The Influence on Hybridization Between *Drosophila melanogaster* Females and *D. simulans* Males of Early Exposure to the Other Species

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Abstract

(1) Under the conditions of these experiments, early exposure of young (0-24) *Drosophila melanogaster* females to *simulans* males had no effect on interspecific isolation when the same females were three to four days old. (2) An insemination of a *melanogaster* female by a *simulans* male significantly reduces the probability of an insemination by a second *simulans* male. (3) To score small mass mating vials without determining exactly what percentage of the females is inseminated results in an overestimate of hybridization, since the insemination of just one female can cause a small mass mating vial to be scored as a mating. (4) Isolation between these two species varies widely from one experiment to the next, even under supposedly uniform conditions.

Introduction

Various factors influencing the isolation between *Drosophila melanogaster* and *D. simulans* have been studied. These include differences between strains (1, 13), mixed cultures (5, 8), being reared in isolation rather than in conspecific cultures (9), artificial selection (6, 7), and age (2, 3, 14). The purpose of the research reported here is to determine whether *melanogaster* females between three and four days of age hybridize more readily with *simulans* males if they have been in the company of *simulans* males for the three preceding days than if they are exposed to *simulans* males for the first time at three to four day of age.

Methods and Materials

I made stock bottles of yellow *melanogaster*, wild type *simulans*, and yellow *simulans*. Yellow is a sex-linked recessive gene, and the stock was made from a multiple sex-linked marker stock (y ct^6 ras f). The *simulans* stocks were lab stocks which had been maintained for years by mass-culturing.

Stock bottles of a particular type were always begun with eggs laid by the same number of flies during any particular experiment, in order to maintain population density relatively constant. However, more *simulans* were always used to lay eggs in *simulans* bottles in order to compensate for either lower fecundity (4, 17) or the greater loss of *simulans* pupae which are more often formed on the surface of the medium and suffocated if submerged (15).

All crosses were made in eight-dram food vials plugged with cotton and cultured at about 27° C. in a constant temperature incubator in which trays of water maintained high humidity. Constant light was provided by a 40-watt bulb located a few inches above the same shelf on which the vials were placed and between one and three feet from them. The food was made from tap water, methyl parasept, brewer's yeast, molasses, and cornmeal, and was always autoclaved a day or two before being used.

In the crosses described below, I judged whether mating had occurred by the presence or absence of unisexual broods of appropriate phenotype (5, 6, 7).

In order to determine the influence of early exposure to the other species, the following procedure was followed: female *melanogaster* collected on day 1 (and therefore between zero and twenty-four hours old) were divided into equal groups: one group (the counter-conditioning group) was placed, five per vial, in vials each of which contained five yellow *simulans* males (four to five days old), any offspring by which would be yellow; the other group (later to be used for control crosses) was placed, ten per vial, in vials for storage at room temperature with alternating periods of light and dark, until three to four days of age. In this way all vials, both for storage and mating, contained ten flies. Since mating behavior may be influenced by the number of stimuli received previously from other flies (10), it is important to equalize the numbers of flies in the two sets of vials.

At the end of the fourth day, the *melanogaster* females which were stored without *simulans* males were etherized and placed, five per vial, with five wild type *simulans* males (four to five days old) for four days. These were the control crosses. Also at the end of the fourth day, the *melanogaster* females which had been together with yellow *simulans* males on days two through four were removed from those vials (designated the counter-conditioning crosses), etherized, and mixed together before being redistributed, five per vial with five wild type *simulans* males (four to five days old) for four days. Any offspring by these males would be wild type. These constituted the experimental crosses.

At the end of four more days, all flies were etherized and, in the first experiment, discarded. In the second experiment, all surviving females were placed singly in food vials in order to determine exactly what percentage of flies had been inseminated in the controls. In the experimentals, because progeny by the two types of males would be different, placing females singly in food vials allowed for an exact determination of what percentage had been inseminated by the "first" (yellow) males, the "second" (wild type) males, and by both.

At this time I also recorded the number of dead flies of each sex, if any, since the alteration of the sex ratio during the experiment might alter the outcome. Statistical tests were later made to determine whether alteration of the number of sex ratio of flies in a vial influenced the mating results. Because preliminary crosses showed such different results from one experiment to the next, experimental and control crosses were always set up simultaneously.

All of the flies used in the above crosses were examined for macroscopic abnormalities (shrivelled wing, etc.) because of the importance of various body parts, especially the wings, in courtship, and only normal-appearing flies were used.

Results

The results of these two experiments are shown in Table I. Except when stated otherwise, all statistical tests mentioned were 2 x 2 con-

TABLE 1. *Number of crosses set up and number of five pair matings and individual females yielding progeny.*

Type of cross	No. of 5 pr. matings	Number yielding progeny	%	No. of females tested	No. of females inseminated	%
Experiment 1						
control	120	32	26.7	0	---	---
		3 (by 1st males)	2.4			
experimental	124	20 (by 2nd males)	16.1	0	---	---
		0 (by both)	0.0			
counter-conditioning	134	4	3.0	---	---	---
Experiment 2						
control	106	34	32.1	516	110	21.3
		21 (by 1st males)	19.8		24 (by 1st)	4.7
experimental	106	32 (by 2nd males)	30.2	512	98 (by 2nd)	19.1
		6 (by both)	5.7		1 (both)	.2
counter-conditioning	125	28	22.4	---	---	---

tingency tables with one degree of freedom, and Yates' correction factor was employed whenever the total sample was less than 40 or the expected number for any class was less than ten. Differences described as significant yielded probability values of less than 0.05.

In the first of these experiments only the results of the small mass matings are available, and there is a significant difference ($.05 > p > .01$) between the numbers of vials which yield progeny (of wild *simulans* males) in the experimental and control crosses (20 and 32, respectively). This indicates decreased hybridization after counter-conditioning. One should keep in mind, however, that the results of a small mass mating vial (having five females) may not reflect accurately the mating activities of individual females.

In the second experiment individual females were scored for inseminations. For both the five-pair matings and the female tests there is no significant difference between the numbers yielding progeny by wild type males in the control and experimental crosses (34 vs. 32 and 110 vs. 98, respectively). In the female tests, there is only one female which yielded progeny of both "first" and "second" males, compared to the nearly five expected if the two types of insemination were independent events. A test for independence indicates that an insemination by a "first" male significantly reduces, though barely so, the chances of insemination by a "second" male ($X^2 = 3.91$, $.05 > p > .01$).

As mentioned in the Methods and Materials, I recorded the number of flies in each sex which died during a cross in order to study the possible effect upon hybridization of altering the number or sex ratio of the flies. When, for a much larger number of crosses of the same sort (not reported upon here), the number of females inseminated in vials with all flies remaining alive was compared with the number of females inseminated in vials in which one or more flies had died, no significant differences were found.

Two things should be noted about the experiments as a whole. One is the difference between the first and second experiments in frequency of hybridization, especially in the counter-conditioning vials (3% and 22.4%, respectively). The other point is the large difference between the percentage of females inseminated and the percentage of small mass mating vials which yield offspring (21.3 vs. 32.1, respectively, for the controls, and 19.1 vs. 30.2, respectively, for the experimentals). To not determine exactly what percentage of the females is inseminated results in an overestimate of hybridization, since the insemination of just one female can cause a five-pair mating vial to be scored as a mating.

Discussion

Counter-conditioning the young (one- to four-day) *melanogaster* females with *simulans* males had no effect on later isolation, judging from the experiment in which individual females were tested singly. The results of my first experiment, which showed significantly increased isolation (after counter-conditioning) for the five-pair mating vials, is difficult to interpret since the individual females were not tested for insemination, and not enough of these experiments were performed to determine how the time of year might influence these types of crosses (especially since the two experiments were performed in a very different time of the year, March and September, respectively, for the first and second experiments). I have earlier shown (6, 7), in a much more extensive series of experiments, that isolation between these two species can vary widely from one season to another.

When Mayr and Dobzhansky (11) counter-conditioned *D. pseudoobscura* males with *persimilis* females, the males later showed greater isolation from *persimilis* (under poorly controlled conditions); *persimilis* males counter-conditioned with *pseudoobscura* females later showed significantly decreased isolation with *pseudoobscura*. It should be noted that Mayr and Dobzhansky studied counter-conditioning of males, whereas I studied counter-conditioning of females.

Pontecorvo (14) found that once a young *melanogaster* female mates with a *simulans* male, successive matings occur till old age, as though the mating reaction of the young female is not yet fully determined but still liable to conditioning by foreign males. By contrast, I have found that an insemination of a *melanogaster* female by a *simulans* male significantly reduces the chances of an insemination by a second *simulans* male. For different races of *D. paulistorum*, it has been found (12) that previous heterogamic copulations did not change the degree of sexual isolation but that previous homogamic copulations were followed by significantly higher female preferences for homogamic males.

The results presented above may be discussed in light of the relation between the two species in nature. Although there are some differences between the two species as regards their preferences for oviposition sites (16), both *simulans* and *melanogaster* females lay their eggs in rotting fruit, and it is likely that newly emerged flies of the two species are on occasion temporarily close together; it takes an hour or so for the wings to become functional. One would expect, in the light

of these experiments, that such early contacts with the other species would have no significant effect on the later discrimination by *melanogaster* females against *simulans* males.

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