STUDIES OF SELECTIVE MATING USING THE YELLOW MUTANT OF DROSOPHILA MELANOGASTER

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IN analyses of sexual isolation and selective mating in Drosophila, a number of methods have been used. The most common, particularly in studies of interspecific sexual isolation, is "male choice", where one kind of male is placed with two female types. The use of "choice" in the name of this method is perhaps unfortunate, as in most cases it is a test of which of two types of females will most readily accept the males, rather than the preferential choice of one type of female by the males (MERRELL 1960). Generally, ventral receptacles or spermathecae are examined for presence of spermatozoa to determine which females have been inseminated (DOBZHANSKY and KOLLER 1938). STURTEVANT (1915) used direct observation exclusively, while other workers (STALKER 1942; RENDEL 1945; MAYR 1946a) supplemented their other data by this method. Pair matings (a male of one type with a female of the other) have been used extensively by workers at the University of Texas in studies of sexual isolation between species (PATTERSON, STONE and GRIFFEN 1940). DIEDERICH (1941) used two methods, called "female choice" (one type of female with two male types) and "multiple choice" (two types of females with the two types of males). Male choice and female choice were used by MERRELL (1949) to study selective mating between each of a number of mutants and their combinations, and wild type in *D. melanogaster*. Deviations from random mating occurred more often and were greater with female choice than with male choice. RENDEL (1951) and KOREF-SANTIBAÑEZ and WADDINGTON (1958) also used both male and female choice. Male choice, female choice, and multiple choice were all used by MERRELL (1954) to study the sexual isolation between *D. persimilis* and *D. pseudoobscura*. Female and multiple choice experiments gave essentially the same result, a much higher degree of sexual isolation than male choice. An adaptation of the multiple choice method was used by ELENS (1957), where up to 400 males and females of two types were introduced into a cage and copulations directly observed.

Unless it is consistently mutual, selective mating between genotypes within a species will lead to changes in gene frequency, i.e. it will affect the relative fitness of the alleles and the genotypes, and will need to be considered as a factor in natural selection. REED and REED (1950); MERRELL (1953); and PETIT (1958) have suggested that selective mating is mainly responsible for the elimination from populations of certain sex-linked mutants, with replacement by their wild-type alleles. However, MORPURGO and NICOLETTI (1955) claimed that selective mating is not important in this selection process. Because of the variation in

degree of isolation indicated by different methods in the studies of MERRELL (1949, 1954), and the conflicting results obtained by different workers on the importance of selective mating in particular cases of selection between genotypes, two experiments have been carried out to analyse selective mating. In the first experiment, four of the methods of analysis mentioned above, *viz.* pair mating, male choice, female choice, and multiple choice, were used. However, MORPURGO and NICOLETTI (1955) found strong selective mating between white and wild type when two males of different types were put with one female of either type (i.e. female choice), but there was no evidence of selective mating when the sex ratio was 1:1. Therefore in this experiment two further methods were used, called male choice (E) and female choice (E), in which the sex ratio in the mating vials was 1:1. In the second experiment, multiple choice only was used, and the effect of certain experimental conditions on the degree of selective mating was analysed.

Results obtained with the different methods may be compared using the isolation estimate (I), the male mating ratio (M_m), and the female mating ratio (M_f), devised by MERRELL (1950). These are the most useful indices for this comparison, as others that have been proposed (STALKER 1942; LEVENE and DOBZHANSKY 1945; BATEMAN 1949; LEVENE 1949) were all designed for use with male choice experiments. However, some comments on the calculation of the isolation estimate are necessary.

MERRELL (1950) states that the isolation estimate "is designed for use when equal numbers of the types compared are present (for example, equal numbers of each female type in male choice experiments)." The isolation estimate is then calculated as the ratio of the number of heterogamic matings to the number of homogamic. However, consider male choice experiments involving two types A and B. A males are placed with equal numbers of A and B females, and B males are placed with equal numbers of A and B females. It is not necessary, and often is not the case, that the total number of females tested with A males is the same as the total number with B. MERRELL uses as an example some of his own data (1949) involving the mutant cut and wild type in *D. melanogaster*, where the numbers of females with each type of male are not the same, *viz.* 26 each of homozygous and heterozygous cut females with wild-type males, and 30 of each with cut males. He then calculates:

$$I = \frac{24 + 11}{22 + 15} = 0.95.$$

But this takes no account of the different numbers of females. The estimate should be calculated on the basis of the fraction (or percentage) of females fertilized in each mating type, i.e.:

$$I = \frac{80.0 + 42.3}{84.6 + 50.0} = 0.91.$$

The difference in this case is quite small, but the error in I as calculated from the numbers fertilized, rather than the fraction (or percentage) fertilized, will be greater as the difference between the total numbers of females used increases. MERRELL also uses as an example some data of DOBZHANSKY and STREISINGER (1944) on sexual isolation between geographic strains of *D. prosaltans*. If the numbers fertilized in each mating type were used:

$$I = \frac{23 + 41}{69 + 15} = 0.76.$$

However, I is given as 0.78. This has apparently been arrived at as follows:

$$I = \frac{23 + 41}{70 + 47} \Big/ \frac{69 + 15}{72 + 48} = 0.78.$$

In fact, if the percentage of females fertilized in each mating type is used:

$$I = \frac{32.9 + 87.2}{95.8 + 31.3} = 0.94.$$

Therefore the isolation estimate is much closer to unity than was originally indicated. To take account of variation in the numbers of females tested in the different mating types, the isolation estimate should be calculated as:

$$I = \frac{\text{percent successful } AB + \text{percent successful } BA.}{\text{percent successful } AA + \text{percent successful } BB}$$

EXPERIMENT 1—COMPARISON OF METHODS USED TO ANALYZE SELECTIVE MATING

Materials and Methods: The stocks used were the wild-type Oregon-R-C and the sex-linked mutant yellow (body color, *y*) of *D. melanogaster*. This mutant was chosen because previous workers (STURTEVANT 1915; DIEDERICH 1941; MERRELL 1949; BASTOCK 1956 in *D. melanogaster;* RENDEL 1945 in *D. subobscura*, and TAN 1946 in *D. pseudoobscura*) demonstrated selective mating between it and wild type. All matings were done under continuous light at 25 ± 0.5°C.

MERRELL (1949), and RENDEL (1951) have pointed out the need to reduce environmental variables and have used techniques to ensure that in any particular experiment, all the flies used came from the same culture bottle. This was considered necessary because of the effect of state of nutrition on mating behaviour. Thus, for a sex-linked mutant, four of the five genotypes can be produced in the one bottle by mating mutant males to heterozygous females. However, it was considered desirable to obtain some information on the relative mating behavior of +/+ females, even though they could not be produced from the same bottle as the other genotypes. Flies for the mating experiments were thus obtained by the above mating, and by mating wild-type males and females

of the same age in bottles prepared in the same batch. The complete mating program used to produce flies for the mating experiments was:

		Males	Females		Males	Females	
I.	(a)	+	y/y	(b)	+	+/+	(Flies from stocks—ten pairs/bottle.)
		↓			↓		
		y	+/y		+	+/+	
		Collected as virgins, aged two days.					
II.	(a)	y	+/y	(b)	+	+/+	(Five pairs/bottle.)
		↓			↓		
		+	+/y				
		y	y/y		+	+/+	

All progeny from the (II a) mating and +/+ females from the (II b) mating were used in the selective mating tests. These flies were collected when not more than four hours old and males and females from the same culture (except for +/+ females) placed together in vials containing food. These were the "young fly" mating tests. Flies emerging overnight were collected as virgins each morning, and males and females aged separately for seven days, when the mating tests were repeated. These were the "aged fly" tests. For all mating experiments, the young flies were left together in the vials for 23 hours, and the aged flies for five hours.

The methods of analysis used were:

(a) Pair matings—for each mating type, one male and one female were placed in a vial containing food. At the end of the mating period, males were removed. Presence of larvae and pupae in a vial was taken as evidence of a successful mating.

(b) Male choice (E)—Three males of one genotype and one female of each of the three female genotypes were placed in a vial. At the end of the mating period, each female was placed in a separate vial. Progeny were inspected to determine the original female genotype in each vial of the group of three.

(c) Male choice—Five males of one genotype and five females of each of the three female genotypes were placed in a vial. The determination of successful matings was as for Male choice (E).

(d) Female choice (E)—Two females of one genotype and one male of each male genotype were placed in a vial. The females were put in separate vials at the end of the mating period and the progeny examined to determine the genotype of the successful male. However, for +/+ females, all female progeny are phenotypically wild type regardless of the genotype of the male parent. To determine the successful male in this case, these wild-type female progeny were mated to their + male sibs, one pair per vial. The genotype of the original successful male was determined by examination of their progeny.

(e) Female choice—Five females of one genotype and five males of each male genotype were placed in a vial. Subsequent treatment was as for Female choice (E).

(f) Multiple choice—Three males of each male genotype and two females of each female genotype were placed in a vial. Subsequent treatment was as for Female choice (E).

In all methods, if any flies died during the mating period, that vial was discarded. If, after isolation in a vial, a female died without leaving eggs, that vial was discarded (or the series in the case of male and multiple choice experiments). *Results:* Table 1 shows the numbers of females tested and the percentage fertilized for each mating type. Where double inseminations occurred in the female and multiple choice methods, each male has been scored one half. The isolation estimate *(I)*, male mating ratio *(M_m)*, and female mating ratio *(M_f)* (MERRELL 1950), and the chi-squared appropriate to each have been calculated using the fraction fertilized in each mating type (Table 2). If there is no sexual isolation, I will be one, while if sexual isolation is complete, I will be zero. If mating is at random, both M_m and M_f will be one.

For all methods, the results show that y males were distasteful to wild-type females, although the percentages of wild-type females fertilized by y males were higher for aged flies than for young flies. This low percentage of success of y males with wild-type females was to be expected from the results of STURTEVANT (1915); DIEDERICH (1941); MERRELL (1949); BASTOCK (1956) in *D. melanogaster*, RENDEL (1945) in *D. subobscura*, and TAN (1946) in *D. pseudoobscura*. These workers also showed wild-type males to be more successful than yellow with y/y females. In the present experiments, however, this comparison is strictly

TABLE 1

Numbers of females tested (N) and percentages successful fertilization observed (percent) in experiments of different type

Females	Young flies						Aged flies					
	+/+		+/y		y/y		+/+		+/y		y/y	
Males	N	Percent	N	Percent	N	Percent	N	Percent	N	Percent	N	Percent
Pair matings												
+	114	71.1	148	85.1	108	78.7	170	90.6	137	96.4	148	93.9
y	145	1.4	102	7.8	108	50.0	100	22.0	128	39.8	111	82.0
Male choice (E)												
+	46	84.8	46	95.7	46	89.1	62	98.4	62	98.4	62	100.0
y	49	8.2	49	6.1	49	81.6	62	32.3	62	29.0	62	98.4
Male choice												
+	50	52.0	50	72.0	50	78.0	70	98.6	70	98.6	70	100.0
y	75	2.7	75	12.0	75	52.0	85	22.4	85	21.2	85	96.5
Female choice (E)												
+	146	59.6	194	78.4	104	42.3	120	87.5	128	78.9	106	48.6
y	146	3.4	194	0.5	104	38.5	120	5.0	128	11.7	106	44.8
Female choice												
+	143	89.5	136	89.0	90	65.6	170	94.1	135	93.3	142	51.1
y	143	2.8	136	0.0	90	25.6	170	3.5	135	5.2	142	46.8
Multiple choice												
+	70	90.0	70	91.4	70	72.1	112	79.0	112	83.0	112	57.1
y	70	1.4	70	2.9	70	25.0	112	19.2	112	15.2	112	40.2

TABLE 2

Estimates and calculated χ^2 of the isolation estimate (I), *female mating ratio* (M_f), *and male mating ratio* (M_m) *derived from the data of Table 1*

Female comparison	Young flies						Aged flies					
	I	χ^2	M_f	χ^2	M_m	χ^2	I	χ^2	M_f	χ^2	M_m	χ^2
Pair matings												
+/+ +/y	1.10	0.00	0.78	25.04***	16.98	295.24***	0.91	8.32**	0.83	0.91	3.03	221.31***
+/+ y/y	0.66	8.57**	0.56	51.89***	2.91	134.66***	0.67	65.03***	0.64	41.20***	1.77	105.49***
+/y y/y	0.64	33.34***	0.72	6.09*	2.83	133.12***	0.75	32.88***	0.77	30.58***	1.56	99.06***
Male choice (E)												
+/+ +/y	1.14	0.80	0.91	0.34	...	....	1.03	0.07	1.03	0.07	...	....
+/+ y/y	0.58	24.97***	0.54	33.48***	...	....	0.67	44.19***	0.66	48.72***	...	....
+/y y/y	0.54	36.29***	0.60	27.66***	...	....	0.66	47.10***	0.64	51.70***	...	....
Male choice												
+/+ +/y	1.17	0.82	0.65	5.59*	...	....	1.01	0.01	1.01	0.01	...	....
+/+ y/y	0.78	3.35	0.42	40.98***	...	....	0.63	57.95***	0.62	75.50***	...	....
+/y y/y	0.73	6.94**	0.65	17.42***	...	....	0.62	59.16***	0.61	77.00***	...	....
Female choice (E)												
+/+ +/y	1.36	8.50**	...	....	35.38	347.12***	0.85	2.93	...	....	9.96	278.23***
+/+ y/y	0.47	36.46***	...	....	2.43	64.88***	0.41	70.05***	...	....	2.73	94.26***
+/y y/y	0.37	77.22***	...	....	3.09	167.96***	0.49	47.01***	...	....	2.26	69.79***
Female choice												
+/+ +/y	1.03	0.07	...	....	63.75	433.76***	0.97	0.09	.	....	21.54	488.72***
+/+ y/y	0.59	24.30***	...	....	5.46	221.52***	0.39	115.42***	...	....	2.89	164.10***
+/y y/y	0.57	26.29***	...	....	6.04	220.78***	0.40	97.48***	...	....	2.78	112.82***
Multiple choice												
+/+ +/y	1.00	0.00	0.97	0.06	42.19	220.54***	1.08	0.72	1.00	0.00	4.71	182.58***
+/+ y/y	0.64	12.06***	0.94	0.23	6.14	129.45***	0.64	20.57***	1.01	0.01	2.29	66.04***
+/y y/y	0.64	12.01***	0.97	0.06	5.86	128.93***	0.59	29.01***	1.01	0.01	2.53	80.58***

* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

valid only for female and multiple choice where the two types of males were in direct "competition" for the y/y females. For multiple choice and female choice young flies, the percentage of y/y females fertilized by + males was significantly greater than that for y males. However, for these two methods with aged flies, and for female choice (E) with both young and aged flies the difference was not significant, although the percentage fertilized by + males was always higher. The pair mating results also showed a higher percentage of y/y females fertilized by + males than by y males, indicating a lower mating ability of y males. On the other hand, in the male choice and multiple choice experiments, the wild-type males mated at random with all three female genotypes, except for multiple choice aged flies ($\chi^2 = 6.22$, $P<0.05$). In these experiments also, there were no significant differences between the percentages of $+/+$ and $+/y$ females fertilized by either + males or y males, although with male choice young flies, significantly fewer $+/+$ than $+/y$ females were fertilized by both male types combined ($M_f = 0.65$, $\chi^2 = 5.59$, $P<0.05$). There was a general tendency in this direction with all methods, but the differences are small and are most likely due to differences in culture conditions, the $+/+$ females being raised in bottles separate from all other genotypes. However, RENDEL (1945) found in *D. subobscura* that the reaction of non-y females to y males was not as dominant over y as is the effect on body color of the gene, the heterozygous female being intermediate between the two homozygotes in its reaction to y males. The present data are inadequate to confirm or discount this effect in *D. melanogaster*, although it appears unlikely.

It would be reasonable, therefore, to combine the data for $+/+$ and $+/y$ females, although this has not been done and isolation estimates and male and female mating ratios have been calculated separately for $+/+$ and y/y, and for $+/y$ and y/y. In these comparisons, the isolation estimate is significantly less than one in every case except for male choice, young flies, $+/+$ and y/y. This isolation results from the failure of y males to successfully fertilize wild-type females, but there are differences in the extent of isolation as indicated by the different methods. In general, the male choice methods and pair mating indicate less isolation than female and multiple choice, although for young flies with male choice (E), the results suggest greater isolation than do those of female or multiple choice. Such differences between the methods have been reported by MERRELL (1954), who found that for *D. persimilis* and *D. pseudoobscura*, the isolation estimates calculated from female and multiple choice data were much the same, but both were considerably less than the estimate obtained from male choice data. In a study of sexual isolation between a number of strains of *D. melanogaster*, KOREF-SANTIBAÑEZ and WADDINGTON (1958) found that the tendency towards isolation between inbred lines was more marked in male choice experiments than in female choice, i.e. the inverse of the results of MERRELL (1954) and this study.

The female mating ratios (M_f) for wild-type females compared to yellow females are all significant in the male choice and pair mating experiments, although not significant in the multiple choice. The male mating ratios (M_m),

however, are significant in all cases in the pair mating, female choice, and multiple choice experiments.

EXPERIMENT 2—EFFECT OF EXPERIMENTAL TECHNIQUES ON DEGREE OF SELECTIVE MATING

Materials and Methods: The stocks of *D. melanogaster* used were the Oregon-R–C wild type, and a stock of the yellow mutant that had been made isogenic with Oregon-R–C by backcrossing to it for 18 generations. The only genetic difference between the stocks should thus be the yellow locus and closely linked loci. Multiple choice only was used, with flies less than five hours old when the mating bottles were set up. All matings were done under continuous light at 25 ± 0.5°C.

The flies used were collected by the same mating schedule as in Experiment 1. In setting up mating bottles, equal numbers of the three female genotypes, +/+, +/*y*, and *y*/*y*, were placed together with the same total number of males, one half of which were + and the other half *y*. The experimental variates tested were:

(a) Numbers of flies in each mating bottle, 36 and 108 being used,

(b) Mating period, i.e. the time that males and females remain together in the mating bottles. Periods of 18 and 24 hours were used.

The experiment was designed as a 2×2 replicated factorial and 17 mating bottles were set up for each treatment combination. Some of these had to be discarded because of death of flies during the mating period, and because some females died without laying eggs after being set out in individual vials.

Seven mating bottles were scored in three treatment combinations, and eight in the other. One of these latter was discarded at random, giving seven replications for the experiment.

Results: The results for each mating bottle were scored into nine categories, according to the genotype of the female, the genotype of the male, and the number of sterile females of each genotype (Table 3). One *y*/*y* female was fertilized by males of both genotypes, and this double insemination was scored as one half to each male genotype. The data have been treated as a $3 \times 3 \times 2 \times 2 \times 7$ contingency table, and analysed by the partition of chi-square method developed by CLARINGBOLD (1961). The model used is such that chi-squares testing significance of differences between main effect comparisons are identically zero. Thus, this is an analysis of interactions only. The partition of chi-square is given in Table 4. The single degree of freedom chi-squares in the left-hand column relate to the appropriate interactions listed under source of variation. The right-hand column of chi-squares gives the interaction of the treatment variates with replication. Although a number of these are significant and their sum with 144 degrees of freedom is highly significant, the 24 chi-squares are homogeneous. BARTLETT's test of homogeneity gives:

$$\chi^2_{(23)} = 15.48,\ P > 0.05,$$

so the heterogeneity observed cannot be ascribed to any particular source of variation. Presumably it is inherent in the mating process within bottles. MAYR (1946a) has suggested that time of day plays some role in determining sexual activity in Drosophila, that this is greater in the morning and evening than during

TABLE 3

Results of Experiment 2, presented as the 3 × 3 × 2 × 2 × 7 contingency table appropriate to the method of statistical analysis used

			Treatment											
			No. flies/mating bottle											
			36			36			104			104		
			Mating period (hrs)											
			18			24			18			24		
Replication	Mated	Male genotype	Female genotype											
			+/+	+/y	y/y	+/+	+/y	y/y	+/+	+/y	y/y	+/+	+/y	y/y
0	Yes	+	1	0	1	5	4	3	12	6	5	17	14	13
	Yes	y	0	0	0	1	2	3	0	0	4	0	2	2
	No		5	6	5	0	0	0	6	12	9	1	2	3
1	Yes	+	2	5	1	5	4	6	8	5	7½	16	17	10
	Yes	y	0	0	4	0	2	0	0	0	4½	2	1	4
	No		4	1	1	1	0	0	10	13	6	0	0	4
2	Yes	+	2	2	0	6	5	2	5	7	5	15	16	10
	Yes	y	0	0	0	0	0	3	0	0	2	2	0	7
	No		4	4	6	0	1	1	13	11	11	1	2	1
3	Yes	+	2	2	1	1	5	2	11	9	0	16	17	14
	Yes	y	0	0	0	3	1	4	0	1	3	0	1	3
	No		4	4	5	2	0	0	7	8	15	2	0	1
4	Yes	+	3	3	1	4	4	1	7	6	8	15	13	8
	Yes	y	0	0	1	1	0	5	1	0	1	3	5	7
	No		3	3	4	1	2	0	10	12	9	0	0	3
5	Yes	+	1	1	2	4	4	4	9	8	3	11	13	11
	Yes	y	0	0	1	2	2	1	1	2	5	1	1	5
	No		5	5	3	0	0	1	8	8	10	6	4	2
6	Yes	+	4	5	2	6	5	1	13	10	5	12	15	7
	Yes	y	0	0	2	0	1	4	1	0	4	3	2	10
	No		2	1	2	0	0	1	4	8	9	3	1	1

the middle of the day, even when examined under controlled experimental conditions in the laboratory. This effect may be important here as all the significant chi-squares in the right-hand column of Table 4 involve the difference between mating periods, and the mean time of setting up of the seven replications finally scored in each of the four treatment combinations were as follows:

Mating period 18 hrs, no. of flies/mating bottle 36– 4:24 P.M.
Mating period 18 hrs, no. of flies/mating bottle 108– 4:34 P.M.
Mating period 24 hrs, no. of flies/mating bottle 36–12:44 P.M.
Mating period 24 hrs, no. of flies/mating bottle 108–12:37 P.M.

Because of this heterogeneity, the chi-squares given in the left-hand column will be correspondingly enlarged, and the test of significance therefore is based on the F distribution using the error mean square (or heterogeneity factor, FINNEY 1952). This analysis shows:

(a) As expected from Experiment 1, mating was markedly nonrandom. Wild-

TABLE 4

The partition of chi-square of the data of Table 3. Chi-squares identically zero on the model used are omitted

Source of variation				Chi-square	
C_1	C_2	C_3	C_4†	df=1‡	df=6
1	1	0	0	69.65***	5.62
1	2	0	0	19.24***	6.77
2	1	0	0	0.83	4.29
2	2	0	0	0.00	4.64
1	0	1	0	−5.83*	6.32
2	0	1	0	−0.95	6.48
1	0	0	1	1.79	14.17*
2	0	0	1	−238.91***	11.85
1	1	1	0	−0.65	9.00
1	2	1	0	−0.42	5.86
2	1	1	0	0.82	5.48
2	2	1	0	−0.48	2.09
1	1	0	1	1.33	12.89*
1	2	0	1	0.67	5.38
2	1	0	1	−0.55	13.55*
2	2	0	1	1.21	4.39
1	0	1	1	−5.45*	12.24
2	0	1	1	2.31	12.99*
1	1	1	1	−0.49	7.12
1	2	1	1	0.02	13.87*
2	1	1	1	−0.19	7.07
2	2	1	1	1.16	4.45
1	0	0	0	....	7.08
2	0	0	0	....	11.57
Total heterogeneity chi-square df = 144					195.17**
Mean square					1.356

* $P<0.05$
** $P<0.01$.
*** $P<0.001$.
† The contrasts listed are as follows:
C_1: 1=relative success of + and y males in fertilization.
2=mean fertility of males, i.e. mated *vs.* not mated.
C_2: 0=mean effect of female genotype.
1=difference between +/+ and y/y females.
2=dominance, i.e. $+/y$ *vs.* +/+ and y/y.
C_3: 0=mean effect of no. of flies/mating bottle.
1=difference between 36 and 104 flies/mating bottle.
C_4: 0=mean effect of mating period.
1=difference between mating periods of 18 and 24 hours.
‡ This column of significance tests is based on the $F_{(1,144)}$ distribution.

type males were far more successful than y with +/+ females, but only slightly more successful with y/y females (1100).

(b) The relative success of + and y males with $+/y$ females was not intermediate between the results with +/+ and y/y females, but was indistinguishable from their relative success with +/+ (the contrast 1200 measures the discrepancy of the heterozygote from the mean of +/+ and y/y, and is highly

significant). A supplementary test of the difference between +/+ and +/y gives $\chi^2_{(1)} = 0.14$.

(c) The total number of females fertilized was significantly greater in the 24 hour mating period than in the 18 hour period (2001).

(d) The relative success of the + and y males varied with the number of flies per mating bottle and with the mating period (1010 and 1011).

As this analysis shows no difference in the relative success of + and y males with +/+ and +/y females, the results for these two female genotypes are averaged (Table 5a). The isolation estimates and male and female mating ratios for each of the four treatment combinations are given in Table 5b. The degree of isolation was higher for the shorter mating period, and with the smaller number of flies per mating bottle. The effect of the latter on the relative success of + and y males with y/y females is marked, no difference with the smaller number, but + males more successful with the larger number. This is not consistent with multiple choice, young flies in Experiment 1, where there were only 12 flies per mating bottle, yet + males were significantly more successful with y/y females. However, y males were more successful with wild-type females in Experiment 2 than they were in Experiment 1. It is difficult to relate differences between the two experiments to particular effects, because they were done at different times and because of differences in the yellow stock used (nonisogenic *vs.* isogenic).

DISCUSSION

As an analysis of the selective mating conditioned by the mutant gene for yellow

TABLE 5

(a). Numbers of successful fertilizations, averaging results for the +/+ and +/y female genotypes, in each of the four treatment combinations in Experiment 2

Mating period (hrs)	Male genotype	No. of flies/mating bottle 36 — Female genotype +/—	y/y	No. of flies/mating bottle 104 — Female genotype +/—	y/y
18	+	16½	8	58	33½
	y	0	8	3	23½
24	+	31	19	103½	73
	y	7½	20	11½	38

(b). The isolation estimate (I), *female mating ratio* (M_f), *and male mating ratio* (M_m) *for each of the four treatment combinations in Experiment 2*

Treatment: Mating period (hrs)	Treatment: No. of flies/ mating bottle	I	χ^2	M_f	χ^2	M_m	χ^2
18	36	0.33	10.39**	1.03	0.01	3.06	10.39**
	104	0.45	22.45***	1.07	0.18	3.45	46.83***
24	36	0.52	14.41***	0.99	0.01	1.82	12.15***
	104	0.60	26.10***	1.04	0.13	3.57	129.56***

* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

body color, this study generally confirms the results of RENDEL (1945); MERRELL (1949); and BASTOCK (1956). The apparent complete dominance of the normal allele in terms of the reaction of nonyellow females to yellow males has been referred to above.

Although previous workers have found that more yellow females are fertilized by + males than by *y* males, this result has not been observed here with all the methods where this comparison is valid, i.e. female choice (E), female choice, and multiple choice. In the female choice experiments, there were twice as many males as females in each vial, i.e. equal numbers of each genotype. If, as suggested by BASTOCK (1956), the sexual motivation of yellow males is less than that of wild type, then in these vials, one would expect the wild-type males to fertilize most of the females, provided of course that the females did not show any preferences for males of their own type. In these experiments, as well as a choice of males, the female has a choice of mating or not mating following any particular courtship. The females therefore could be partly responsible for the differential success of the males in that the courtship by yellow males may not always stimulate them sufficiently to accept the male. In other words, it is an interaction between the sexual activity of the male and the receptivity of the females, rather than any particular male or female preference, that determines whether or not mating will occur. With young flies, significantly more yellow females were fertilized by + than by *y* males, although with aged flies, the difference was not significant. One factor that may contribute to the greater success of + males with young flies is the age at sexual maturity. It is conceivable that + males reach sexual maturity earlier than *y* males, which would allow them to fertilize more yellow females. With aged flies, all males would be fully matured, and as the males had been aged in the absence of females for seven days, the sexual activity of the *y* males may be increased and be not very different from that of + males. Also, the aged females may show a lower threshold of response to male courtship, so that the first male to court any female will probably be successful. The latter is the more likely in view of BASTOCK's finding (1956) that with four to five day old flies, the + males do have some advantage in that following introduction to a vial containing wild-type females, they commence courting significantly sooner than do *y* males. Presumably they would have a similar advantage with yellow females. In any case, one might expect, as was observed, little difference in the proportions of yellow females fertilized by + and *y* males. The total percentages of females fertilized were generally higher for aged flies, but it does not appear that this could account in any way for the different relative success of + and *y* males as young flies compared with aged flies.

Similar arguments may apply to the multiple choice experiments, where with young flies, significantly more *y*/*y* females were fertilized by + than by *y*, while with aged flies, the difference was not significant. With young flies, another factor in addition to those already mentioned might be partly responsible for the greater success of + males. In these experiments, the numbers of each sex in each mating vial were the same. The extreme lack of success of *y* males with wild-type females has been noted, but BASTOCK (1956) has shown that this is not due to their failure

to court these females. Thus, while the + males will be accepted by most of the females they court (either wild type or yellow), the y males will spend time unsuccessfully courting wild-type females. Under these conditions, one would expect more y/y females to be fertilized by + than by y. With aged flies, the presumed lower threshold of response of females to male courtship could again account for the lack of significant difference between the percentages of yellow females fertilized by + and y males, particularly as in this case, y males were more successful with wild-type females than they were as young flies. However, as shown by Experiment 2, other factors affect this relative success of + and y males. In the female choice (E) experiments, where the numbers of each sex in the mating vials were the same (two y/y females, one + and one y male), no significant differences were found for either young or aged flies. On the basis of the arguments presented, one might have expected for young flies, a significantly greater success of + males. Apparently, other unaccounted factors are operating that affect their relative success. It is possible that the difference in sexual activity between the two types of males is not as great as is suggested by the female and multiple choice experiments. In the former, where there were twice as many males as females in each vial, but equal numbers of the three genotypes, any slight advantage of + males would be accentuated as practically all the yellow females could have accepted + males before the y males even commenced courting. Similarly, in multiple choice experiments, any advantage of + males would be accentuated by the y males wasting effort in courting wild type females.

Sexual isolation: The significant isolation estimates for wild type and yellow are of interest, particularly as MERRELL (1949) states that his results agree with those of STURTEVANT (1915), and DIEDERICH (1941) in indicating no such isolation. In interspecific crosses, sexual isolation is generally understood as a preference for homogamic mating. That is, species A and B are sexually isolated if males of A mate mainly with A females, and B males with B females. However, in intraspecific crosses of strains, races, or subspecies (DOBZHANSKY and MAYR 1944; DOBZHANSKY and STREISINGER 1944; PATTERSON, MCDANALD and STONE 1947) "one-sided mating preferences" (DOBZHANSKY 1944) have often been found. In this case, males of A mate more often with A than with B females, but B males either mate at random or mate more often with A females. This is the situation found with yellow and wild type, and consideration of the isolation estimates, together with the male and female mating ratios, shows that the significance of the former is spurious, and does not indicate true sexual isolation. If two populations, one homozygous for y and the other homozygous for the wild-type allele, came in contact with each other, there would be no sexual isolation as the wild-type males would inseminate more of all the females than the y males, and the wild-type allele would increase in frequency, y being eliminated.

The differences between the isolation estimates obtained with the various methods have been noted. In these experiments, a higher degree of isolation is indicated by the female and multiple choice experiments, yet in MERRELL's (1949) data, male choice indicated higher isolation than female choice. In most studies of sexual isolation, the male choice method has been used exclu-

sively (STALKER 1942; DOBZHANSKY 1944; MAYR 1946b; TAN 1946; PATTERSON *et al.* 1947). As MERRELL (1949) points out, this is unfortunate because with this method, the female only has a choice of mating or not mating, and in addition, males are more likely to mate at random than are females. In view of the differences obtained with the different methods in these experiments, it is suggested that if possible, multiple choice should be used because this is the only method in which all genotypes are present in the mating vials. That is, this method will give the closest approach to the conditions one would find in a population containing these genotypes. However, male and female choice would still be useful to provide information that may assist in interpretation. It is doubtful if pair mating is of much value as it allows no interactions between males or between females, although it may sometimes be of value in analysing the relative sexual activity of different genotypes. It is conceivable that much of the work on sexual isolation and selective mating where only male choice was used could profitably be repeated using multiple choice. In view of the significant effects in Experiment 2, it is suggested that such experiments should be designed to test the isolation or selective mating over a range of experimental conditions, allowing an adequate statistical analysis and a more complete evaluation of the situation.

Prediction of changes in gene frequency: If the isolation estimate indicates no sexual isolation between types A and B, which differ in a single allele, then the male and female mating ratios can be used to estimate the changes in gene frequency in a population due to selective mating (MERRELL 1950). This calculation depends on the assumptions that there is a fixed probability that an individual of a given type will mate and that the probability of a given mating is then the product of these separate probabilities. As MERRELL points out, these assumptions may not be completely valid. However, when multiple choice is used, the probability of each mating type is given directly, so that results obtained should be most satisfactory for predictive purposes.

In the multiple choice results of the present experiments (Tables 2 and 5), none of the estimates of M_f are significant so that the probability of being fertilized is the same for each female genotype. Changes in gene frequency will depend therefore on the relative success of the two types of male with each type of female. However, the percentages of $+/+$ and $+/y$ females fertilized by $+$ and by y males are not significantly different so that only the overall relative success of $+$ and y males with phenotypically $+$ females need be considered. Therefore, changes in gene frequency can be predicted by calculating male mating ratios separately for wild type and for yellow females. Thus, if M_{m1} is the male mating ratio with wild-type females, and M_{m2} with yellow females, and using the notation of MERRELL (1950) where:

RR = recessive females
RD = heterozygous females
DD = dominant females
RY = recessive males

$$DY = \text{dominant males}$$
$$RR + RD + DD = 1.0$$
$$RY + DY = 1.0,$$

then the frequencies of each mating type will be as given in Table 6, where the subscript n on each genotype refers to the proportions in the nth generation. Equations for the proportions of each genotype in the following generation then may be obtained as follows:

$$RY_{n+1} = \frac{\frac{1}{2}RD_n(M_{m1}\cdot DY_n + RY_n) + RR_n(M_{m2}\cdot DY_n + RY_n)}{(DD_n + RD_n)(M_{m1}\cdot DY_n + RY_n) + RR_n(M_{m2}\cdot DY_n + RY_n)}$$
$$DY_{n+1} = 1 - RY_{n+1}$$
$$\text{or} \quad = \frac{(DD_n + \frac{1}{2}RD_n)(M_{m1}\cdot DY_n + RY_n)}{(DD_n + RD_n)(M_{m1}\cdot DY_n + RY_n) + RR_n(M_{m2}\cdot DY_n + RY_n)}$$

$$RR_{n+1} = \frac{RY_n(\frac{1}{2}RD_n + RR_n)}{(DD_n + RD_n)(M_{m1}\cdot DY_n + RY_n) + RR_n(M_{m2}\cdot DY_n + RY_n)}$$

$$DD_{n+1} = \frac{M_{m1}\cdot DY_n\,(DD_n + \frac{1}{2}RD_n)}{(DD_n + RD_n)(M_{m1}\cdot DY_n + RY_n) + RR_n(M_{m2}\cdot DY_n + RY_n)}$$
$$RD_{n+1} = 1 - RR_{n+1} - DD_{n+1}$$
$$\text{or} \quad = \frac{RY_n(DD_n + \frac{1}{2}RD_n) + (\frac{1}{2}M_{m1}\cdot DY_n\cdot RD_n) + (M_{m2}\cdot DY_n\cdot RR_n)}{(DD_n + RD_n)(M_{m1}\cdot DY_n + RY_n) + RR_n(M_{m2}\cdot DY_n + RY_n)}$$

Taking the results of multiple choice, young flies (Experiment 1):

$$M_{m1} = \frac{90.0 + 91.4}{1.4 + 2.9} = 42.19,$$
$$M_{m2} = \frac{72.1}{25.0} = 2.88.$$

Using these values and the equations above, the expected changes in gene frequency in a population initiated with equal numbers of the two male types and with all females heterozygous have been calculated (Table 7). It is seen that this degree of selective mating results in rapid decrease in the frequency of the yellow mutant. The accuracy of this prediction, however, must be questioned.

TABLE 6

Frequencies of each mating type expected when the female mating ratio is 1:1:1, M_{m1} *the male mating ratio with homozygous dominant and heterozygous females, and* M_{m2} *the male mating ratio with homozygous recessive females*

Male genotype frequency	Male mating ratio: With *DD* or *RD* females	Male mating ratio: With *RR* females	Female genotype frequencies: DD_n	RD_n	RR_n
DY_n	M_{m1}	M_{m2}	$M_{m1}\cdot DY_n\cdot DD_n$	$M_{m1}\cdot DY_n\cdot RD_n$	$M_{m2}\cdot DY_n\cdot RR_n$
RY_n	1	1	$RY_n\cdot DD_n$	$RY_n\cdot RD_n$	$RY_n\cdot RR_n$

TABLE 7

Expected changes in gene frequency and the frequency of each genotype in a population, given selective mating as estimated from the multiple choice, young flies experiment

	Genotype					
	Males		Females			
Generation	+	y	+/+	+/y	y/y	q_y(females)
0	0.500	0.500	0.000	1.000	0.000	0.500
1	0.500	0.500	0.488	0.500	0.012	0.262
2	0.746	0.254	0.729	0.265	0.006	0.139
3	0.866	0.134	0.859	0.140	0.001	0.071
4	0.930	0.070	0.927	0.073	0.000	0.037
5	0.964	0.036	0.962	0.038	0.000	0.019

The degree of selective mating indicated by the multiple choice, aged flies results ($M_{m1} = 4.71$, $M_{m2} = 1.42$) would result in considerably slower decrease in the frequency of yellow. In a population, most females will be inseminated in the first few days after eclosion, so that the results using young flies probably give the better prediction. However, Experiment 2 showed changes in M_{m1} and M_{m2} with experimental conditions, so that even though prediction is theoretically possible, it is doubtful how accurate it could be. Better prediction should be obtained from experiments more closely simulating population conditions by using large numbers of flies per mating bottle (say at least 100), and using a mating period of say 24 hours, to allow most females to be inseminated. From the results, expected changes in gene frequency could be calculated using the equations above. This prediction then could be compared with observed changes in frequency in experimental populations, allowing a more complete understanding of the importance of selective mating than is possible at present. Even in this case, though, some inaccuracy in prediction might be expected because the frequencies of each mating type will probably change as genotype frequencies change (PETIT 1951, 1958). There is one further problem because in comparing results of experimental populations with predicted, one has to translate the time scale of the former (days) to that of the latter (generations) (see BARKER 1962). If the true generation interval in the experimental populations were something other than the estimate used, then of course the comparison of observed and predicted would be invalid.

SUMMARY

Generally, four methods have been used in studies of sexual isolation and selective mating, *viz.* pair mating, male choice, female choice, and multiple choice. Various workers have shown variation in degree of isolation indicated by different methods, and have obtained conflicting results on the importance of selective mating in particular cases of selection between genotypes. Two experiments have been done using the yellow mutant of *D. melanogaster* to compare

these methods, and to determine the effect of experimental techniques on the degree of selective mating.

Male choice and pair mating indicate less isolation between mutant and wild-type phenotypes than do female and multiple choice. The degree of selective mating is significantly affected by mating period and the number of flies per mating bottle. These results are discussed in terms of factors affecting mating activity. It is suggested that multiple choice should be used in analyses of selective mating, because this method gives the closest approach to population conditions. Prediction of changes in gene frequency due to selective mating is discussed, and the possibility of obtaining more accurate predictions considered.

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