SEXUAL ISOLATION BETWEEN *DROSOPHILA MELANOGASTER* AND *DROSOPHILA SIMULANS*

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The sexual isolation between the sibling species *Drosophila melanogaster* and *Drosophila simulans* has been studied by a number of investigators since the species were distinguished by Sturtevant (1919). Sturtevant (1929) reported that offspring were obtained in ten to 40 per cent of *D. melanogaster* ♀ × D. *simulans* ♂ crosses and in less than two per cent of *D. simulans* ♀ × *D. melanogaster* ♂ crosses. The first cross produced females and a few exceptional males, the second males and a few females. All hybrids were sterile. Further, Sturtevant found that males will court females of the opposite species, but females prefer to mate with males of their own kind. Essentially similar results were obtained by Biddle (1932) and Uphoff (1949). However, almost 100 per cent fertile crosses were obtained by Pontecorvo (1943), using wild type or *forked D. simulans* males aged several days in the absence of females, and mated with newly emerged triploid *D. melanogaster* females that were homozygous or heterozygous for a number of mutants. Manning (1959a) found that the percentages of three-day-old males showing full courtship to three-day-old females of their own species were 100 for both *D. melanogaster* and *D. simulans*, while those for three-day-old *D. melanogaster* males with one-, two-, three-, and four-day-old *D. simulans* females were 83, 87, 45, and eight respectively. The percentages for the reciprocal cross with one-, two-, and three-day-old *D. melanogaster* females were 25, zero, and zero respectively. Thus, although *D. simulans* males court foreign females less well than do *D. melanogaster* males, they are apparently eventually more successful. However, even though it is generally accepted that the cross succeeds more readily when *D. simulans* is the male parent, at least one exception has been noted by Ronen (1957).

This paper presents the results of an analysis of the sexual isolation between *D. melanogaster* and various *D. simulans* mutants. These are of interest because of the high degree of sexual isolation observed and because most of the successful cross-fertilization was obtained when *D. melanogaster* was the male parent.

MATERIALS AND METHODS

The stocks used were:

(1) *D. melanogaster:*
    Oregon-R-C—Or-R-C; wild type.
    ebony—e^{11}; III, 70.7 (Bridges and Brehme, 1944).
(2) *D. simulans:*
    Orbost—$(+^0)$ a wild type strain originating from a single fe-

male captured at Orbost, Victoria, in 1951 and since maintained in the laboratory by mass culture.

vermilion—v; I, -.

scarlet —st; III, 42.

peach—p; III, 106.

yellow—y; I, 0.

white—w; I, 4.1.   (Sturtevant, 1929).

Two experiments were done to study the occurrence and extent of sexual isolation. Experiment 1 used the pair mating method with flies of two ages. Virgin flies were collected at intervals of three to four hours and placed as single pairs in vials as soon as they recovered from etherization. The pairs were removed from the vials exactly seven days later, and only those vials in which both members of the pair were still alive were retained for scoring. Vials were later scored as successful or unsuccessful fertilization according to whether progeny were present or not. For six of the eight stocks of *D. simulans* so mated as young flies, virgin flies were collected at 12 hour intervals and stored in their separate species and sex groups on yeasted culture medium for at least three days. Then, after recovery from etherization, single pairs were placed in vials. Their subsequent treatment was as for the young flies. These flies were three to seven days old when the pair matings were set up, but the members of most pairs were the same age. The maximum age difference was one day and in almost all such cases, the female was older than the male. In all matings with young flies, and with the six stocks of *D. simulans* mated as aged flies, Or-R-C was the *D. melanogaster* stock used. In addition, aged fly matings were done using *D. melanogaster* e^{11} with *D. simulans* $+^0$ and p.

Experiment 2 was designed to detect the extent of hybridization in large populations maintained in population cages. Results of Experiment 1 had shown that hybrids between *D. melanogaster* e^{11} and *D. simulans* p were wild type as to eye and body color. Cages 1 and 2 were initiated each with 100 zero- to 24-hour-old virgin flies of each sex of each of these stocks. Daily egg samples were taken from the cages, allowed to develop in uncrowded conditions on dead yeast fortified medium, and the emerging adults scored as ebony, peach, or wild type. The daily egg samples were taken for 67 days, then allowed to lapse for 31 days, whence further samples were taken daily for 24 days. The cages were then terminated and the populations scored. By this time, the *D. melanogaster* e^{11} flies were practically eliminated. Because of the chance of a higher probability of interspecific mating when one of the species was at a low frequency in the cage, Cages 3 and 4 were then initiated, each with 1,000 zero- to 24-hour-old virgin flies of each sex of *D. simulans* p and 100 zero- to 24-hour-old virgin flies of each sex of *D. melanogaster* e^{11}. Daily egg samples were taken from these cages as before for 118 days, excluding some weekends. Again at termination, the populations in the cages were scored.

All experiments were done at $25 \pm 0.5°C$ in a room lit for periods of nine to 12 hours each day.

TABLE 1

Numbers of females tested (N), percentage fertilized (%), and isolation estimate (I) for intra- and inter-specific pair matings with *D. melanogaster* (Oregon-R-C and e^{11}) and *D. simulans* (various stocks) using young flies (less than four hours old) and aged flies (at least three days old)

| Mating type | | Young flies | | | Aged flies | | |
Females	Males	N	Per cent	I*	N	Per cent	I*
D. melanogaster (Or-R-C)	*D. melanogaster* (Or-R-C)	165	98.8		135	100.0	
D. simulans (v)	*D. simulans (v)*	124	94.4		179	82.7	
D. simulans (v)	*D. melanogaster* (Or-R-C)	131	0.0	0.000	347	0.0	0.000
D. melanogaster (Or-R-C)	*D. simulans (v)*	160	0.0		336	0.0	
D. simulans (st)	*D. simulans (st)*	147	93.2		369	90.8	
D. simulans (st)	*D. melanogaster* (Or-R-C)	154	0.0	0.003	178	4.5	0.026
D. melanogaster (Or-R-C)	*D. simulans (st)*	190	0.5		244	0.4	
D. simulans (p)	*D. simulans (p)*	122	96.7		293	86.0	
D. simulans (p)	*D. melanogaster* (Or-R-C)	119	1.7	0.009	171	7.0	0.038
D. melanogaster (Or-R-C)	*D. simulans (p)*	120	0.0		244	0.0	
D. simulans (st p)	*D. simulans (st p)*	155	89.0		280	72.9	
D. simulans (st p)	*D. melanogaster* (Or-R-C)	111	0.0	0.000	321	0.3	0.002
D. melanogaster (Or-R-C)	*D. simulans (st p)*	134	0.0		320	0.0	
D. simulans (y)	*D. simulans (y)*	130	90.8		207	88.4	
D. simulans (y)	*D. melanogaster* (Or-R-C)	117	0.0	0.000	169	5.9	0.031
D. melanogaster (Or-R-C)	*D. simulans (y)*	102	0.0		315	0.0	
D. simulans (w)	*D. simulans (w)*	265	73.6		301	74.4	
D. simulans (w)	*D. melanogaster* (Or-R-C)	161	0.0	0.000	294	1.0	0.006
D. melanogaster (Or-R-C)	*D. simulans (w)*	177	0.0		266	0.0	
D. simulans (y w)	*D. simulans (y w)*	103	83.5				
D. simulans (y w)	*D. melanogaster* (Or-R-C)	87	6.9	0.038			
D. melanogaster (Or-R-C)	*D. simulans (y w)*	69	0.0				

*I = Isolation estimate (Merrell, 1950)

$$= \frac{\Sigma \text{ per cent successful heterogamic matings}}{\Sigma \text{ per cent successful homogamic matings}}$$

TABLE 1 *(continued)*

Mating type		Young flies			Aged flies		
Females	Males	N	Per cent	I*	N	Per cent	I*
D. simulans $(+^0)$	D. simulans $(+^0)$	131	57.3				
D. simulans $(+^0)$	D. melanogaster (Or-R-C)	118	0.9	0.005			
D. melanogaster (Or-R-C)	D. simulans $(+^0)$	181	0.0				
D. simulans $(+^0)$	D. melanogaster (e^{11})				299	0.7	
D. melanogaster (e^{11})	D. simulans $(+^0)$				234	0.4	
D. simulans (p)	D. melanogaster (e^{11})				277	0.4	
D. melanogaster (e^{11})	D. simulans (p)				249	0.4	

RESULTS

Experiment 1

For all stocks of D. simulans using young flies, the degree of sexual isolation was extremely high and was complete for v, $st\,p$, y, and w (table 1). The highest percentage successful cross-fertilization was 6.9 for the D. simulans $y\,w \times D.$ melanogaster Or-R-C. However, three of the four D. simulans stocks that did give successful crosses did so for the cross D. simulans ♀ × D. melanogaster ♂. For aged flies, sexual isolation was complete between D. simulans v and D. melanogaster Or-R-C. The cross D. simulans $p \times D.$ melanogaster Or-R-C gave the highest percentage successful cross-fertilization, while no successes were obtained for the reciprocal cross. In all crosses, the hybrid progeny obtained were as expected with regard to sex, phenotype, and sterility.

The results in table 1 suggest that the percentage successful cross-fertilization was higher for aged flies than for young flies, and higher for the

TABLE 2

Analysis of variance of the data in table 1 for the six D. simulans mutant stocks v, st, p, $st\,p$, y, and w

Source of variation	D.F.	Mean square	F
Mutants	5	0.660	
Ages	1	2.272	0.95
Crosses	1	3.151	1.32
Mutants × ages	5	0.206	
Mutants × crosses	5	0.539	
Ages × crosses	1	2.381	10.76*
Mutants × ages × crosses	5	0.221	

*P < 0.05.

cross *D. simulans* ♀ × *D. melanogaster* ♂ than for the reciprocal. Data for the six *D. simulans* stocks tested both as young and as aged flies were subjected to analysis of variance after square root transformation (table 2). This transformation approximates the inverse sine root proportion with small percentages. The only significant effect was the ages × crosses interaction. For the cross *D. simulans* ♀ × *D. melanogaster* ♂, the mean percentage cross-fertilization was much higher for aged flies (3.13) than for young flies (0.28), but for the reciprocal cross, there was practically no difference due to age (0.07 and 0.09 for aged and young respectively). However, for each of the combinations except *D. simulans* ♀ × *D. melanogaster* ♂ using aged flies, only one or two successful cross-fertilizations were obtained (see table 1).

The significances of the difference between crosses for the other two *D. simulans* stocks tested as young flies ($+^0$ and *y w*) and for the crosses with aged flies between *D. melanogaster* e^{11} and *D. simulans* $+^0$ and *p* were tested by the *t* test. The differences were significant for all but the *D. simulans p, D. melanogaster* e^{11} matings.

Experiment 2

The numbers of *D. melanogaster* e^{11}, *D. simulans p*, and hybrid flies scored from the population cages are shown in table 3. A total of 125,037 flies were scored from cages 1, 2, and 4, but no hybrids were observed. But in the daily

TABLE 3

Numbers of *D. melanogaster* e^{11}, *D. simulans p*, and hybrid flies scored in daily samples taken from population cages, and in the adult populations of the cages at termination.

Cage no.	Total flies scored from daily egg samples			Cage populations at termination		
	e^{11}	*p*	Hybrid	e^{11}	*p*	Hybrid
1	8,519	26,774	...	18	7,625	...
2	6,497	27,929	...	43	7,706	...
3	539	35,011	7	10	7,165	1
4	713	31,264	...	42	7,907	...

TABLE 4

Distribution of the hybrid flies observed in the daily egg samples from Cage 3

No. of days from initiation of cage	No. of hybrid flies emerging from egg sample	Per cent e^{11} flies emerging from egg sample
24	1	0.28
38	1	0.50
76	2	0.00
97	1	0.00
103	1	0.00
117	1	0.19

egg samples from cage 3, seven hybrids were observed in 35,557 flies, while at termination, one of the 7,176 flies was a hybrid. The hybrids were found at various intervals throughout the history of the cage, although the percentage of e^{11} flies emerging from the egg samples was very small by the time the first hybrid was observed (table 4).

DISCUSSION

Sturtevant (1920, 1929) noted variation between different mutants in the degree of successful hybridization, yellow females of both species being easier to cross-mate than were wild type. This was especially noticeable in the cross *D. simulans* ♀ × *D. melanogaster* ♂, where most of the successful matings were from yellow females. Mutants such as black, ruby, peach, and scarlet rarely were successful. However, Biddle (1932) found that yellow *D. simulans* stocks gave below average success and that the highest percentage of successful crosses was obtained with *D. simulans* black. In the present experiments, wild type and all the mutants showed extremely low percentages of successful cross-fertilization in both reciprocal crosses, but no significant differences between mutants were found.

Pontecorvo (1942, 1943), Uphoff (1949), and Manning (1959a) have pointed out the importance of female age in determining the success of hybridization, this being greater with young females, particularly if the males are older. In this case, mating apparently occurs before the female develops full mating discrimination. This would be accentuated by the older male being more persistent in courting young females than older ones (Manning, 1959a). In Experiment 1, the young flies were less than four hours old when placed together, and this could be the main factor accounting for the lower hybridization here as compared with the results of Sturtevant (1920, 1929), Biddle (1932), Pontecorvo (1943), Uphoff (1949), and Ronen (1957). Two factors need to be considered, the development of female mating discrimination, and the male's persistency in courtship. The receptivity of females to males of their own species (three-day-old) increases to a peak at an age of three days, decreases slightly at four days, and remains fairly stable thereafter, at least to ten days (Manning, 1959a). When very young females are placed with foreign males, their initial receptivity is even less than with their own males and will be expected to decrease as mating discrimination develops. It is likely that a young male will be less persistent in courtship than an older one. Rendel (1945) showed for *D. subobscura* that, although males will mate from the first day after emergence, they become more active with age to about the third day. It would seem that when very young flies are placed together, full mating discrimination by the female usually develops sufficiently to prevent mating before the male becomes persistent enough to achieve success.

Thus, the apparent differences in percentage successful cross-fertilization between the *D. simulans* stocks need to be accounted for in terms of intensity or rate of development of mating discrimination by the female or the persistency of the male. Sturtevant (1929) pointed out that a relatively weak

and inactive *D. simulans* female is more likely to accept a *D. melanogaster* male. This may mean that such a female shows less mating discrimination, or is less likely to be able to reject a male. The latter is suggested by Merrell (1949a), who considered that females with some reduction in vigor (hybrids or mutants) have an increased chance of being mated with, presumably because their lesser activity prevents their avoiding the males. Thus, in Experiment 1 (young flies), the highest percentage successful cross-fertilization was found with *D. simulans* yellow white females, which are probably the "weakest" females of those tested. However, consider another possibility: Sturtevant (1915) showed yellow females of *D. melanogaster* were more easily mated with (by *D. melanogaster* males) than were wild type, while Reed and Reed (1950) found that white *D. melanogaster* females mated preferentially with wild type *D. melanogaster* males rather than with white males. If the situation is similar with these mutants in *D. simulans*, one might expect a greater breakdown of the sexual isolation with a yellow white stock than with other mutants that do not show this selective mating.

For both young and aged flies, all but two of the successful crosses (with *D. melanogaster* Or-R-C) were obtained when *D. simulans* was the female parent. Ronen (1957) has observed a similar situation with one strain of *D. simulans*, although his percentages of success were much higher than here. As he used two strains of *D. melanogaster* with similar results for each, he concluded that the reversal of the usual situation was due to the genetic constitution of the *D. simulans* strain. This explanation is untenable here since the *D. simulans* stocks that were used originated from a number of laboratories and have probably been genetically isolated for long periods of time. There is no obvious reason for this reversal of the usual pattern of interspecific mating. In fact, one might anticipate the pattern found here. Manning (1959a) suggested that the isolation between mature males and young foreign females (for *D. melanogaster*\and *D. simulans*) is due largely to the females. It is suggested above that with young males and young females, the males also influence the success or otherwise of the interspecific mating. Although it is now recognized that the behavior of females is generally responsible for occurrence of non-random mating (Bateman, 1948; Streisinger, 1948; Merrell, 1949a, b, 1954), the behavior of the male during courtship has been found important in some cases of interspecific mating (Spieth, 1949; Miller, 1950). Within species, male vigor may be important. Sturtevant (1915) noted that the lesser activity of mutant males as compared with wild type reduced the numbers of females with which each mutant male copulated. Maynard Smith (1956) found the mating success of inbred males to be lower than that of outbred ones. He suggested that this was due not to a lower intensity of courtship but to lower athletic ability. Nicoletti and Solima (1956) also reported that mutant males (*ssa*) of *D. melanogaster* showed lower sexual activity than wild type. Thus, mutant *D. simulans* males might be expected to be less successful in interspecific mating than the more vigorous *D. melanogaster* wild type males. This could be accentuated by differences between *D. melanogaster* and *D. simulans* females. Mayr (1946) proposed

that the more active the female, the more likely she is to be courted. However, if the female is trying to avoid mating, the more active she is, the more successful she will be. Thus, in the interspecific matings, the more active *D. melanogaster* female is paired with the less vigorous *D. simulans* male, while the less active *D. simulans* female is paired with the more vigorous *D. melanogaster* male. The latter cross might then be expected to be the more successful. Manning (1959a) found the percentage of *D. melanogaster* males showing full courtship to *D. simulans* females was higher than in the reciprocal mating. If one could relate this courtship behavior to successful fertilization, those results are in agreement with these here.

The higher frequency of interspecific mating with aged flies is difficult to account for in terms of any effect of age on mating behavior. As flies at least three days old were used, the results of Pontecorvo (1943) and Manning (1959a) suggest that mating should be less successful than with young flies. Increased sexual excitement might account for the higher frequency of interspecific mating with aged flies as compared with young flies. Aged flies are certainly more sexually excited, as courting and, in the intraspecific matings, copulating pairs can be seen in the vials within a few minutes after the flies are placed together. This effect has been noted by a number of investigators (for example, Rendel, 1945). Results of Dobzhansky and Koller (1938), using *D. pseudoobscura* and *D. miranda*, suggest that males aged in the absence of females are less efficient in discriminating between their own and foreign females than are males pro-conditioned with their own females. This could be due either to sexual excitement of the males aged without females or to the pro-conditioning of the other group of males. However, Mayr and Dobzhansky (1945), using *D. pseudoobscura*, *D. persimilis*, and four strains of *D. prosaltans* aged in isolation from the opposite sex for about seven days, found no change in the degree of sexual isolation when *D. pseudoobscura* males were used, while with *D. persimilis* males, aging seemed to lead to a strengthening of the isolation. For one strain of *D. prosaltans* (Chilpancingo), aged males showed some decrease in sexual isolation as compared with that found with young males. Males of *D. prosaltans* (Iporanga strain) preferred Zopilote strain females to their own, and this preference seemed to be enhanced by aging in the absence of females. There is, therefore, some evidence that the use of aged flies may lead to a breakdown in the degree of sexual isolation, but these experiments do not show whether this is due to an increased sexual excitement of such aged flies.

It has been suggested above that using flies of the same age may be a major factor (particularly with young flies) accounting for the lower percentage successful cross-fertilization obtained here as compared with the results of previous workers. But one difference in experimental technique also may be important. In these experiments, single pair matings were used, while Biddle (1932), Pontecorvo (1943), Uphoff (1949), and Ronen (1957) used 15 or more flies in each mating bottle, generally with an excess of males. In this case, some sort of facilitation may be involved, one courtship stimulating other males in the same bottle to increased activity. This

effect was observed by Manning (1959b), who found that placing two *D. simulans* males with one female accelerated the tempo of male courting as compared with a single pair. Morgan (1929) found interspecific hybridization (*D. melanogaster* and *D. simulans*) was more successful in small mass cultures than in pair matings. For certain species of the *melanogaster* group, Spieth and Hsu (1950) found that an increased percentage of copulations occurred if a large number of flies were used in one mass mating than if the same number were used in smaller groups (five to ten flies of each sex per bottle). The high degree of sexual isolation observed here is unlikely to be due to the genetic constitutions of the *D. melanogaster* or *D. simulans* strains, as similar results have been obtained with two strains of *D. melanogaster* (Or-R-C and e^{11}) and a number of strains of *D. simulans*.

The results of Experiment 2 suggest that when conspecific individuals are available to flies of both sexes, sexual isolation is very nearly complete. Because of the low frequency of *D. melanogaster* in these cages for much of the time, it is surprising that more interspecific mating did not occur, as one might imagine that they would have difficulty in finding conspecific mates. Presumably, in this situation, they do not mate at all. However, the degree of sexual isolation is not necessarily so extreme in nature, as Mourad and Mallah (1960) found that, of some 1600 females of these two species captured in the wild, two had been fertilized by a male of the other species.

SUMMARY

Single pair matings, using two strains of *D. melanogaster* (Oregon-R-C and e^{11}) and a number of strains of *D. simulans*, have shown a considerably higher degree of sexual isolation than has been previously observed. Using young flies (less than four hours old) only ten successful cross-fertilizations were obtained from 2,131 interspecific pairs, while aged flies (at least three days old) gave 40 successes out of 4,264 such pairs. With both young and aged flies, most of the successful cross-fertilizations were obtained for the *D. simulans* ♀ × *D. melanogaster* ♂ cross. In population cages, using *D. melanogaster* e^{11} and *D. simulans* p, only eight interspecific hybrids were found in a total of 167,762 flies examined. The higher degree of sexual isolation observed as compared with previous results, the generally more successful *D. simulans* ♀ × *D. melanogaster* ♂ cross, and the higher degree of sexual isolation in young as compared with aged flies are discussed in terms of the effects of age on mating behavior, of male and female vigor, of sexual excitation, and of facilitation of mating in bottles containing more than one pair of flies.

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