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Inheritance of female mating propensities for *yellow* locus genotypes in *Drosophila melanogaster*

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SUMMARY

Females from different wild-type laboratory populations of *Drosophila melanogaster* differ genotypically in their degree of mating discrimination against mutant *yellow* males. The chromosomal organization of this difference was examined in two wild-type laboratory strains by experimental observation of the mating propensities of hybrid females in a mass-mating, multiple choice situation. The results indicate that the strain difference is polygenic in origin, involving loci on both the *X*-chromosome and autosomes. Reciprocal crosses revealed no maternal/cytoplasmic effects. The mating scores of parental, F_1 and backcross females fit well to a model of additive chromosomal effects, with *X*-linked loci being recessive, and autosomal loci overdominant, for increased mating with *yellow* males. However, interactions, arising most probably from recombination, led to increased mating with *yellow* on the part of F_2 females. In addition to the difference in female discrimination against *yellow* males, male/female interaction was found for the mating speed of flies from the two strains. These results are discussed in the light of previous studies of mating preferences in *D. melanogaster*. It is suggested that genetic variation in female mating preferences may be an important source of variation in the reported mating success of mutant *yellow* males.

1. INTRODUCTION

The genus *Drosophila* has figured importantly in studies of selective mating, probably more so that any other group of organisms. The role of female mating preferences in the maintenance of reproductive isolation between populations and in the selective elimination of genetic variants is well established. While a fair amount is known about the existence of mating preferences in *Drosphila* populations, much less is known about their genetic basis. The genetic relationship between mating preferences and the traits toward which they are directed will influence the evolution of reproductive characters in populations. An adequate theory for the evolution of reproductive traits therefore depends as much on genetical knowledge about mating preferences as it does on knowledge of the genetics of sexually selected traits themselves.

Most research on the inheritance of mating preferences in *Drosophila* has been concerned with prezygotic reproductive isolation between closely related species (Tan, 1946; Ehrman, 1961; Ewing, 1969; Kawanishi & Watanabe, 1981; Zouros, 1981) or with partial isolation between divergent populations of the same species (Kilias & Alahiotis, 1982). These studies, in general, are concerned with the genetic basis of assortative mating. The genetics of other types of mating preferences, such as female 'choice' of male characters and frequency dependent ('rare male') mating advantages, is virtually unknown.

The research I report here is concerned with the inheritance of female discrimination among potential mates. In particular, I describe experiments designed to elucidate some major genetic features of female mating propensities for wild-type versus mutant *yellow* males of *Drosophila melanogaster*.

Mutant alleles at the *yellow* locus have pleiotropic effects on components of male courtship that are known to be important in stimulating females to accept copulation (Bastock, 1956; Bastock & Manning, 1955; Wilson *et al.* 1976). Thus, the *yellow* locus potentially could be used to detect genetic variation in female responsiveness to male courtship. Furthermore, the inheritance of variation in the receptivity of wild-type females to *yellow* males may help explain the enhanced receptivity to *yellow* males observed among *yellow* females themselves (Sturtevant, 1915; Merrell, 1949; Bastock, 1956; Barker, 1962; Dow, 1976).

The genetic features I examine are (1) maternal effects versus Mendelian inheritance, (2) *X*-linked versus autosomal factors, and (3) the presence or absence of dominance. For traits expressed only in females, these three factors can be distinguished by comparing different parental strains with their two reciprocal F_1 hybrids and the four possible backcrosses of F_1 males. This method thus provides considerable information with only two generations of preparatory crosses. It is a simplified version of that used by Tan (1946) and subsequent workers to dissect the chromosomal basis for behavioural isolation between closely related *Drosophila* species (Ehrman, 1961; Zouros, 1981).

2. MATERIALS AND METHODS

(i) *Strains*

Twelve wild-type laboratory strains of *Drosophila melanogaster* were screened for variation in female responsiveness to *yellow* males. From these twelve strains, two were selected for further detailed study: Mt Carmel, Illinois (abbreviated *MC*), whose females mated relatively frequently with *yellow* males, and Niobrara, Nebraska (abbreviated *NB*), whose females rarely mated with *yellow* males. Both strains are derived from single wild females collected in 1970 by Dr Lynn H. Throckmorton and maintained in his laboratory for 11 years prior to these experiments.

Unless otherwise noted, all male flies used in tests were wild-type Oregon-R (abbreviated *OR*) and *yellow* (*y*) flies from long-standing laboratory stocks. In these two 'tester' strains, no attempt was made to randomize genetic factors that may have influenced competitive male mating success in addition to the effects of the *yellow* locus. Hence measures of the relative mating success of Oregon-R and *yellow*

males in these experiments do not measure effects from the *yellow* locus alone, but rather overall differences between tester stocks. Most of this difference should be owing to the action of the *yellow* locus, however, since differences in mating success between wild-type and mutant males on the same genetic background are generally much larger than are differences among genetic backgrounds within a given genotype (Wilson *et al.* 1976).

All flies were reared in 3 dr shell vials on standard cornmeal–molasses–agar medium. Developing larvae were kept at room temperature (21–23 °C). At the onset of eclosion, vials producing test females were shifted to 18 °C to simplify the collection of virgins. Test males and virgin females were then aged 5–12 days on fresh medium at room temperature prior to testing. Vials were randomized within racks during the period of larval development.

(ii) *Mating tests*

Fly mating behaviour was observed using mating chambers constructed from clear Plexiglas, measuring 5 × 10 cm in area by 2 cm in height, and equipped with four entry ports plugged with cotton. Chamber floors were covered with a thin layer of medium. Thin, removable acetate partitions divided each Chamber into two 5 × 5 × 2 cm compartments, permitting the sexes to be segregated prior to the beginning of observation.

Flies were aspirated into chambers 1 day prior to testing. This permitted them to adjust thoroughly to the test environment. A total of 24 virgin females, 24 *yellow* males, and 12 wild-type males participated in each test. (It was necessary to provide *yellow* males with a numerical advantage in tests in order to obtain a sufficient range of scores for their mating success, since the success of *yellow* in competition with wild-type males is low even among those females that are most receptive to them.)

Mating tests took place under uniform fluorescent light filtered through white Plexiglas. Tests were initiated simply by removing the acetate partition from a chamber. Flies were continuously observed for 1 h, during which more than half of the females usually mated. Copulating pairs were removed with an aspirator and the phenotype of the mating male and time of mating (in 2·5 min intervals) were recorded. Three to five chambers were generally observed simultaneously, with test initiation staggered at thirty second intervals.

(iii) *Crosses tested*

In addition to the two parental strains, ten hybrid crosses between *MC* and *NB* were tested for female mating behaviour. The twelve groups thus compared consisted of female offspring from (1) matings within the two parental strains, (2) the two reciprocal crosses between *MC* and *NB* (abbreviated *F1MN* and *F1NM*). (3) the four possible crosses between F_1's (abbreviated *F2ij*, $i,j = M,N$), and (4) the four possible backcrosses of F_1 males to one or the other parental line (abbreviated *Bij*; $i,j = M,N$). Table 2 summarizes these crosses, the cross designations used, and the genetic characteristics (with respect to strain origin) of resulting female progeny.

(iv) *Experimental design*

Female mating propensities were tested using a randomized block design, thereby insuring that differences between females could be unequivocally attributed to genotypic effects. A block consisted of a group of test chambers that were tested on the same day. The 12 original wild-type strains were subdivided into three groups, each consisting of four strains tested together in a single randomized block experiment. In tests of hybrids, each block included tests of both parental strains and both reciprocal F_1's. In addition, some blocks contained groups of F_2 females (Experiment 2), while others contained the four types of backcross daughters (Experiment 1).

An additional experiment investigated differences in the competitive mating success of males from the two strains. In this, either *MC* or *NB* males competed against mutant males for matings with *MC*, *NB* or F_1 females. Six replicate blocks were conducted, each consisting of the six possible combinations of wild-type males and females.

Within any block of tests, the age and origin (by vial) of test females and tester males were controlled, while the developmental environment, chamber assignment and rank order of testing were randomized. The major potential sources of variation among blocks were fly age (range 5–12 days), temperature (range 20–24 °C), and between-generation differences owing to replication of the overall experiment.

3. RESULTS

(i) *Variation among wild-type strains*

The twelve strains tested for female mating propensities are listed in Table 1, along with the means and standard deviations of the proportion of matings by *yellow* males (denoted *f*). Variation among all twelve strains was highly significant by one-way analysis of variance ($F = 4{\cdot}30$, D.F. $= 11,36, P < 0{\cdot}001$). On average, 15·4% of all females that mated within 1 h mated with *yellow* males. This is equivalent to a per-male success rate for Oregon-R males about eleven times as great as that for *yellow* males.

(ii) *Variation among hybrid females*

This section presents the results of observations on the mating propensities of hybrid females, whose behaviour was used to examine the pattern of inheritance of the difference between females from the *MC* and *NB* strains. The chosen measure of male mating success was the proportion of all matings within 60 min that involved *yellow* males, henceforth denoted *f*. Overall means and standard deviations for this value are presented in Table 2.

Variation among hybrid females was tested by two-way analysis of variance (cross × block). The distributional requirements for this test were met by employing the generalized logarithmic transformation $f' = \ln(0{\cdot}2813 + f)$ (Wright, 1968). Both Experiment 1, which compared mating by parental, F_1 and backcross females, and Experiment 2, which compared mating by parental, F_1 and F_2

females, revealed significant variation ($F = 5{\cdot}667, 4{\cdot}116$; $P < 0{\cdot}001, 0{\cdot}01$, respectively). This confirmed the validity of further analysis to determine whether this variation could be ascribed to maternal effects, *X*-chromosomal inheritance or autosomal inheritance.

In Table 2, there are 13 pairs of crosses in which two of these three components of inheritance are held constant while the third is permitted to vary. (For example,

Table 1. *Variation among wild-type strains in the proportion of matings by* yellow *males*

(The three groups are separate randomized block experiments. Listed proportions are the mean proportions of all mating that involved *yellow* males. Sample size equals 4 for all 12 strains.)

Strain tested	Mean proportion of mating by yellow (f)	Standard deviation of proportion
Group 1*		
Amherst, Mass. (*AM*)	0·0609	0·0407
Stillwater, Oklahoma (*ST*)	0·2741	0·1202
Austin, Texas (*AS*)	0·0942	0·0825
Margarita, Venezuela (*MG*)	0·0885	0·1039
Group 2		
Clearwater, Florida (*CW*)	0·0385	0·0769
Madison, Wisconsin (*MD*)	0·1410	0·0806
Ilan, Taiwan (*IL*)	0·1964	0·1669
Nan Kang, Taiwan (*NK*)	0·1546	0·1619
Group 3†		
Australia (*AU*)	0·2396	0·0420
Oregon-R (*OR*)	0·1662	0·0325
Mt. Carmel, Illinois (*MC*)	0·3841	0·0521
Niobrara S.P., Nebraska (*NB*)	0·0758	0·0586

* Variation among strains within this group is significant at the $P < 0{\cdot}05$ level by 2-way analysis of variance ($F = 4{\cdot}052$); data transformed as $f' = \ln(1+f)$.

† Variation among strains within this group is significant at the $P < 0{\cdot}001$ level by 2-way analysis of variance ($F = 25{\cdot}576$); data transformed as $f' = \ln(1+f)$.

a difference between the parent *MC* and the backcross *BMM* is due solely to autosomal effects, since both groups have the same type of female parent and are homozygous for the *MC* *X*-chromosome.) These 13 pairs were tested by paired comparisons of the differences in the untransformed proportion (f) against the null hypothesis of no difference. The results are summarized in Table 3.

No evidence was found for the occurrence of cytoplasmic or environmental maternal effects among reciprocal F_1 and F_2 crosses (comparisons 1–3 in Table 3). Among chromosomal comparisons, three differences are significant.

The differences, $f(BMM)$–$f(BMN)$ and $f(F2\text{–}M)$–$f(F1)$ (comparisons (4) and Pooled (5) and (6)), indicate a phenotypic difference between females homozygous for the *MC* *X*-chromosome and females heterozygous for the two strains. By contrast, the phenotypic differences that compare females homozygous for the *NB* *X*-chromosome with heterozygous females (comparisons 7–9) neither are significant nor are their magnitudes appreciably different from zero. This suggests that the

Table 2. *Hybrid crosses, genetic constitution, and mean proportion of females mating with* yellow *males*

Cross dam × sire	Designation used	Origin of cytoplasm	Expected proportion of *MC* chromosomes: *X*	Expected proportion of *MC* chromosomes: Autosomes	$\hat{f}$	S.D.	*n*
MC × *MC*	*MC*	*MC*	2·00	2·00	0·314	0·123	18
MC × *F1MN*	*BMM*	*MC*	2·00	1·50*	0·261	0·123	10
MC × *F1NM*	*BMN*	*MC*	1·00	1·50*	0·158	0·109	10
MC × *NB*	*F1MN*	*MC*	1·00	1·00	0·233	0·145	18
NB × *MC*	*F1NM*	*NB*	1·00	1·00	0·212	0·144	22
NB × *F1MN*	*BNM*	*NB*	1·00	0·50*	0·118	0·086	14
NB × *F1NM*	*BNN*	*NB*	0·00	0·50*	0·086	0·080	14
NB × *NB*	*NB*	*NB*	0·00	0·00	0·091	0·086	22
F1MN × *F1MN*	*F2MM*	*MC*	1·50*	1·00*	0·320	0·111	8
F1NM × *F1MN*	*F2NM*	*NB*	1·50*	1·00*	0·370	0·056	4
F1MN × *F1NM*	*F2MN*	*MC*	0·50*	1·00*	0·253	0·142	8
F1NM × *F1NM*	*F2NN*	*NB*	0·50*	1·00*	0·320	0·038	4

* Value is an expectation rather than an exact value.

Table 3. *Paired comparisons tests of hybrid females*

Difference used in comparison	*n*	Mean difference	Standard deviation	Probability of zero difference
Maternal effects*				
(1) *F1MN–F1NM*	18	−0·0180	0·148	0·61
(2) *F2MM–F2NM*	4	0·0071	0·036	0·72
(3) *F2MN–F2NN*	4	0·0250	0·062	0·48
X-chromosome effects†				
(4) *BMM–BMN*	10	0·1039	0·140	0·022‡
(5) *F2MM–F1MN*	8	0·1018	0·248	0·140
(6) *F2NM–F1NM*	4	0·092	0·185	0·392
Pooled (5) and (6)	12	0·099	0·168	0·034‡
(7) *BNM–BNN*	14	0·0319	0·099	0·125
(8) *F1MN–F2MN*	8	−0·0352	0·311	0·380
(9) *F1NM–F2NN*	4	−0·043	0·122	0·534
Pooled (8) and (9)	12	−0·0377	0·140	0·185
Autosomal effects†				
(10) *MC–BMM*	10	0·0138	0·792	0·300
(11) *BMN–F1MN*	10	−0·0662	0·141	0·445
(12) *F1NM–BNM*	14	0·0471	0·106	0·060
(13) *BNN–NB*	14	0·0325	0·061	0·035‡

* Two-tailed paired comparisons *t*-test.
† One-tailed paired comparisons *t*-test.
‡ Difference significant at $P < 0·05$.

X-chromosomal factors that increase the receptivity of *MC* females to *yellow* males act in a net recessive manner.

The third significant difference (comparison 13) suggests that autosomal loci also contribute to the strain difference. This is supported by the observation that the difference between F_1 flies and the *NB* parent is large ($f'(F1NM–f'(NB) =$

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$0{\cdot}317 \pm 0{\cdot}0914$ S.E.), highly significant ($t = 3{\cdot}47$, 63 D.F., $P < 0{\cdot}005$), and cannot be accounted for by X-linked factors. The estimated contribution of the X-chromosome to this difference is $0{\cdot}051 \pm 0{\cdot}914$ S.E., which is not significantly different from zero. In contrast, the contribution of the autosomes is $0{\cdot}266 \pm 0{\cdot}129$ S.E., which is significantly non-zero ($t = 2{\cdot}059$, $P < 0{\cdot}05$).

It is possible to estimate the relative contributions of the X-chromosome and autosomes to the trait if certain simplifying assumptions are made. If one assumes that the X-chromosome and autosomes act additively, the expected phenotypic value of any hybrid female can be expressed as a sum of two separate genotypic values. Let **g** denote the expected deviation of an individual from the mid-strain value for the trait (the average of *MC* and *NB* parental types), and let **x** and **a** be the genotypic deviations of *MC* X-chromosomal and autosomal homozygotes, respectively. The values of individuals from *MC* and *NB* parental strains can then be written as,

$$\mathbf{g}(MC) = \mathbf{x} + \mathbf{a},$$

and,

$$\mathbf{g}(NB) = -\mathbf{x} - \mathbf{a}.$$

Likewise, if $\mathbf{d}_x$ and $\mathbf{d}_a$ are the respective genotypic deviations of X and autosomal heterozygotes, the deviation of F_1 females from the mid-strain value can be written,

$$\mathbf{g}(F1) = \mathbf{d}_x + \mathbf{d}_a.$$

Although backcross progeny will vary owing to segregation and assortment of autosomes, and F_2 females will further be affected by crossing over within chromosomes, their expected genotypic values can be similarly expressed:

$$\begin{aligned}
\mathbf{g}(BMM) &= \mathbf{x} + \mathbf{a}/2 + \mathbf{d}_a/2, \\
\mathbf{g}(BMN) &= \mathbf{d}_x + \mathbf{a}/2 + \mathbf{d}_a/2, \\
\mathbf{g}(BNM) &= \mathbf{d}_x - \mathbf{a}/2 + \mathbf{d}_a/2, \\
\mathbf{g}(BNN) &= -\mathbf{x} - \mathbf{a}/2 + \mathbf{d}_a/2, \\
\mathbf{g}(F2-M) &= \mathbf{x}/2 + \mathbf{d}_x/2 + \mathbf{d}_a/2, \\
\mathbf{g}(F2-N) &= -\mathbf{x}/2 + \mathbf{d}_x/2 + \mathbf{d}_a/2.
\end{aligned}$$

Least squares regression was employed to compute the best fit of the four unknown genotypic values, **x**, $\mathbf{d}_x$, **a** and $\mathbf{d}_a$, to this linear model. The logarithmic transformation, $f' = \ln(0{\cdot}2813 + f)$, was again used, rescaled by subtraction of the mid-strain value of the appropriate test block. The entire data set of 136 tests was used in the computations. The fitted genotypic values are:

$$\begin{aligned}
\mathbf{x} &= 0{\cdot}127 \pm 0{\cdot}0566 \text{ S.E.}, \\
\mathbf{d}_x &= -0{\cdot}0811 \pm 0{\cdot}0716 \text{ S.E.}, \\
\mathbf{a} &= 0{\cdot}0750 \pm 0{\cdot}0657 \text{ S.E.},
\end{aligned}$$

and

$$\mathbf{d}_a = 0{\cdot}143 \pm 0{\cdot}0893 \text{ S.E.}$$

Of these, only the coefficient **x** is significantly different from zero ($t = 2{\cdot}24$, $P < 0{\cdot}05$). The overall regression, however, is highly significant ($F = 11{\cdot}07$, 4/131 D.F., $P < < 0{\cdot}001$, $r^2 = 0{\cdot}253$). Thus, in the absence of more detailed information

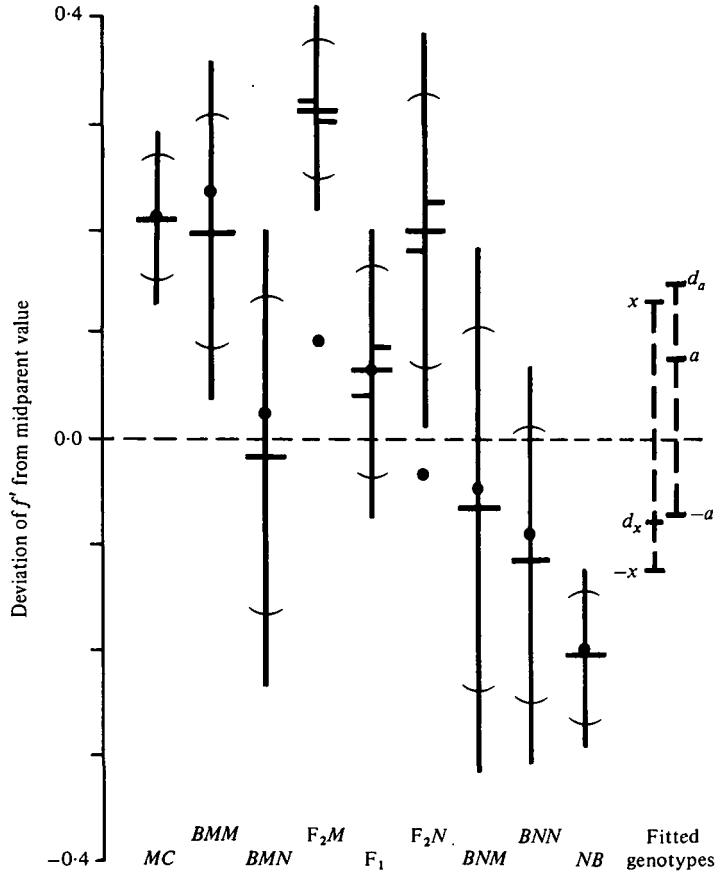

Fig. 1. Mating by *MC*, *NB* and hybrid females with *yellow* males. The mean (horizontal bar), 95 % (in parentheses) and 99 % confidence intervals for the deviation of each cross from the midparental (average of *MC* and *NB*) score in any block of tests. Data are the proportion, f, of all mating by *yellow* males transformed as ln (0·2813+f). Short horizontal bars for F_1 and F_2 crosses are reciprocal means, and point in the direction of origin of the cross cytoplasm. Circles give the genotypic values fitted using an additive model of chromosomal action. Dashed bars on the right illustrate the fitted homozygous (x and a) and heterozygous (d_x and d_a) autosomal and X-chromosomal values, respectively.

about the inheritance of this trait, the observed behaviour of hybrid females can be accounted for by a model in which the *MC* strain carries recessive, *X*-linked alleles that increase the receptivity of females to mutant males, while the two strains also carry autosomal alleles that, in heterozygous condition, exhibit overdominance for increased receptivity to *yellow* males.

Fig. 1 shows the relationship between the observed and fitted values for the nine classes of females. The fitted values match the observed means extremely well, with the notable exception of the behaviour of F_2 females. As can be seen in the figure, F_2 progeny have unusually high mating frequencies with *yellow* males, and are distinctive within the relatively graded series formed by the other crosses. The

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scores of F_2 females are significantly higher than those of F_1 females ($t = 2{\cdot}21$, 23 D.F., $P < 0{\cdot}05$, by pooled paired comparisons). Furthermore, *F2MM* females have significantly higher scores than even the *MC* parental type ($t = 2{\cdot}609$, 11 D.F., $P < 0{\cdot}05$, by paired comparisons). These observations cannot be accounted for using the simple model proposed above. While additive chromosomal effects account very well for the behaviour of F_1 and backcross females, a more complex model is required to explain the behaviour of F_2 females.

Several possible mechanisms could be responsible for the high F_2 scores. Primary among these are interactions among alleles at different loci arising through crossover recombination in F_1 females and segregation of genotypes that promote increased receptivity to mutant males. Such interaction would have to be a general consequence of recombination rather than specific to a few recombinant genotypes, since the effect of a few unusual individuals on the total proportion should be small. A more probable explanation is that there exists interaction, not between loci within individuals, but among individuals within the test chambers. Each data point measures the overall behaviour of a group of 24 females rather than the phenotype of a single individual. One may infer that the proportion of hybrid females mating with *yellow* males estimates the probability that any single female will so mate, but this is subject to the validity of using group behaviours as measures of individual propensities. The unusually high scores of F_2 groups suggest that this inference is not justified in the case of these flies. A few unusual genotypes arising through recombination may have affected the mating behaviour of entire groups of F_2 females, perhaps through the production of chemical stimuli (Shorey & Bartell, 1970; Averhoff & Richardson, 1974, 1976).

Regardless of the actual basis for the high scores of F_2 females, the contrast between the simple picture of inheritance that arises from observation of hybrids that lack recombinant chromosomes, and the complex situation apparent when recombinants are included, suggests that circumspection is important in interpreting the results of experiments based on the observation of hybrid phenotypes.

(iii) *Between-strain differences among males*

Studies of the mating behaviour of different strains, subspecies and species of *Drosophila* have often revealed patterns in the relationship between male and female mating behaviour within populations. These include positive assortative mating among populations that have diverged in nature or in the laboratory (Solar, 1966; Dobzhansky & Pavlovsky, 1971; Ehrman & Parsons, 1981; Markow, 1981; Kilias & Alahiotis, 1982), negative assortative mating (Averhoff & Richardson, 1974; Ehrman & Parsons 1981), and one-sided mating preferences, in which females of one strain discriminate against 'foreign' males, but females of the other strain do not (Kaneshiro, 1980; Watanabe & Kawanishi, 1979; Markow, 1981). The following experiment investigated the possibility that the genetically based differences in the behaviour of *MC* and *NB* females are associated with corresponding differences in the mating behaviour of males from the two strains.

Figure 2 graphs the mean cumulative number of wild-type males mating as a function of time for each of the six combinations of *MC* or *NB* males in competition

with mutant males for matings with *MC*, *NB* or F_1 females. Data were tested by three-way analysis of variance; the results appear in Table 4. Males of the two strains were not found to differ significantly in their mating success, either during early matings or over the entire hour. Analysis of the number of wild-type matings within 30 and 45 min also revealed no significant difference between *MC* and *NB* males ($F = 1{\cdot}438$, $0{\cdot}051$, respectively). Females of different types, however, did

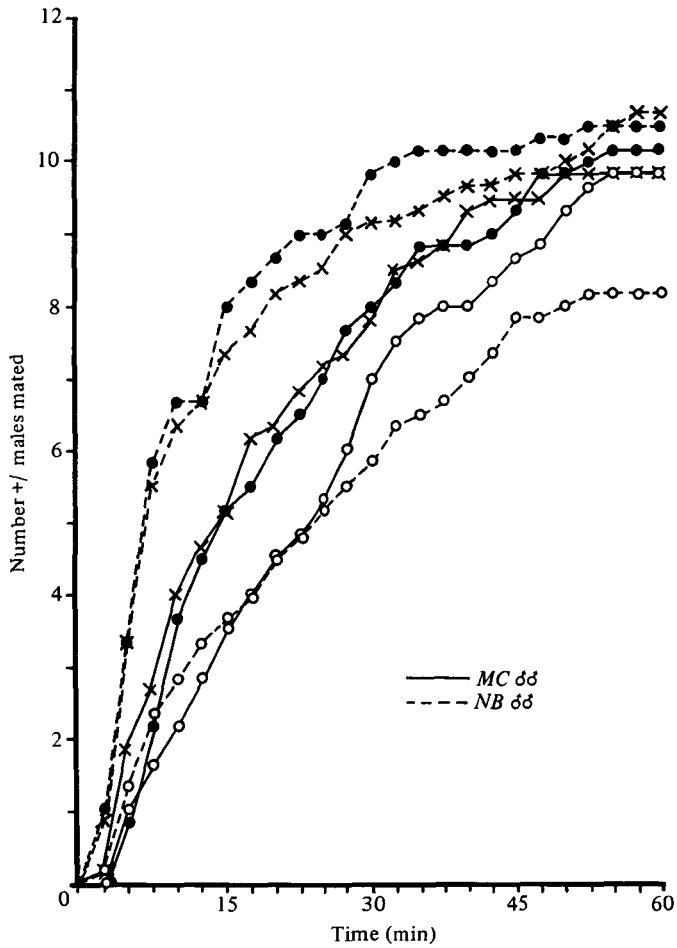

Fig. 2. Time course of mating by wild-type males. Each line plots the mean of six replicate tests in the number of wild-type males mating as a function of time since the onset of observation. Dotted lines represent mating by *NB* males; solid lines mating by *MC* males. Results with three types of females are shown: *MC* (●), *NB* (○) and F_1 (+).

differ significantly in their propensity to mate with wild-type males. As can be seen in Fig. 2, *MC* and F_1 females mated faster and with a larger total number of wild-type males than did *NB* females. Furthermore, significant male × female interaction was found for both the 15 and 60 min periods (Table 4). *MC* and F_1 females mated more readily than expected with *NB* males, while *NB* females mated more readily than

Table 4. *Analysis of variance of strain males tested with MC,* F_1 *and NB females*

(Each test involved 12 *MC* or *NB* males in competition with 24 *yellow* males for matings with 24 females that were either *MC*, *NB* or F_1. Data were transformed as $f' = \ln(1+f)$.)

Source of variation	D.F.	Sum of squares	Mean square	*F*	
		Number wild-type males mating in 15 min			
Male	1	26·69	26·69	2·41	
Female	2	64·89	32·44	5·49	($P < 0·05$)
Block	5	7·47	1·49	2·58	
Male × Female	2	11·56	5·78	9·97	($P < 0·005$)
Male × Block	5	55·47	11·09	19·12	($P < 0·001$)
Female × Block	10	59·11	5·91	10·19	($P < 0·001$)
Remainder	10	5·78	0·58		
Total	35	230·97			
		Number wild-type males mating in 60 min			
Male	1	0·25	0·25	0·11	
Female	2	13·39	6·69	8·80	($P < 0·025$)
Block	5	10·81	2·16	1·73	
Male × Female	2	10·50	5·25	4·20	($P < 0.05$)
Male × Block	5	11·25	2·25	1·80	
Female × Block	10	7·61	0·76	0·61	
Remainder	10	12·50	1·25		
Total	35	66·31			
		Proportion of mating by *yellow*			
Male	1	0·0000	0·0000	0·000	
Female	2	0·2868	0·1434	33·35	($P < 0·001$)
Block	5	0·1427	0·0285	23·75	($P < 0·001$)
Male × Female	2	0·0114	0·0057	4·75	($P < 0·05$)
Male × Block	5	0·0249	0·0050	4·17	($P < 0·05$)
Female × Block	10	0·0434	0·0043	3·58	($P < 0·05$)
Remainder	10	0·0122	0·0012		
Total	35	0·5214			

expected with *MC* males. Hence, some degree of negative assortative mating occurred. F_1 females are indistinguishable from *MC* females in their tendency to mate with wild-type males, suggesting the *MC* alleles governing this aspect of female mating behaviour are dominant.

The proportion of mating by *yellow* males was also analysed for this experiment. As before, females from the two strains differ in their propensity to mate with mutant males, regardless of the origin of the wild-type males. On average, 37·3 % of *MC* females, 20·4 % of F_1 females and 10·6 % of *NB* females mated with *yellow* males. This compares well with the results of tests using Oregon-R males. The overall success of *yellow* males was not dependent on the type of wild-type male present, but was affected by particular male–female combinations (male × female interaction significant at $P < 0·05$). Mutant males did better than expected if they competed against *NB* males for matings with *MC* or *NB* females, but did less well than expected when competing for F_1 females.

(iv) *Female 'choice' versus overall receptivity*

In this section, I will present evidence that the genetic differences between *MC* and *NB* females can be regarded, at least in part, as a difference in 'mating preference'. The term 'mating preference' has been subject to a multiplicity of definitions. I use it here to refer to the broad class of female characters that, in net effect, produce a force of sexual selection on male traits. This definition

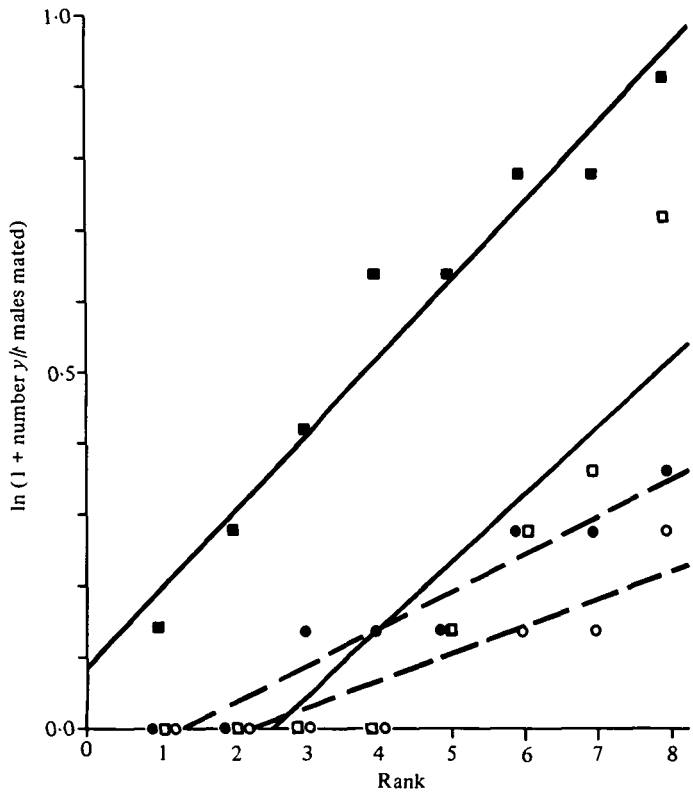

Fig. 3. Time course of early mating by *yellow* males. Each point represents the mean of five replicate tests in the number of *yellow* males mating as a function of the total number of females that have mated so far, through the first eight matings of each test. Lines plot the regression of ln $(1 + Y)$, where Y is the number of mutant males mated, on the total number of mated males. The magnitudes of the regression coefficients are: $0{\cdot}1069 \pm 0{\cdot}0263$ S.E., for *MC* females with *MC* males (■), $0{\cdot}0926 \pm 0{\cdot}0204$ S.E., for *MC* females with *NB* males (□), $0{\cdot}0513 \pm 0{\cdot}0206$ S.E., for *NB* females with *MC* males (●), and $0{\cdot}0380 \pm 0{\cdot}0150$ S.E., for *NB* females with *NB* males (○).

includes, but is not restricted to, behavioural traits of females during courtship, such as differential rejection of different types of males. It specifically excludes, on the other hand, female traits of any sort that do not affect the way that mating success is distributed among males of different types. Thus, 'mating preferences', in this sense, are defined by their effects on male fitness rather than by their behavioural expression.

My particular aim is to show that the difference between *MC* and *NB* females is not simply a difference in their overall willingness to mate, but in the probability that they will mate with a *yellow* male, given that they are mating. That is, I wish to consider the relative success of *yellow* males with the two types of females independent of possible differences in overall receptivity.

Throughout the tests reported here, variation in the proportion of all mating by *yellow* males resulted largely from variation in the *numbers* of matings by *yellow* males, since nearly all of the 12 wild-type males present in any test mated. This raised the possibility that the difference in the mating success of *yellow* males with *MC* and *NB* females arose solely from a reluctance on the part of *NB* females to mate within the observation period, regardless of male phenotype. Since wild-type males are more successful at courting females of both types than are *yellow* males, they typically mated early in the observation period. As they became rare, only *yellow* males were left as potential mates. If a smaller proportion of *NB* females are willing to mate during the observation period, fewer receptive females would then be available to mate with *yellow* males.

These two hypotheses can be distinguished by analysing the sequential pattern of mating within individual tests. If the receptivity hypothesis is correct, *yellow* males should show the same sequential pattern during early matings with both types of female. That is, their mating rate in competition with wild-type males should be invariant. The excess of mating by *yellow* males with *MC* females would be entirely owing to the increased total number of matings. By contrast, if the discrimination hypothesis is correct, *yellow* males should have, from the beginning of the observation period, a higher mating rate with *MC* females than with *NB* females.

Fig. 3 plots the mean cumulative number of *yellow* males mating against the total number of matings over the course of the first eight matings. The data are from tests in which mutant males competed against *MC* and *NB* males. The two variables have a curvilinear relationship, in which the mating rate of *yellow* males increases as more matings occur (i.e. as wild-type males become rare). In order to test the hypothesis that the pattern of early mating was identical for *MC* and *NB* females, the relationship between the two variates was rectified using the transformation, $Y' = \ln [1 + Y]$, where Y is the number of matings by mutant males. A line was then fitted by least squares regression of the transformed variate, Y', for five replicates of each male–female combination. The four regression coefficients were compared by analysis of covariance. *Yellow* males are indeed more successful during early mating with *MC* females than with *NB* females ($F = 30{\cdot}7$, 1,76 D.F., $P \ll 0{\cdot}001$ when competing against *MC* males; $F = 7{\cdot}615$, 1,76 D.F., $P < 0{\cdot}01$ when competing against *NB* males). This confirms that mating 'preference' contributes, at least in part, to the difference between *MC* and *NB* females, since different slopes correspond to differences in the rate at which the mating success of *yellow* males increases; this should be invariant if *MC* and *NB* females do not differ in the magnitude of their discrimination against mutant males.

4. DISCUSSION

The study reported here shows that a difference in the mating success of *yellow* males with females from two different wild-type strains is caused, at least in part, by a genetic difference in mating 'preference', that is, a behavioural difference in the response of females to at least one of the special characteristics of *yellow* males. Furthermore, this difference is controlled by alleles at a minimum of two loci, at least one being *X*-linked. This may be the first genetic analysis of a directional (non-assortative) mating preference. It is likely that directional mating preferences form the basis for the evolution of assortative mating among divergent populations, since assortment should arise from linkage disequilibrium when preferred traits in males are phenotypically expressed in females. Hence, further studies of this sort would be of value not only to the study of sexual selection within populations, but also to our understanding of the evolution of ethological isolation between populations.

Reported studies of the *yellow* locus reveal considerable variation in the competitive mating success of males. Success of pair matings with wild-type females ranges from as low as 1·9% mated in 9 days (Dow, 1976) to more than 30% mated in less than 1 h (Bastock, 1956; Wilson *et al.* 1976). Similarly, in 'female choice' experiments involving one or two females with one each of wild-type and mutant males, *yellow* males obtained from as few as 3–5% of the matings (Merrell, 1949; Barker, 1962; Wilson *et al.* 1976) to as many as 15–17% (Sturtevant, 1915; Threlkeld *et al.* 1974). This variation is not likely to be the result of allelic differences: although most *yellow* alleles used in mating studies are not designated, they are probably type-1 mutants (Green, 1961). Rather, variation among reported studies in the mutant's mating success most likely results from the combined effects of male genetic background, variation among females used in mating tests, and possibly inbreeding.

Experiments in which genetic background is carefully controlled typically report very low mating scores for *yellow* males (Merrell, 1949; Barker, 1962; Dow, 1976; Wilson *et al.* 1976). These studies involved crossing strains in such a way as to insert the mutant allele into a wild-type stock background, while simultaneously inbreeding the experimental lines. Inbreeding itself may in part be responsible for the low scores of *yellow* males, if the courtship of mutants is more severely affected by inbreeding than is that of wild-type males. High mating scores by *yellow* appear to be obtained only if, as in the present experiments, outbred strains are used and the mutant allele is on the genetic background in which it existed as a laboratory stock (Sturtevant, 1915; Bastock, 1956; Threlkeld *et al.* 1974). This suggests that 'compensatory' evolution in laboratory stock populations is modifying the behavioural expression of the mutant allele, as originally suggested by Bastock (1956).

Female mating preferences have not been discussed as a potential cause of variation in the mating success of *yellow* males. The experiments reported here suggest that they may be important. This view is further supported by two previous studies of the *yellow* locus. First, in her original work on the behavioural effects of *yellow*, Bastock (1956) intercrossed y and y^+ stocks, 'in such a way that

the wild type stock became genetically similar to the *yellow* except for *yellow* and closely linked genes' (1956, p. 436). Before intercrossing, the success of pair matings involving wild-type females differed significantly from that of pair matings involving *yellow* females, both with wild-type and with *yellow* males. After intercrossing, the two types of females ceased to be statistically distinguishable in their mating with either type of male, although *yellow* females remained slightly more receptive to both types. At the same time, although the mating success of the two types of males with particular female genotypes changed, their average success in pair matings changed only negligibly. Hence, the impact of changing the genetic background on the mating success of *yellow* males was largely via its effects on female, rather than on male, behaviour.

In a later study, Threlkeld *et al.* (1974) observed considerable variation in the propensity of females from different wild-type stocks to mate with *yellow* males. By repeated backcrossing of females from a 'low rejection' strain into a mutant *yellow* stock, they obtained y/y^+ females whose propensity to mate with *yellow* males exceeded that of both the original wild-type and mutant stocks. These females, in fact, mated more frequently with *yellow* males than with wild-type males: the only reported case of a net 'preference' for *yellow*.

In summary then, it is clear from this and other studies that both the *yellow* locus and other loci, some autosomal, influence female mating propensities for *yellow* locus genotypes. Genotypic differences among females used to test competitive male mating success may be an important cause of variation in the reported mating success of *yellow* males. Studies of *Drosophila* behavioural mutations should bear in mind this potential source of variation in male mating success.

The behavioural basis for the difference between *MC* and *NB* females was not investigated in this study. A number of possibilities exist, since *yellow* males deviate from the normal courtship pattern of *D. melanogaster* in numerous respects (Bastock, 1956; Wilson *et al.* 1976; Burnet & Wilson, 1980).

It is possible that the recessive *X*-linked element I have observed that increases the receptivity of *MC* females to *yellow* males is in fact a wild-type allele at the *yellow* locus itself. If, as suggested by Burnet & Connolly (1974) the *yellow* locus is involved in the pathway leading to catecholamine synthesis, then wild-type variation at this locus should also be expected to affect behaviour. In discussing their work on the behaviour of type-2 mutant males, Burnet & Wilson (1980) predicted that,

> pattern mosaic expression of the type-2 mutants suggests the possible existence of other *yellow* mutants, which are wild-type in their cuticular phenotype but nevertheless show some of the behavioural effects of the mutant gene (1980, p. 245).

Perhaps the *MC* strain is fixed for such an allele, one which has a small effect on male mating success, evidenced by a reduction in the mating speed of *MC* males, while simultaneously exhibiting the pleiotropic effects of the allele on female receptivity to mutant male courtship. Mapping of the region of the *MC X*-chromosome that carries the 'preference' factor(s) would obviously be desirable.

Finally the association between female and male differences in the mating behaviour of *MC* and *NB* flies raises the question of whether female variation in acceptance of *yellow* males reflects differences in adaptation during the evolutionary

history of the two strains. *MC* females are relatively receptive to *yellow* males and mate rapidly with wild-type males, while *MC* males mate relatively slowly. *NB* females, on the other hand, rarely mate with *yellow* males and mate slowly with wild-type males, while *NB* males appear to be fast maters. Inverse relationships between female receptivity and male 'vigor' have been repeatedly observed in comparisons between *Drosophila* populations (Speith, 1952, 1968; Kaneshiro, 1980). These are consistent with the theory that male and female behaviour evolve jointly, with male traits evolving in response to the level of stimulation required by females, and female discrimination evolving in response to the compound requirements of relatively rapid mating and the advantages of obtaining a superior mate (Fisher, 1915, 1958; Bastock, 1956). It is probable that the inverse relationship between female and male mating speeds in the *MC* and *NB* strains is owing to just such a process of joint evolution.

The genetic basis of variation in female mating preferences has been largely neglected in studies of mating behaviour in *Drosophila*, with the exception of a small body of literature on the genetic basis of reproductive isolation (Tan, 1946; Ehrman, 1961; Zouros, 1981; Kilias & Alahiotis, 1982). The *yellow* locus may provide an excellent research tool for uncovering genetic variation in female mating preferences. Its manifold effects on male behaviour enable it to reveal variation in female responsiveness to a large variety of courtship components. Moreover, the experiments reported here suggest that such variation can be easily found, if sought, and lends itself readily to genetic analysis.

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