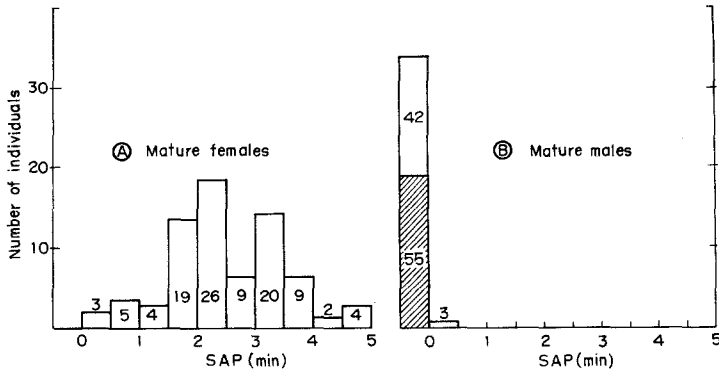


Fig. 1. Histograms comparing ability of either mature females (A) or mature males (B) to induce wing vibrations from male testers. A: Wild-type female, 3 days after eclosion, was placed with a fresh, mature male, and the total time the male vibrated his wings in a characteristic courtship pattern was measured during a 5-min test interval. The same female was tested similarly with five other tester males. The cumulative wing vibration times were averaged and called the "sex appeal parameter" (SAP) of the female. Seventy-one mature females were tested, and the distribution of their SAP is shown in this histogram. Note that there was not a single female with a zero SAP value. B: The same measurement was made with 35 wild-type males, 3 days after eclosion. Among them, only 3% showed significantly non-zero SAP values (SAP < 30 sec). Among the other 97%, however, there were 55% which induced a single very short vibration only once, which lasted only about 1 sec. These individuals with the abortive vibration are indicated as a shaded column in the histogram. The two histograms clearly show presence and absence of the sex appeal in mature females and males, respectively.

2 and 3 min. Although the SAP varied from individual to individual, not a single female had a score of zero. Figure 1 also shows the results of similar tests on the sex appeal of mature wild-type males (more than 3 days after eclosion); 97% of them induced either no vibration at all or a single vibration (SAP \approx 1 sec). This single vibration was repeated in only 3% of the cases, but the SAP even in these cases was less than 15 sec. It is clear that the SAP can be used to differentiate mature females from mature males without ambiguity.

Ontogeny of Sex Appeal

Young and old wild-type females (1–20 hr after eclosion) were courted by tester males with equal vigor. As shown in Fig. 2A, the average value of the sex appeal parameter did not vary significantly with female's age through at least 5 days after eclosion. However, SAP was somewhat more variable and on the average much lower during the first 10 min after emergence than later.

We also investigated whether males lacked sex appeal at all stages of the development and found that young wild-type males, soon after emergence, were highly attractive. Most of them were chased very

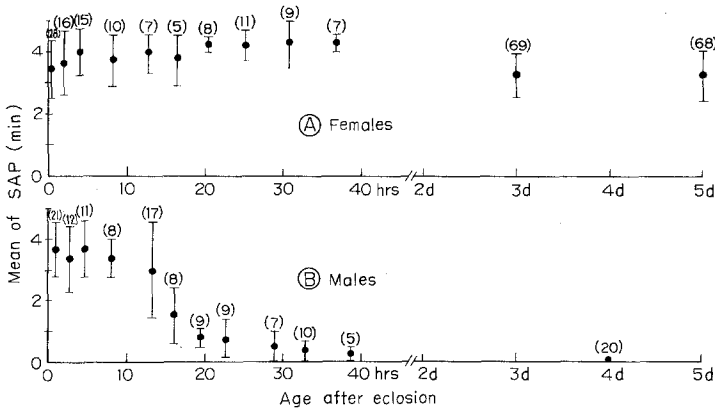


Fig. 2. Temporary femalelike sex appeal of immature males (B) compared with the age-independent sex appeal of females (A). Flies were isolated soon after emergence and kept individually until the courtship test. Several flies, either male or female, of the same age, were tested individually with male testers, and their SAPs were scored. Age dependence of sex appeal in young females (A) or young males (B) is shown. The ordinates indicate mean SAP (SAP); the abscissas show age in hours and days after eclosion. For each point, number of flies examined (*n*) is given in parentheses and standard deviations are shown with bars. Standard deviations (SD) have been calculated with the formula $SD^2 = \Sigma(SAP - \bar{SAP})^2 / (n - 1)$.

vigorously by mature males, which performed the sequence of action patterns they usually display toward females, very often to the point of attempted copulation. About 10 hr after emergence, the young males started to resist homosexual courtship by fleeing, kicking, and flicking their wings. The mature males, however, persisted in their courtship. During this period, the SAP value was not significantly different from that of mature females (Fig. 2).

By 1 day after emergence, however, males lost most of their sex appeal, and by 3 days of age they induced only a weak courtship response. This residual sex appeal, observed in 55% of cases (Fig. 1), involved a brief pursuit and a very short wing vibration (SAP \approx 1 sec), repeated within the test period in only 3% of the cases (Fig. 1).

Maturation of the Male's Vibrating Ability

In the first hours after emergence, young males showed no reaction toward a nearby female. Later in the first day, they started some orientation and short bouts of vibration directed toward females. At 30 hr of age there was a sharp increase in the duration of wing vibration (Fig. 3). Attempted copulation had a similar age dependence. Ability to perform wing vibrations and to attempt copulation was thus fully developed by 40 hr after eclosion.

In an experiment involving a set of 42 males aged between 15 and 30 hr, we recorded both the vibration time they induced from a mature male mate and their own vibration time toward a female. The disappearance of the femalelike sex appeal of young males preceded the appearance of their courting ability. Males having both abilities were very rare.

Male "Fatigue"

When a mature wild-type male was tested with a series of females, the cumulative wing vibration time decreased with the number of tests. Figure 4A illustrates this "fatigue" observed in an experiment in which each of 25 males was allowed to court a female for 5 min, respectively, each encounter being followed by a 5-min rest interval. The males showed no more response after the seventh trial. It took almost a day for the males to recover from the fatigue (Fig. 4B).

Females courted by a series of fresh mature males never lost their sex appeal in a systematic fashion.

Sex Appeal Focus

The sex appeal of gynanders was analyzed to identify the sex appeal focus, that is, a structure which must be female for a mosaic to have sex appeal.

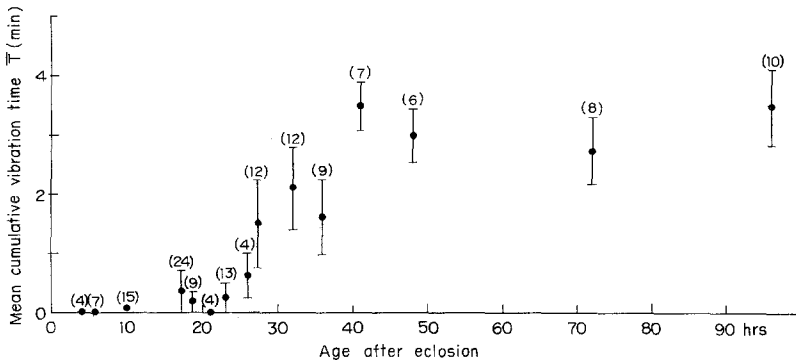


Fig. 3. Maturation of the male's ability to display wing vibrations in response to female sex appeal. Males, isolated soon after emergence, were tested at various stages for their ability to respond to the sex appeal of mature wild-type females. For each male the cumulative wing vibration time (T) was measured during 5 min, and then the individual values obtained for males of the same age were averaged. Calculated mean cumulative wing vibration time, \bar{T} , is plotted against males' age after eclosion. Numbers of flies examined (n) are shown in parentheses, and bars attached indicate standard deviations of the observations (SD). $SD^2 = \Sigma(T - \bar{T})^2 / (n - 1)$.

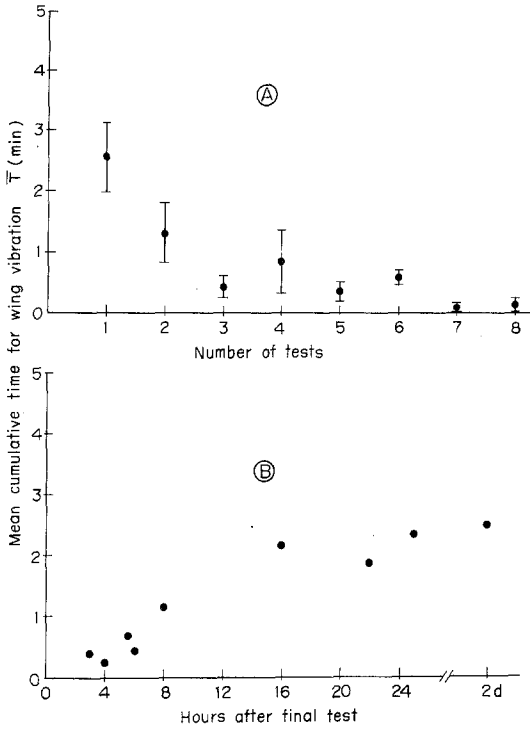


Fig. 4. Male "fatigue." A: Normal males were isolated soon after emergence and were not allowed to meet a female until the beginning of the experiment. When the male was 4 days old, he was introduced into a courtship chamber and was exposed individually to a normal female (3 days old) for 5 min. The time he spent vibrating his wings was scored, and then the female was removed. The male was left alone for 5 min, and a new normal female was introduced. The behavior of the male during the rest period was tested but never was any wing vibration observed. Such courtship tests were repeated 8 times in succession. Twenty-five males were examined, and the cumulative times of wing vibration for each test were averaged and shown. B: After the eighth test, these males were isolated again and the recovery from the "fatigue" was followed through time. Each point for the first 25 hr is the mean score of three males. Males were not reused.

Analysis of the Sex Appeal of Gynandromorphs

As expected from the fact that immature individuals of both sexes have high sex appeal, all gynanders less than 1 day after eclosion were found to be able to induce long wing vibrations from mature, fresh tester males (25 young mosaics were tested). Such gynanders were then kept in isolation for 4 days and their sex appeal was reexamined. Each tester male was first checked with a normal female to confirm its normal courtship behavior. Then the male was transferred into a courtship chamber with a gynandromorph, and their behavior was observed for 5 min. This test was repeated the following day using a different tester male. When the results

were not consistent, a third test was performed the next day. Among 267 sex mosaics examined, 123 (46%) induced "long wing vibrations" (this refers to SAPs greater than 30 sec), 88 of them in a reproducible way and 35 in only one of the tests; 101 (38%) failed in all of the tests. Among the latter, 31 gynanders induced a single vibration, which we decided not to count, since such brief vibrations are often observed among pairs of normal males. The remaining 43 mosaics (16%) always induced "short vibrations" (SAP values below 30 sec).

Among normal females, such low SAPs were very rare (4%), while they were common among gynanders (16%) (Fig. 5). We therefore divided the gynanders into three behavioral classes: (1) positive, those inducing "long vibrations" from tester males; (2) intermediate, those inducing only "short vibrations"; and (3) negative, which could not induce significant wing vibrations from tester males. When these criteria are applied to normal flies, 96% of normal females would belong to the positive class, while 97% of normal males would be classified in the negative class.

Correlation Between Behavior and External Mosaic Pattern of Gynanders

After the behavioral tests, every gynander was examined under a binocular microscope to score the surface mosaicism by means of the X-linked, recessive marker genes expressed only in male parts. Randomly chosen examples of the surface patterns in mosaics belonging to each of the three behavioral classes are shown in Fig. 6.

Before calculating distances, we tried to determine the approximate location of the sex appeal focus, by correlating the gynanders SAPs with the genotypes of their heads, thoraces, and abdomens (Table I). It appears that the presence of sex appeal in gynanders is more closely related to genotype of the abdomen than to that of head or thorax. There were five gynanders which had a completely male abdomen externally but which belonged to the positive class. There were also five mosaics which had an abdomen

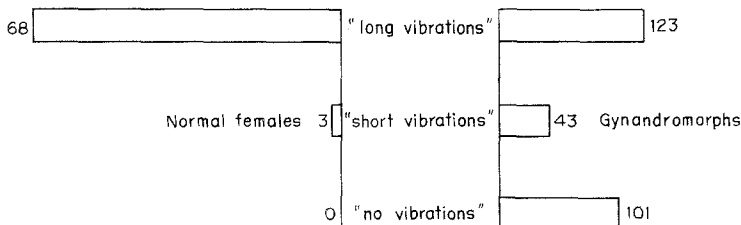


Fig. 5. Comparison of sex appeal in normal females and gynandromorphs. "Long vibrations" refer to SAPs greater than 30 sec, "short vibrations" refer to SAPs less than 30 sec, and "no vibrations" refer to SAPs \approx 1 sec. Bars have lengths proportional to the percentage of each group within both ensembles. The number attached to each bar shows actual number of individuals belonging to the group.

Table I. Matrices for Preliminary Mapping of Female Sex Appeal Focus^a

	Head			Thorax			Abdomen		
	Entirely female	Mixed	Entirely male	Entirely female	Mixed	Entirely male	Entirely female	Mixed	Entirely male
Positive	46	38	39	33	66	24	48	70	5
Intermediate	11	15	17	1	22	20	5	27	11
Negative	30	52	19	2	38	61	5	37	59

^a Surface genotypes (sexes) of gynanders' head, thorax, and abdomen are correlated to their ability to induce long wing vibrations (positive class), short wing vibrations (intermediate class), or no wing vibrations (negative class) from male testers.

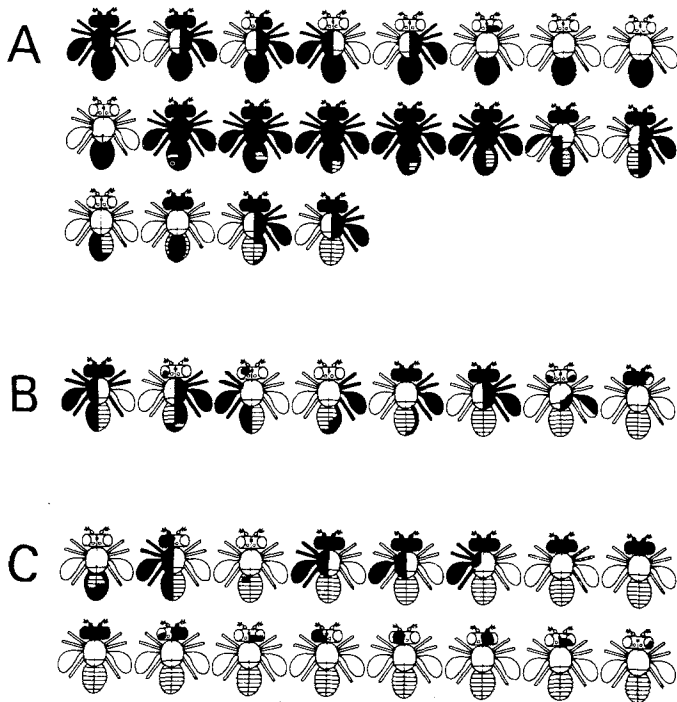


Fig. 6. Surface mosaicism patterns of 44 randomly chosen gynandromorphs belonging to the three behavioral classes. A: Positive class. B: Intermediate class. C: negative class. (See text.) Darkened areas show female genotype while open areas are male. Note the correlation between presence or absence of the sex appeal and the sex of the abdominal surface.

externally completely female but which belonged to the negative class. This suggests that the focus is not on the abdominal surface.

By taking two homologous, bilateral surface landmarks and correlating their genotypes (sexes) with the behavioral class to which the mosaic individual belongs, mosaics can be classified according to 3×3 matrix as shown in Table II.

Table II. 3×3 Matrix for Classification of Mosaics^a

Sex appeal	Bilateral surface landmarks <i>A</i> and <i>A'</i>		
	Both sides female	One side female One side male	Both sides male
Positive	P_{11}	P_{21}	P_{31}
Intermediate	P_{12}	P_{22}	P_{32}
Negative	P_{13}	P_{23}	P_{33}

^a P_{ij} represents fraction of mosaics belonging to group.

Assuming that the blastoderm is made up of two symmetrical hemispheres, the sex appeal foci should show bilateral symmetry. One cannot evaluate the sexes of the foci on both sides separately but can only detect the presence or absence of sex appeal in each individual. We used the bilateral, symmetrical, pointlike foci model of Hotta and Benzer (1972) to calculate fate map distances between the sex appeal focus and surface landmarks from such matrices.

We assumed that intermediate cases are mosaics with one female and one male sex appeal focus, since a significant fraction of gynanders have partial (intermediate) sex appeal. Under this assumption, the fate map distances between sex appeal foci and ipsilateral surface landmarks (\overline{Af}) and the interfocal distance ($\overline{ff'}$) can be deduced from the matrix given above. They are

$$\overline{Af} = (P_{13} + P_{31}) + \frac{1}{2} (P_{12} + P_{32}) + \frac{1}{2} (P_{21} + P_{23})$$

$$\overline{ff'} : P_{12} + P_{22} + P_{32}$$

We constructed the 3 × 3 matrices for all 40 surface landmarks scored. Examples of these matrices are presented in Table III. The distances between the sex appeal foci and the primordia of all landmarks were calculated and are shown on the fate map of Fig. 7. The results indicate that the sex appeal foci are very distant from all head primordia (ca. 50 sturts) and

Table III. Examples of Matrices Correlating Genotype of External Landmarks with Presence or Absence of Sex Appeal^a

AO	53	25	45	AN	50	28	45
	13	6	24		13	5	25
	41	33	27		33	36	32
ANP	37	56	30	W	38	58	27
	1	18	24		1	19	23
	2	33	66		2	30	69
4t	57	49	17	5t	62	40	21
	5	16	22		6	19	18
	6	27	68		10	21	70
3s	75	31	17	4s	74	32	17
	6	21	16		5	22	16
	9	17	75		9	17	75

^a Presence or absence of sex appeal is compared with sex genotype of 40 cuticular surface landmarks for each gynander tested. The results have been tabulated in matrices similar to those in Table I. Among them, a few examples are shown in this table. They are for two head landmarks, AO (anterior orbital bristle) and AN (antenna); two thoracic landmarks, ANP (anterior notopleural bristle) and W (wing); four abdominal landmarks, 4t (fourth tergite), 5t (fifth tergite), 3s (third sternite), and 4s (fourth sternite).

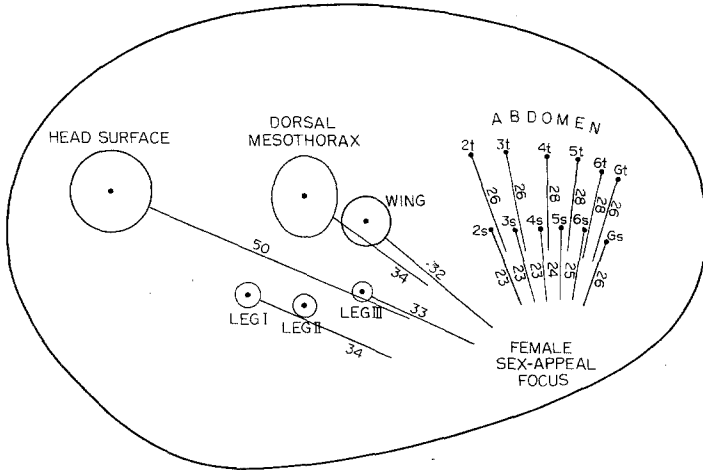


Fig. 7. Fate map of *Drosophila* female sex appeal focus. Gynanders were collected and their surface mosaic patterns were scored for about 40 pairs of surface landmarks. Each gynander was kept individually in a vial until its sex appeal was examined 4–7 days after emergence. The correlation between surface mosaic pattern and presence of sex appeal was tabulated in a matrix form as in Table III. The matrices were then converted to the embryonic fate map distances between sex appeal foci and surface landmarks. A distance between two points on the blastoderm fate map is defined as the probability with which the mosaic dividing line falls between them. It is expressed in “sturt” units, 1 sturt representing a probability of 1% that, among the entire set of adult mosaics, the two structures in question will be of different genotype.

closer to those of abdomen (ca. 26 sturts) than to those of thorax (ca. 33 sturts). The sex appeal foci are in the ventroposterior region of the blastoderm fate map closest to the primordia of the anterior sternites (23 sturts), at a significant distance from the midline (13 sturts). Another calculation, using a domineering focus model (Hotta and Benzer, 1972), gave a very similar position for the foci, suggesting that the conclusion we drew is not sensitively dependent on the interpretation of the “intermediate” cases. These results also suggest that the foci are internal, in agreement with the fact that there were several gynanders whose abdominal surface was entirely of a single sex and yet whose SAP values were characteristic of the opposite sex.

Amputation of Female's Abdomen

Fate mapping the sex appeal focus provides information about the site of sex-specific gene action for production of sex appeal, but it does not determine which structure actually emits sex appeal. Since the sex appeal focus lies in the posterior part of the blastoderm, we tested whether a female whose abdomen had been amputated would still possess sex appeal.

Table IV. Sex Appeal of Flies Without Abdomens^a

	Number of flies examined	Sex appeal positive
Females	46	40
Immature males	18	15
Mature males	24	1

^a Sex appeal was tested on wild-type flies whose abdomens had been surgically amputated. Sex appeal was scored as positive when an abdomenless fly could induce long wing vibrations from a male tester during the 5-min test interval. Abdomenless males had sex appeal when they were immature (less than 20 hr after emergence) but did not when mature.

Anesthetized females were severed between thorax and abdomen. The abdomenless flies recovered and were able to walk. Each of the two halves was presented to males. The males gave wing vibrations toward the isolated abdomen but also courted the abdomenless females very vigorously, performing the usual repertoire, especially licking the cut end frequently. Some males even tried to copulate with the cut thorax. The male's excitation must be caused by the true sex appeal of abdomenless females and not by a surgical artifact since 87% of the abdomenless females were able to excite males. By comparison, only 4% (1/24) of abdomenless males older than 3 days were exciting for males of the same age (Table IV). We performed the same experiment with young males possessing femalelike sex appeal (less than 20 hr after emergence). Abdomenless young males were as attractive as intact ones of the same age, as also were abdomenless young females of the same age (Table IV). When we cut females at the neck, the posterior parts were found to have sex appeal, but we never observed wing vibration of males directed toward isolated female heads.

These experiments show that the sex appeal bearing structure is extended over both thorax and abdomen.

DISCUSSION

Ontogeny of Sex Appeal

We demonstrated that adult females of *D. melanogaster* possess almost equal amounts of sex appeal throughout their adult life. We also found that immature wild-type males have sex appeal and can elicit long wing vibrations from mature wild-type males, characteristic of heterosexual courtship.

About 1 day after emergence, however, normal males no longer excite tester males. We consider the following hypotheses to explain the ontogeny of sex appeal in males: (1a) femalelike sex appeal of young males is abolished, or (1b) it is reduced to a level undetectable by wild-type males; (2) sex appeal is maintained but mature males produce an additional signal of repulsive nature.

We were, however, unable to find evidence favoring the existence of such an antagonistic signal. It cannot be mediated by the visual sense, since blind males (25 *tan*, or *norp A^{EE5}* hemizygotes examined) behaved in the same way as normal males. An etherized or decapitated mature male presented to a normal mature male (we checked 12 pairs of each type) also failed to elicit courtship, so behavioral rejection does not seem to be involved. Finally, the presence of anesthetized or decapitated males in close vicinity to a male courting a female did not interfere with the courtship. In contrast, if two mature males competed to court a female, their excitation was much enhanced. Therefore, the last hypothesis seems unlikely.

In the course of the present study, many strains bearing various mutations on the X chromosome have been screened for anomalous sexual behavior. We found one strain in which males were strongly attracted toward mature wild-type males (more than 4 days old). The majority of them showed long wing vibrations toward these males; some were even seen licking frequently and attempting copulation. However, the average cumulative time they spent for wing vibration within the 5-min test interval was much lower when they courted other males than when they courted females (SAP values 69 and 155 sec, respectively). A preliminary chromosomal mapping suggests that this is due to a gene near *chocolate*.

This fact leads us to favor hypothesis (1b), namely that at about 1 day after emergence, the sex appeal of males is decreased to a level below the threshold needed to excite mature wild-type males. This low level, however, could be responsible for the very short vibration often observed when two mature males meet each other. The suppression of the femalelike sex appeal of young males could be under neural control—for example, by a neurohormone; this is suggested by our preliminary observation that a few young males decapitated at about 8 hr after emergence were found to be attractive as long as they lived (five of them up to 5 days). Such protracted sex appeal was never observed when decapitation was performed on the fourth day.

A similar mutation on the third chromosome *fruitless (fru)*, causes homozygous males to court other males, as females (Gill, 1963). Homozygous males vigorously court each other and wild-type males; they are, on the other hand, vigorously courted by wild-type males (Hall, 1978). Hall's interpretation is that males have a femalelike sex appeal which

induces courtship in both themselves and wild-type males. There was no significant courtship by wild-type males toward males of our mutant strain. Therefore, our bisexual mutants may be hypersensitive to sex appeal stimuli and so are able to detect a low level of residual sex appeal in males.

Focus Mapping of Sex Appeal

By analyzing the sex appeal of gynandromorphs, we located the sex appeal focus in the ventroposterior region of the blastoderm fate map closest to the primordia of the anterior sternites (ca. 23 sturts). Using gynandromorphs produced by a different procedure (with the mutant gene *pal*), Hall (1977) also mapped the female focus to the abdomen. Furthermore, among 267 gynandromorphs, five had a completely male abdomen externally but were fully attractive. There were also five mosaics with an externally female abdomen but lacking sex appeal entirely. Thus the sex appeal focus seems to be an internal structure which arises from ventroposterior portion of the blastoderm fate map.

Although the approximate position of the sex appeal focus was established, a close look at the gynander analyses revealed unusual properties of the system. First, calculated sturt distances between the foci and various ipsilateral surface landmarks did not add up to show the unequivocal location of the focus by the triangulation method (Hotta and Benzer, 1972). Second, the matrices were asymmetrical with respect to the diagonal terms, especially those involving thoracic landmarks. The latter could be partly attributed to the fact that ratio of male to female tissues was significantly higher with thoracic landmarks. We occasionally encountered such a skewed mosaic ensemble, especially when we selected $\text{In}(1)^{w^{vC}}$ sublines with higher frequency of mosaic-producing ability. Such a selection would also enhance the semidominant lethal character of the $\text{In}(1)^{w^{vC}}$ chromosome. If we assume that the $\text{In}(1)^{w^{vC}}$ lethality focus is in the thorax, the higher frequencies of maleness in thoracic landmarks are readily explained, since those mosaics which have lost the $\text{In}(1)^{w^{vC}}$ chromosome from thoracic area must have an advantage in survival. This should affect the symmetry in the thorax matrices.

Another possible cause for the asymmetry is that the sex appeal focus is not a point on the blastoderm fate map but corresponds to a number of cells. Under such circumstances, the calculation would indicate only the center of a focus but not its actual shape and size. The existence of a large or diffuse focus can introduce some error into the calculations, since the mosaic boundary will occasionally divide the focus in various orientations to produce mosaics with an intermediate amount of sex appeal. Indeed, we observed that 16% of the gynanders were able to induce, repeatedly, only

short bouts of wing vibration in testers. It seems likely that these intermediate cases result from foci with sex mosaicism. Such gynanders would produce insufficient sex appeal to induce sustained bouts of wing vibrations.

Nissani (1977), investigating courtship behavior of *Drosophila* gynandromorphs, interpreted his results with a courting unit hypothesis. According to this hypothesis, sex appeal is produced by female's tergites, sternites, and thoracic integument cells, but these cells do not produce it in equivalent amount; the presence of a minimum number of courting units is required to determine male's response. However, in our study, two gynandromorphs had sex appeal, although their tergites, sternites, and thoracic integuments had completely male genotype. We also found eight gynandromorphs which possessed enough courting units according to Nissani's criteria but had low SAPs (including one with thorax and abdomen integuments completely female). Finally, we found several cases with similar genotype for their external structures but with or without sex appeal (compare, for example, in Fig. 6, the last mosaics of series A with the fourth and fifth of series C). Therefore, under our experimental conditions, we could not confirm Nissani's assignment of sex appeal focus to cuticular surface structures such as tergite, sternite, and thoracic integument. A possible cause of the discrepancy may be the differences in our experimental conditions. For example: (1) he measured female attractiveness by the whole series of male responses while we used the vibration response and measured it in well-defined conditions using more objective criteria; (2) he did not isolate tester males soon after eclosion, so the "fatigue" phenomenon we demonstrated in this study might have affected his results.

Identification of the Sex Appeal Focus

In Poulson's (1950) blastoderm fate map, the location of the sex appeal focus coincides with part of the presumptive mesoderm. It is also not far from the posterior midgut rudiment or from the abdominal nervous system. The fat body may be a good candidate for investigation, for the following reasons: fat body cells are derived from a large area of mesoderm (Janning, 1976) and are scattered widely within the imaginal body cavity; they are most abundant in the abdomen. The fat body has been regarded as the major center for metabolism of fatty acids, to which a number of pheromones, especially in Diptera, are structurally related and to be under complex neuroendocrine control. Fat bodies of female and male imagoes are chemically different, especially in their lipid contents (Butterworth and Bodenstein, 1968). The larval fat body, which seems more similar to the female's fat body than the male's, is known to persist in adults of both sexes until about 2 days after eclosion (Butterworth, 1972). Therefore, most of the

experimental results are consistent with the idea that the sex appeal stimulus (possibly a pheromone) is produced in both larval and female fat bodies, while male fat bodies do not synthesize it. To test such hypothesis, we have examined the sex appeal of males homozygous for the mutant gene apterous (ap^4). This mutation was first characterized by the defective wings but was also demonstrated to cause larval fat body to remain in imagoes of both sexes (Butterworth, 1972; Postlewaith *et al.*, 1976). Our preliminary observation showed that adult ap^4/ap^4 males maintain sex appeal for more than 3 days. Thus a correlation seems to exist between the disappearances of sex appeal and larval fat body.

That there are sex appeal foci does not necessarily imply a structure which actually synthesizes or releases that sex appeal stimulus (sex appeal bearing structure). It is possible that the female fat body produces a certain factor (e.g., pheromone precursor or hormone) which is used by other structures to synthesize the sex appeal or to control its synthesis.

In this discussion, we have implicitly assumed that the sex appeal focus is a femininity center which must have female genotype for the gynandromorphs to possess sex appeal. However, the existence and ontogeny of sex appeal in young males suggest another possible interpretation: the focus could be a center which controls the decrease of the young male's sex appeal, so that the focus would have to be of female genotype for the sex appeal to be maintained. At present, we do not have an effective method to discriminate between these two possibilities.

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