MATING SUCCESS IN THE DROSOPHILA VICTORIA SPECIES GROUP, SUBGENUS PHOLADORIS (DIPTERA: DROSOPHILIDAE)*

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Reproductive success in Drosophila depends both upon mating propensity and upon discrimination in each sex, not only in specific and subspecific crosses, but also in strain crosses of the same species (Dobzhansky and Koller, 1938; Stalker, 1942; Mayr, 1946; Streisinger, 1948; Spieth, 1949, 1950; Miller, 1950, 1958; Merrell, 1954; summary of other authors in Patterson and Stone, 1952). The polygenic nature of mating behavior was established by Tan (1946) for differences between *D. persimilis* and *D. pseudoobscura*; by Ehrman (1961) for differences between members of the *D. paulistorum* subspecific group; by Petit (1958) for differences between certain mutant and wild type laboratory strains of *D. melanogaster*, and in the selection experiments of Mather and Harrison (1949), using the same species. A superiority in mating success on the part of males homozygous for the Whitney rearrangement of chromosome III in *D. persimilis* compared with male karyotypes homozygous for the Klamath rearrangement has been reported by Spiess and Langer (1961).

A study of mating success exhibited by members of the victoria species group was undertaken as a background for an investigation of mating activity on the part of different genotypes in cage populations segregating for a single pair of alleles which determine a mesonotal color polymorphism in natural populations of *D. l. lebanonensis*. The work on mating activity in these cage populations will be reported in a future paper.

MATERIALS AND METHODS

The species available for investigation included *D. l. lebanonensis* and *D. pattersoni* (Beirut, Lebanon), *D. l. casteeli* (Prescott, Arizona), and *D. brooksae* (Tucson, Arizona). The hybridization relationships between these species have been described elsewhere (Pipkin, 1961).

Single choice reciprocal mating tests among the four species were made, using 20 virgin males and 20 virgin females per 95 × 25 mm vial containing corn meal culture medium. Tests were continued until 100 females had been examined for each type of cross. The flies were aged from three to four days and were not etherized for 24 hours before mating. Care was taken to use no flies with injured wings. The laboratory temperature was 21–23 °C. Overhead lighting was used, with no especial source of bright light near the

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flies. In one series of tests the males and females were kept together for one hour; in a second series, for two days. Twenty-four hours after the separation of the sexes, the females were dissected, and an examination was made for the presence of sperm in the spermatheceae and in the simple sac-like ventral receptacle characteristic of this group.

Male choice tests were also conducted for *l. lebanonensis, l. casteeli,* and for *l. lebanonensis/l. casteeli* hybrids. Here 20 males of one kind and 20 females of each of two kinds were put together for three hours before the sexes were separated. Otherwise the procedure was continued as in single choice tests. From 70 to 100 females of each kind were dissected for each cross. Certain other single choice tests will be described later. In these, the flies were likewise virgin and from three to four days old when used.

RESULTS AND DISCUSSION

Table 1 gives the per cent insemination in crosses of four members of the victoria species group, using the single choice method. Clearly sexual isolation is weak in this group, the members of which, with the exception of *l. lebanonensis* and *l. casteeli*, are isolated by hybrid mortality (Pipkin, 1961), since insemination occurs in reciprocal crosses of all possible combinations of the species. The rates of insemination for the one-hour exposure period are for each cross significantly different from the insemination rate of the corresponding two day exposure period. For example, the chi square from the fourfold table for the cross *brooksae* females with *l. casteeli* males is 7.04; for the cross *l. lebanonensis* females with *pattersoni* males, 8.00;

TABLE 1.

Percentage of insemination among members of the victoria group: single choice method

Females		Males		
	pattersoni	*l. lebanonensis*	*l. casteeli*	*brooksae*
pattersoni	26 1 hour	0	0	2
	95 2 days	57	43	36
l. lebanonensis (beirut)	4 1 hour	77	50	20
	16 2 days	100	68	41
l. casteeli	0 1 hour	27	16	13
	3 2 days	75	58	56
brooksae	0 1 hour	0	4	5
	51 2 days	57	15	74

for the cross *l. lebanonensis* females with *l. casteeli* males, 7.40. With one degree of freedom, in each case P is less than 0.05.

In the reciprocal crosses of *l. lebanonensis* and *l. casteeli*, there is a higher percentage of insemination than in the homogamic cross of *l. casteeli* males and females; that is, there is negative isolation. According to male choice tests to be described shortly, the coefficient of joint isolation of *l. lebanonensis* and *l. casteeli*, K_1 and $_2$, is -0.56, following the method of Levene (1949). The wide geographical separation between the two subspecies accounts for absence of selective pressures which might have led to positive sexual isolation. Negative isolation has been observed in geographically distant strain crosses of *sturtevanti* by Dobzhansky (1944); *willistoni* (Burla, da Cunha, et al., 1949); and *prosaltans* (Dobzhansky and Streisinger, 1944).

In other crosses between victoria group members (with the exception of the comparison of the heterogamic cross *l. casteeli* females with *brooksae* males), the percentage of insemination for two-day exposures is significantly lower for heterogamic than for homogamic matings, demonstrating discrimination in the former. For example, 57 per cent *brooksae* females inseminated by *l. lebanonensis* males is lower than the 74 per cent inseminated in the homogamic cross *brooksae* females with *brooksae* males (chi square, 6.39, $P < 0.02$.). Similarly the 56 per cent *l. casteeli* females inseminated by *brooksae* males is lower than the 74 per cent insemination in the homogamic mating of *brooksae* females with *brooksae* males (chi square, 9.20; $P < 0.01$). For the two-day period, the lowest percentage insemination occurs for matings of *l. casteeli* females with *pattersoni* males (3 per cent), *l. lebanonensis* females with *pattersoni* males (16 per cent), and *brooksae* females with *l. casteeli* males (15 per cent). Results of the second cross indicate strong discrimination since *l. lebanonensis* females accept males of other species readily. The reciprocal matings of each of these crosses yield more than 40 per cent insemination. *L. lebanonensis* and *pattersoni* are sympatric species in the Lebanon, but it is not known if *l. casteeli* and *brooksae* are sympatric in Arizona.

The lowered percentage of females inseminated in the cross of *l. lebanonensis* females with *brooksae* males, respectively, of the two-day exposure series must be the result of a "learned" repugnance such as described by Spieth (1949) from courtship observation of five members of the willistoni group between which there is regularly no insemination and also by Haskins and Haskins (1949) for three species of poeciliid fishes. Thus, according to table 1, the initially high insemination rate of 20 per cent in the first hour of the cross between *l. lebanonensis* females and *brooksae* males becomes only 41 per cent for the two-day exposure, compared with 5 per cent *brooksae* females inseminated by *brooksae* males in the first hour and 74 per cent inseminated during the two-day exposure period. The difference between the insemination rates of *l. lebanonensis* females by *brooksae* males compared with the insemination rates of *brooksae* females by *brooksae* males for the one hour and two-day exposure periods, respectively, is not

due to a chance fluctuation. A chi square of 14.94 ($f = 1$; $P < 0.05$) is obtained from the comparison of these proportions in a contingency table. The cross of *brooksae* females and *l. casteeli* males giving three per cent insemination for the one-hour series and only 15 per cent for the two-day exposure series probably represents a similar learned repugnance.

The mating success differences between *l. lebanonensis* and *l. casteeli* are owing chiefly to a much lower mating propensity of the latter according both to table 1 and to direct observation. It is possible for a previously unmated *l. lebanonensis* male to inseminate a *l. lebanonensis* virgin female in half a minute after introduction into the mating vial when two pairs were being observed. In this case there was practically no courtship. Copulation usually lasts from a half to two minutes. In *l. casteeli* courtship is begun twenty minutes or more after introduction of virgin flies into a vial, but the duration of copulation is likewise from half to two minutes. The time periods obtained by direct observation agree with results obtained by exposing males and females for different time periods followed by searching for sperm in the receptacles of the females. In one series, when 20 pairs of virgin males and females of *l. lebanonensis* were introduced into a mating vial, 11 of 20 females were inseminated five minutes later. None of 53 virgin *l. casteeli* females put with an equal number of virgin *l. casteeli* males for five minutes was inseminated. Two of 46 such females were inseminated when the time was twenty-five minutes. Fluctuations observed from vial to vial in *l. casteeli* homogamic crosses exposed for an hour were greater than for homogamic crosses of other species for this period.

Table 2 shows the percentage of females inseminated during a three-hour period using the so-called male choice method. Chi square tests comparing the observed rates of insemination of two classes of females used in each test with rates expected in the absence of selective mating show no significant difference in insemination rates of *l. lebanonensis* vs. *l. lebanonensis*/

TABLE 2.

Percentage insemination among females of *D. l. lebanonensis*, *D. l. casteeli*, and their hybrids: male choice method

Females	Males		
	l. lebanonensis	*l. casteeli*	*l. l./l. c.*
l. lebanonensis	66	53	55
l. casteeli	46	31	11
chi square	3.57	5.76	29.33
$f = 1$	$0.1 < P < 0.05$	$P < 0.05$	$P < 0.05$
l. lebanonensis	72	12	64
l. l./l. c.	59	18	56
chi square	1.29	1.20	0.53
$f = 1$	$0.3 < P < 0.2$	$0.3 < P < 0.2$	$0.5 < P < 0.3$
l. casteeli	15	2	32
l. l./l. c.	43	30	35
chi square	5.76	24.50	0.13
$f = 1$	$P < 0.05$	$P < 0.05$	$0.8 < P < 0.7$

l. casteeli hybrid females exposed to any of the three types of males. In addition, the test of *l. casteeli* and *l. lebanonensis/l. casteeli* hybrid females with *l. lebanonensis/l. casteeli* hybrid males shows no evidence of selective mating. Selective mating exists in the five other tests according to chi squares and their corresponding probabilities given in table 2.

Table 2 also suggests that the high mating propensity of *l. lebanonensis* females has a positive influence upon the percentage inseminated of *l. casteeli* females in crosses involving the same type of male. For example, in the presence of *l. lebanonensis* females, *l. lebanonensis* males inseminate 46 per cent of *l. casteeli* females, whereas only 15 per cent *l. casteeli* females are inseminated by the same type of male when hybrid females (*l. lebanonensis/l. casteeli*) replace the *l. lebanonensis* females. The probability that the ratio of the proportion of *l. casteeli* females inseminated to the proportion of "other type" females inseminated in these two male choice tests are homogeneous is as low as between 0.1 and 0.05 (chi square, 3.79).

Similarly, 31 per cent *l. casteeli* females are inseminated by *l. casteeli* males when equal numbers of *l. lebanonensis* females are present, compared with only 2 per cent *l. casteeli* females inseminated by the same type of male in the presence of *l. lebanonensis/l. casteeli* hybrid females. Here the ratios of the proportion of *l. casteeli* females inseminated to the proportion of "other type" females inseminated in the two male choice tests are clearly heterogeneous (chi square, 10.70; $P < 0.05$).

Miller (1958) found a higher percentage of insemination in male choice experiments with the Wyoming and Michigan strains of *D. athabasca* than in the single choice experiments between these strains. A somewhat different influence of one class of female on the percentage of insemination of the other female class in male choice experiments was described by Koref-Santibañez and del Solar (1961). A *D. gaucha* male put with equal numbers of *D. gaucha* females and *D. pavani* females from Mendoza showed no preference. However, a *D. gaucha* male put with equal numbers of *D. gaucha* females and *D. pavani* females from Chile directed attention preferentially and inseminated preferentially its own females. Also, Barker (1962), discussing various methods of studying selective mating, using the yellow mutant of *D. melanogaster*, reported that for well aged flies, male choice and the pair mating method give lower estimates of isolation than do female choice and multiple choice experiments. Finally, the positive influence of one class of female upon the insemination rate of the other in male choice experiments may be compared with the finding by Petit (1958) of a positive influence of one type of male upon the mating success of another type in female choice experiments. She states that direct observation shows that males of a strain of *D. melanogaster* marked with the mutant *white* can profit from excitation of a female courted by a wild type strain suitor.

Single choice tests prepared in series of 20 females and 10 males per mating vial for three hours show an insemination percentage of 88 for the *l. lebanonensis* homogamic cross, 39 for the *l. casteeli* homogamic cross, and 85 for *l. lebanonensis/l. casteeli* hybrid females crossed with males of the

same composition. The hybrids arose from a cross of *l. lebanonensis* females with *l. casteeli* males. Although the mating success in single choice tests of these hybrids is as high as that of homogamic crosses of the *l. lebanonensis* parents, male choice tests show differences between *l. lebanonensis/l. casteeli* females vs. *l. lebanonensis* females and also between *l. lebanonensis/l. casteeli* males vs. *l. lebanonensis* males. Hybrid males and females do not appear to have as strong a mating propensity in certain combinations as males and females of *l. lebanonensis*. For example, *l. lebanonensis* males inseminated 56 per cent of the female combination *l. lebanonensis* and *l. casteeli*, but hybrid males inseminated only 35 per cent of this female combination, a significantly different proportion with chi square, 8.89; $P < 0.01$. Further, hybrid males inseminated equal numbers of *l. casteeli* and hybrid females, whereas *l. lebanonensis* males inseminated 43 per cent of hybrid females and only 15 per cent of *l. casteeli*. The probability that this difference is due to chance is less than 0.01 (chi square, 6.35).

Hybrids coming from the reciprocal cross of *l. casteeli* females and *l. lebanonensis* males were also studied in single choice experiments. Here tests were made with 20 virgin males and 20 virgin females to the vial, previously unmated, aged three to four days, and exposure time was one hour. Tests were continued until 100 females had been examined. The *l. casteeli/l. lebanonensis* hybrids crossed together show an insemination rate of 27.8 per cent, close to that which was obtained in similarly conducted single choice tests of the homogamic cross of *l. casteeli* males and females according to table 1. The low mating propensity of *l. casteeli/l. lebanonensis* hybrids is thus a maternal effect.

Previously unmated male progeny of the backcross *l. lebanonensis/l. casteeli* females with *l. lebanonensis* males mated with virgin sibling females give a rate of insemination of 92 per cent for a one-hour exposure, using 20 of each sex per mating vial. Here the increase in percentage of insemination over the 78 per cent found for the Beirut strain of *l. lebanonensis* in table 1 is probably due to the effect of heterosis and is thus in agreement with the work of Bösiger (1958). On the other hand, single choice tests of male and female progeny of the reciprocal backcross (*l. lebanonensis/l. casteeli* females and *l. casteeli* males) show an insemination rate of 41 per cent for a one-hour exposure, a figure well above the 16 per cent characteristic of homoganic crosses of *l. casteeli* for one hour, according to table 1. The tendency of mating propensity of each of the reciprocal backcross progeny to approach that of the respective backcross parent subspecies is in keeping with the theory of a polygenic inheritance of this trait.

SUMMARY

Sexual isolation was found to be weak in four members of the victoria group, three of which are isolated by hybrid mortality. The difference in mating success between *D. l. lebanonensis* and *D. l. casteeli*, which are widely separated geographically, is mainly one of propensity; the reciprocal

heterogamic matings between these subspecies resulting in a higher mating success than in the homogamic cross of *D. l. casteeli* males and females. Heterogamic crosses between other members of the group (with one exception) give a lower percentage of insemination than corresponding homogamic crosses of the two-day exposure period. Certain heterogamic crosses demonstrate "learned" repugnance since the insemination rate for the one-hour exposure is higher than that of one of the corresponding homogamic crosses, but the insemination rate of the same heterogamic cross for the two-day exposure is far below that of the aforesaid homogamic cross. Single choice tests of male and female hybrids of the composition *D. l. lebanonensis/D. l. casteeli* show a per cent insemination practically the same as that of *D. l. lebanonensis* females crossed with the same males. Reciprocal hybrid females (*D. l. casteeli/D. l. lebanonensis*) crossed with males of the same composition in single choice tests, on the other hand, display almost as low a mating success as that of homogamic crosses of *D. l. casteeli*. Mating propensity in the latter cross therefore shows a strong influence of a "maternal effect."

Progeny of the backcross of *D. l. lebanonensis/D. l. casteeli* females with *D. l. lebanonensis* males show an even higher rate of insemination in single choice tests than homogamic crosses of *D. l. lebanonensis*, possibly owing to heterosis. On the other hand, progeny of the reciprocal backcross, *D. l. lebanonensis/D. l. castaeli* females with *D. l. casteeli* males, mated together in single choice tests, have a rate of insemination intermediate between that of the homogamic cross of *D. l. casteeli* and that of the homogamic cross of *D. l. lebanonensis*. The tendency of mating propensity of the progeny of these reciprocal backcrosses to approach that of the respective backcross parent subspecies is in keeping with a polygenic inheritance of mating propensity. A positive influence of the high mating propensity of *D. l. lebanonensis* females on the per cent insemination of *D. l. casteeli* females in male choice tests was found, emphasizing the importance of the female in mating success.

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