Behavioural pleiotropy of the *yellow* gene in *Drosophila melanogaster*

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SUMMARY

The *yellow* mutant ($y-1:0\cdot0$) of *Drosophila melanogaster* shows a reduction in body pigmentation associated with a decrement in locomotor activity and in male competitive mating ability. The effects of the mutant gene are specific to locomotor activity in the adult fly, measures of larval activity being unaffected. In the presence of active females *yellow* males offer a reduced intensity of sexual stimulation because they are less able to maintain contact during courtship. However, the impaired locomotor performance of *yellow* males is not the general cause for their reduced competitive mating ability, since the stimuli provided by *yellow* mutants courting inactive females appear to be both quantitatively and qualitatively indistinguishable from those of their wild-type male sibs. Nor is there any good evidence, as measured by the frequency of rejection responses, that the courtship stimulation offered by the *yellow* males is less acceptable to the females. The mutant males are nevertheless unsuccessful in achieving copulation with such females. It is suggested that impairment of mating ability in *yellow* males may be caused by changes in the efficiency for mating of their secondary sexual structures due to the effect of the *yellow* gene on the properties of the cuticle.

1. INTRODUCTION

The *yellow* mutant ($y-1:0\cdot0$) shows a reduction in the black pigmentation normally present in certain areas of the adult body cuticle and its derivative structures (Lindsley & Grell, 1968). Sturtevant (1915) first reported that *yellow* males are usually unsuccessful in a competitive mating situation and it seemed possible that this might be because the courtship behaviour of the mutant males was different or inadequately stimulating to the female. In a comparison of the courtship behaviour of *yellow* and wild-type flies Bastock (1956) reported that mutant males appeared less active, and that they were deficient with respect to the average duration of bouts of wing vibration display which forms an important element in the stimulation of the female. She suggested that the effects of the mutation on the sexual behaviour of the *yellow* males may be due to reduction in their sexual motivation.

The wing display of the male provides both visual and auditory stimuli. If the

antennae are removed from females they cannot perceive auditory stimuli. Bastock observed that a difference in success rate between mutant and wild-type flies remained in courtships involving antennaless females in light, and in darkness, which suggests that the reduced level of wing display offered by the mutants may not be a primary cause of the decrement in their mating success. Bastock seems not to have made a quantitative measure of activity in her flies, but Burnet & Connolly (1974) reported preliminary findings showing that *yellow* males have a consistent and significantly lower level of locomotor activity than their wild-type sibs. They suggested that this may cause breaks in courtship leading to a lower frequency of contact stimuli required to bring the female to a threshold of acceptance.

The aim of this investigation is to test whether there is a causal connexion between locomotor activity and sexual behaviour in *yellow* mutants, and to examine whether the *yellow* gene has effects on courtship behaviour *per se*, or whether the decrement in mating success of mutant males is a secondary consequence of some other pleiotropic effect of the gene.

2. MATERIALS AND METHODS

(i) *Stocks*

The *yellow* mutant allele described here is a type-1 mutant. Special care was taken to remove the effects of residual genetic variation within stocks used for behavioural studies by placing the *yellow* allele into each of two standardized genetic backgrounds provided by the isogenic wild-type stocks Novosibirsk and Oregon-K. Repeated backcrossing into and reisolation of the mutant from each wild-type background was performed for 20–25 generations prior to the beginning of the investigation.

(ii) *Experimental procedures*

(*a*) *Behavioural measures* are notoriously sensitive to environmental variables associated with conditions of culture. Subjects for behavioural tests were obtained from a backcross in which $+/y$ heterozygous females were crossed to their hemizygous wild-type male sibs. The male progeny consist of equal numbers of wild-type and *yellow* mutants, which were collected daily and transferred to freshly yeasted culture vials. Comparisons of performance of *yellow* and wild-type males in behavioural tests were made using full sibs raised under identical culture conditions. All experiments were performed at 25 °C under standardized conditions of illumination in the room in which the flies were cultured. Unless stated otherwise, all flies were aged 3 days after eclosion from the pupa, and were transferred to the apparatus for observation without anaesthetization.

(*b*) *Courtship behaviour* was observed in cells, 19 mm in diameter and 7 mm deep, moulded from opaque white plastic and fitted with clear lids. Flies were introduced into the cell via an entry port which could be closed by a plug. Mating speed was measured from the time of introduction of the male to the female in the cell until copulation occurred, or for a total period of observation of 20 min.

(*c*) *Open field locomotor activity* was measured in a 10 × 10 × 0·8 cm deep box

with walls and base of opaque white plastic fitted with a clear lid on which was engraved a lattice of fine lines forming 1 cm squares. Flies were introduced into the chamber through an entry port which was then closed by a plug.

Flies were observed under a binocular dissecting microscope and their behaviour recorded using a multichannel serial time/event recorder which gives simultaneous permanent records of separate behavioural measures against a continuous time base.

A single fly placed in an activity chamber shows an initially high level of open field locomotor activity which rapidly decreases to a more or less stable value. This is caused by the reaction of the fly to its surroundings. Reactivity and its relationship to locomotor activity have been analysed by Connolly (1967). In practical terms the significance of the distinction is that an initial settling down period is required before consistent measures of the spontaneous open field behaviour of a given subject can be made. The behaviour of each subject was observed for a continuous period of 100 sec beginning 2 min after the fly entered the activity chamber.

(*d*) *Larval locomotor activity and feeding rate* were measured following the procedure described by Sewell, Burnet & Connolly (1975). Larvae were transferred singly to a 10 cm diameter petri dish containing a water agar gel overlaid by a freshly made suspension of 1 % live baker's yeast in water. After a settling down period of 1 min the number of cephalopharyngeal retractions in 1 min of continuous feeding was recorded. Larval locomotor activity was recorded by counting the number of forward and reverse movements made by the larva during the observation period.

(*e*) *Acoustic recordings* were made using a Reslosound ribbon microphone and Tandberg 3341 X stereophonic AM tape recorder following the procedure described by Burnet, Eastwood & Connolly (1976). Recordings were made at 7·5 i.p.s. and the sounds monitored through headphones and visually displayed on an oscilloscope. Permanent visual records for measurement with a continuous time base were made using a Medelec u.v. fibre optics recorder. Flies used for recordings were aged 3 days after emergence from the pupa. Each male was placed in a single pair mating situation in the recording chamber with a wild-type virgin female. Females homozygous for the mutant genes *aristaless* and *thread* were also used. The mutant females lack a functional arista and have a high sexual response threshold (Burnet, Connolly & Dennis, 1971) which allows extended courtships to be sampled. Acoustic recordings were made of the first 8 min of courtship at a constant temperature of 25 ± 0.5 °C.

3. RESULTS

(i) *Adult locomotor activity*

The open field behaviour of individual males is shown in Table 1. On both genetic backgrounds the *yellow* males spend significantly more time standing still and significantly less time walking and running than their wild-type sibs. All subjects fly rather infrequently in the activity chamber. There are no significant

differences between mutant and wild-type flies with respect to time spent preening. Dyskinesis following loss of foothold on the sides or roof of the chamber is about equally frequent in mutant and wild-type flies indicating that there is no gross distubance of motor coordination in *yellow* males (Burnet, Connolly & Mallinson, 1974).

Table 1. *Open field behaviour of 3-day-old males, expressed as the mean time spent (sec) in performing each activity*

(Thirty males of each genotype were observed for a continuous observation period of 100 sec.)

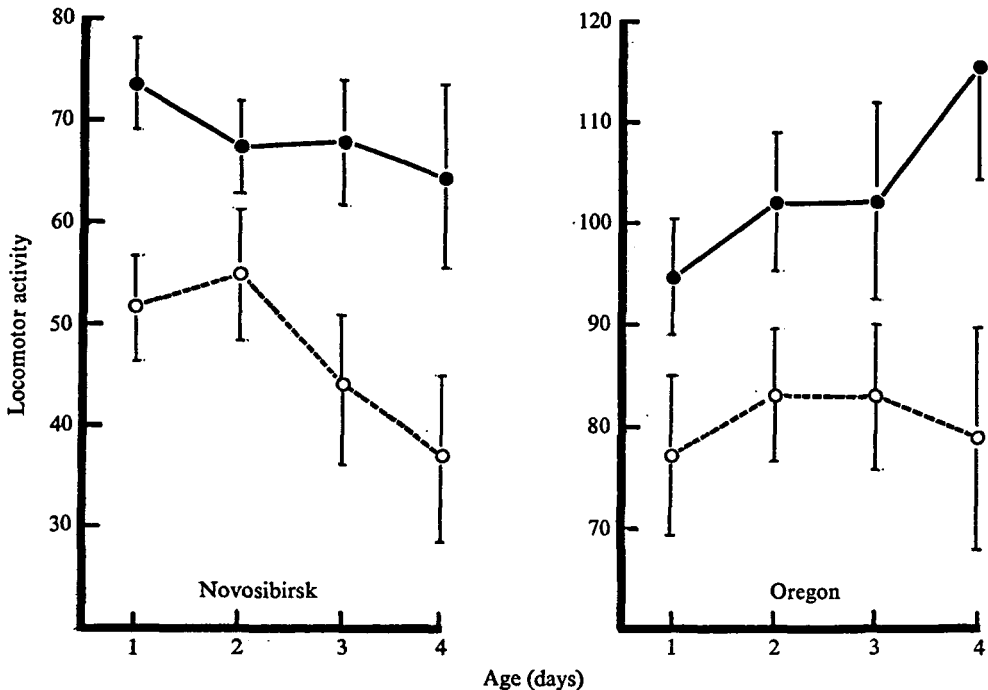|	Novosibirsk			Oregon		
	Wild type	*yellow*	*P*	Wild type	*yellow*	*P*
Standing	1·50	12·9	< 0·01	1·85	6·93	< 0·01
Preening	10·1	10·4	N.S.	9·31	8·09	N.S.
Flying	1·77	1·54	N.S.	0·33	0·25	N.S.
Dyskinesis	0·68	0·65	N.S.	0·07	0·16	N.S.
Walking/running	85·7	75·3	< 0·01	88·5	84·3	< 0·05

Fig. 1. Locomotor activity in the open field situation measured as the number of lines crossed per minute in the activity chamber. The mean activity score is given for the same group of subjects in each genotype group measured on successive days after eclosion from the pupa on day 0. ●, Wild-type males; ○, *yellow* males. The vertical bars indicate the 95 % confidence interval for each mean score.
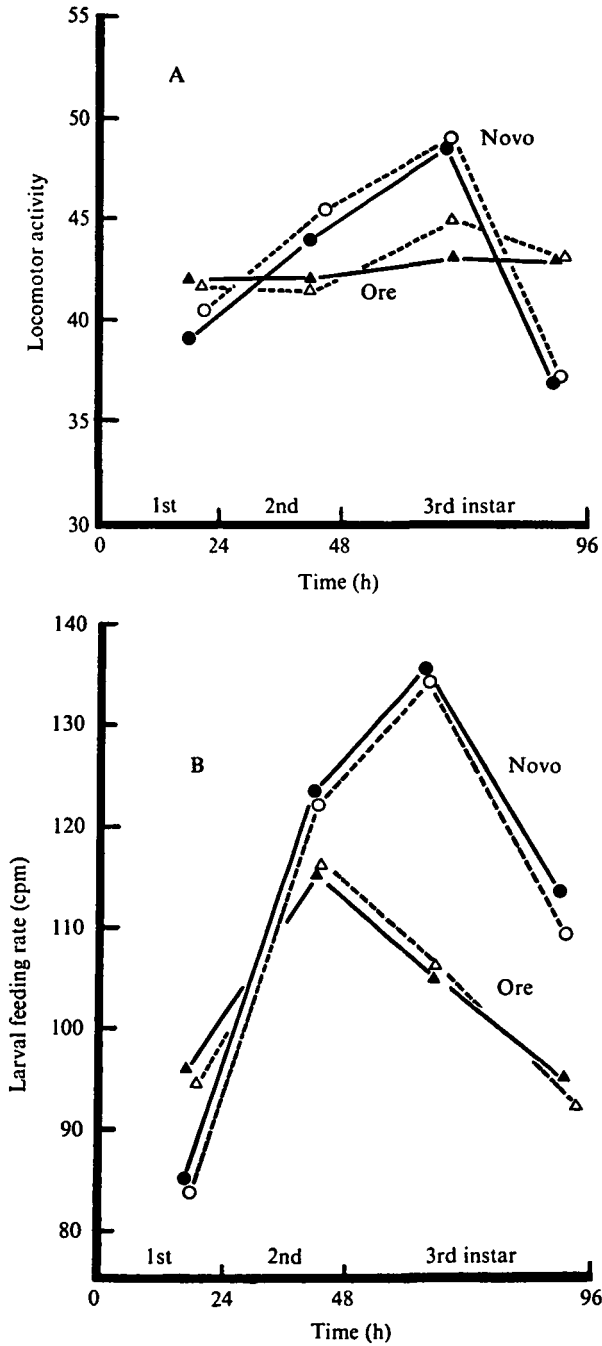
Fig. 2. Locomotor activity (A) and feeding rate (B) in larvae measured over a standardized 96 h larval period from the time of eclosion from the egg. Solid symbols, wild type; open symbols, *yellow*.

The pattern of activity in relation to age is illustrated in greater detail in Fig. 1, which shows that spontaneous locomotor activity is significantly higher in the Oregon than in the Novosibirsk background. There is also a systematic change in the mean activity score in relation to age which is in opposite directions in the two backgrounds. Within both backgrounds, and at each age, *yellow* males show a significantly lower level of spontaneous open field locomotor activity than their wild-type sibs.

(ii) *Larval activity*

An interesting feature of the *yellow* mutant is that larval as well as adult cuticular structures such as the cephalopharyngeal sclerites and spiracles are affected, and this raises the possibility that the pattern of gene expression may also extend to measures of motor performance in larvae. A genetic and developmental analysis of larval behaviour is given by Sewell *et al.* (1975). Larvae normally show a rather low level of spontaneous locomotor activity *in situ* on the culture medium but they feed continuously. The rate of larval feeding can be measured by the rate of cephalopharyngeal retraction, which is coupled to the pumping action of the muscular pharynx. The results illustrated in Fig. 2 show that, although the developmental trajectory of feeding rate in relation to larval age is different for the two genetic backgrounds, there are no significant differences between *yellow* and wild-type larvae, with respect either to feeding rate or locomotor activity. In contrast to its effects on the cuticular phenotype the expression of *y* evidently does not extend to larval activity. This agrees with previous findings (Sewell *et al.* 1975) that different patterns of motor activity are under separate genetic control in the larval and adult stages.

(iii) *Mating speed and competitive mating ability*

Males of each genotype group were placed in the single pair mating situation with a wild-type female of the corresponding genetic background. There is a small but significant difference in mating speed between the Oregon and Novosibirsk strains illustrated in Fig. 3. Novosibirsk wild-type males nearly all copulated with the females within 5 min, whereas less than 40 % of the *yellow* mutant males had successfully copulated within the 20 min observation period. In the Oregon background the result is similar but the mutant males are even less successful with the Oregon wild-type females.

The consequences of these differences in performance with respect to mating speed when males are in competition can be examined using a female choice situation involving a single wild-type virgin female with a mutant and a wild-type male. The outcome of 50 independent trials of this kind for each background is shown in Table 2. As expected, *yellow* mutant males are at a severe disadvantage relative to their wild-type sibs.

The deficit in mating speed and in competitive mating ability shown by *yellow* mutant males is not a consequence of lack of response to the females, or failure to initiate courtship. On the contrary, many of the mutant males court actively.

Their lack of success may therefore indicate that the courtship of *yellow* males is less vigorous and inadequately stimulating to the females, or that the quality of the courtship stimuli they provide may be altered in some respect which makes them unacceptable.
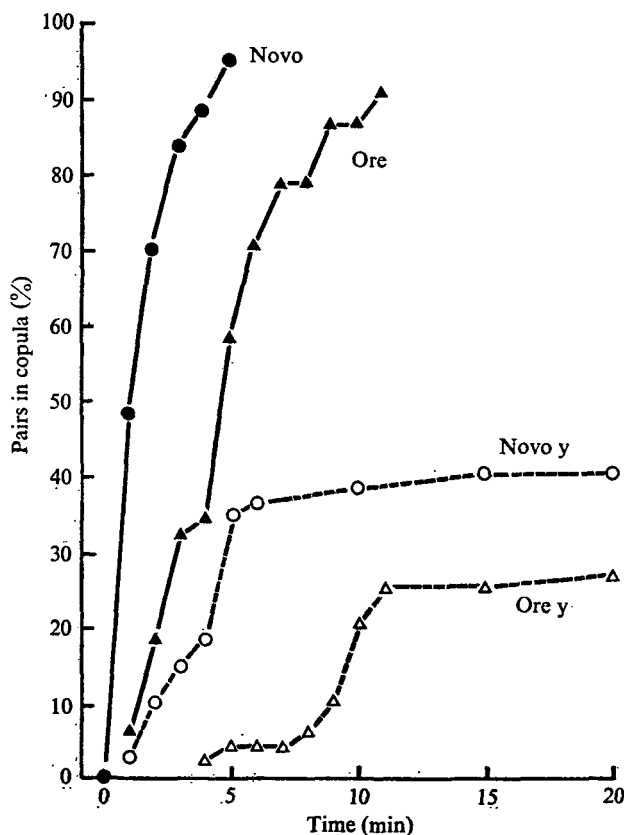

Fig. 3. Cumulative percentage of matings as a function of time for three-day-old wild-type and *yellow* males in a single pair mating situation with a wild-type virgin female from the appropriate background.

Table 2. *Mating success in a competitive situation involving a wild-type and a* yellow *male with a single wild-type virgin female*

(Fifty competitive mating tests were made within each genetic background, and observed until copulation occurred or for a period of 20 min.)

	Wild type	*yellow*	P
Novosibirsk	46	2	< 0.01
Oregon	45	3	< 0.01

(iv) *Courtship behaviour*

A description of the courtship behaviour of *D. melanogaster* is given by Burnet & Connolly (1974), so that it is not necessary to give a detailed account here.

The courting male approaches the female and takes up a position near and with his body axis oriented directly towards her. If she moves, he follows. The male then extends the wing nearest to the female and vibrates it up and down for brief periods. The male frequently repeats this wing display. Moving towards the posterior end of the female, he licks her genitalia, after which he will attempt to copulate.

Table 3. *Comparison of courtship behaviour for 3-day-old adult males in single pair matings with wild-type virgin females*

(Twenty-five single pair matings were observed for each male genotype group until copulation occurred or for a total observation period of 1000 sec. Orientation is measured as the mean percentage (angular transformation) of the total courtship time during which the male was oriented to the female. Wing vibration is expressed as the mean percentage (angular transformation) of orientation time. Licking and attempted copulation, respectively, are each expressed as the mean number of events per orientation bout.)

	Novosibirsk			Oregon		
	Wild type	*yellow*	*P*	Wild type	*yellow*	*P*
Orientation	55·9	52·0	N.S.	59·0	32·9	< 0·01
Vibration	44·7	47·5	N.S.	42·3	31·3	< 0·01
Licking	1·77	4·33	< 0·01	3·30	1·13	< 0·05
Attempted copulation	1·38	1·88	N.S.	1·22	0·40	< 0·05

The courtship behaviour of *yellow* males and that of their wild-type sibs in the single pair mating situation was compared using wild-type virgin females with the same genetic background. The results, which are summarized in Table 3, reveal a striking effect of genetic background on the performance of the mutant. In the Oregon stock *yellow* males spend a significantly smaller proportion of time oriented to the female than their wild-type sibs. Since each of the other courtship elements is superimposed over the orientation component this, in effect, means that the mutant males spend less time courting. The wing vibration display forms a significantly smaller proportion of mutant male courtship and they lick, and attempt copulation, less frequently. Evidently, on the Oregon background the mutant males court less, and offer a lower intensity of courtship stimuli, than their wild-type sibs.

In the Novosibirsk stock mutant and wild-type males do not differ in the proportion of time spent courting, even though the period of observation is on average longer for the mutant males because they usually fail to achieve copulation (Fig. 3). There is no significant difference in the amount of wing vibration offered by the mutant males, or in the frequency of attempted copulation, but they show a significant increase in the frequency of licking (Table 3).

Burnet, Connolly & Harrison (1973) observed a significant increase in the mean duration of breaks between bouts of orientation, and wing vibration, in the courtship of *yellow* mutant males on the Amherst wild-type background. This evidence that *yellow* mutant males offer a less intense courtship than their wild-type sibs is in agreement with the results for the Oregon strain shown in Table 3, and might

Table 4. *Structure of the male courtship song in terms of mean phrase length and the mean duration of the intervals between phrases*

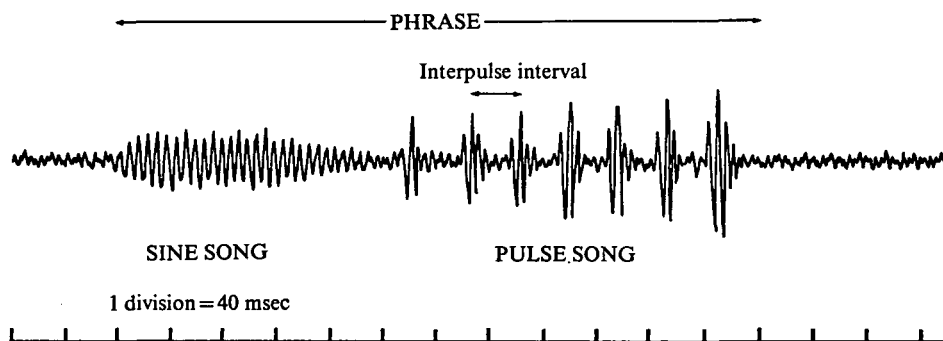|	Phrase length (sec)			Inter-phrase interval (sec)		
	Wild type	*yellow*	P	Wild type	*yellow*	P
Novosibirsk	2·79	2·44	N.S.	3·66	3·57	N.S.
Oregon	1·87	2·22	N.S.	3·24	3·26	N.S.

Fig. 4. The two wave-form elements, sine song and pulse song, forming the courtship song of *D. melanogaster*. A phrase is defined as a continuous period of song with breaks not exceeding 0·5 sec. Phrases are variable in length and usually contain both wave-form elements.

well account for the decrement in mating success shown by the mutant. But such an interpretation is quite contradicted by the results obtained using the Novosibirsk background on which the *yellow* mutant males show no significant difference from wild-type in the proportion of time invested in courtship, nor any apparent reduction in the performance of its separate elements. Nevertheless they have reduced mating success. Virgin females of the Novosibirsk stock are very receptive and mate readily with normal males (Connolly, Burnet, Kearney & Eastwood, 1974), which suggests that although the courtship of Novosibirsk *yellow* males is quantitatively adequate it may be qualitatively different in some way which makes them less acceptable or less stimulating to the females.

(v) *The courtship song*
The wing vibration display provides the female with patterned auditory stimuli that are received through her antennae. These auditory signals, known as the courtship song, form a species specific code by which the female can identify a conspecific male. They also stimulate the female to copulate with the male

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(Bennet-Clark, Ewing & Manning, 1973). Detailed description of the auditory characteristics of the courtship song is given by Bennet-Clark (1975). The patterned auditory stimuli are made up of two basic wave form elements illustrated in Fig. 4. These consist of a tone burst in the form of a modified sine wave referred to here as sine song, and a train of discrete pulses referred to here as pulse song (von Schilcher, 1976). The gross structure of the song can be described in terms of the mean duration of song phrases and the mean length of the intervals between them.
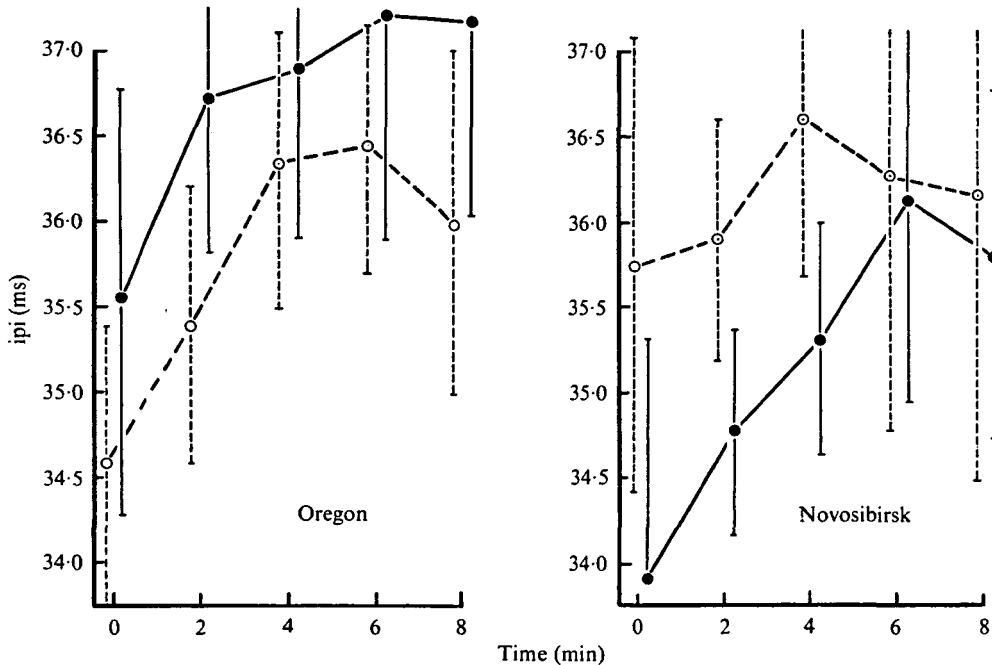

Fig. 5. Mean interpulse interval (msec) for the courtship song of wild-type and *yellow* males, measured over the first 8 min after initiation of courtship. ●, Wild-type males; ○, *yellow* males. The 95 % confidence interval for each mean is indicated by the vertical bars.

The structure of the courtship song produced by wild-type and *yellow* mutant males was studied using single pair matings with 3-day-old virgin females homozygous for the mutants *aristaless* and *thread*. These females which lack a functional arista are effectively deaf and are very unreceptive. They require a lengthy period of courtship before they will copulate with the male (Burnet *et al.* 1971). Consequently they allow extended samples of male courtship song to be made. The results of an analysis of the acoustic records of the first 8 min of courtship by each male are shown in Table 4. There are no significant differences in mean phrase length or duration of the interval between phrases between *yellow* and wild-type males within either the Novosibirsk or Oregon backgrounds. The gross composition of phrases in terms of the relative proportions of sine song and pulse song was closely similar in the two genotype groups.

The interpulse-interval is regarded by Bennet-Clark & Ewing (1969) as the critical parameter for species discrimination, and also appears to affect the sexual response threshold of the females (Bennet-Clark, *et al.* 1973). The results illustrated in Fig. 5 show that the mean interpulse interval tends to increase as courtship proceeds. There is a consistent difference between the wild-type strains, Oregon males having a longer mean interpulse interval than Novosibirsk males. However, the differences in phenotype scores between *yellow* and wild-type males within a given genetic background are generally less than the differences between wild-type scores between backgrounds. There are no significant differences in mean interpulse interval between *yellow* and wild-type males within either background.

(vi) *Responses of the female*

Females exercise an influence over the outcome of courtship either by decamping from the vicinity of the courting male or by making rejection movements described in detail by Connolly & Cook (1973). These serve to keep the male at bay until sufficient courtship stimulation has been received by the female to bring

Table 5. *Rejection responses of females to courtship by wild-type or* yellow *males expressed as the mean number of responses per orientation bout by the male*

(Wild-type virgin females with the same genetic background as the males were used in single pair matings within each genotype group.)

	Decamping			Kicking and fending		
	Wild type	*yellow*	P	Wild type	*yellow*	P
Novosibirsk	0·68	0·76	N.S.	1·65	1·74	N.S.
Oregon	1·51	1·06	N.S.	1·46	0·41	< 0·01

her to a threshold of acceptance. The results summarized in Table 5 show that there is no marked difference in the response of Novosibirsk females to courtship by wild-type and *yellow* males. On the Oregon background females decamp less often from *yellow* males, and make significantly fewer rejection movements towards them. The reason for this is probably because the Oregon *yellow* males offer, as shown by the results in Table 3, a lower intensity of courtship stimulation than their wild-type sibs.

4. DISCUSSION

Males hemizygous for *y* show a deficit in mating ability, and are at a disadvantage in a competitive mating situation. The courtship behaviour of males with an Oregon background agrees with the earlier observations of Bastock (1956) and Burnet *et al.* (1973), in showing that the mutants produce shorter bouts of wing vibration spaced at longer intervals than their wild-type sibs. This feature of mutant male courtship proves, however, to be background-dependent, since Novosibirsk *yellow* males, which also show a decrement in courtship success, do

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not show any significant quantitative reduction in the wing vibration component. The decrease in average bout length of the wing vibration component is evidently variable even within a given genetic background, since acoustic recordings for Oregon *yellow* males which courted *al; th* females show no significant differences in mean phrase length, or duration of inter-phrase intervals, expected on the basis of their performance with Oregon wild-type females. Unlike *al; th* females which remain stationary for long periods, the Oregon females have a high level of loco-motor activity, and the higher average duration of breaks between bouts of orien-tation and vibration shown by mutant males is a secondary consequence of loss of contact due to their inability to keep up with them.

Although she did not make any quantitative measures, Bastock (1956) had the impression that *yellow* males appeared to be less active than normal males and ascribed this, together with the reduction in wing vibration bout length, to a reduction in sexual motivation in the mutant males. It is inappropriate here to argue the precise meaning of motivation as it might apply to a fly, but our results suggest that a decrement in *yellow* male courtship activity can follow from their lower level of locomotor activity in a way which depends on the behaviour of the female. The *yellow* males show no obvious reduction in the duration or intensity of their courtship of accessible females with which they can successfully maintain contact, and in this sense their sexual motivation seems equal to that of wild-type males.

Threlkeld, Procwat, Abbot & Yeung (1974) have reported the development of female preference for *yellow* males in response to selection in a female choice mating situation. The exercise of an active female preference for *yellow* males is, as the authors point out, difficult to reconcile with the view that the mutants only offer a reduced level of courtship stimuli. Rather it would seem likely that such a female preference would depend on some positive qualitative feature of mutant male courtship which distinguishes it from wild-type. The mating system em-ployed by these authors involved selection of those females which copulated with *yellow* males. The daughters of the females were then tested in the mating choice situation with males of the two phenotype groups. However, the males used in the choice situation were always drawn from the same two stocks – one recently caught wild-type isolate, and a laboratory *yellow* mutant stock. Since these stocks were apparently not standardized to remove differences in residual genetic background outside the *yellow* locus they are likely to have shown other relevant phenotypic differences affecting male courtship behaviour, and it is consequently not clear that the female preference demonstrated by these authors was for the *yellow* mutant phenotype as such.

The fact that *yellow* males are unsuccessful even in a situation where they are courting receptive Novosibirsk virgin females which remain relatively inactive suggests that the reduced level of locomotor activity, although an interesting aspect of the expression of *y*, cannot be the general cause underlying the reduced mating success shown by the mutant males. The courtship stimulation offered by the mutant males appears to be, both qualitatively and quantitatively, indistin-

guishable from that of their wild-type sibs, although it is always possible that there may be some special feature of the acoustic stimuli produced by mutant males which has not been detected in our analysis. Nor is there any sign that the mutant male courtship is less acceptable to the females, since there was no good evidence to indicate an increase in the frequency of rejection responses which they made toward mutant males. What is curious, is that frequency of attempts to copulate is similar in mutant and normal males – the total number of attempts is of course much larger for *yellow* males because their courtships last much longer – yet the mutants hardly ever seem to succeed in securing effective genital engagement before mounting. This suggests that there may be a structural basis for the behavioural effects of the mutant. It is already known (Kalmus, 1941) that *yellow* mutants are less resistant to desiccation than wild-type flies, indicating a difference in the permeability of their cuticles. The *yellow* locus is also known from the work of Dobzhansky & Holz (1943) to have pleiotropic effects on the structure of the female genital apparatus, and it is possible that the locus may also affect the male genitalia in some manner which makes copulation and intromission more difficult to achieve. The sex-combs located on the fore-tarsi of the male also play an important role in copulation. Removal of these structures greatly impairs the copulatory behaviour of males (Cook, 1975), and impairment of the function of the sex-combs in *yellow* males would be expected to have similar effects. There are no gross morphological differences apparent in the sex-combs or genitalia of *yellow* mutant males, but there may be functional impairment of these structures due to changes in the mechanical properties of the integument.

Different mutant alleles of independent mutational origin at the *yellow* locus are by no means equivalent in their effects on the pigmentary phenotype. Nash & Yarkin (1974) distinguish between type-1 mutants in which all the cuticular structures are *yellow* and type-2 mutants which are variable in expression. From their comparative studies on these mutants Nash and Yarkin suggested that *yellow* is a pattern-forming responder gene, but these authors did not consider the effects of the gene on behaviour. In an attempt to account for the pigmentary and behavioural effects of the mutant Burnet & Connolly (1974) have offered a metabolic model based on the suggestion that the *yellow* gene may be involved in controlling one of the steps in the conversion of tyrosine to 3,4-dihydroxy-phenylalanine which is utilized in three biosynthetic pathways leading to the synthesis of (i) melanin, (ii) sclerotin, and (iii) the catecholamines dopamine and noradrenalin. Pathways (i) and (ii) are concerned with the hardening and pigmentation of the cuticle, whilst the catecholamines subserve a function related to neural transmission. It is possible that the behavioural and pigmentary effects of the mutant may all be referrable to the same underlying cause – a change in some property of the integument. Evidence presented here suggests that mating success and the yellow body phenotype may indeed be related in this way, and a direct test of this suggestion may be possible from studies on the courtship and sexual behaviour of males hemizygous for type-2 mutant alleles such as, y^{bl} which has yellow sex combs but the tip of the abdomen is normally pigmented, and y^{bab}

which has normally pigmented sex combs and yellow abdominal tip. However, the possibility that the *yellow* gene affects locomotor activity by a different route, namely by an effect mediated through the catecholamines rather than by a presumably mechanical effect of the integument, is currently being investigated.

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