THE CHROMOSOMAL BASIS OF SEXUAL ISOLATION IN TWO SIBLING SPECIES OF DROSOPHILA: *D. ARIZONENSIS* AND *D. MOJAVENSIS*

E. ZOUROS

Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

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ABSTRACT

The chromosomal determination of interspecific differences in mating behavior was studied in the interfertile pair, *Drosophila arizonensis* and *Drosophila mojavensis*, by means of chromosomal substitutions. Interspecific crossing over was avoided by crossing hybrid males to parental females, and identification of the origin of each chromosome in backcrossed hybrids was possible by means of allozyme markers. It was found that male mating behavior is controlled by factors located in the PGM-marked chromosome (which, in other Drosophila species, is part of the *X* chromosome) and in the *Y* chromosome. The other chromosomes influence male sexual behavior through their interactions with each other and with the PGM-marked chromosome, but their overall effect is minor. Female mating behavior is controlled by factors located in the ODH-marked and AMY-marked chromosomes, with the other chromosomes exercising a small additive effect. Hence, the two sex-specific behaviors are under different genetic control. Cytoplasmic origin has no effect on the mating behavior of either sex. There appears to be no correlation between a chromosome's structural diversity (*i.e.*, amounts of inversion polymorphism within a species or numbers of fixed inversions across species) and its contribution to sexual isolation. These findings are in general agreement with those from similar Drosophila studies and may not be specific to the species studied here.

SEXUAL (or ethological) isolation is among the most important and most widespread forms of reproductive isolation in the animal kingdom (MAYR 1963). According to the classical view (DOBZHANSKY 1940), its development is the product of selection against reproductive waste resulting from hybridization between populations that have already developed some form of postzygotic isolation.

There are, however, many known cases of allopatrically developed ethological isolation (ANDERSON and EHRMAN 1967; CRADDOCK 1974a; KANESHIRO 1976; OHTA 1978). For a number of such cases, there exists evidence that ethological isolation was not preceded by postzygotic isolation (CRADDOCK 1974b; CARSON 1978). These observations have forced CARSON (1978) to call attention to the importance of "mutual adjustment of the sexes to what may be called the intraspecific sexual environment" that "in the early phases of divergence may far outweigh, in evolutionary importance, other types of adjustment to ambient en-

vironment." The need for sexual adjustment may result from founder effect or from random drift following a population flush-crush cycle through which novel modes of behavior may appear (CARSON 1975; POWELL 1978).

The development of these theories stresses the need for a better understanding of the genetic basis of sexual behavior. However, the strong influence of environment, age, previous experience (PRUZAN *et al.* 1979) and other nongenetic factors makes it very difficult to evaluate the contribution of the various genetic factors to sexual behavior. At best, one can hope to identify only those genetic elements that exercise a major effect on its expression. Moreover, one may question whether the findings from a study of a given species would be applicable to other species, even within the same genus. In spite of these limitations, the importance of sexual isolation in speciation is such that genetic studies of it are worth undertaking.

Here, I report the findings from a study designed to evaluate the contribution of each chromosome to the determination of sexual behavior of two closely related species of Drosophila. The method used was chromosomal substitution. The interfertile species pair, *Drosophila mojavensis* and *Drosophila arizonensis*, was used to obtain male and female flies carrying various interspecific chromosomal combinations. These flies were subsequently tested for mating preference. The technique was basically similar to that of TAN (1946) and EHRMAN (1961), but differed from both in a number of ways. First, I used naturally occurring electrophoretic variants as chromosomal markers. The use of phenotypic markers is a serious disadvantage in many studies of the genetic basis of sexual isolation. Such mutants may seriously influence the results because either they directly interfere with mating choice or they adversely affect the mating competitiveness of their carriers (EHRMAN and PARSONS 1976). Second, I have studied the mating preference of each sex separately. Third, I have allowed all chromosomes to vary independently, so that I could estimate each one's contribution to sexual isolation, not only in terms of main effects, but also in terms of interactions with the other chromosomes.

MATERIALS AND METHODS

Materials: The materials used in this study consisted of two strains of *Drosophila arizonensis* from Tucson, Arizona (a83 and a88), and two strains of *Drosophila mojavensis* from San Ignacio, Baja California (m422 and m426). These strains were established as multifemale lines and maintained for years in the laboratory prior to their use in these experiments. Strains of *D. mojanensis* from Baja California were used in this study because they carried the fast allele of the alcohol dehydrogenase locus (*Adh*), which marks chromosome *3* (see below). Populations of *D. mojavensis* from elsewhere are indistinguishable from *D. arizonensis* at the *Adh* locus. Information concerning the chromosomal variation, geographical distribution, ecology and reproductive isolation of these species can be found in HEED (1978).

Electrophoretic markers: The two species have the standard *D. repleta* karyotype consisting of 5 pairs of rod-like chromosomes and a pair of small dot-like chromosomes. WHARTON (1942) has assigned number *1* to the *X* chromosome, numbers *2* to *5* to the four long acrocentric chromosomes and number *6* to the small dot-like chromosome.

I have used the following loci-markers: octanol dehydrogenase (*Odh*), alcohol dehydrogenase (*Adh*), phosphoglucose mutase (*Pgm*), and amylase (*Amy*). These four loci reside on different autosomes. Using inversions as markers, it was possible to show that *Odh* is in chromosome *2*, and

Adh in chromosome 3 (ZOUROS 1976). *Pgm* is either in chromosome 4 (in which case *Amy* is in chromosome 5), or in chromosome 5 (in which case, *Amy* is in chromosome 4). The fact that the 2 species bear no cytological differences in these 2 chromosomes makes it impossible to distinguish between the two possibilities. It is very unlikely that *Pgm* or *Amy* marks chromosome 6. These loci have been mapped in other species of Drosophila and were found to reside in chromosomes other than the sixth (CAVENER 1977; LOUKAS *et al.* 1979). In the rest of this paper, the 4 marked chromosomes will be denoted as C-ODH (2), C-ADH (3), C-PGM (4 or 5), and C-AMY (5 or 4). The 4 loci were chosen among others mainly because their electrophoretic variants are species-diagnostic, *i.e.*, the 2 species carry in high frequencies alleles that have different electrophoretic mobilities. The 4 stocks used here were monomorphic for the diagnostic electromorphs, except a88, which segregated for 2 alleles at the *Pgm* locus; these alleles have different mobilities from that of the allele of the *D. mojavensis* stocks. The first 3 columns of Table 1 summarize this information.

Electrophoresis was carried out on adult flies. Half of the homogenate was inserted in a starch gel and run according to POULIK's (1957) system. Three slices were obtained from this gel, one stained for ODH, one for ADH and one for PGM. The other half was put in a polyacrylamide gel, which was stained for AMY according to the technique of PRAKASH, LEWONTIN and HUBBY (1969). Thus, it was possible to identify electrophoretically the specific origins of all 8 autosomes of a backcrossed fly. The origins of its sex chromosomes were known from its parents and from its sex. Thus, with the exception of the sixth chromosome pair, the complete chromosomal constitution of the fly could be identified.

Mating preference experiments: When females from one species are confined with males from the other species, viable offspring of both sexes will appear, but the males from the cross "female *D. arizonensis* × male *D. mojavensis*" are sterile. For this reason, the cross "female *D. mojavensis* × male *D. arizonensis*" was invariably used to provide hybrid males for further crosses. In all crosses, the female parents were *D. arizonensis* or *D. mojavensis*; the male parents were either *D. arizonensis* or *D. mojavensis*, or else carried a chromosomal combination of the 2 species. Because there is no crossing over in the male, it was possible to transfer the parental chromosomes intact from one generation to the next.

In all tests for mating preference, one of the 2 sexes was a pure species, *i.e.*, it came directly from the stocks. The opposite sex was either pure species, or F_1, or progeny from a backcross of F_1 males to one of the parental species. In one test, the individuals involved were progeny of males from a double backcross.

TABLE 1

The chromosomes, their markers and their effects on sexual isolation between D. arizonensis *and* D. mojavensis

Chromosome*	Marker in		Effect on S.B. of		Number of inversions†
	D. arizon.	*D. mojav.*	Male	Female	
Y	n.m.	n.m.	+	n.a.	n.a.
X	n.m.	n.m.	?	—	9
2	*Odh*(S)	*Odh*(F)	—	+	103
3	*Adh*(S)	*Adh*(F)	—	—	18
4 or *5*	*Pgm*(F,S)	*Pgm*(I)	+	—	4 or 10
5 or *4*	*Amy*(S)	*Amy*(F)	—	+	10 or 4

* Numbering of chromosomes according to WHARTON (1942).

† Number of inversions observed in 46 species of the repleta group (WASSERMAN 1963).

S.B.: sexual behavior; *Odh*: octanol dehydrogenase locus; *Adh*: alcohol dehydrogenase locus; *Pgm*: phosphoglucose mutase locus; *Amy*: amylase locus; S, I, F: slow, intermediate, and fast electrophoretic mobilities; n.m.: no marker; n.a.: not applicable; +: major effect; —: minor or no effect; ?: not tested.

Mating preference was measured in two ways:

(1) *Direct observation of matings*: One virgin female was confined in a vial with 2 virgin males of different origins. Because the males were morphologically indistinguishable, one of them was marked with a small spot of ink on the thorax. Marking was done one day prior to testing. Pilot experiments showed that the marking did not affect mating frequencies. For this reason, and to simplify the procedure, the male from the parental stock was always marked. All flies involved in the tests came from uncrowded cultures treated in as much the same way as possible. The flies were between 11 and 14 days old when tested for mating preference. This was the age that, under our experimental conditions, produced the highest number of copulations. The 11- to 14-day age variation was among trios. Within each trio the competing males were of same age (measured in days). The trio was observed for 1 hr. If no copulation occurred, all 3 flies were discarded (pseudocopulations were recognized from the fact that they did not last more than 30 sec). If a copulation occurred, observation of that trio was terminated and the flies were processed as will be described for each particular test (see RESULTS). All observations were carried out in the afternoon hours under constant temperature and light conditions.

(2) *Detection of sperm in females*: Twenty to 25 virgin females varying in age from 11 to 14 days were introduced into a Drosophila population cage, together with an equal number of virgin males, all of the same age. The cage was set up at an afternoon hour and was left for 24 hr, after which the females were removed. Two to 3 days later, the sperm receptacles of each female were removed and examined for presence of sperm. The rest of the fly's body was frozen at —75° for electrophoresis.

The results from the 2 methods are not strictly comparable. The "detection of sperm" method gives a higher number of inseminations than does the "direct observation" method. For this reason, only results obtained by the same method will be compared to each other.

RESULTS

The mating tests to be discussed here fall in two categories: those designed to understand the chromosomal basis of the male mating behavior and those designed to understand the chromosomal basis of the female mating behavior.

Tests of male mating behavior

(1) *Pure males*: Rows 1 to 4 of Table 2 give the results of four "direct observation" tests in which a single female was offered a choice between a male from her

TABLE 2

Mating scores in tests involving two males each from a different species or a male from one species and an F_1 hybrid male

	Trio	Chromosomes of "foreign" male	Total matings	Frequency of heterogamic matings (S.E.)
1	♀ a83, ♂ a83, ♂ m422	*Ym/Xm Am/m*	170	0.023(0.011)
2	♀ m422, ♂ m422, ♂ a83	*Ya/Xa Aa/a*	104	0.471(0.049)
3	♀ a88, ♂ a88, ♂ m426	*Ym/Xm Am/m*	81	0
4	♀ m426, ♂ m426, ♂ a88	*Ya/Xa Aa/a*	101	0.485(0.050)
5	♀ a83, ♂ a83, ♂ (♀ m422 × ♂ a83)	*Ya/Xm Aa/m*	103	0.281(0.044)
6	♀ a83, ♂ a83, ♂ (♀ a83 × ♂ m422)	*Ym/Xa Aa/m*	105	0.267(0.043)
7	♀ a88, ♂ a88, ♂ (♀ m426 × ♂ a88)	*Ya/Xm Aa/m*	100	0.250(0.043)
8	♀ a88, ♂ a88, ♂ (♀ a88 × ♂ m426)	*Ym/Xa Aa/m*	103	0.194(0.039)

a: *arizonensis*; m: *mojavensis*; *Y*: the *Y* chromosome; *X*: the *X* chromosome; *A*: autosomes; S.E.: standard error.

own stock and a male from a stock of the other species. The results are given in terms of frequencies of heterogamic matings. Note that the *D. mojavensis* female does not discriminate against the *D. arizonensis* male, but the *D. arizonensis* female discriminates strongly against the *D. mojavensis* male. This is consistent with previous observations (ZOUROS and D'ENTREMONT 1974, 1980; WASSERMAN and KOEPFER 1977). Because the *D. arizonensis* female is a more sensitive discriminator of male mating behaviors than is the *D. mojavensis* female, it has been used exclusively in tests designed to discriminate between the behaviors of males carrying various combinations of chromosomes.

(2) *F_1 hybrid males*: Rows 5 to 8 of Table 2 give the results from tests in which a *D. arizonensis* female was offered a choice between a male from her own stock and a male of which half of the chromosomes were from her stock. These F_1 hybrids were produced in both reciprocal crosses. The first observation is that the F_1 males are more successful in copulating with *D. arizonensis* females than are the *D. mojavensis* males. None of the F_1 hybrid scores is significantly different from 0.25, which is the mid-point value between complete rejection of the heterogamic male (zero) and complete lack of discrimination (0.50). Another observation is that the direction of the parental cross did not matter. Thus, there is no evidence of any cytoplasmic effects.

(3) *Backcross males*: F_1 males from the cross "female *D. mojavensis*-422 × male *D. arizonensis*-83" were backcrossed to *D. mojavensis*-422 females. Males from this cross (which I call *B* males) represent a collection of sixteen different chromosomal combinations. Each one of the *B* males was matched for age with a *D. arizonensis*-83 male, and the two males were placed together with a *D. arizonensis*-83 female. Each trio was observed in the usual way; after a mating had occurred, the *B* male was removed and characterized as "successful" if it was the male involved in the copulation, or "unsuccessful" if the mating occurred with the *D. arizonensis male*. The so-characterized *B* males were frozen at $-75°$ and subsequently put into electrophoresis for the determination of their chromosomal constitution.

The results are shown in Table 3, from which several points of interest emerge. First, note that the males of the first row are "*D. mojavensis*" with respect to all autosomes, the *X* chromosome and cytoplasmic origin. The only difference between these males and parental *D. mojavensis* males is in the *Y* chromosome, which in the *B* males is of *D. arizonensis* origin. Yet, these males have a much higher rate of success than the *D. mojavensis* males, as a comparison with the first row of Table 2 will show. The test for homogeneity of the two scores gives a chi-square value of 26.76 ($d.f. = 1, P \simeq 0$). This result clearly implicates the *Y* chromosome in the determination of the male's mating behavior. I have, of course, no control over the very small sixth chromosome. Half of the *Ya/Xm Am/m* males (where *A* stands for "autosomes") must contain one *D. arizonensis* chromosome *6* and, in principle, it is possible that all eleven successful ones were carriers of that chromosome. If this is true, then chromosome *6* is the most important chromosome in the determination of the male's mating behavior, but this appears quite unlikely. Another possibility is that the foreign *Y* chromosome did not affect the

TABLE 3

Mating scores of Ya/Xm Am/(m or a) males (or B males) with D. arizonensis *females when competing with* D. arizonensis *males*

	C-ODH	C-ADH	C-PGM	C-AMY	S	U	S/N (S.E.)
1	m/m	m/m	m/m	m/m	11	34	0.244(0.064)
2	m/m	m/m	m/m	m/a	2	20	0.091(0.061)
3	m/m	m/m	m/a	m/m	7	26	0.212(0.071)
4	m/m	m/a	m/m	m/m	1	18	0.053(0.051)
5	m/a	m/m	m/m	m/m	7	27	0.206(0.069)
6	m/m	m/m	m/a	m/a	2	14	0.125(0.083)
7	m/m	m/a	m/m	m/a	3	18	0.143(0.076)
8	m/a	m/m	m/m	m/a	3	16	0.158(0.084)
9	m/m	m/a	m/a	m/m	6	16	0.273(0.095)
10	m/a	m/m	m/a	m/m	12	17	0.414(0.091)
11	m/a	m/a	m/m	m/m	1	35	0.028(0.027)
12	m/m	m/a	m/a	m/a	6	17	0.261(0.092)
13	m/a	m/m	m/a	m/a	7	14	0.333(0.103)
14	m/a	m/a	m/m	m/a	0	16	0
15	m/a	m/a	m/a	m/m	3	17	0.150(0.080)
16	m/a	m/a	m/a	m/a	8	15	0.348(0.099)
	Total				79	320	

C: chromosome; S: successful; U: unsuccessful; N: S+U; m: *mojavensis*; a: *arizonensis*.

mating behavior of the *D. mojavensis* males, but rather increased their mating drive. By necessity, the mating tests in this study are such that they cannot distinguish between discriminatory and nondiscriminatory factors. No matter how unlikely the hypothesis is that a foreign *Y* will increase mating drive, it cannot be discarded without further evidence.

The next four rows (2 to 5) of Table 3 give the mating scores of males that differ from the males of the first row in that one of their autosomes is of *D. arizonensis* origin. Because these males contain more *D. arizonensis* genes than do those of the first row, one might expect that they would have a higher mating success with *D. arizonensis* females, but this is not so. The score is lower in all four cases. For row 4, the difference in score from row 1 is nearly significant (from FISHER's exact test, the probability that it might have occurred by chance is 0.067), and the probability that all four scores are lower than the score of row 1 by chance is 0.062. It appears that a single *D. arizonensis* chromosome in a Ya/Xm background has a negative, rather than a positive, effect on the mating success with a *D. arizonensis* female.

In order to evaluate the effect of any single chromosome on the mating success of its carrier, one must examine the mating scores of that chromosome in all possible combinations with the other chromosomes. For this reason, the columns S and U of Table 3 were submitted to a discrete multivariate analysis according to the log-linear models developed by BISHOP, FIENBERG and HOLLAND (1975). The computer program used was BMDP3F of the Health Sciences Computing Facility of the University of California in Los Angeles.

The results are shown in Table 4. Two chromosomes, C-ADH and C-PGM, directly affect the male's chance to be accepted by a *D. arizonensis* female. The effect of C-ADH is barely significant and, furthermore, it appears to work opposite from the expected direction, *i.e.*, *Ya/Xm* flies that carry a *D. arizonensis* ADH-marked chromosome are more rigorously discriminated against by *D. arizonensis* females than are males in which both third chromosomes are of *D. mojavensis* origin. The only autosome with a clear effect on the male's mating success is the PGM-marked one. Males with a *D. arizonensis* PGM-marked chromosome are accepted by *D. arizonensis* females at a much higher rate than are males in which both PGM-marked chromosomes are of *D. mojavensis* origin.

Four of the six pair-wise interactions are significant (or nearly significant) at the 5% level. In three cases, the nature of the interaction is such that when the two (nonhomologous) chromosomes are conspecific, then there is a higher chance of mating success than when they are heterospecific. But the C-ODH × C-ADH interaction does not follow this rule. It would appear from this analysis that, in addition to C-PGM, all the other autosomes affect the male's mating success, but only through their interactions with each other. Even so, their effects are minor compared to the effect of C-PGM.

It is possible to consider Table 3 as containing five different categories of males: one category with no *D. arizonensis* autosomes (row 1), one with one *D. arizonensis* autosome (rows 2 to 5), one with two (rows 6 to 11), one with three (rows 12 to 15) and one with four (row 16). In Figure 1, I have plotted the mating scores against the percentage of *D. mojavensis* autosomes in each category. The resulting pattern suggests that males that inherited a mixed set of chromosomes from their father have lower mating scores than males that inherited either a complete *D. mojavensis* or a complete *D. arizonensis* set. This can be tested if rows 1 and 16 are

TABLE 4

Log-linear discrete multivariate analysis of the mating scores of Ya/Xm Am/(m *or* a) *males from columns S and U of Table 3*

Factor	D.F.	Chi-square (% of total)	Probability	Direction
C-ODH	1	0.30(0.8)	0.586	—
C-ADH	1	3.83(11.3)	0.050	a ↓
C-PGM	1	12.29(36.3)	0.000	a ↑
C-AMY	1	0.03(0.1)	0.871	—
C-ODH × C-ADH	1	4.87(14.4)	0.027	(a+a), (m+m) ↓
C-ODH × C-PGM	1	3.80(11.2)	0.051	(a+a), (m+m) ↑
C-ODH × C-AMY	1	0.98(2.9)	0.323	—
C-ADH × C-PGM	1	3.90(11.5)	0.048	(a+a), (m+m) ↑
C-ADH × C-AMY	1	3.69(10.9)	0.055	(a+a), (m+m) ↑
C-PGM × C-AMY	1	0.00(0)	0.997	—
Higher order interactions		Nonsignificant		

↑,↓: the factor in the indicated state increases or decreases the mating score of the *Ya/Xm Am/*(*m* or *a*) male.

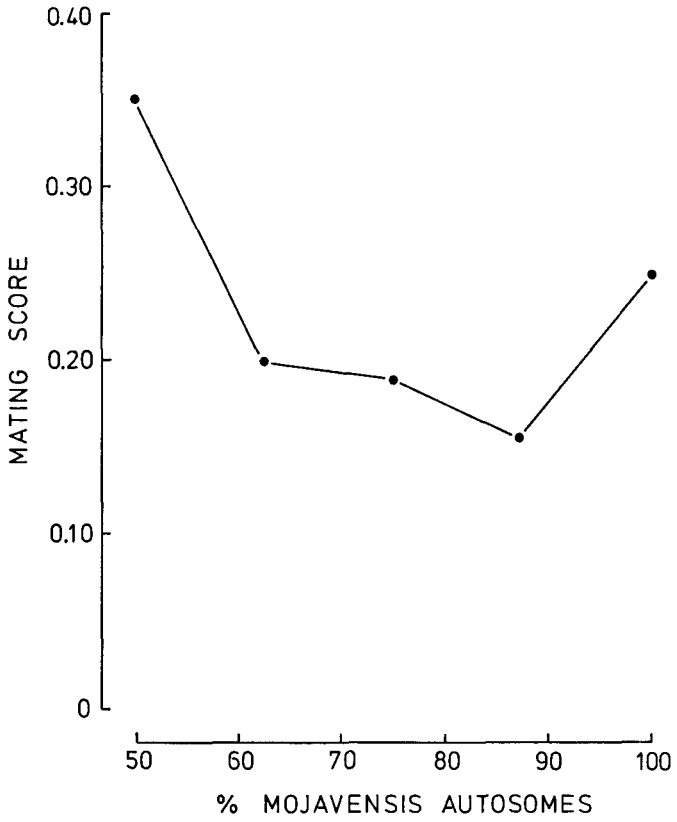

FIGURE 1.—The mating scores of *Ya/Xm Am/(m* or *a)* males (or *B* males) as a function of their content in *D. mojavensis* autosomes. These males were placed in competition with *D. arizonensis* males; the choosing female was *D. arizonensis*.

pooled together into one group, rows 2 to 15 into another and the two sets tested for homogeneity. The test yields a chi-square value of 3.45 ($d.f. = 1, P = 0.063$).

Tests of female mating behavior

(1) *Pure and F_1 females*: Table 5 gives the results from various female-preference tests. In the tests of rows 1 to 4, one F_1 female was placed with one *D. arizonensis* and one *D. mojavensis* male, and its mating choice was recorded by direct observation. In all four tests, the matings with the *D. arizonensis* male outnumbered the matings with the *D. mojavensis* male. This preference for the *D. arizonensis* male was independent of the cytoplasmic origin of the hybrid female: the scores in rows 1 and 2 are not different from each other, and the same applies to the scores in rows 3 and 4. Again, one observes that the cytoplasmic origin has no effect on mating preference, but the combined score of rows 1 and 2 is significantly different from the combined score of rows 3 and 4 (the chi-square value is 33.16, $d.f. = 1, P \simeq 0$). This means that the discrimination of the hybrid female against the *D. mojavensis* male is much stronger in the "a88–m426" com-

TABLE 5

Mating preferences of pure or F_1 hybrid females

	Trio	Chromosomes of female	Total matings	Frequency of matings with ♂ m (S.E.)
1	♀ (♀ m422 × ♂ a83), ♂ a83, ♂ m422	*Xa/Xm Aa/m*	111	0.360(0.045)
2	♀ (♀ a83 × ♂ m422), ♂ a83, ♂ m422	*Xa/Xm Aa/m*	100	0.360(0.048)
3	♀ (♀ m426 × ♂ a88), ♂ a88, ♂ m426	*Xa/Xm Aa/m*	121	0.149(0.032)
4	♀ (♀ a88 × ♂ m426), ♂ a88, ♂ m426	*Xa/Xm Aa/m*	103	0.097(0.029)
	Cage			
5	♀ a88, ♂ m426	*Xa/Xa Aa/a*	124	0.113(0.023)
6	♀ (♀ m426 × a88), ♂ m426	*Xa/Xm Aa/m*	100	0.820(0.038)

* Notation as in Table 1.

bination than it is in the "a83–m422" combination. For this reason, the *D. arizonensis* 88 and *D. mojavensis* 426 stocks were used exclusively in further tests of female mating behavior.

The real interest in the data of Table 5 lies in the comparison between rows 5 and 6, where one sees that females carrying a complete set of *D. mojavensis* chromosomes were inseminated by *D. mojavensis* males at a much higher rate than did flies carrying no such chromosomes. The following experiments were designed to investigate the genetic basis of this observation.

(2) *Backcross and double-backcross females*: Males from the cross "females *D. mojavensis*-426 × males *D. arizonensis*-88" were backcrossed to *arizonensis*-88 females. Female progeny from this cross will be of the type *Xa/Xm Aa/*(*a* or *m*). There will be, then, sixteen types of such females, as listed in Table 6. These females (which I call *B* females) were placed in a cage with *D. mojavensis*-426 males. After their examination for presence or absence of sperm, they were put to electrophoresis. The results appear in Table 6 and the statistical analysis in Table 7.

The males from the backcross (*i.e.*, the brothers of the *B* females) were again backcrossed to *D. arizonensis*-88 females. The resulting females (which I call *BB* females) are of the type *Xa/Xa Aa/*(*a* or *m*). In this case, the probability that one of the chromosomes in a given pair is of *D. mojavensis* origin is only one-fourth. The results are shown in Table 6. Note that the male parents of *BB* females comprise a heterogeneous class of sixteen types. Not all of these types will be equally accepted by the *D. arizonensis* females (those with more *D. arizonensis* chromosomes will be accepted more often than those with fewer *D. arizonensis* chromosomes). Indeed, the observed distribution of the various chromosomal combinations among the *BB* females deviates markedly from the one expected if all *B* males had an equal chance for mating. *BB* females with three or four *mojavensis* chromosomes are entirely missing (their combined expectation is 14.9), and the combined number of *BB* females with one or two *D. mojavensis* chromosomes is 96; whereas, its expected value is 185.4. The results from the multivariate analysis of *BB* mating scores are presented in Table 7.

TABLE 6

Insemination rates of backcrossed (B) *and double-backcrossed* (BB) *females with* D. mojavensis *males*

	C-ODH	C-ADH	C-PGM	C-AMY	B females (*Xa/Xm*) S	NS	S/N	BB females (*Xa/Xa*) S	NS	S/N
1	a/a	a/a	a/a	a/a	3	11	0.214(0.110)	58	139	0.294(0.032)
2	a/a	a/a	a/a	a/m	5	4	0.555(0.166)	18	10	0.643(0.090)
3	a/a	a/a	a/m	a/a	4	12	0.250(0.108)	6	23	0.261(0.081)
4	a/a	a/m	a/a	a/a	5	11	0.312(0.116)	1	2	0.333(0.272)
5	a/m	a/a	a/a	a/a	9	3	0.750(0.125)	19	12	0.613(0.087)
6	a/a	a/a	a/m	a/m	7	2	0.778(0.139)	0	0	—
7	a/a	a/m	a/a	a/m	12	7	0.632(0.111)	0	0	—
8	a/m	a/a	a/a	a/m	8	4	0.667(0.136)	2	0	—
9	a/a	a/m	a/m	a/a	14	5	0.737(0.101)	1	0	—
10	a/m	a/a	a/m	a/a	9	6	0.600(0.126)	1	1	—
11	a/m	a/m	a/a	a/a	11	1	0.917(0.080)	0	0	—
12	a/a	a/m	a/m	a/m	6	4	0.600(0.155)	0	0	—
13	a/m	a/a	a/m	a/m	16	1	0.941(0.059)	0	0	—
14	a/m	a/m	a/a	a/m	11	3	0.786(0.110)	0	0	—
15	a/m	a/m	a/m	a/a	13	6	0.684(0.107)	0	0	—
16	a/m	a/m	a/m	a/m	11	1	0.917(0.080)	0	0	—
	Total				144	81		225	106	

S: presence of sperm, NS: absence of sperm, N: S+NS; rest of notation as in Table 3.

TABLE 7

Log-linear discrete multivariate analysis of the insemination rates of Xa/Xm Aa/(a or m) and of Xa/Xa Aa/(a or m) females by D. mojavensis *males**

Factor	D.F.	Chi-square (% of total)	Probability	Direction
a. *Xa/Xm Aa/*(*a* or *m*) females (columns S and NS of *B* of Table 6)				
C-ODH	1	17.22(38.0)	0.000	a ↓
C-ADH	1	3.73(8.2)	0.053	a ↓
C-PGM	1	2.22(4.9)	0.136	—
C-AMY	1	8.09(17.8)	0.004	a ↓
C-ODH × C-PGM × C-AMY	1	5.35(11.8)	0.021	(a,a a) ↓
Other interactions		Nonsignificant		
b. *Xa/Xa Aa/*(*a* or *m*) females (columns S and NS of *BB* of Table 6)				
C-ODH	1	11.22(41.9)	0.001	a ↓
C-ADH	1	0.11(0.4)	0.744	—
C-PGM	1	1.02(3.8)	0.313	—
C-AMY	1	11.67(43.6)	0.001	a ↓
Interactions		Nonsignificant		

* Notation as in Table 4.

The results of Table 7 are quite clear. Two autosomes, C-ODH and C-AMY, have a definite effect on the female's rate of acceptance of the *D. mojavensis* male. Both chromosomes exercise their effect in the expected way, *i.e.*, they reduce the rate of insemination if they are of *D. arizonensis* origin, and increase the rate if they are of *D. mojavensis* origin. The effect of the ADH-marked chromosome is barely significant among *B* females, and among *BB* females it is clearly not significant. Finally, the PGM-marked chromosome shows no effect on the female's ability to discriminate between *D. arizonensis* and *D. mojavensis* males. This chromosome is the main determinant of the male's mating success. Also note that there is no evidence of chromosomal interactions in the determination of the female's ability to discriminate between males of different origins (one three-way interaction is significant, but it may be fortuitous; with 22 interaction terms for both *B* and *BB* analyses, one significant interaction may appear by chance alone). This is another difference between the genetic modes of determination of sexual behavior in the two sexes.

When the data in Table 6 are organized in groups according to the number of *D. mojavensis* chromosomes contained and the score of each such group is plotted

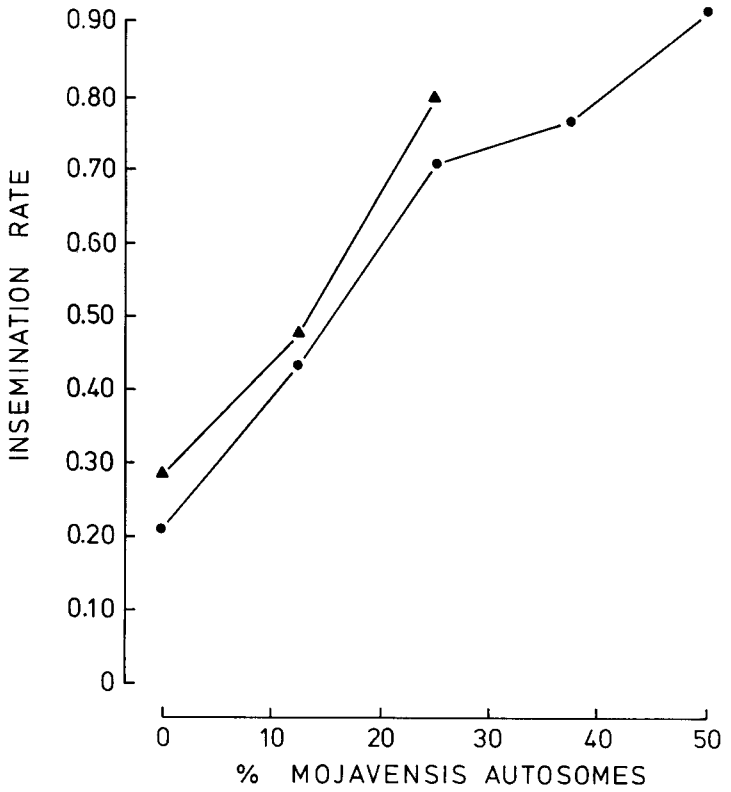

FIGURE 2.—The insemination rates of *Xa/Xm Aa/(a* or *m)* females (or *B* females) and the insemination rates of *Xa/Xa Aa/(a* or *m)* females (or *BB* females) by *D. mojavensis* males as a function of their content in *D. mojavensis* autosomes. Circles: *B* females; triangles: *BB* females.

against its proportion of *D. mojavensis* chromosomes, one obtains the patterns of Figure 2. One sees that the percentage of backcross females inseminated by *D. mojavensis* males increases almost linearly with the number of *D. mojavensis* autosomes contained by the females. The trend is very similar among *B* and *BB* females.

Finally, it is interesting to note that *B* and *BB* females produced very similar results. The two classes of females are quite different in their sex chromosomes, those of the first class carrying *X* chromosomes from two different species and those of the second, from one species. The similarity in insemination scores suggests either that the *X* chromosome carries no factors affecting the female's ability to discriminate between male mating behaviors or that the *D. arizonensis X* chromosome is dominant over the *D. mojavensis X* chromosome.

The results from both the male and female series of experiments are summarized in the fourth and fifth columns of Table 1.

DISCUSSION

The discussion will be based on the following model. It will be assumed that the male emits a sequence of signals that collectively will be called male-mating behavior; he is the emitter. The female's mating behavior consists in sensing this sequence of signals, analyzing it and making a decision to accept or reject the courting male; she is the receptor. This model may suffer from all the shortcomings of oversimplification, but it provides a framework within which to examine the results. It appears to be basically correct for most species of Drosophila and is certainly applicable to the two species studied here. In all trios that were observed directly, courting was initiated by the male. Both males did not court in all trios, so that it is possible that in those trios the noncourting male had "decided" not to court. If this occurred, it would be compatible with the notion that males, too, exercise a preference. But our observations provide no evidence for this, and in no event did females initiate the courting process.

Departing from the assumptions of the model, the experiments that I have described may be interpreted as follows. In the male series of experiments, the rationale was to start with an "all *D. mojavensis*" male and substitute *D. arizonensis* chromosomes for *D. mojavensis* ones, in order to see which chromosomes (or combinations of chromosomes) would modify the male's behavior in such a way that its probability of being accepted by the *D. arizonensis* female is increased to the extent that this probability is increased, we say that these chromosomes (or combinations) affect the mating behavior of the male. In the female series of experiments, the rationale was to replace a number (up to half) of a *D. arizonensis* female's chromosomes with *D. mojavensis* ones, and see which such substitutions would lower the female's ability to reject the *D. mojavensis* male (or, inversely, to raise the rate of acceptance of the *D. mojavensis* male).

The basic findings from the male mating behavior experiments are that it is mainly determined by two chromosomes, the *Y* and the one marked with the PGM locus. The other chromosomes appear to act in concert, but their overall effect is minor.

How do these results compare with other Drosophila studies in which the technique of chromosomal substitution was used? It appears that this is the first study in which the *Y* chromosome is clearly implicated in the determination of sexual behavior in Drosophila. The work of TRACEY and ESPINET (1976) also involved the *Y* chromosome, but in their experiments it was not possible to tell whether the effect on mating behavior came from the *Y* itself or from the piece of the *X* chromosome that was translocated onto the *Y* chromosome.

TAN (1946) experimented with F_1 and backcross females of the sibling species pair *D. pseudoobscura* and *D. persimilis*, but not with males. As a result, his study provides little information about the role of the various chromosomes in determining male mating behavior. EHRMAN (1961) produced such males from crosses between semispecies *D. paulistorum*. She placed these males with two kinds of females, one from each parental semispecies. This experimental scheme makes it difficult to compare her results with mine since, in the *D. paulistorum* case, males, not females, were given a mating choice.

Most comparable with the present results are those of EWING (1969). He recorded the mating songs of F_1 and backcross males of *D. pseudoobscura* and *D. persimilis* and concluded that these songs were to a large extent determined by genes located in the *X* chromosome, which is metacentric. On the basis of homologies of enzyme loci, it can be shown that one of its arms, *XR*, corresponds to the chromosome of the *D. arizonensis-D. mojavensis* pair that is marked with the PGM locus (ZOUROS 1976). It is not known whether the genes affecting the moting song characteristics of *D. pseudoobscura* and *D persimilis* are in *XR* or *XL* (or both). The experiments reported here indicate that they are in the *XR*. In any event, one may note that had I worked with the *D. pseudoobscura-D. persimilis* pair, I would have found that the male's performance is affected by the *X* chromosome.

The main conclusion from the female series of experiments is that two chromosomes, one marked with the ODH locus and one marked with the AMY locus, affect the female's ability to discriminate between conspecific and heterospecific male mating behaviors. The other autosomes contribute to a much lesser extent and in an additive, rather than synergistic, fashion. In TAN's (1946) paper, there is also evidence that chromosome *2* of the *D. pseudoobscura-D. persimilis* pair contains the genetic factors most responsible for the female's ability to discriminate between heterospecific male mating behaviors. Chromosome *2* in this pair of species corresponds to the ODH-marked chromosome *2* of the *D. arizonensis-D. mojavensis* pair (ZOUROS 1976). The evidence comes from TAN's Tables 5 and 6, from which one can see that the proportion among backcross females that accepted the *D. persimilis* male is lower among females with two *D. pseudoobscura* second chromosomes than it is among females with one *D. pseudoobscura* and one *D. persimilis* second chromosome (the corresponding chi-square value in Table 5 is 11.46, d.f. $= 1$, $P = 0.007$; in Table 6 it is 21.71, d.f. $= 1$, $P \simeq 0$). Such effects are not apparent for the other chromosomes, including the *X*. This effect of chromosome *2* was also noted by TAN.

A major finding of the present study is that the genetic determination of sexual behavior is quite different in the two sexes. This is contrary to findings in crickets,

where the production of songs in males and its detection by females apparently have a common genetic basis (HOY, HAHN and PAUL 1977), which has not been elucidated in crickets, however, apart from the fact that the *X* chromosome is implicated in the determination of the male's song. In Drosophila, not only are different chromosomes involved, but also the way they interact in the determination of sexual behavior is different in the two sexes.

Finally, one may ask whether there is a correlation between a chromosome's contribution to sexual isolation and its rate of cytological evolution. Such a correlation may be expected on the grounds that genes responsible for differences in sexual behavior will have a higher probability of being driven to high frequencies (and, eventually, to fixation) in different populations, if they are linked to genes that are responsible for adaptation to the ambient environment of the populations. Inversions provide an effective device for such linkages.

WASSERMAN (1963) compared cytologically 46 species of the *repleta* group of Drosophila (of which *D. arizonensis* and *D. mojavensis* are members). It may be seen from the last column of Table 1 that the rate of fixation of paracentric inversions during the evolution of this group varied quite markedly among the chromosomes. Chromosome *2* is by far the most differentiated among species. On the other extreme, chromosomes *4* and *5* are the most conservative. In terms of intraspecific inversion polymorphism, *D. mojavensis* is known to carry such a polymorphism in chromosome *2*, and to a lesser extent in *3*, but none in chromosomes *4* and *5* (JOHNSON and HEED, in press). Although the role of chromosome *2* in the determination of female mating behavior correlates well with its high degree of interspecific structural differentiation, this correlation does not hold for the AMY-marked chromosome, which also affects the female's mating behavior. The same is true for the PGM-marked chromosome, the main autosomal determinant of male mating behavior. This apparent lack of correlation between structural diversity of a chromosome and its contribution to sexual isolation is more in line with the notion that the beginnings of speciation may not always be found in the process of adaptation to local environments (CARSON 1978).

The tests included in this study are only a few of many that could be done, and in retrospect it may appear that some of the omitted tests might have provided more information. Even so, some knowledge about the chromosomal basis of sexual behavior (and, by extention, sexual isolation) in Drosophila has been gained. None of the findings is incompatible with pre-existing information, and some of the obsrvations described here help put pre-existing information in more specific terms. The observation that the PGM-marked chromosome is the main determinant of male mating behavior agrees with EWING's (1969) results, and the observation that the ODH-marked chromosome *2* is one of the major chromosomes affecting the female's mating behavior agrees with TAN's (1946) results. EHRMAN's (1961) main conclusion that all chromosomes are involved in sexual isolation is correct in a broad sense, but it may be seen from this study that the role of a given chromosome may be important in one sex and insignificant in the other. The fact that the findings from three different species groups of Drosophila (*D. obscura*, *D. willistoni*, *D. repleta*) are in broad agreement suggests that the main

genetic elements for sexual isolation are the same in all species of Drosophila, and that species-specific variations in mating behavior may be recent acquisitions involving little genetic differentiaton.

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