

# GENETICS OF SEXUAL ISOLATION BETWEEN *DROSOPHILA PSEUDOOBSCURA* AND *DROSOPHILA PERSIMILIS*

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## INTRODUCTION

NOTWITHSTANDING their morphological similarity, *Drosophila pseudoobscura* and *Drosophila persimilis* are distinct species. No hybrids between them have been recorded in natural populations, although the two species frequently occur together in the same habitats. Although in laboratory experiments species hybrids may be obtained without much difficulty, intraspecific matings succeed more easily than interspecific ones. The principal if not the only cause of this is the pronounced sexual isolation between the species. If females of the two species are placed together with males of one of them, a greater proportion of conspecific than of alien females are inseminated (DOBZHANSKY and EPLING 1944).

Although the degree of sexual isolation between *D. pseudoobscura* and *D. persimilis* depends upon the environment in which they live (MAYR and DOBZHANSKY 1945), there can be no doubt that the principal cause of the isolation is genetic. The genetic basis of sexual isolation has never been studied. The experiments reported in the present article represent an attempt to explore this little known field. Since female hybrids between *D. persimilis* and *D. pseudoobscura* are fertile when backcrossed to either parental species, it is, theoretically, possible to obtain individuals with any combination of chromosomes of the parents. The sexual preferences of such individuals of mixed parentage may, then, permit inferences regarding the genetic mechanisms underlying the isolation between the species. It may be noted that differences in sexual preferences sometimes occur between mutants and the wild type of the same species (SPETT 1931; NIKORO, GUSSEV, PAVLOV, and GRIASNOV 1935; RENDEL 1945), as well as between geographic strains of a single species (DOBZHANSKY and KOLLER 1938; STALKER 1942; DOBZHANSKY and MAYR 1944; DOBZHANSKY and STREISINGER 1944). However, these differences in sexual preferences seldom assume the character of a true sexual isolation—that is, of a regular preference for endogamic, as opposed to exogamic, mating. This of course, is what would be expected on theoretical grounds if, as is usually supposed, species arise from geographic races by a process of gradual divergence. The genetic elements from which an isolation may be built are available within a species, but these elements are combined to form a functional isolating mechanism only during the process of speciation—that is, during the transformation of the diverging races into distinct species.

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## MATERIALS AND METHODS

The following mutant strains of *D. pseudoobscura* were used: (1) yellow, singed, vermilion, compressed, short (*y, sn, v, co, sh*), (2) orange (*or*), (3) orange purple (*or pr*), (4) white (*w*), (5) aristapedia (*ast*), and (6) Bare Curly (*Ba Cy*). Mutants affecting different parts of the body are included in this list: a body color mutant yellow; eye color mutants vermilion, orange, purple, and white; bristle mutants singed and Bare; wing mutants short and Curly; and an antenna mutant aristapedia. With the exception of Bare and Curly, which are dominant and lethal when homozygous, all other mutants are recessive and viable in homozygous condition. The wild type flies used were obtained from a Piñon Flats (California) strain.

In the experiments on different classes of backcross hybrids it was necessary to obtain strains of the two species, *D. pseudoobscura* and *D. persimilis*, which carried appropriate mutant markers in each chromosome except the small fifth which is not under genetic control. *D. pseudoobscura* females carrying sex-linked recessives yellow (*y*), singed (*sn*), vermilion (*v*) compressed (*co*), and short (*sh*), and the second chromosome dominant Bare (*Ba*), were mated to males of the same species heterozygous for Curly (*Cy*) associated with an inversion in the fourth chromosome. The  $F_1$  daughters which showed *Ba* and *Cy* were selected and mated to *y sn v co sh* males. Among the progeny, only *y co sh Ba Cy* flies were selected and crossed to *D. persimilis* homozygous for a third chromosome mutant orange (*or*). The hybrids that showed *Ba* and *Cy* were heterozygous for the recessive mutant *y* located in the left limb of the X chromosome, *co* near the centromere, and *sh* in the right limb of the X chromosome, dominant *Ba* in the second chromosome, recessive *or* in the third chromosome, and dominant *Cy* in the fourth chromosome. The hybrids are heterozygous for five different inversions, one each in the right limb and in the left limb of the X chromosome, in the second chromosome, in the third chromosome, and in the fourth chromosome. The first four of the above inversions are the characteristic differences between the two species (TAN 1935), and the fifth inversion has been introduced with the Curly strain of *D. pseudoobscura*. The hybrids thus obtained were then backcrossed to *D. pseudoobscura* males carrying sex-linked recessives *y, sn, v, co, sh*, and the third chromosome recessive *or*.

According to the setting of the experiments, every one of the chromosomes of the hybrid females with the exception of the small fifth chromosome, in which no genes are available, is marked by one or more genes, which should make the different classes of the backcross progeny phenotypically distinguishable from each other. The 16 different classes of individuals each representing a specific combination of the four major pairs of chromosomes of the two species are shown schematically in the accompanying figure. They range from a type in which all chromosomes belong to *D. pseudoobscura* to the other extreme in which one chromosome of each pair comes from *D. pseudoobscura* and the other from *D. persimilis*. This, of course, leaves out an account of the crossing over that may occur despite the inversions and that escapes detection in these experiments.

Since different mutant strains may vary in sexual behavior, tests were made by comparing each class of backcross hybrid females with females of *D. pseudoobscura* carrying the corresponding mutants. For this purpose, mass cultures of the following four mutant stocks were made from which the 16 required classes of flies were obtained. One of these cultures contained *Ba* and *Cy*; it produces *Ba*, *Cy*, *Ba Cy*, and wild type. Another cross gave *Ba or*, or *Cy*,

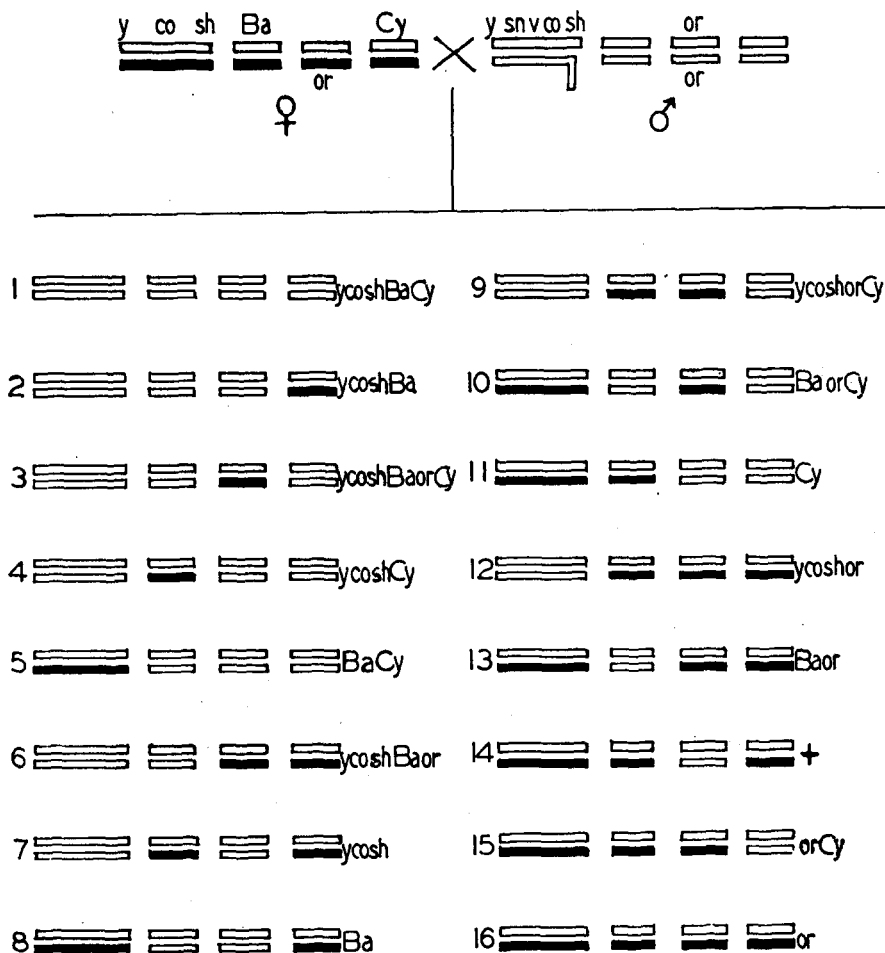


FIGURE 1. The chromosome constitution and the phenotype of the 16 different classes of females appearing in the backcross of the  $F_1$  hybrid female heterozygous for yellow (*y*), compressed (*co*), short (*sh*), Bare (*Ba*), and Curly (*Cy*) to yellow (*y*), singed (*sn*), vermillion (*v*), compressed (*co*), short (*sh*), and orange (*or*) *D. pseudoobscura* males. *D. pseudoobscura* chromosomes—white; *D. persimilis* chromosomes—black.

*or*, and *Ba or Cy* flies. Still another cross furnished *y co sh Ba*, *y co sh Cy*, *y co sh*, and *y co sh Ba Cy*. Finally, a fourth culture gave rise to *y co sh Ba or*, *y co sh or Cy*, *y co sh or*, and *y co sh Ba or Cy* flies.

All flies used in these experiments were raised on a cream of wheat-molasses medium (SPASSKY 1943) at 22.5°C. In order to avoid crowding, the parent flies were transferred repeatedly to fresh culture bottles. When the adults in any culture began to emerge, the bottle was removed from the incubator and kept at room temperature. Newly emerged adults of both sexes were isolated every day and aged from five to seven days in separate vials with food at room temperature. In tests with *D. persimilis*, all flies were aged from seven to ten days, because they require a longer time to become sexually mature.

In the tests involving backcross hybrid progenies, it was frequently necessary to use flies with an age difference of as much as three days. In such cases, to minimize the possible effect of the age difference, control females with ages as heterogeneous as the hybrid females were used.

When two kinds of females involved in a given experiment are phenotypically alike, they are distinguished by clipping of wings, one type of females having the right and the other the left wing clipped. In flies carrying the *Cy* mutant, which may obscure the effect of wing clipping, a leg was clipped instead.

For each individual experiment, usually ten mature females of each of two kinds and five mature males of one kind were placed together in a vial containing food. Sometimes, when a sufficient number of properly aged virgin females of one or of both kinds was not available, the number of females used was reduced, but never to fewer than five of each kind. In these cases, the number of males used was also proportionately reduced, keeping the ratio of two females of each kind to one male. All mating experiments were carried on in an incubator of 25.5°C.

The length of time during which the females and males were kept together varied in individual cases, depending largely on which species of males were used. When *D. pseudoobscura* is used as male, two to three hours are usually sufficient to have more than 50 percent of the females inseminated. In the case of *D. persimilis* males, it takes sometimes even four or five days to reach a 50 percent insemination level. The experimental vials in which too few or too many (less than 10 percent, more than 90 percent) of the females were inseminated were discarded.

#### PREFERENTIAL MATING IN *D. PSEUDOBSCURA*

Table 1 summarizes the results of the sexual preference tests between different strains of *D. pseudoobscura*. For each cross, the percentage of homogamically (matings among members of the same strain) and heterogamically (matings between members of different strains) inseminated females is given, together with the  $\chi^2$  which measures the statistical significance of the difference observed between these frequencies. The isolation index was originally proposed by STALKER (1942), and has since been used as a standard measure of the degree of randomness of mating. It is obtained by dividing the difference by the sum of the percentages of homogamic and heterogamic inseminations. If the matings are at random, the isolation index is 0 or close to it. If homo-

TABLE I

Number of females dissected (*N*) and percentage carrying sperm (%) in different crosses of *Drosophila pseudoobscura*.

NO.	MALES	FEMALES	HOMOGAMIC		HETERO-GAMIC		$\chi^2$	INDEX
			N	%	N	%		
1	wild type	wild type, <i>or pr</i>	62	79.0	67	73.4	0.57	+0.04
2	wild type	wild type, <i>or</i>	43	58.1	41	90.2	11.07	-0.21
3	wild type	wild type, <i>ast</i>	38	76.3	39	66.7	0.91	+0.07
4	wild type	wild type, <i>w</i>	53	77.4	52	63.5	2.44	+0.10
5	wild type	wild type, <i>Ba Cy</i>	53	79.2	52	59.6	4.77	+0.14
6	wild type	wild type, <i>y sn v co sh</i>	49	69.4	53	79.2	1.29	-0.07
7	<i>or pr</i>	<i>or pr</i> , wild type	84	84.5	81	55.6	16.46	+0.20
8	<i>or pr</i>	<i>or pr</i> , <i>or</i>	34	41.2	36	44.4	0.08	-0.04
9	<i>or pr</i>	<i>or pr</i> , <i>ast</i>	19	78.9	19	57.9	1.96	+0.15
10	<i>or pr</i>	<i>or pr</i> , <i>w</i>	26	34.6	26	42.3	0.32	-0.10
11	<i>or pr</i>	<i>or pr</i> , <i>Ba Cy</i>	35	65.7	36	66.7	0.00	-0.01
12	<i>or pr</i>	<i>or pr</i> , <i>y sn v co sh</i>	29	79.3	27	74.1	0.19	+0.03
13	<i>or</i>	<i>or</i> , wild type	41	80.5	42	61.9	3.57	+0.12
14	<i>or</i>	<i>or</i> , <i>or pr</i>	43	60.5	45	77.8	3.07	-0.13
15	<i>or</i>	<i>or</i> , <i>ast</i>	37	86.5	37	37.8	11.66	+0.39
16	<i>or</i>	<i>or</i> , <i>w</i>	35	74.3	33	78.7	0.21	-0.03
17	<i>or</i>	<i>or</i> , <i>Ba Cy</i>	29	79.3	30	56.7	2.80	+0.17
18	<i>or</i>	<i>or</i> , <i>y sn v co sh</i>	76	46.1	76	57.9	2.14	-0.11
19	<i>ast</i>	<i>ast</i> , wild type	33	60.6	38	60.5	0.00	0.00
20	<i>ast</i>	<i>ast</i> , <i>or pr</i>	18	16.7	18	38.9	1.99	-0.40
21	<i>ast</i>	<i>ast</i> , <i>or</i>	39	53.8	35	80.0	3.00	-0.20
22	<i>ast</i>	<i>ast</i> , <i>w</i>	34	41.2	37	35.1	1.89	+0.08
23	<i>ast</i>	<i>ast</i> , <i>Ba Cy</i>	42	0.0	45	40.0	21.23	-1.00
24	<i>ast</i>	<i>ast</i> , <i>y sn v co sh</i>	38	60.5	39	66.7	1.23	-0.05
25	<i>w</i>	<i>w</i> , wild type	74	64.9	74	78.4	3.32	-0.09
26	<i>w</i>	<i>w</i> , <i>or pr</i>	27	37.0	27	11.1	4.98	+0.54
27	<i>w</i>	<i>w</i> , <i>or</i>	44	54.5	43	55.8	0.02	-0.01
28	<i>w</i>	<i>w</i> , <i>ast</i>	43	58.1	42	42.9	1.92	+0.15
29	<i>w</i>	<i>w</i> , <i>Ba Cy</i>	30	13.3	32	25.0	0.94	-0.30
30	<i>w</i>	<i>w</i> , <i>y sn v co sh</i>	55	36.4	54	37.0	0.01	-0.01
31	<i>Ba Cy</i>	<i>Ba Cy</i> , wild type	30	86.7	30	86.7	0.00	0.00
32	<i>Ba Cy</i>	<i>Ba Cy</i> , <i>or pr</i>	45	68.9	44	77.3	0.82	-0.06
33	<i>Ba Cy</i>	<i>Ba Cy</i> , <i>or</i>	29	79.3	29	96.6	4.00	-0.10
34	<i>Ba Cy</i>	<i>Ba Cy</i> , <i>ast</i>	33	81.6	35	51.4	9.57	+0.23
35	<i>Ba Cy</i>	<i>Ba Cy</i> , <i>w</i>	35	77.1	35	71.4	0.70	+0.04
36	<i>Ba Cy</i>	<i>Ba Cy</i> , <i>y sn v co sh</i>	28	82.1	30	76.7	0.57	+0.03
37	<i>y sn v co sh</i>	<i>y sn v co sh</i> , wild type	110	83.6	104	59.6	15.19	+0.17
38	<i>y sn v co sh</i>	<i>y sn v co sh</i> , <i>or pr</i>	57	66.7	61	36.1	10.99	+0.30
39	<i>y sn v co sh</i>	<i>y sn v co sh</i> , <i>or</i>	71	70.4	65	26.2	26.53	+0.46
40	<i>y sn v co sh</i>	<i>y sn v co sh</i> , <i>ast</i>	44	34.1	46	4.3	13.03	+0.78
41	<i>y sn v co sh</i>	<i>y sn v co sh</i> , <i>w</i>	64	35.9	66	7.6	15.45	+0.65
42	<i>y sn v co sh</i>	<i>y sn v co sh</i> , <i>Ba Cy</i>	45	37.8	46	26.1	1.48	+0.18

gamic or heterogamic matings are preferred, the index lies between 0 to +1, or between 0 and -1, respectively. An index +1 indicates complete isolation.

As shown in table 1, most of the crosses that involve wild type, *or pr*, *or*, *ast*, *w*, or *Ba Cy* males show no significant deviations from randomness of mating. The few exceptional cases are experiments Nos. 2, 7, 15, 23, and 34. Preferences for heterogamic inseminations are exhibited by wild type males in the crosses to *or* females (No. 2), and by *ast* males in the cross to *Ba Cy* females (No. 23). It is interesting to note that while *ast* males prefer *Ba Cy* females to their own, *Ba Cy* males prefer *Ba Cy* to *ast* females (No. 34). This relationship seems to hold true also for *or* and wild type. Wild type males mate with *or* more frequently than with wild type females (No. 2,  $I = -0.21$ ), whereas *or* males prefer *or* to wild type females (No. 13,  $I = +0.12$ ). Likewise, *or* males prefer *or* to *ast* females (No. 15,  $I = +0.39$ ), while *ast* males seem to prefer *or* females to their own (No. 21,  $I = -0.20$ ). This, however, is not true in the case of *or pr* and wild type flies: *or pr* males prefer *or pr* to wild type females (No. 7,  $I = +0.20$ ), but wild type males mate at random with wild type and *or pr* females (No. 1,  $I = +0.04$ ).

When yellow singed vermilion compressed short (*y sn v co sh*) flies are used as males, there is a strong preference for homogamic matings (Nos. 37 to 42). The isolation index varies in individual cases from +0.17 (No. 37) to +0.78 (No. 40). Statistically, all these values are significant with the exception of the case (No. 42) involving *Ba Cy* females. It is obvious that *y sn v co sh* males mate with females of their own strain more frequently than with females of other strains. The reciprocal crosses (Nos. 6, 12, 18, 24, 30, and 36), however, do not show any preference for either homogamic or heterogamic matings.

The mating preferences found in the cultures containing *y sn v co sh* females and males and wild type females (see above), may be caused directly by one of the mutant genes involved, or they may be due to genetic factors independent of these visible mutants. To clarify this point, *y sn v co sh* males were crossed to wild type females and the  $F_1$  hybrid females were backcrossed to wild type males; *y sn v co sh* and *y* (crossover) males were selected in the progeny of the backcross. These males were crossed to their female sibs in small mass cultures, and in the following generation *y sn v co sh* and *y* strains were re-established. The sexual preferences of these "extracted" *y sn v co sh* and *y* flies were compared with those of the original *y sn v co sh* strain and of wild type and of *or* flies. The results are summarized in table 2. In this table, the designations of the "extracted" are underlined, and those of the original strain are shown in the usual type. It is evident that the behaviors of the original and the "extracted" *y sn v co sh* strains are alike within limits of experimental error. Furthermore, extracted *y* males inseminate more females of their own strain than that of wild type or *or* strains. Wild type males seem to show a slight preference for females of the "extracted" *y sn v co sh* and *y* strains in comparison to wild type females. These results are in accord with the

view that the peculiar mating preferences of the *y sn v co sh* strain are caused by the yellow mutant gene.

HYBRIDS OF *D. PERSIMILIS* AND *D. PSEUDOOSCURA*

Tables 3 to 7 summarize the results of tests involving the pure species and different classes of backcross hybrids of *D. persimilis* and *D. pseudoobscura*. In the crosses involving hybrid females and males and females of the same species, the term "homogamic" refers to matings within the species and "heterogamic" to matings with hybrid females (Tables 3, 4, and 6). If the pure species females used belong to a species different from the male (Table

TABLE 2  
Number of females dissected (*N*) and percentage carrying sperm (%) in crosses with "extracted" *y* and *y sn v co sh* strains of *Drosophila pseudoobscura*.

NO.	MALES	FEMALES	HOMOGAMIC		HETEROGAMIC		$\chi^2$	INDEX
			N	%	N	%		
1	<i>y sn v co sh</i>	<i>y sn v co sh</i> , wild type	72	76.4	62	58.1	4.96	+0.14
2	<i>y sn v co sh</i>	<i>y sn v co sh</i> , or	59	78.0	58	50.0	9.99	+0.22
3	<i>y sn v co sh</i>	<i>y sn v co sh</i> , <i>y sn v co sh</i>	32	46.9	32	40.6	0.21	+0.07
4	<i>y</i>	<i>y</i> , wild type	70	81.4	70	58.6	8.72	+0.16
5	<i>y</i>	<i>y</i> , or	135	72.6	134	59.0	5.24	+0.10
6	<i>y</i>	<i>y</i> , <i>y sn v co sh</i>	66	69.7	71	69.0	0.00	+0.01
7	wild type	wild type, <i>y sn v co sh</i>	25	44.0	27	77.8	6.60	-0.28
8	wild type	wild type, <i>y</i>	48	56.3	47	74.5	3.38	-0.14
9	or	or, <i>y sn v co sh</i>	32	46.9	34	52.9	0.24	-0.06
10	or	or, <i>y</i>	96	33.3	102	43.1	1.06	-0.13

5 and 7), the genetic constitution of the hybrid females is closer to that of the males used than to that of the alien females. Hence, "homogamic" refers to matings with hybrid females and "heterogamic" to those with alien females.

The first experiment consisted in crossing *y co sh Ba Cy D. pseudoobscura* females to or *D. persimilis* males. The  $F_1$  hybrid females were backcrossed to *y sn v co sh* or *D. pseudoobscura* males. The resulting backcross hybrid females have *D. pseudoobscura* cytoplasm, at least one complete set of *D. pseudoobscura* chromosomes, and from none to four *D. persimilis* chromosomes. The genetic composition of the different backcross females is symbolized in tables 3 to 7 by means of formulae in which the letter "a" stands for *D. pseudoobscura* and the letter "b" for *D. persimilis* chromosomes. Starting from the left, the first letter symbolizes the X chromosome, and the second, third, and fourth letters the second, third, and fourth chromosomes respectively. Thus, the formula  $\frac{a}{b} \frac{a}{a} \frac{a}{b} \frac{a}{a}$  indicates a female having one *D. persimilis* X chromosome and one *D. persimilis* third chromosome, the other chromosomes being those of *D. pseudoobscura*. Altogether 16 classes of backcross females were tested

TABLE 3

Number of females dissected (*N*) and percentage carrying sperm (%) in crosses between backcross hybrid females (with *pseudoobscura* cytoplasm), *D. pseudoobscura* females, *D. pseudoobscura* males.

NO.	MALES <i>pseudo-</i> <i>obscura</i>	FEMALES <i>pseudoobscura</i> , HYBRIDS	HYBRID CHROMOSOMES				HOMO- GAMIC		HETERO- GAMIC		$\chi^2$	INDEX	
			X	II	III	IV	N	%	N	%			
1	<i>y co sh Ba Cy</i>		$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	11	36.4	10	60.0	1.70	-0.24	
2	<i>y co sh Ba</i>		$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	15	40.0	16	56.3	0.72	-0.17	
3	<i>y co sh Ba or Cy</i>		$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	10	50.0	10	60.0	0.22	-0.09	
4	<i>y co sh Cy</i>		$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	23	43.5	23	65.2	2.04	-0.20	
5	<i>Ba Cy</i>		$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	28	64.3	29	72.4	0.32	-0.06	
6	<i>y co sh Ba or</i>		$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	16	31.3	16	75.0	6.18	-0.41	
7	<i>y co sh</i>		$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	30	60.0	30	76.7	1.78	-0.12	
8	<i>Ba</i>		$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	32	43.8	32	84.4	11.46	-0.32	
9	<i>y co sh or Cy</i>		$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	6	66.7	6	50.0	0.34	+0.14	
10	<i>Ba or Cy</i>		$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	34	58.8	33	72.7	1.54	-0.11	
11	<i>Cy</i>		$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	36	72.2	38	65.8	0.36	+0.05	
12	<i>y co sh or</i>		$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	20	60.0	20	60.0	0.00	0.00	
13	<i>Ba or</i>		$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	41	75.6	40	70.0	0.30	+0.04	
14	+		$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	34	67.6	34	79.4	2.10	-0.04	
15	<i>or Cy</i>		$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	30	83.3	32	75.0	0.66	+0.05	
16	<i>or</i>		$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	60	60.0	62	74.2	2.62	-0.11	
Total								426	60.3	431	71.5	11.97	-0.08

(table 3). Each test consisted in placing together backcross females, *D. pseudoobscura* females, and *D. pseudoobscura* males carrying the same visible mutants.



TABLE 4

Number of females dissected (*N*) and percentage carrying sperm (%) in crosses between backcross hybrid females (with *persimilis* cytoplasm), *D. pseudoobscura* females, and *D. pseudoobscura* males.

NO.	MALES <i>pseudo-</i> <i>obscura</i>	FEMALES <i>pseudoobscura</i> , HYBRIDS	HYBRID CHROMOSOMES				HOMO- GAMIC		HETERO- GAMIC		$\chi^2$	INDEX
			X	II	III	IV	N	%	N	%		
1	<i>y co sh Ba Cy</i>		$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	10	30.0	9	22.2	0.18	+0.15
2	<i>y co sh Ba</i>		$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	18	22.2	18	50.0	3.04	-0.39
3	<i>y co sh Ba or Cy</i>		$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	7	28.6	9	22.2	0.06	+0.13
4	<i>y co sh Cy</i>		$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	23	52.2	25	60.0	0.22	-0.07
5	<i>Ba Cy</i>		$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	24	50.0	23	60.9	0.49	-0.10
6	<i>y co sh Ba or</i>		$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	22	36.4	22	54.5	1.32	-0.20
7	<i>y co sh</i>		$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	39	20.5	43	60.5	12.90	-0.49
8	<i>Ba</i>		$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	28	39.3	29	34.5	0.10	+0.07
9	<i>y co sh or Cy</i>		$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	54	40.7	57	63.2	5.37	-0.22
10	<i>Ba or Cy</i>		$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	20	55.0	19	43.1	0.57	+0.13
11	<i>Cy</i>		$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	30	63.3	33	75.8	1.20	-0.09
	<i>y co sh or</i>		$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	35	42.9	38	71.1	6.30	-0.25
13	<i>Ba or</i>		$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	39	61.5	38	76.3	0.00	-0.11
14	+		$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	35	60.0	36	61.1	0.04	-0.01
15	<i>or Cy</i>		$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	37	67.6	41	58.5	0.63	+0.07
16	<i>or</i>		$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	47	51.1	47	59.6	0.62	-0.08
Total							468	47.2	487	69.3	14.06	-0.19

As shown in table 3, in most of the tests the matings took place either at random or else there was a slight preference for hybrid females.

The second experiment (table 4) paralleled the first, except that the original

TABLE 5

Number of females dissected (*N*) and percentage carrying sperm (%) in crosses between backcross hybrid females, *D. pseudoobscura* females, and *D. persimilis* males.

NO.	MALES <i>persimilis</i>	FEMALES <i>pseudoobscura</i> , HYBRIDS	HYBRID CHROMOSOMES				HETERO- GAMIC ALIEN SP.		HOMO- GAMIC HYBRID		$\chi^2$	INDEX
			X	II	III	IV	N	%	N	%		
1	orange	<i>y co sh Ba Cy</i>	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	44	22.7	42	33.3	1.22	+0.19
2	orange	<i>y co sh Ba</i>	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	49	36.7	50	48.0	1.11	+0.13
3	orange	<i>y co sh Ba or Cy</i>	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	59	39.0	59	37.3	0.06	-0.02
4	orange	<i>y co ch sh Cy</i>	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	66	15.2	70	54.3	22.12	+0.56
5	orange	<i>Ba Cy</i>	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	50	22.0	49	57.1	12.24	+0.44
6	orange	<i>y co sh Ba or</i>	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	43	39.5	43	58.1	3.13	+0.19
7	orange	<i>y co sh</i>	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	56	12.5	57	57.9	24.58	+0.64
8	orange	<i>Ba</i>	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	62	12.9	63	34.9	8.34	+0.46
9	orange	<i>y co sh or Cy</i>	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	72	19.4	76	48.7	13.21	+0.43
10	orange	<i>Ba or Cy</i>	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	56	21.4	58	43.1	5.52	+0.34
11	orange	<i>Cy</i>	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	82	11.0	84	56.0	18.50	+0.67
12	orange	<i>y co sh or</i>	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	78	19.2	82	50.0	27.40	+0.45
13	orange	<i>Ba or</i>	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	59	16.9	61	44.3	10.77	+0.45
14	orange	+	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	99	30.3	100	53.0	10.79	+0.27
15	orange	<i>or Cy</i>	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	70	17.1	73	64.4	32.20	+0.58
16	orange	<i>or</i>	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	78	19.2	77	57.1	23.63	+0.50
Total							1023	21.6	1044	50.5	186.06	+0.42

cross involved *or D. persimilis* females and *y co sh Ba Cy D. pseudoobscura* males. Hence, the backcross hybrids had *D. persimilis* cytoplasm. The result obtained is the same as in the first experiment—namely, the mating is either

TABLE 6

Number of females dissected (*N*) and percentage carrying sperm (%) in crosses between backcross hybrid females, *D. persimilis* females, and *D. persimilis* males.

NO.	MALES <i>persimilis</i>	FEMALES <i>persimilis</i> , HYBRIDS	HYBRID CHROMOSOMES				HOMO- GAMIC		HETERO- GAMIC		$\chi^2$	INDEX
			X	II	III	IV	N	%	N	%		
1	orange ( <i>or</i> )	<i>y co sh Ba Cy</i>	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	18	50.0	17	41.2	0.22	+0.10
2	orange ( <i>or</i> )	<i>y co sh Ba</i>	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	23	65.2	23	39.1	3.14	+0.25
3	orange ( <i>or</i> )	<i>y co sh Ba or Cy</i>	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	16	50.0	17	17.6	3.09	+0.48
4	orange ( <i>or</i> )	<i>y co sh Cy</i>	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	35	54.3	38	76.3	2.09	-0.17
5	orange ( <i>or</i> )	<i>Ba Cy</i>	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{a}$	34	47.1	34	76.5	7.62	-0.24
6	orange ( <i>or</i> )	<i>y co sh Ba or</i>	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	28	50.0	29	44.8	0.18	+0.05
7	orange ( <i>or</i> )	<i>y co sh</i>	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	31	48.4	31	54.8	0.31	-0.06
8	orange ( <i>or</i> )	<i>Ba</i>	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	$\frac{a}{b}$	26	42.3	29	34.5	0.37	+0.10
9	orange ( <i>or</i> )	<i>y co sh or Cy</i>	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	30	56.7	30	66.7	0.73	-0.08
10	orange ( <i>or</i> )	<i>Ba or Cy</i>	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{a}$	13	69.2	14	42.9	1.57	+0.23
11	orange ( <i>or</i> )	<i>Cy</i>	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	27	33.3	32	56.3	3.19	-0.26
12	orange ( <i>or</i> )	<i>y co sh or</i>	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	30	50.0	32	62.5	0.84	-0.11
13	orange ( <i>or</i> )	<i>Ba or</i>	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	$\frac{a}{b}$	25	36.0	25	28.0	0.38	+0.13
14	orange ( <i>or</i> )	+	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	38	34.2	39	51.3	2.31	-0.20
15	orange ( <i>or</i> )	<i>or Cy</i>	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	40	42.5	45	62.2	3.34	-0.19
16	orange ( <i>or</i> )	<i>or</i>	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	47	29.8	47	55.3	5.82	-0.30
Total							461	45.6	482	53.7	6.14	-0.08

random or else there is a slight preference for insemination of hybrid females. No striking differences in the behavior of different classes of hybrids is apparent.

The third experiment (table 5) differed from the first in that orange mutant males of *D. persimilis* were used in the final test instead of *D. pseudoobscura* males. Hence, in this experiment orange *D. persimilis* males had the choice of mating with pure *D. pseudoobscura* females and with hybrid females having *D. pseudoobscura* cytoplasm, from one to two complete sets of *D. pseudoobscura* chromosomes, and from none to four *D. persimilis* chromosomes. The result obtained is quite clear (table 5). Namely, *D. persimilis* males mate with hybrid females in preference to the pure *D. pseudoobscura* females; this is found in all the tests except Nos. 1, 2, 3, and 6. The four exceptional tests are those in which the backcross hybrid females carried only *D. pseudoobscura* chromo-

TABLE 7

Number of females dissected (N) and percentage carrying sperm (%) in crosses between backcross hybrid females, *D. persimilis* females, and *D. pseudoobscura* males.

NO.	MALES <i>pseudo-</i> <i>obscura</i>	FEMALES <i>persimilis</i> , HYBRIDS	HYBRID CHROMOSOMES				HOMO- GAMIC HYBRID		HETERO- GAMIC ALIEN SP.		$\chi^2$	INDEX
			X	II	III	IV	N	%	N	%		
1	Ba	or Ba	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{a}$	a b	14	78.6	12	0.0	16.49	+1.00
2	Cy	or Cy	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	a a	15	100.0	17	11.8	24.70	+0.79
3	Ba or	or Ba or	$\frac{a}{b}$	$\frac{a}{a}$	$\frac{a}{b}$	a b	7	57.1	5	0.0	4.56	+1.00
4	+	or +	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{a}$	a b	34	97.1	34	8.8	53.12	+0.83
5	or Cy	or or Cy	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	a a	16	100.0	17	11.8	26.08	+0.79
6	or	or or	$\frac{a}{b}$	$\frac{a}{b}$	$\frac{a}{b}$	a b	29	82.6	27	14.8	25.84	+0.70
Total							115	89.6	112	9.8	144.00	+0.80

somes (No. 1), or a fourth chromosome of *D. persimilis* (No. 2), or a third chromosome of *D. persimilis* (No. 3), or a third and a fourth chromosome of *D. persimilis* (No. 6).

In the fourth experiment, *y co sh Ba Cy* females of *D. pseudoobscura* were crossed to *or* males of *D. persimilis*. The  $F_1$  hybrid females were backcrossed to *y sn v co sh or D. pseudoobscura* males. The resulting backcross hybrids had, therefore, *D. pseudoobscura* cytoplasm, from one to two sets of *D. pseudoobscura* chromosomes, and from one set to no *D. persimilis* chromosomes. Such backcross hybrid females were placed in vials with orange *D. persimilis* females and orange *D. persimilis* males (table 6). Consequently, the *D. persimilis* males had the choice of pure *D. persimilis* females and of females which

were predominantly *D. pseudoobscura* but which had some *D. persimilis* chromosomes. The observed matings mostly failed to deviate significantly from randomness. However, there seems to be a slight tendency for homogamic mating in the tests (Nos. 1, 2, and 3) in which the hybrid females had either none or only one third or fourth chromosome from *D. persimilis*.

The fifth experiment started like the fourth (see above), but the backcross hybrid females were tested with orange *D. persimilis* females and with *D. pseudoobscura* males carrying the same mutant genes as the hybrid females in the same vial. The results (table 7) show a striking preference for mating with hybrid females.

#### DISCUSSION

Among the results obtained in the intraspecific sexual preference tests, the behavior of the mutant yellow deserves some consideration. Whenever males carrying yellow, either alone or in combination with other mutant genes, are placed with a mixture of yellow and non-yellow females, there is always a preference for homogamic matings. Experiments with outcrossing and "extracting" yellow (table 2) indicate that the above behavior is caused by the change at the yellow locus rather than by associated modifying genes. Furthermore, this behavior of yellow is not confined to *Drosophila pseudoobscura* but occurs also in other species in which apparently homologous mutants are known and have been tested for sexual preference—namely, in *D. melanogaster* (SPETT 1931; NIKORO, GUSSEV, PAVLOV, and GRIASNOV 1935) and in *D. subobscura* (RENDEL 1945). The causes of this peculiar behavior of the yellow mutant are not clear. According to MAYR (1946), mating preferences may be controlled by specific attractions, degree of activity, and by physical compatibility of the genitalia. Yellow flies are hardly more active sexually than the non-yellow ones, since STURTEVANT (1915) and DIEDERICH (1941) reported that in *D. melanogaster*, yellow and yellow white males copulate with fewer females than the normal males when the two kinds of males are mated to yellow or yellow white females. RENDEL (1945) found that in *D. subobscura*, yellow males are discriminated against in mating by females of some, but not of all, wild type strains. The chitinous genitalia of yellow and non-yellow males seem to be morphologically similar. A specific attraction between yellow females and males, or a repulsion between yellow males and non-yellow females, may be present, but it must be noted that when non-yellow males are placed with yellow and non-yellow females, the matings are usually at random.

MAYR (1946) found that when *D. persimilis* males have a choice of mating with females of their own species and with  $F_1$  hybrid females from the cross *D. persimilis*  $\times$  *D. pseudoobscura*, more hybrids than conspecific females are inseminated. When *D. pseudoobscura* males are offered a choice of their own and of  $F_1$  hybrid females, there is a slight preference for homogamic matings; but this preference is much less pronounced than that found when *D. pseudoobscura* males have a choice of *D. pseudoobscura* and *D. persimilis* females. MAYR attributes these results to the  $F_1$  hybrid females being more vigorous and active than females of the pure species, presumably on account of hetero-

sis. This explanation does not fit our data. Males of *D. pseudoobscura* mate with females of their own species and with backcross hybrid females either at random or else show a slight preference for the hybrids (tables 3 and 4). The backcross hybrid females range in genetic constitution from almost pure *D. pseudoobscura* (class No. 1) to a class having equal number of chromosomes of *D. pseudoobscura* and *D. persimilis* (class No. 16). In contrast to the F<sub>1</sub> hybrid females, which are at least as vigorous as those of the pure species, the backcross hybrid females exhibit every sign of constitutional weakness (DOBZHANSKY 1936; DOBZHANSKY and EPLING 1944). The failure of *D. pseudoobscura* males to discriminate against hybrid females carrying from one to four *D. persimilis* chromosomes (classes Nos. 2 to 16 in tables 3 and 4) suggests, then, a dominance of the *D. pseudoobscura* genes which cause a female to be acceptable to a *D. pseudoobscura* male. Indeed, all the backcross hybrid females in these experiments carried at least one full set of *D. pseudoobscura* chromosomes (fig. 1).

Much clearer are the results obtained when *D. persimilis* males are placed together with mixtures of backcross hybrid females and pure *D. pseudoobscura* females (table 5). The backcross hybrid females in these experiments represent the same range of genetic constitutions as in the experiments dealt with above (fig. 1). Now, *D. persimilis* males definitely prefer backcross hybrid females having whole sets of the chromosomes of both parental species (class No. 16) to pure *D. pseudoobscura* females. On the other hand, backcross hybrid females which have only, or mainly, *D. pseudoobscura* chromosomes (class No. 1) are no more acceptable to *D. persimilis* males than are pure *D. pseudoobscura* females. A more detailed analysis of the data in table 5 discloses that the genes responsible for the acceptability of females to *D. persimilis* males seem to be dominant and to lie in probably all chromosomes, with X and second chromosomes being more important than the third and the fourth. Thus, females having one second chromosome of *D. persimilis*, and the remainder of the chromosomes from *D. pseudoobscura* (class No. 4), are definitely more acceptable to *D. persimilis* males than are pure *D. pseudoobscura* females. The same holds for females having a single *D. persimilis* X chromosome in an otherwise pure *D. pseudoobscura* complex (class No. 5).

Essentially the same conclusions follow from the experiments in which *D. persimilis* males had to discriminate between backcross hybrid females and pure *D. persimilis* females (table 6). Here the backcross hybrids having equal numbers of chromosomes of the two species (class No. 16) are preferred to pure conspecific females. This may seem paradoxical, but this result agrees with those of MAYR (1946), who, as mentioned above, found that F<sub>1</sub> hybrid females are inseminated by *D. persimilis* males more often than are pure *D. persimilis* females. On the other hand, backcross hybrids having only *D. pseudoobscura* chromosomes (class No. 1), or a single fourth or third chromosome of *D. persimilis* in an otherwise pure *D. pseudoobscura* chromosome complex (classes Nos. 2 and 3), are discriminated against in favor of pure *D. persimilis* females. The X and second chromosomes seem to be most important.

The data at hand therefore support the view that the sexual isolation between *D. pseudoobscura* and *D. persimilis* is caused by a system of at least partly dominant factors carried by each species. *D. pseudoobscura* may be assumed to carry the gene complexes *TT*, and *D. persimilis* the corresponding, but nonallelic, gene groups *SS*; the  $F_1$  hybrids between these species are, then, *TtSs*. The genetic mechanism of sexual isolation may thus be similar to that producing the sterility of the hybrids between them (DOBZHANSKY 1936). In both cases the gene divergence has arisen presumably during the stage when *D. pseudoobscura* and *D. persimilis* were geographically isolated races, and it became accentuated and built up to form reproductive isolating mechanisms when the stage of speciation was reached. The most important difference appears to be that, whereas the sterility genes bring about a complete sterility of  $F_1$  hybrid males, the genes responsible for the sexual attraction are dominant and make the  $F_1$  hybrid females acceptable to males of either pure species. From the standpoint of maintenance of the separation of the two species in nature, the mechanism of sexual isolation is thus insecure. Nevertheless, it probably serves well its biological function as a part of a system of isolating mechanisms.

#### SUMMARY

Sexual preference tests were made using different mutant strains of *Drosophila pseudoobscura*—namely, orange purple (*or pr*), orange (*or*), white (*w*), aristapedia (*ast*), Bare Curly (*Ba Cy*), yellow singed vermilion compressed short (*y sn v co sh*), and one wild type strain. The *y sn v co sh* males inseminate more females of their own kind than females of other mutant strains or of wild type. This effect depends upon the mutant yellow. Other tests showing preferential matings include: (1) wild type males inseminate more *or* than wild type females, (2) *or pr* males inseminate more *or pr* than wild type females, (3) *ast* males inseminate more *Ba Cy* than *ast* females, (4) *Ba Cy* males inseminate more *Ba Cy* than *ast* females and (5) *or* males inseminate more *or* than *ast* females.

Hybrids of *D. pseudoobscura* and *D. persimilis* were obtained ranging in chromosomal constitution from individuals having all chromosomes of *D. pseudoobscura* to individuals having equal numbers of *D. pseudoobscura* and *D. persimilis* chromosomes. When representatives of the different classes of backcross hybrid females were placed together with *D. pseudoobscura* females and *D. pseudoobscura* males, slightly more hybrid than *D. pseudoobscura* females were inseminated.

When a mixture of backcross hybrid females and *D. persimilis* females are exposed to *D. persimilis* males, the results also show a slight preference for mating with the hybrids. But those classes of hybrid females which do not carry the X chromosome, or the second chromosome, of *D. persimilis* are preferred by *D. persimilis* males to a smaller extent than those females which carry these chromosomes.

When *D. persimilis* males are placed with *D. pseudoobscura* females and with backcross hybrid females, the results show a significant preference for mating

with the hybrids, with the exception of the four classes of hybrids which do not carry either an X chromosome or a second chromosome of *D. persimilis*. This suggests that the main factors which distinguish the mating behavior of *D. persimilis* from *D. pseudoobscura* lie in the X and second chromosomes.

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