The Reproductive Behaviour of Gynandromorphic *Drosophila melanogaster*

Robert Cook *

Department of Genetics and Human Variaion, La Trobe University, Bundoora, Victoria, Australia

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Reproductive behaviour was studied in 192 gynandromorphs with female genitalia and reproductive system, produced by ring-X chromosome loss. Male and female behaviour patterns were frequently found to coexist in the same individuals, and male courtship behaviour, when it occurred, retained its characteristic hierarchical organisation. Sexually receptive individuals were found to be an almost perfect subset of those ovipositing, and the control of both of these behaviours mapped to the head, as did male orientation (courtship). High rates of wing flicking, a response of males to courtship, mapped rather to the thorax, although a quantitative analysis demonstrated that the frequency of flicking behaviour was also influenced by male tissue in the head. In non-ovipositing individuals mature oocytes were retained in the ovary. An egg held in the uterus is not deposited by a fly without female tissue in the head and all but one sexually receptive individuals laid eggs. It is therefore concluded that both of these behaviours depend upon closely related neural circuitry operating the genital musculature under control from the brain.

Introduction

Interest has long been present in the developmental analysis of the structure and behaviour of *Drosophila* using gynandromorphs [1, 2], but only recently, with the means of producing sex mosaics in quantity, have specific studies become possible. The work of Hotta and Benzer [3, 4], Hall [5] and Nissani [6] has already yielded clear indications as to the gross sites from which neural events leading to reproductive behaviour are initiated. The data to be reported here are, where comparable, largely consistent with those of these workers.

In *Drosophila* sex specific behaviour patterns may be quite clearly distinguished, although exceptions to this may be found [7]. This property fits *Drosophila* for exploitation by gynandromorph techniques, which have so far tended to concentrate mainly on the blastodermal "mapping" of sites controlling behaviour, from which deductions about the location of sites controlling behaviour in adults may be made. The current study hopes additionally to emphasize the potential role of gynandromorphs in the system analysis of the behaviour itself. Ultimately we seek an understanding of the logical organisation of the neural systems controlling behaviour. Questions such as the following may be clarified by gynandromorph studies: to what extent are the neural control systems exclusively involved in one behaviour pattern, or do they participate rather in a number of behaviour patterns? Do the neural control systems show mutual exclusiveness between the sexes, that is, can unlike sexed behaviours be identified which cannot coexist? Are there sex differences in input-output channels and transducers?

The current study was aimed to aimed to bear on these questions and to reveal the areas where further study would be most rewarded. To this end a compromise was necessitated between the number of individuals studied and the tests performed on each, leading to a relative loss of precision in fate mapping. High precision in the latter is anyway a nearly unattainable aim without the use of markers of internal tissue genotype, as used and developed for example by Kankel and Hall [8].

Materials and Methods

Stocks

Gynandromorphs were generated using the unstable Ring-X stock (In(1) W^{VC}) kindly provided by S. Benzer. Mosaicism is produced by the loss of In(1) W^{VC} (X^R) early in embryogenesis. Such loss usually occurs at the first division of the X/X^R nucleus, such that clones of X/X^R and X/O cells are produced. The course of subsequent events, insofar as they are known, is described by Hotta

* Present address: Max-Planck-Institut für biologische Kybernetik, Spemannstraße 38, D-7400 Tübingen 1, BRD.

and Benzer [3]. In the current experiments the rod X carried the markers *y w spl.*

Control experiments were run using X/X^R females from this stock (in which X^R had not been lost) and with homozygous *y w spl* females. Pacific strain males (3 – 4 days old) were used in all cases. Stocks were reared and observations performed at 25 °C.

Since the phenotypically male portions of the gynandromorph are produced by elimination of one X chromosome from a female (X/X^R) zygote, they lack a Y chromosome. Although no detailed studies of the behaviour of X/O flies have been reported, sterile (X/O) males show apparently normal sexual behaviour. The genetical markers employed give rise to other problems. The yellow body colour mutant can have clear effects on courtship behaviour [9, 10], although not all alleles have this effect (Green, cited by Burnet and Connolly [11]). The white mutant has reduced contrast sensitivity and increased light sensitivity (Hengstenberg and Götz [12]); Connolly *et al.* [13] observed the occurrence of "inappropriate" wing vibration in the double mutant *v; bw* which lacks the ommochrome and pterin pigments which serve to isolate adjacent ommatidia optically. The visual system is involved in the control of the tracking of the female by the male. No report of behavioural deficit associated with the mutant *spl* is known. These markers thus pose problems for a fine grained analysis of the courtship of the gynandromorphs. For this reason detailed recording of the behaviour of the gynandromorphs were not made and a control experiment was conducted to exclude the possibility of pleiotropic effects for certain crucial points.

Selection of flies

The experimental design imposed the constraint that the external genitalia and the abdominal reproductive organs be female. After behavioural measurement the flies were dissected to ascertain the sex of the reproductive system. Only those having normal ovaries and a structurally normal female reproductive system were included in the behavioural analysis, although the rest were included in the construction of the fate map for surface landmarks. Of a total of 224 gynandromorphs measured, 192 fulfilled the criterion for inclusion in the behavioural analysis. Selection of the animals in this way has a biassing effect on the distribution of mosaic tissue in the head and thorax.

Design of experiment and behavioural measures

Gynandromorphs selected for measurement at eclosion were aged singly in 10 cm glass tubes with live yeast medium. On the day of initial measurements (between days 3 and 6) the tube was inspected for the presence of eggs.

The gynandromorph was then tested for its ability to show male courtship behaviour. It was placed in a plastic observation cell (7 mm deep, 18 mm diameter), with a female of the Pacific strain. The pair was watched for 10 min, and any occurrence of the behaviours (as defined by Bastock and Manning [14]) orientation, wing vibration, licking, and attempted copulation was noted. Any courtship behaviour directed to the side of the cell was included.

The Pacific wild type female was then removed from the cell, and replaced by a Pacific wild type male. A detailed record of the courtship of the male to the gynandromorph was made. Each pairing was observed for a minimum of 10 min (up to 20 min for some of the first gynandromorphs), or until copulation occurred.

Behaviour of gynandromorphs

Additionally it is possible to measure the response of the gynandromorphs to courtship. Connolly and Cook [15] have studied the so-called rejection responses of *Drosophila* in some detail. On the day of eclosion virgin females show high levels (80% of rejection responses) of wing flicking in response to courtship, but this declines by days 3 and 4 to 20% of the total rejection responses. This response however is shown vigorously by males in response to courtship [14] and is thus a suitable behaviour with which to assess the sex of the mechanism responding to courtship. Since females may also show a low flicking rate in response to courtship it was necessary to adopt statistical criteria (see results section) for discriminating male from female flicking rates.

After this testing procedure the gynandromorph was returned to a fresh vial. Gynandromorphs which had copulated were allowed to separate from the male, which was then discarded. They were stored

singly for from 3 to 10 days and remating tests were conducted during this period. For this test the gynandromorph was observed with a male for a 10 min period and the presence of certain rejection responses noted: The aim of these tests was to ascertain whether or not the behavioural consequences of mating in gynandromorphs were comparable to those of normal flies, both in terms of rejecting behaviour and the re-appearance of receptivity [16].

The gynandromorphs not receptive in the initial behavioural tests were stored together with the male. After three days their vials were examined for the presence or absence of eggs and larvae. 145 of the 192 gynandromorphs which fulfilled the criteria for inclusion were also subjected to a test of their reflex oviposition [17]. They were immobilized with CO_2, and the presence or absence of egg extrusion noted.

Results

Behaviour of gynandromorphs

The frequencies of all the observed combinations of male and female behaviour were expressed as percentages, such that a Venn type diagram (Fig. 1 a – c) could be pieced together to describe the relationships in the behavioural data. The shape of the diagram is arbitrary, but the areas are based on calculated percentages. The outer broken line represents the whole sample of 192 gynandromorphs.

Fig. 1 a demonstrates that courtship behaviour in gynandromorphs did not suffer a breakdown in its characteristic hierarchical organisation. All flies which licked (20%) also showed wing vibration and orientation. Since the behaviour was not recorded in detail, it is possible that disturbances in sequence and relative frequency occurred. No abnormalities of patterning were observed in the behaviours orientation, wing vibration and licking although the intensity of courtship was often low. Attempted copulation, defined as the genitalia passing under the midline of the body, was rare and abnormal in form. It often resembled rather the "curling" response to courtship [15] of unreceptive females, consisting of a downward deflexion of the whole abdomen. This may be accounted for by the presence of bulky ovaries in the abdomen, rather than by differences in innervation or musculature.

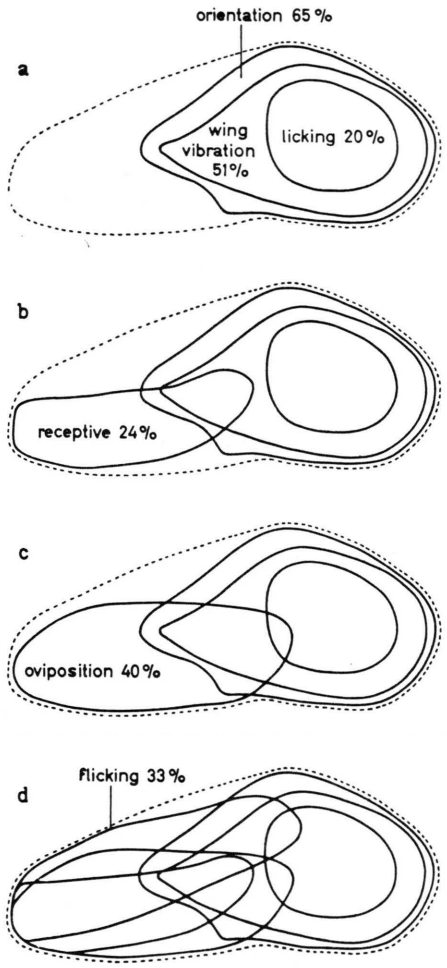

Fig. 1 a – d. Venn diagrams demonstrating the pattern of co-existence of behaviours in the gynandromorphs. All numbers are percentages of the total 192 gynandromorphs.

Receptivity

Twenty-four percent of the individuals were receptive. The intersection of receptivity with male courtship behaviours (Fig. 1 b) represents 7 percent of the sample. None of these flies also showed licking.

Oviposition

Superimposing oviposition onto this (40% of the gynandromorphs laid at least one egg), Fig. 1 c,

yields a greater intersection with courtship behaviour (13.5 percent of total), including licking, and encompasses all receptive individuals but one. Receptivity is thus an almost perfect subset of oviposition, which strongly suggests that the controlling sites are closely linked physiologically.

Since all gynandromorphs were observed to contain mature eggs and had no abnormalities of the reproductive system this clearly indicates that sites outside the abdominal reproductive system are involved in ovulation and/or oviposition. Dissection sometimes disclosed the presence of an egg in the uterus in non-ovipositing individuals, but not always. A clear characteristic of the ovaries of flies which had not oviposited was the presence of at least two mature filamented eggs per ovariole. These eggs had an indented appearance probably due to the extreme distention of the ovaries caused by egg retention.

Flicking

Flicking was first expressed as a rate per 100 seconds of wing vibration of the courting male (Fig. 2 a). Shown in Fig. 2 b and c are the flicking rates to courtship for X/X^R females and homozygous *yellow, white, split* females. The former resemble genotypically the female portions of the gynandromorphs the latter the male portions insofar as they express the marker genes. A strong bimodality is observed in the distribution for the gynandromorphs which is absent in the control females. The cut-off point between male and female behaviour was taken immediately above the antimode of the bimodal distribution, that is above a flicking rate of 8. On this criterion 67 percent of the gynandromorphs showed male flicking behaviour. (The other cut-off points shown, above 6 and above 16, yielded 71% and 54% male flicking respectively). In Fig. 1 d the female flicking set is superimposed on those for the other behaviours. Individuals outside this set showed a flicking rate above 8 per 100 seconds of wing vibration. Of the receptive individuals 35 percent gave a male flicking rate. Moreover, 24 percent of the individuals showing male courtship gave a female flicking rate. This degree of independence of flicking type from both male and female behaviours signifies a separation of the sites controlling flicking from those controlling the other behaviours.

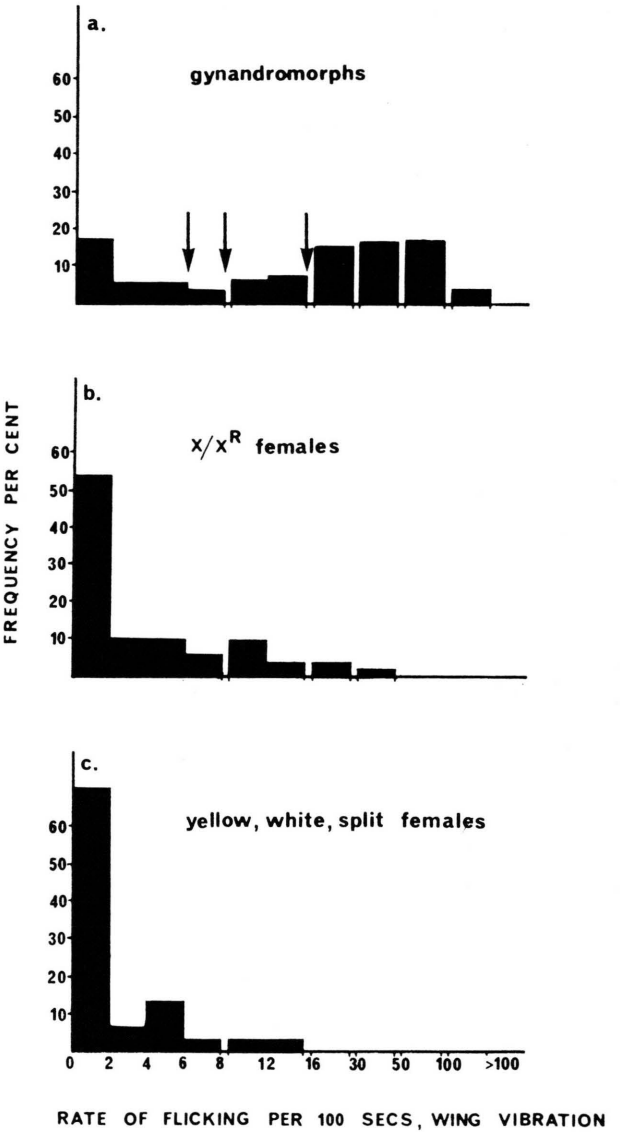

Fig. 2 a–c. Distributions of rate of wing flicking per 100 sec, of wing vibration for the types indicated.

Reflex oviposition

This test was conducted to complement the oviposition tests, although it was expected to correspond closely with them.

The data, dichotomised on both tests, are shown in Table I. There is very little correspondence between these tests. Only 49% of those ovipositing were positive on the reflex test, and more importantly, 48% of the individuals not ovipositing were positive on the reflex test. Few of the Ring-X

Table I. Contingency table classifying oviposition and reflex oviposition.

		Oviposition in Vial	
		positive	negative
Reflex Oviposition	positive	25	45
	negative	26	49

(15.8%) and *y w spl* (6.5%) control females showed reflex oviposition, although they invariably showed extensive oviposition.

It may be assumed that the egg extruded in reflex oviposition was held in the uterus immediately prior to the test. Thus at least 48% of the non-ovipositing gynandromorphs had an egg in the uterus, and the lack of oviposition was therefore not due to absent ovulation. Presumably the neuromuscular systems controlling the oviducts were functional, but not those controlling oviposition.

Whilst the possibility of physical blockages in the duct system, uterus and vaginal plates can not be excluded (see Holzworth *et al.* [18], for an example of this type of sterility) few signs of this were found on dissection. Light pressure on an egg in the uterus of a freshly killed female will usually expell the egg. In four cases where an egg was held in the uterus this would not occur and thus a structural blockage could have been present. In most cases therefore the retention must be associated with functional changes elsewhere in the fly.

Courtship elicited by gynandromorphs and controls

Measuring the behaviour directed to gynandromorphs by wild type (Pacific) males yields information on their "attractiveness" to males [19, 20]. The courtship elicited by control females of two types was also recorded: 1. Ring-X (x/x^R) females, approximating genotypically the female portions of the gynandromorphs. 2. Homozygous *y w spl* females, controlling for the effects of expression of the genetic markers in the male portions of the gynandromorphs.

Courtship may be analysed in terms of rates of occurrence of its component elements. Comparisons are here based only on the data from receptive individuals, since most of the controls copulated. Means and their standard errors, where appropriate, are presented in Table II. The percentage of receptive individuals in each group, their mean courtship duration (time from start of courtship to copulation) and the mean latency to the start of courtship are also shown in this table. Paired comparisons were made (Mann-Whitney U test) between the three experiments for each behaviour, significance levels being shown in the final column.

Few of the gynandromorphs were receptive during the test by comparison with the controls, al-

Table II. Means and significance levels for courtship directed to gynandromorphs and control females.

	Gynandromorphs	X/X^R	*yellow, white, split*	Significance on U test
	1	2	3	
Number measured	192	57	31	
% Receptive during measure	14.06	87.71	96.77	
Orientation/courtship time X 100	87.81 ± 3.00	91.68 ± 2.00	98.32 ± 0.98	$1-2 < 0.05$ $1-3 < 0.01$ $2-3 < 0.05$
Vibration/orientation time X 100	34.81 ± 1.64	31.03 ± 1.55	28.36 ± 1.64	$1-3 < 0.05$
Licking/vibration X 100	15.48 ± 2.39	18.98 ± 1.47	19.27 ± 1.98	$1-2 < 0.05$ $1-3 < 0.05$
Attempted copulation/vibration X 100	10.75 ± 2.31	8.79 ± 1.00	9.72 ± 1.42	NS
Flicking/vibration X 100	14.55 ± 4.94	4.28 ± 1.02	1.84 ± 0.64	$1-3 < 0.05$
Extrusion/vibration X 100	6.33 ± 2.08	5.93 ± 0.95	0.62 ± 0.31	$1-3 < 0.01$ $2-3 < 0.001$
Courtship duration	207.07 ± 22.94	195.24 ± 18.78	182.50 ± 16.13	NS
Latency (for all individuals)	88.19 ± 6.87	78.47 ± 7.35	82.58 ± 11.28	NS

Table III. Percentage of cases in which landmarks and behaviours were haplo-X. For head, thorax, and behaviours this is the mean of the percentages for each component item.

Percentage of cases haplo-X:	left		right
Head (including proboscis)	66·01		69·04
Thorax (including humerals)	45·79		46·72
First leg	51·56		51·04
Second leg	44·79		44·72
Third leg	39·58		42·19
Behaviour	—	46.42	—

though the mean duration of courtship for those receptive did not differ significantly amongst the three groups.

All three groups were distinguished from each other in the percentage of courtship time for which orientation occurred. This was highest with the *y w spl* females suggesting that they provided strong stimuli for courtship. In spite of this, the males spent a significantly greater percentage of orientation time in wing vibration with the gynandromorphs than with the *y w spl*. The gynandromorphs were licked significantly less than the two controls, although attempted copulation rate did not distinguish the groups.

Flicking rate differed in the anticipated direction for the gynandromorphs, which flicked at a higher rate than females. The overall mean flicking rate for the gynandromorphs (that is, including all 192 individuals) was 31.7 ± 3.36.

Ring-*X* controls and gynandromorphs did not differ significantly in the rate of genital extrusion, although the *y w spl* females were significantly lower than either. This result cannot at present be explained.

In summary, the gynandromorphs elicited a different pattern of courtship to the controls; it was less persistent and contained a lower rate of licking, but a higher rate of wing vibration.

Switch-off and return of receptivity in gynandromorphs and controls

Of the 27 gynandromorphs receptive in the courtship test, 23 were re-tested at varying intervals afterwards. Of those, 11 (47.8%) became receptive again in a mean of 6.9 days. The minimum time to recovery of receptivity was 3 days, the maximum 10 days. Of the 49 X/X^R females retested, 17 (34.7%) became receptive again in a mean of 7.1 days (minimum 4 days, maximum 11 days). Neither the proportions recopulating nor the number of days to recopulation differed significantly between these two groups.

Preliminary mapping of behaviours to head and thorax

An initial localisation of blastoderm regions controlling the behaviours may be gained by gross mapping to the head and thorax [3] which also obviates the problem of bilateral interacting foci. Of the 192 gynandromorphs studied, 116 had entirely male or entirely female external head landmarks. For the thorax only 102 could be classified as all male or all female. Using only these individuals the "sturt" [3] and "sturtoid" [21] distances from the six behaviours were calculated (Table IV). The "sturtoid" method is most appropriate for these data since there is an unequal distribution of male and female tissue (24 entirely female heads to 92 entirely male heads).

The gross distribution of mosaic tissue is also uneven, showing a male-female gradient from head to abdomen (Table III). In calculating fate map distances for behaviour several other difficulties must be borne in mind. As Hotta and Benzer [4] observe, sequentially occurring behaviours may be controlled by separate but dependent foci. The behavioural data presented here confirm those of Hotta and Benzer [4] in that subsets of individuals performing successive elements of courtship were entirely nested within each other. This could indeed be interpreted as representing a series of foci which are in some sense sequentially dependent upon one another. However, since courtship is labile and liable to shifts in the relative frequencies of its elements it is possible that such a pattern could be produced by differential activation of one single focus. The higher elements in the behavioural hierarchy, such as licking and attempted copulation, may be the most susceptible to reductions in frequency of occurrence as a result of differences in the activitation of the focus. Such differences in the activation of the system, being of diverse origins and possibly interacting with one another in unpredictable ways, may not localise to specific activating foci.

In Table IV the data for vibration and licking are presented for the two situations, assuming independence amongst controlling foci, line (a), and

Table IV. Sturt and sturtoid distances of the behaviours from the head (116 cases) and thorax (102 cases). Line b) (vibration and licking) gives distances based only on individuals performing preceding element (orientation and vibration respectively).

	Distance from:			
	Head		Thorax	
	sturts	sturtoids	sturts	sturtoids
oviposition	7	5	42	37
receptivity	4	2	45	36
flicking	20	13	30	28
orientation	18	12	50	44
vibration a)	25	18	49	50
b)	17	10	54	44
licking a)	53	50	44	71
b)	51	36	47	60

assuming sequential dependence, line (b). In case (a), as expected, vibration and licking are both more distant from the head than orientation. In case (b) wing vibration is brought back to a position equivalent to that for orientation. Licking however, although drawn closer to the head, is still clearly isolated from the orientation focus. This is consistent with either the existence of a second focus for licking or a greater rate of false negatives for this behaviour.

Oviposition and receptivity map very close to the head region, whereas orientation and wing vibration are somewhat further. Both of these are, however, distant from the thorax. Flicking is closer to the thorax than the other behaviours, whilst still mapping close to the head.

Further light on the location of the region(s) controlling flicking behaviour may be gained by classifying the head and thorax of each gynandromorph as either wholly male, wholly female, and half male and half female, and casting the flicking rates into the three by three table produced by this cross-classification. 169 individuals were cross classifiable on these criteria. One cell of the matrix had no entires, and two cells had less than three entires each. The remaining cells all had more than 10 entires each. The mean flicking rates generated by this procedure are shown in Fig. 3 a and b. Increasing maleness of the head, holding thorax constant (Fig. 3 a) leads to increased flicking rate (extremes significant on Mann Whitney U test at $p<0.01$). Also, increasing maleness of the thorax, holding head constant (Fig. 3 b) leads to increased flicking rate (extremes significant on U test at $p<0.05$). These data suggest that flicking rate is influenced by site(s) in the head and in the thorax, which have an at least partially additive relationship to each other.

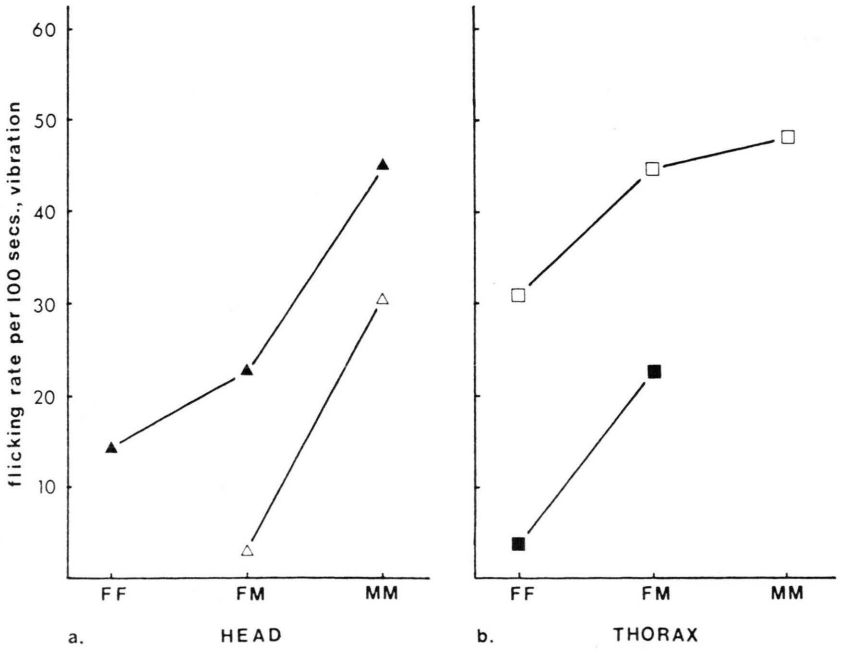

Fig. 3, a, b. Relationship of flicking rate to gross distribution of male and female tissue in head (a) and thorax (b). ▲, Thorax FM. △, Thorax FF. □, Head MM. ■, Head FM.

Fate mapping of structures and behaviours

A fate map for surface landmarks was constructed using the methods described by Hotta and Benzer [3], again modifying the calculation of distances by the sturtoid method of Gelbart [21]. No abdominal sites could be mapped since this tissue was largely female in the animals studied. The fate map for surface landmarks is based on 224 individuals (448 sides).

Behaviour may be controlled by a single focus or by several foci. A special case of multifocal control has been dealt with by Hotta and Benzer (op. cit.). With a symmetrical brain it is possible that behaviour may be controlled by neural mechanisms on either side. These mechanisms may interact as a logical "or" or a logical "and". A blastoderm focus behaving in the former way is said to be domineering, in the latter way submissive. These terms are usually applied to the behaviour corresponding to haplo-X tissue. The models of Hotta and Benzer [3] were applied to try and distinguish these cases. Blastoderm sites for the landmarks and behaviours are mapped in Fig. 4. Initially a map was made by hand using the triangulation method. Subsequently a computer program (kindly provided by J. Flanagan) improved this mapping [22]. This is a least squares procedure which, on the basis of the derivatives of an error function, successively shifts the coordinates of the foci mapped in the direction of a map which fits the observed distances better. When the initial guesses as to the coordinates of the behavioural foci were varied systematically it was possible sometimes to find more than one minimum. In most cases such ambiguity can be resolved by comparison of the error terms ($\Sigma((r-o)^2/o)$ [22]) associated with the placement. Representative error terms for each site are shown in parenthesis in the legend to Fig. 4. Not oviposition was submissive to oviposition, and not receptivity was domineering to receptivity. These two sites are very closely linked but cannot at present be regarded as sequentially dependent foci, since in one case an individual was receptive, but laid no eggs.

The putative foci for orientation and flicking are also shown in Fig. 4. Orientation was domineering to not orientation and flicking domineering to not flicking.

All of these sites fall into the region of the blastoderm shown by Kankel and Hall [8] to yield ganglia in the brain or thoracic ganglion. In conjunction with the previous simpler mapping to head and thorax (Table IV) it may be suggested with some certainty that oviposition and receptivity are controlled by mechanisms in the brain. Orientation

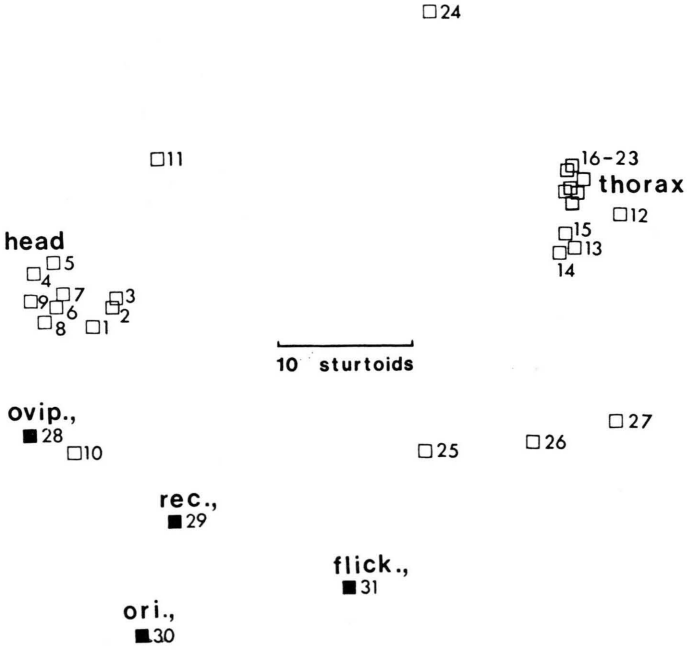

Fig. 4. Sturtoid, fate map of blastoderm for surface landmarks and behaviours. Mapped by least squares iterative procedure of Flanagan [22]. In parentheses: For landmarks, final error after 80 iterations mapping oviposition. For behaviours, actual final error. 1, Arista (0.03). 2, Palp (0.05). 3, Vibrissae (0.05). 4, Inner vertical (0.02). 5, Outer vertical (0.02). 6, Postvertical (0.02). 7, Occiput (0.02). 8, Ocellar (0.02). 9, Orbitals (0.02). 10, Eye (0.24). 11, Proboscis (0.04). 12, Wing (0.02). 13, Presutural (0.02). 14, Anterior notopleural (0.03). 15, Posterior notopleural (0.02). 16, Anterior Supra-alar (0.02). 17, Posterior supraalar (0.02). 18, Anterior post-alar (0.02). 19, Posterior post-alar (0.02). 20, Anterior scutellar (0.02). 21, Posterior scutellar (0.02). 22, Anterior dorso-central (0.02). 23, Posterior dorso-central (0.02). 24, Humeral (0.03). 25, First leg (0.10). 26, Second leg (0.09). 27, Third leg (0.06). 28, Oviposition (0.12). 29, Receptivity (0.14). 30, Orientation (0.19). 31, Flicking (0.17).

is probably also a brain site, taking into account its distance from the thoracic structures. Flicking however maps to the region corresponding most closely to thoracic ganglion sites in the map of Kankel and Hall [8]. When vibration and licking were taken as sequentially dependent foci they were found to fit domineering and submissive models respectively. However, when this is done the distribution of mosaic tissue for the contributing individuals becomes so unequal (*e. g.* 83% male for vibration focus from arista, 86% male for licking focus from arista) that plotting such values may have little meaning.

Coexistence of male and female behaviour

It is of interest in attempting to understand the possible logical organisation of the systems controlling sexual behaviour to seek individuals demonstrating the behaviour of both sexes, and to assess the extent to which the behaviour of each sex can operate independently.

How dependent, it may be asked, are male and female control systems in courtship? The Venn diagrams have already demonstrated that the male response mechanism to courtship, whose output is flicking and possibly running, is somehow independent of the female receptivity determining system, whose output is reduction in activity level [23, 24], suppression of kicking off the male, and ultimately the operation of the vaginal plates enabling copulation; the former response mechanism may be male, resulting in high levels of flicking, but the fly may be receptive: male and female response mechanisms would thus appear to be operating on sensory input in a parallel, non-interactive way. Their "catchment" of sensory input may of course not necessarily be the same and it would be of great interest to determine this.

Since insemination has far-reaching effects on the behaviour and reproductive physiology of the female, we may ask whether male or female courtship response systems are affected by this in the gynandromorphs. The results have already suggested that an operative female receptivity system is affected normally by insemination. Further to this, eight individuals with adequate data were found, which showed a male flicking rate, but were also sexually receptive and oviposited. Two of these individuals also showed male courtship behaviour. In all cases the response to courtship after insemination included both extrusion and flicking behaviour. Since flicking *rate* after insemination was not measured the question of possible changes in intensity cannot be answered. Nonetheless, it appears that the male response system has been uninfluenced by the insemination and continues to operate in parallel, but now also synergistically, with the female response system. As yet there is no clear example of a conditional behavioural incompatibility caused by simultaneous operation of male and female systems, although they may interact more subtly, leading, for example, to the appearance of sporadic behaviour [6].

Discussion

Gynandromorphism in *Drosophila melanogaster* produces no remarkable anomalies of behaviour such as those reported by Whiting [25] for *Habrobracon*. With *Drosophila* there are however fewer ways in which mixed responses to environmental stimuli could be produced, since fewer distinctive behaviour patterns are involved. The only behaviour suspected of being anomalous in the gynandromorphs was attempted copulation which was rare and often abnormal in form.

When courted, gynandromorphs will frequently turn and court the courter head-on. This response was observed in 80% of the flies classified as capable of male courtship behaviour, at a mean frequency of 6.2 ± 0.5 bouts per 10 min sampling period. This could be construed as a crossed-wires response [25] since the usual response of a male to courtship is wing flicking and running. However, males are stimulated to courtship by hearing the wing vibration of other males [24]. Additionally, a gynandromorph having a male head and female abdomen may be aroused to courtship activity by its own pheromones, which Nissani [6] finds map mainly to abdominal tissue.

The data on the attractiveness of the gynandromorphs to males are only partially comparable to those of Nissani [6], since the range of tissue distribution is here relatively limited. However, from Nissani's results we would expect the gynandromorphs to be courted less than the controls, and after a long initial latency. The latency is in fact longer for the gynandromorphs, but not significantly so. Orientation occurred for a significantly short-

er percentage of the total courtship duration, consistent with Nissani's results. However, the causality of differences in attractiveness can only be unambiguously assessed if the locomotor activity of the assayed fly is either controlled, for example by decapitation [19], by mechanical means (experiments currently in progress), or directly measured: activity levels may influence both the frequency and structure of courtship behaviour.

The results reported here are in agreement with those of Hotta and Benzer [4], Hall [5] and Nissani [6] concerning the site of control of female receptivity. In these experiments the genitalia of all gynandromorphs were female, so structural incompatibilities may be excluded from consideration. The sex of tissue in the head is clearly the most important determinant of receptivity. Such a finding is consistent with previous suggestions that the female processes courtship before allowing a male to copulate [16, 26, 27]. There are at least three sites at which receptivity could be inhibited by the absence of female tissue: Primarily, in the decision making system, and secondarily in the system which produces a juvenile hormone titre adequate for the switch-on of receptivity [16, 26]. There is circumstantial evidence from mutants ([20]; Cook and Grossfield, unpublished experiments) that feedback from the ovaries is important in attaining a sufficient juvenile hormone titre. Unless there are other sex-dependent links in the feedback loop this system is probably not important in the present experiments, since ovaries were normal; thirdly, receptivity controlling sites may lie in the system responsible for enacting the decision of the processing system, for example in controlling the operation of the vaginal plates, or in reducing locomotor activity sufficiently to allow copulation.

The high degree of correspondence between the behavioural subsets oviposition and receptivity (Figs. 1 b and c) makes it tempting to speculate that a common requisite for both systems is the ability to operate the genitalia such that eggs may be extruded or the male genitalia accommodated.

In normal females a decision making system is also involved in oviposition, since eggs are laid only in appropriate sites. It may therefore be considered as highly significant that the site controlling oviposition maps to the head, since this is also the site where diverse sensory information is likely to be integrated concerning the suitability of oviposition substrates. "Brain output" is clearly more important in unoperated flies than suggested by the decapitation studies of Grossfield and Sakri [17].

Absent oviposition was due largely to an absence of egglaying response, rather than to a lack of ovulation. As noted there was no correspondence between reflex oviposition tests, such that many flies laying no eggs were nonetheless positive in the reflex test, demonstrating the presence of an egg in the uterus. Apart from the possibility that four of these individuals had blockages in the posterior reproductive tract, absence of the necessary neural control for egg deposition is indicated.

The release of mature oocytes from the ovarioles is partially controlled by negative feedback from the uterus, such that the presence of an egg in utero inhibits ovulation [28]. In the gynandromorphs there was no breakdown in this negative feedback system, since even in ovaries heavily swollen with mature eggs no additional release of eggs into the ducts occurred. This is thus consistent with the hypothesis that ovulation is under local control within the abdomen.

Some doubt however exists as to the integrity of the system controlling vitellogenesis in gynandromorphs not laying eggs. Ovarioles were found to be supernormally packed with mature oocytes, most of which had an indented appearance suggesting abnormally high density. Such a build up of mature oocytes does not occur in normal females allowed to oviposit, but we have little information the situation arising when food is available but oviposition is not permitted. Again, hormonal feedback systems are implicated in controlling the processes leading to the production and uptake of yolk proteins in insects, in particular juvenile hormone [29] and possibly an oostatic hormone [30] although the latter has been disputed [31]. Alternatively a resorption process may be inhibited in the gynandromorphs. For example, cell death, which normally occurs in ovarian chambers [32] may not occur.

Flicking in response to courtship was examined in three ways to try and locate the influential factors, and the results suggest a somewhat complex system. The preliminary mapping to head and thorax suggested the relative proximity of the site to thoracic structures, and the fate mapping, with an error for flicking within the range of the other behaviours, strengthens the conclusion that a single focus in the thorax can be considered the major

site in determining the gross rate of wing flicking shown (*i. e.* male or female); this also seems to vindicate such an approach to a continuous variable as has been made here. Nonetheless, the sex of tissue in the head and rest of the thorax helps to account for the high variability in the flicking rates. It is unknown whether such influence is by neutral or humoral factors. The results are therefore not entirely discordant with those of Nissani [6] who suggested that flicking is controlled from the head.

Whereas localisation to the head of sites controlling oviposition, receptivity and orientation is clearly indicated, their classification as "submissive" or "domineering" bilateral foci unfortunately implies nothing about how the control system may operate. Inter-lateral commisures were demonstrated in the brain of *D. melanogaster* by Power [33]; further it has been demonstrated, for example by Hausen [34], that, for the visual system at least, considerable interlateral functional interactions may occur in the brain of *Musca* via transversely projecting neurones. Thus a site that is domineering over the corresponding contralateral site during the neural processing underlying a particular behaviour may achieve this in at least two ways: The outputs from the two sites may independently enter a further system which unites them as a logical "or", or the activity of the submissive site may be directly influenced by neuronal projections from the domineering, such that it is effectively driven by it. Only with more detailed localisation of nervous system sites controlling behaviour, such as that started by Hall [5], can such ambiguities begin to be resolved.

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