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THE GENETIC BASIS OF SEXUAL ISOLATION BETWEEN *DROSOPHILA MELANOGASTER* AND *D. SIMULANS*

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Abstract.—The genetic analysis of sexual isolation between the closely-related species *Drosophila melanogaster* and *Drosophila simulans* involved two experiments with no-choice tests. The efficiency of sexual isolation was measured by the frequency of courtship initiation and interspecific mating. We first surveyed the variation in sexual isolation between *D. melanogaster* strains and *D. simulans* strains of different geographic origin. Then, to investigate variation in sexual isolation within strains, we made F_1 diallel sets of reciprocal crosses within strains of *D. melanogaster* and *D. simulans*. The F_1 diallel progeny of one sex were paired with the opposite sex of the other species. The first experiment showed significant differences in the frequency of interspecific mating between geographic strains. There were more matings between *D. simulans* females and *D. melanogaster* males than between *D. melanogaster* females and *D. simulans* males. The second experiment uncovered that the male genotypes in the *D. melanogaster* diallel significantly differed in interspecific mating frequency, but not in courtship initiation frequency. The female genotypes in the *D. simulans* diallel were not significantly different in courtship initiation and interspecific mating frequency. Genetic analysis reveals that in *D. melanogaster* males sexual isolation was not affected by either maternal cytoplasmic effects, sex-linked effects, or epistatic interaction. The main genetic components were directional dominance and overdominance. The F_1 males achieved more matings with *D. simulans* females than the inbred males. The genetic architecture of sexual isolation in *D. melanogaster* males argues for a history of weak or no selection for lower interspecific mating propensity. The behavioral causes of variation in sexual isolation between the two species are discussed.

Key words.—Biometric genetics, *Drosophila,* intraspecific variability, sexual isolation.

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In spite of the advances in genetics, we are still almost entirely ignorant as to what happens genetically during speciation (Mayr, 1963, 1982, 1988; Lewontin, 1974; Bush, 1975; Endler, 1989). Notwithstanding, evolutionary models of species formation have been constructed and extensively debated ever since Darwin's major publication *The Origin of Species* in 1859. Before we can construct evolutionary models of species formation we first need to understand the genetics and evolution of those mechanisms that maintain the separation of gene pools.

Hybrid sterility and inviability are the most rigorous barriers to gene flow. However, postzygotic mechanisms of reproductive isolation between sympatrically distributed species cause relatively high costs in the form of gametes and energy. Hence, distinctive courtship messages in signalling interaction between species are more likely isolating devices because they could limit and prevent waste of materials and time (Futuyma, 1986; Spiess, 1987). Fully efficient sexual isolation might have arisen as a byproduct of genetic divergence (Muller, 1940; Mayr, 1963) or through natural selection against hybridization (Fisher, 1930; Dobzhansky, 1937).

What little is known about the genetics of sexual isolation comes from interspecific studies with *Drosophila* species pairs (Tan, 1946; Koopman, 1950; Ehrman, 1961; Kessler, 1966; Futch, 1973; Zouros, 1981; Ahearn and Templeton, 1989; Coyne, 1989) and from one study with two closely-related *Colias* species (Grula and Taylor, 1980). The common feature of these studies is that they have been done with species pairs that produce at least one fertile hybrid sex. They show that sexual isolation is maintained by genetic factors distributed over all chromosomes.

Genetic analysis of the differences between species that produce sterile or invi-

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able hybrids of both sexes is difficult. Nevertheless, some information has been obtained on the genetics of sexual isolation in two types of experiments. These are all studies with the closely-related species pairs *Drosophila melanogaster* and *D. simulans.* Intraspecific studies reveal that mutant strains (Sturtevant, 1920, 1929; Barker, 1962), isofemale lines of different geographic origin (Parsons, 1972; Watanabe et al., 1977), and isofemale strains of the same geographic origin (Carracedo and Casares, 1985; Casares and Carracedo, 1985; Carracedo et al., 1987) significantly differ in interspecific crossability yielding hybrid offspring. Artificial selection for and against sexual isolation between *D. melanogaster* yellow mutant females and *D. simulans* males has been successful (Eoff, 1975, 1977). Recently, Carracedo et al. (1989) suggested an intermediate inheritance mode (i.e., directional dominance and maternal effects were absent) for receptivity of *D. melanogaster* females to mate with *D. simulans* males. Interspecific hybrid studies reveal that male hybrids carrying a *D. simulans* X chromosome and a *D. melanogaster* Y chromosome clearly prefer to court *D. simulans* females (Wood and Ringo, 1980), while hybrid males carrying a *D. melanogaster* X chromosome prefer to court *D. melanogaster* females, implying that the preference is sex-linked (Kawanishi and Watanabe, 1981).

Knowledge of the genetic basis of metric variation in traits is fundamental for an understanding of the genetic mechanisms of evolutionary change (Mather, 1966; Mather and Jinks, 1982; Lewontin, 1985; Falconer, 1989). Regarding sexual isolation as a metric trait varying between genotypes within a population, primary information on its genetic basis is obtained by quantitative genetic analysis. This paper reports a quantitative genetic investigation of the genetic architecture of sexual isolation between the species *D. melanogaster* and *D. simulans.* First, experiments were done to take a survey of the variation in sexual isolation between and within strains of different geographic origin. We measured the efficiency of sexual isolation by the frequency of courtship initiation and interspecific mating in no-choice tests. Then, variation within strains was analyzed by the method of a full-diallel cross table (Hayman, 1954*a,* 1954*b*; Mather and Jinks, 1982; Crusio et al., 1984). This method enabled us to gather more specific data on the mode of inheritance of sexual isolation by indicating which genetic components are involved.

Materials and Methods

Flies, Culture, and Test Conditions

The two cosmopolitan species were represented each by four strains of different geographic origin (Table 1). These strains were derived from flies that were kindly made available by Jean-Marc Jallon at the *Centre National de la Recherche Scientifique* (Gif-sur-Yvette, France). In our laboratory, the strains were maintained in three bottles and each generation was started with 10 pairs per bottle that were randomly drawn from the mixed bottle populations. The four strains of *Drosophila melanogaster* were combined with the four strains of *D. simulans* in a 4 × 4 cross table (experiment 1).

The *D. melanogaster* strain Draveil and the *D. simulans* strain Yaoundé were taken as base populations for setting up inbred lines. Each series was started with 15 single pairs of full brothers and sisters and it was continued by full brother-sister matings. The inbred lines of Draveil and Yaoundé were cultured for 15 generations. The inbred lines were used in experiment 2.

The two experiments were performed in 1987. The flies of the geographic strains and inbred lines were maintained at 25 ± 1°C on a 12:12 hr light–dark cycle in half-pint bottles and in vials, respectively. Each bottle held approximately 60 ml and each vial contained approximately 12 ml of the standard cornmeal-yeast-agar-medium. A droplet of living yeast was added to encourage mating and egg laying.

Virgin adults were collected within 8 hr after eclosion from bottles and vials and separated by sex. Females and males, which were used in tests, were marked under CO_2-anesthesia with a droplet of black, white, or yellow nontoxic Pelican ink onto the top of the thorax. Groups of one black, one white, and one yellow marked individual of the same sex were stored for 2–4 days in vials containing 6 ml standard medium, before they were used in tests.

During the tests, room temperature was 23 ± 1°C and relative humidity was 50 ±

TABLE 1. Strains of different geographic origin of the two sibling species used in the crossing experiments. Indicated are the years of collection.

Drosophila melanogaster	*Drosophila simulans*
1. Draveil (France, 1985)	1. La Sirole (France, 1981)
2. Swaziland (1984)	2. Seychelles (1984)
3. Guadeloupe (Carribean, 1983)	3. Yaoundé (Cameroon, 1983)
4. Brazzaville (Congo, 1983)	4. Brazzaville (Congo, 1983)

5%. After the tests the inseminated females were immediately removed and separately kept for two days in vials with the standard medium and a droplet of living yeast. On the 12th day after testing, we scored how many female and male hybrid offspring were produced.

Experiment 1: Variability between Strains

Experiment 1 was a survey of the variability of interspecific mating between the four *D. melanogaster* strains and the four *D. simulans* strains in no-choice tests. This experiment was designed to observe directly the proportion of females that were involved in copulation within 1 hr after commencement. We paired the strains of the two species in all possible interspecific combinations (Fig. 1). The two reciprocal combinations were observed simultaneously. At the same time, we also observed control matings within the strains, which were involved in the interspecific tests. The objective of intraspecific control matings was to determine any day-to-day effects on the frequency of mating within the eight strains.

The sequence of testing of the 2 × 16 interspecific strain combinations was randomized by cards. Each combination was observed on two different days (Fig. 1). We recorded three sessions of three replicate tests per strain combination per day. Each replicate consisted of three pairs of flies. The total sample size of each interspecific combination of strains was 54 pairs of flies. The total sample size of intraspecific pairs of flies was 4 × 54 for each strain.

Experiment 2: Variability within Strains

Experiment 2 consisted of two (4 × 4) diallel cross tables, one with inbred lines of

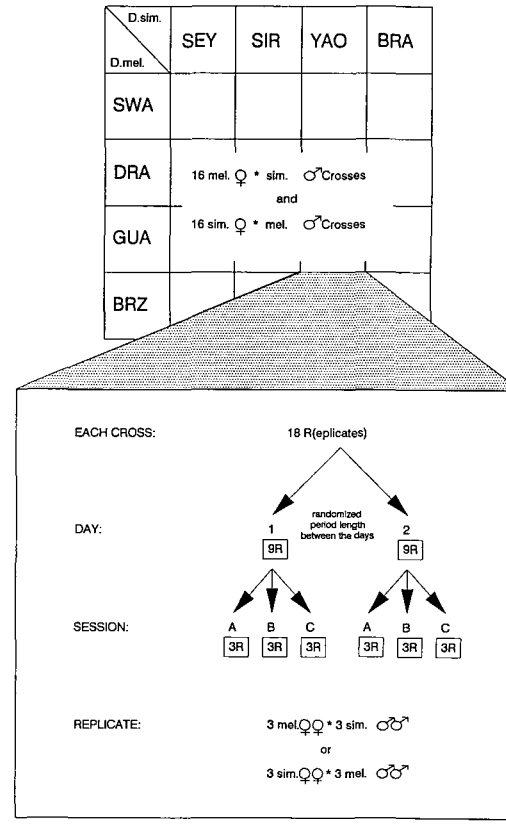

FIG. 1. Upper part: table of interspecific combinations between the geographic strains of *D. melanogaster* (rows) and *D. simulans* (columns) used in experiment 1. Lower part: the scheme of no-choice tests (replicates) is outlined for one interspecific combination of strains (see text for explanation). SWA = Swaziland; DRA = Draveil; GUA = Guadeloupe; BRZ = Brazzaville; SEY = Seychelles; SIR = La Sirole; YAO = Yaoundé; BRA = Brazzaville.

D. melanogaster Draveil (Draveil diallel) and one with inbred lines of *D. simulans* Yaoundé (Yaoundé diallel). To make one (4 × 4) diallel cross table, we took four inbred lines at random from the sample of 15 lines (see Flies, Culture, and Test Conditions). We crossed virgin females and males of the four lines in p^2 possible ways, four of which were the genotype families of the original inbred lines (P) and the remaining 12 genotype families the six F_1 crosses and their reciprocals.

In the Draveil diallel *D. melanogaster* test males were combined with randomly-drawn females of the *D. simulans* Yaoundé outbred strain. In the Yaoundé diallel *D. simulans* test females were combined with ran-

domly-drawn males of the *D. melanogaster* Draveil outbred strain. In both diallels we investigated in detail the variability of the frequency of interspecific matings across the 16 genotype families. We used only the combinations between *D. simulans* females and *D. melanogaster* males, because in experiment 1 we observed no matings between *D. melanogaster* females and *D. simulans* males, except one.

Experiment 2 was designed to observe directly two quantitative aspects of interspecific encounters. First, we scored from the Draveil diallel the proportion of males of every separate genotype family starting courtship behavior. The first Wing Vibrating directed toward a female by the male was used as an indication of his courtship engagement. In the Yaoundé diallel, the proportion of females courted by a male was registered. A female was considered to be involved in courtship when a male showed Wing Vibrating. Second, the proportion of males involved in interspecific matings was observed for every genotype family in the Draveil diallel. In the Yaoundé diallel we observed the proportion of females that was involved in interspecific mating. The two aspects of the interspecific encounters were recorded within 1 hr of testing. For analysis of variance we transformed all proportions between 0 and 1 into arcsine$\sqrt{p}$ values according to the formulae of Winer (1971; p. 399).

Every genotype family in the diallel was observed in two sessions of six replicate tests (three interspecific pairs per test) and the sequence of testing the 16 genotype families was randomized. The data were analyzed according to the replicated full-diallel cross-breeding method of Hayman (1954*a*, 1954*b*; see also Mather and Jinks, 1982). We used the terminology and symbols of Crusio et al. (1984) and adopted their improvements on the calculations. Briefly, in the Hayman ANOVA, first, the total amount of variance is partitioned into three components: (1) the between-cell variance (i.e., variance among the 16 genotype families), (2) a component stemming from the variance between blocks (the two sessions of observation served as the two blocks), and (3) the interaction component of the between-cell variance with the blocks. Second, the between-cell variance is further partitioned into variance components stemming from additive (item *a*), dominance (item *b*), and reciprocal effects (item *c* and *d*). Item *b* was further divided according to three sources of variance: b_1 tests for directional dominance, b_2 for distribution effects (variance caused when some parents contain considerably more dominant alleles than other parents), and b_3 for residual dominance effects. When item b_2 turns out to be significant, then *a* is not free from dominant effects. We therefore calculated the a_p item after what Walters and Gale (1977) have called the usual sum of squares of the diagonal entries of the diallel table. Third, if the interaction terms between the between-cell variance components and the block component are homogeneous, as verified with the Cochran C-test for mean squares (Winer, 1971; p. 208), the overall residual term (3) is used as common error mean square, thereby increasing the power of the tests (Crusio et al., 1984).

The interpretation of the Hayman ANOVA is straightforward when the linear model of additive-dominance effects is adequate. The validity of the model is based on several assumptions (Hayman, 1954*b*). One important assumption is also testable: If dominance is present, parent-offspring covariance of members of the same array (W_r) plotted against variance within arrays (V_r) should be a straight line of unit slope (W_r and V_r are calculated for each array of the summed diallel cross table). That is, the joint regression coefficient of W_r on V_r values might not significantly deviate from 1, but it must significantly deviate from 0. When the assumption is not held, then a more complex genetic system must be invoked to explain the observed variance. The genetic system should then be built on the presence of epistatic interactions, multiple allelism, and correlated allele distributions.

When the assumptions are not violated, additional information about the nature of dominance is provided by the intercept of the regression slope of W_r on V_r. If the regression line cuts the Y-axis below zero, then overdominant determinants are present; at zero there is complete dominance; above zero there is incomplete dominance. The nature of dominance is identified, when the

V_r and W_r values are corrected for environmental variance (Crusio et al., 1984).

To accept that dominance is unidirectional, the Spearman rank correlation of (W_r + V_r) with the phenotypic value of strain r should be significant in addition to a significant b_1 variance. If the sign of this correlation is negative, then there is unidirectional dominance for high phenotypic values. A positive correlation coefficient indicates that there is unidirectional dominance for low phenotypic values.

We developed a program in TurboBasic (copyright Borland) in which the whole replicated diallel cross analysis is performed, including the tests of the assumptions.

Recording Devices and Procedures

Sessions of multiple no-choice tests were observed with a perspex mating wheel of 20 cm diameter with 12 separate observation chambers (Hotta and Benzer, 1976; Collins et al., 1985), each of 20 mm diameter and 8 mm height. One chamber consisted of two compartments which were disjointed before testing. Three marked females and three marked males were separately transferred from their culture vial to the compartments with an aspirator. The wheel was placed upon a light box with a milky perspex pane. At the beginning of tests, the two corresponding compartments of the 12 chambers were rotated into register to bring female and male flies together. Each chamber was illuminated diffusively with the same intensity (4,200 Lux) by the two fluorescent lamps (25 Watt) of the light box from the bottom. Heating of the chambers was very much reduced. After each session the wheels were dismantled and washed thoroughly with soapy water.

The test procedure of experiment 2 was slightly different from experiment 1. Before testing, the compartments of the 12 chambers were already turned into register but disconnected by plastic covers. Each chamber was initiated separately by pulling away the plastic cover. At the moment that females and males of one chamber started to court, the next chamber was initiated.

The identity of a fly, who started to court or was involved in a copulation, was recorded with the keyboard of a microcomputer (MS-DOS). On pressing a defined key the computer clock was read and the time was saved on disk. The computer program was written in TurboBasic (copyright Borland).

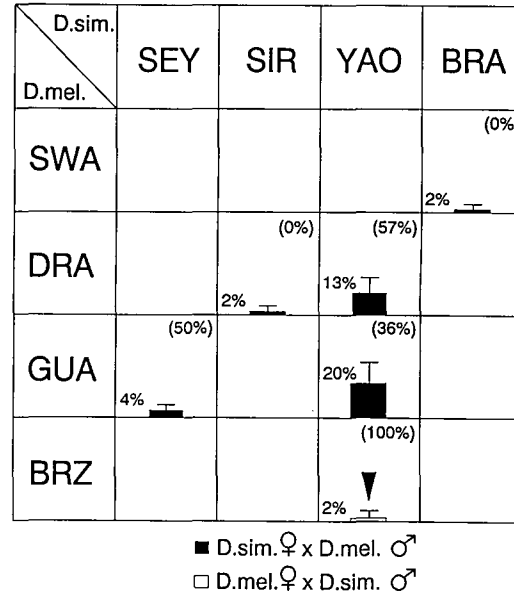

FIG. 2. The bars represent the mean frequency (+SE) of interspecific mating between the *D. melanogaster* and *D. simulans* strains in no-choice tests (for abbreviations, see Fig. 1). The arrow head downwards points to the exceptional mating observed between a *D. melanogaster* female and *D. simulans* male. The number of pairs per reciprocal cross was 54 per cell. The percentages of females, who were involved in interspecific mating that produced hybrid offspring, are given in parentheses.

RESULTS

Experiment 1: Variability between Strains

The no-choice combinations between *D. simulans* females and *D. melanogaster* males showed 23 times more matings than their reciprocals (Fig. 2). Out of 864 pairs between *D. melanogaster* females and *D. simulans* males, only one pair mated (copulation duration was 17.25 min; hybrids were all females). Moreover, there was a clear difference between pairs of strains that mated and those that did not mate interspecifically. Further, a Kruskal-Wallis one-way analysis by ranks (Sokal and Rohlf, 1981; p. 430) was applied to the subset of the five "successful" combinations between *D. simulans* females and *D. melanogaster* males.

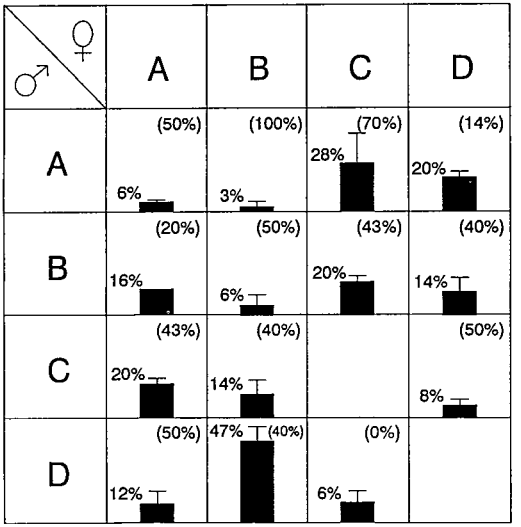

FIG. 3. Diallel cross table of *D. melanogaster* Draveil. Bars represent the mean proportions (+SE) of Draveil F_1 males, who were involved in interspecific matings with *D. simulans* Yaoundé females. The means are based on the two blocks of the diallel. The four inbred lines are symbolized by A, B, C, and D. Number of observed pairs was 36 per cell. Empty table cells indicate lack of interspecific mating. In parentheses are given the percentages of Yaoundé wild-type females, who were involved in interspecific mating and produced hybrid offspring.

There were significant differences between the pairs of strains in the distribution of the interspecific mating frequencies ($H = 9.983$, $df = 4$, $P < 0.05$).

The copulation durations of the interspecific matings ranged from 0.5 min to 16.50 min, whereby 55% were longer than 5 min. On the 12th day after fertilization of the *D. simulans* females, the number of hybrid offspring ranged from 6 to 36 individuals. Two of the nine fertilized females produced both sexes (11% and 13%, respectively, were female hybrids); the rest only produced males. All hybrids were sterile, as they had been checked by backcrosses to both parental strains.

There were no significant differences between the eight days of testing in the frequency of intraspecific control matings in each of the *D. melanogaster* strains ($F_{28,64} = 0.856$, $P > 0.50$). But the strains significantly differed in the average level ($F_{3,28} = 3.68$, $P < 0.05$). The strains Guadeloupe and Brazzaville achieved on average more matings within 1 hr (92.5% and 96.8%, respectively), than Swaziland and Draveil (81.5% and 82.9%, respectively). Inasmuch as the model underlying the nested ANOVA seemed to be inappropriate because of a very small F-value ($F_{3,28} = 0.11$), we tested the differences between the four strains of *D. simulans* with the Kruskal-Wallis test. Further, we compared the eight days of testing within each strain of *D. simulans* (control matings). The four strains achieved the same proportion of intraspecific matings within 1 hr on every day of testing ($P > 0.10$ in all tests). These results suggest similar average intraspecific mating propensity of the tested individuals of a strain.

Experiment 2: Variability within Strains

Draveil Diallel.—The mean proportion of *D. melanogaster* Draveil P and F_1 males, who directed Wing Vibrating towards *D. simulans* Yaoundé females, was 98.1% (SE = 0.55). There were no significant differences between the 16 Draveil genotype families in proportion of males, who started to court Yaoundé females ($F_{15,15} = 0.461$, $P > 0.75$).

The mean frequency of interspecific mating varied between 0% and 47% (Fig. 3). A two-way ANOVA for randomized blocks reveals significant differences between the 16 male genotype families (Table 2A). So, there is ample genetic variance for interspecific mating present in the Draveil population of *D. melanogaster* males.

To test if the diallel set of crosses is compatible with the outbred population of Draveil, we compared the mean proportion of interspecific mating between the F_1 males of the diallel and the males of the original Draveil population tested in experiment 1. Here, we excluded the diagonal entries of the diallel table, because, in general, there are no completely homozygous individuals in a randomly breeding population. The mean proportion of the F_1 males and the males tested in experiment 1 were 17.2% (SE = 3.37) and 13.0% (SE = 6.68), respectively, and they did not differ significantly ($t = 0.905$; $df = 16$, $P > 0.35$). So, the F_1-diallel set of reciprocal crosses appears to be a fairly good representation of the Draveil population.

The variance (V_r)-covariance (W_r) graph of the Draveil diallel provides information on three aspects (Fig. 4). First, the parent-

TABLE 2. Summary of the diallel cross analyses of the interspecific mating propensities of *D. melanogaster* Draveil F_1 males and *D. simulans* Yaoundé F_1 females. A. Analyses of variance of the summed diallel cross-breeding tables (arcsine$\sqrt{p}$-transformed results from Fig. 3 and Fig. 5); B. Test of the assumption of the additive-dominance model by analyzing the regression of covariance (W_r) and variance (V_r); C. Estimates of diallel statistics. *Fs* represents the sample statistic of the *F*-distribution; *t* is the Student's *t*-statistic; *b* is the regression coefficient. (*): $0.01 < P < 0.05$; (**): $0.001 < P < 0.01$; (***): $P < 0.001$.

		Draveil		Yaoundé	
Sources	*df*	MS	*Fs*	MS	*Fs*
A.					
Between blocks	1	0.160	1.960	0.030	0.220
Between genotypes	15	0.334	4.078**	0.285	2.337
Within genotypes (*e*)	15	0.082		0.122	
a	3	0.115	1.410	0.432	3.545*
a_p	3	0.189	2.315	0.395	3.238
b	6	0.602	7.365***	0.266	2.178
b_1	1	2.170	26.536***	0.038	0.314
b_2	3	0.088	1.070	0.160	1.312
b_3	2	0.591	7.223**	0.538	4.411*
c	3	0.011	0.135	0.098	0.805
d	3	0.336	4.113*	0.363	2.978
Sources	*df*	MS	Fs	MS	Fs
B.					
Joint regression	1,6	0.052	22.546**	0.003	1.448
Heterogeneity	1,5	0.0004	0.158	0.001	0.371
Remainder	5	0.003		0.003	
Pooled error	6	0.002		0.002	
	df	*t*		*t*	
$b \neq 0$	5	4.402**		1.139	
$b \neq 1$	5	1.007		2.048	
C.					
Slope ± SE		0.81 ± 0.185		0.36 ± 0.314	
¼(D − H1) ± SE		−0.10 ± 0.052**		0.03 ± 0.050	
√(H1/D)		3.116		1.060	
h^2_{narrow}		0.008		0.177	
h^2_{broad}		0.646		0.368	
$r_{W+V,P}$	6	−0.619		−0.494	

offspring covariance between members of the same array is related to the variance by a straight regression line. The line does not deviate significantly from unit slope, but it does from zero (Table 2B). This result signifies that the main assumptions in the Hayman model, i.e., independent action of non-allelic loci (no epistasis), no multiple allelism, and independent distribution of alleles among parents, are valid. So, the genetic model of additive and dominance factors is appropriate for further diallel analyses. The two-way ANOVA on ($W_r - V_r$) values confirms this deduction, because the ($W_r - V_r$) differences between the arrays of the cross table were small and random ($F_{3,4} = 0.643$; $P > 0.50$).

Second, the intercept of the regression line cuts the ordinate significantly below zero (¼($D - H_1$) ± SE; Table 2C). This could indicate that there is overdominance. But the effects of overdominance are only real, however, if the V_r and W_r values are corrected for environmental variance, by which we obtain an unbiased estimate of ¼($D - H_1$), and this estimate is significantly negative. In the present diallel, this indeed happened to be the case ($z = -3.28$; $P < 0.005$), indicating that there is some striking overdominance.

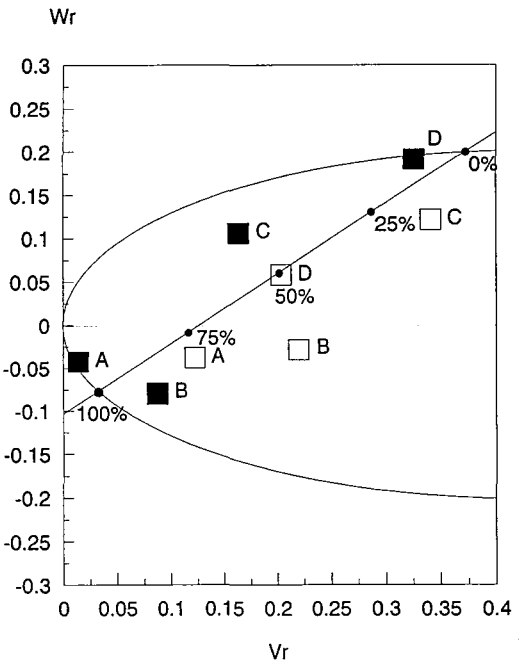

FIG. 4. The variance (V_r) and covariance (W_r) graph for the interspecific mating propensity of *D. melanogaster* Draveil male genotypes. Entered are values for block I (unfilled squares) and for block II (filled squares). A, B, C, and D are the four parental lines. The points are not deviating from a straight line (equation is $W_r = 0.81 \times V_r - 0.10$) inside the limiting parabola $W_r^2 = 0.095 \times V_r$. The percentages indicate the scale of the regression line corresponding to parents with 0% to 100% dominants.

Third, the relative amount of dominant alleles carried by each of the four strains are indicated by the projected positions of their V_r:W_r scores (calculated from the block of means) on the straight line. For scaling between 0% and 100% dominant alleles, first, the highest and lowest theoretical V_r and W_r scores are estimated by calculating the limiting parabola ($W_r^2 = 0.095 \times V_r$). Then, as shown in Figure 4, the higher intersection between the parabola and the straight V_r:W_r line indicates 0% dominant alleles; the lower intersection indicates 100% dominant alleles. Hence, the dominance order of the four strains can be obtained from the positions of the strains relative to each other. The order appeared to be *A-B-C-D*, that is, from a high to a low number of dominant alleles controlling the interspecific mating propensity. However, the dominance order and the relative amount of dominant alleles should be interpreted with caution, because the distribution of the V_r:W_r scores was not completely the same over blocks (Fig. 4).

The Hayman ANOVA reveals that the variance among the genotypes is composed of dominance variance b and variance due to nonsystematic reciprocal effects d (Table 2A). However, when tested against specific block interactions, the item d turned out to be nonsignificant ($F_{3,3} = 3.253$, $P > 0.10$), indicating that there are no consistent differences between reciprocal crosses. The subdivision of the b sum of squares shows that the dominance items b_1 (mean deviation of the F_1's from their midparental values) and b_3 (deviation that is unique to each F_1) are significant. Apparently, there is directional dominance for high interspecific mating propensity of Draveil males, because the Spearman rank correlation coefficient $r_{w+v,p}$ is negative ($P = 0.06$; Table 2C).

The F_1 crosses considerably outscored their parental inbred lines (mean = 3.0%, SE = 1.73). The effects of hybrid vigor on the interspecific mating propensity were also substantiated by the dominance ratio $[\sqrt{(H_1/D)}]$, which is much higher than unity (Table 2C). Heterosis is dependent on directional dominance exceeding the additive component (Falconer, 1989). The last in turn requires that one or both of two genetic conditions be satisfied (Mather and Jinks, 1982), namely: (1) over-dominance at some or all involved loci; (2) accumulation in the heterozygote of favorable dominant genes from each parent. Furthermore, complementary interaction between genes can increase the expression of heterosis whether it be due to condition 1 or 2. Since the analysis of a F_1 diallel set of reciprocal crosses ignores segregation it fails to distinguish between true single-gene overdominance and that due to combinations of favorable dominants and unfavorable recessives in the parents (Hayman, 1957). It is clear though, from the V_r, W_r-graph, that genic interactions played no part in producing the observed heterosis. In addition, genotype-environmental interaction was negligible (Cochran's $C = 0.210$, 1 *df*, $P > 0.10$) and, therefore, the magnitude of heterosis is not affected.

On the 12th day after interspecific insemination, the number of hybrids ranged from 1 to 30. Only 3 out of 31 hybrid progenies

contained both sexes. The ratios of hybrid females to males were 3:30, 2:10, and 2:5. The hybrids were paired with the opposite sex of both parental strains. We found no offspring and thus the hybrids were all sterile.

Yaoundé Diallel.—This diallel showed that 99.3% of the females were involved in courtship behavior. In only 2 out of 32 replicate tests, there were less than 100% of the females courted by Draveil males.

The mean frequency of the female genotype families that was involved in interspecific matings ranged from 0% to 25% (Fig. 5). The differences between the genotype families were not significant (Table 2A), indicating that a corresponding number of females from every family accepted *D. melanogaster* Draveil males as copulating partners.

The mean proportion of the F_1 females (excluding the diagonal) was 9.7% (SE = 2.53). The mean proportion of the females in the geographic strain experiment was 13.0% (SE = 6.68). The difference between the two mean proportions was not significant ($t = 0.568$: $df = 16$; $P > 0.50$). The mean of the F_1 families was also lower than the mean of the P families (10.4%, SE = 4.59), but the difference was fortuitous ($t = 0.353$; $df = 14$; $P > 0.50$).

Although the mean square of the differences between the 16 genotype families did not significantly exceed the error mean square, the Hayman ANOVA still detects a significant additive genetic component and some dominance deviation that is unique to each F_1 (Table 2A). But, the tests of the underlying assumptions for the validity of the additive-dominance model (Table 2B), urge us to neglect these effects. No significant differences mean, however, that two genetic effects were absent in the mating table. First, there were no differences between reciprocal cross vales, so there were no maternal effects. This is also found for *D. melanogaster* female genotypes (Carracedo et al., 1989). Second, there were no significant differences between parental and F_1 genotypes, indicating that inbreeding depression was not important. In addition, apart from the lack of fit of the Yaoundé table to the additive-dominance model, the test of significance on the diagonal entries can still be performed. The a_p item indicates that the four parental genotypes did not differ significantly in the frequency of interspecific mating.

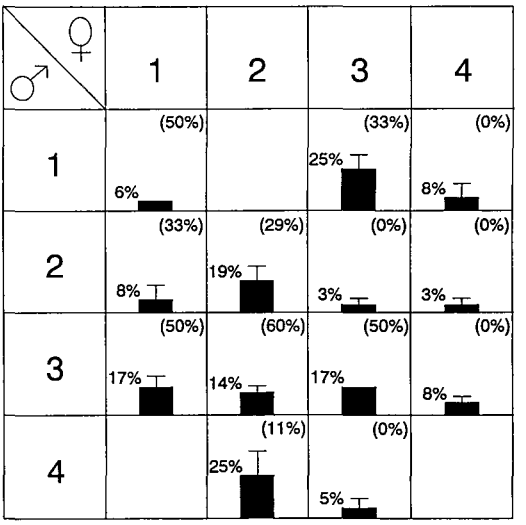

FIG. 5. Diallel cross table of *D. simulans* Yaoundé. Bars represent the mean proportions (+SE) of Yaoundé F_1 females, who were involved in interspecific matings with *D. melanogaster* Draveil males. The means are based on the two blocks of the diallel. The four inbred lines are symbolized by 1, 2, 3, and 4. Number of observed pairs was 36 per cell. Empty table cells indicate lack of interspecific mating. In parentheses are given the percentages of Yaoundé F_1 females, who were involved in interspecific mating and produced hybrid offspring.

The number of progeny, produced by the females in the Yaoundé diallel, ranged from 1 to 44 on the 12th day after insemination. All the females produced only hybrid males with one exception. One female produced 4 hybrid adult females and 37 hybrid males. The hybrids were all sterile.

DISCUSSION

We have shown that there is ample genetic variation in sexual isolation between *Drosophila melanogaster* and *D. simulans.* Our study, in particular, indicated that the frequency of interspecific mating is dependent on the combination of geographic strains. Furthermore, the F_1-diallel set of reciprocal crosses within a *D. melanogaster* strain did disclose significant differences between male genotypes in the frequency of interspecific mating with *D. simulans* fe-

males, but not in courtship initiation frequency.

The quantitative genetic approach has yielded means to elucidate the genetic architecture of sexual isolation between species that produce sterile hybrids. The Hayman analysis of the *D. melanogaster* Draveil diallel reveals two important genetic aspects. First, additive genetic factors do not significantly contribute to genetic variance among *D. melanogaster* males in sexual isolation. Rather, the differences in the diallel were largely influenced by determinants with directional dominance and overdominance effects. Second, sexual isolation was not affected by maternal cytoplasmic or sex-linked effects in the Draveil males, indicating that there is no real departure from autosomal inheritance. These findings are new. There are no other intraspecific studies of sexual isolation designed especially to examine these components of genetic variance in *D. melanogaster* males.

Intraspecific genetic studies with males of other *Drosophila* species are mainly aspired to alter sexual isolation between species. Eoff (1977) was successful in selecting *D. simulans* males for increased and decreased sexual isolation from *D. melanogaster* yellow mutant females. In addition, Koopman (1950) and Kessler (1966) succeeded in selecting males and females for increased and decreased sexual isolation between the species *D. pseudoobscura* and *D. persimilis.* From these studies, additive genetic variance and polygenes for sexual isolation are inferred, because of the continued response to selection. The results of the Draveil diallel are clearly not in agreement with the finding of additive genetic variance in these studies.

In interspecific hybrid studies with *D. melanogaster* and *D. simulans,* Wood and Ringo (1980) did rule out cytoplasmic and maternal inheritance, but they were unable to test for sex-linkage because they had no way of producing males carrying an X chromosome from *D. melanogaster.* Kawanishi and Watanabe (1981), on the other hand, were able to produce this genotype in flies carrying the Lethal hybrid rescue gene. They found that the preference of hybrid males for *D. melanogaster* or *D. simulans* females is sex-linked. The results of Kawanishi and Watanabe (1981), however, do not contradict the results of the present study: there can easily be a sex-linked interspecific difference without sex-linked intraspecific variation.

The conclusions arrived at by the Hayman analyses of variance and the variance-covariance analyses of the Draveil diallel may be interpreted in terms of a possible relationship between the genetic architecture and the adaptive significance of variations of mating propensities. First, the main mode of inheritance was heterotic for male mating propensity. Heterosis and its reverse inbreeding depression are characteristic for those traits which are positively correlated with the fitness of organisms (Bruell, 1964; Wright, 1977; Falconer, 1989). Directional dominance, epistasis of the kind of duplicate gene interaction, and overdominance are diagnostic features of traits that were subjected to directional selection (Mather, 1966; Broadhurst and Jinks, 1974; Mather and Jinks, 1982). Second, the Spearman correlation analyses demonstrated that the direction of dominance was for a high probability of interspecific mating. This suggests that there has been directional selection for male sexual behavior, which just happens to enhance mating rates interspecifically.

Our observations of hybrid vigor for maximum rather than for intermediate or low expression of mating propensity is compatible with the main finding of Fulker (1966), i.e., heterotic effects for increased male mating propensity in intraspecific matings of *D. melanogaster.* Connolly et al. (1974) reported lowered male mating propensity in *D. melanogaster* inbred lines. In addition, Ringo and co-workers gave strong evidence for severely lowered male mating propensity in the sibling species *D. simulans* as a direct result of inbreeding (Ringo et al., 1987*a*) or its equivalent, severe population bottlenecking (Ringo et al., 1986, 1987*b*). Parsons (1974) and Collins and Hewitt (1984) argued that male mating speed in *Drosophila* is under directional selection for rapid mating. In light of these findings and our own observations of *D. melanogaster* males, we suggest that male mating propensities in interaction with conspecific and allospecific females are expressions of the same male character, i.e., the eagerness of male flies to mate (Bateman, 1948).

So, in the evolution of male courtship be-

havior in the Draveil population there was no strong selection for lower interspecific mating propensity. This result has important implications for the evolution of prezygotic reproductive isolation. Efficient sexual isolation could evolve as a byproduct of selection and drift in allopatry or alternatively could develop or could be reinforced by direct selection (Fisher, 1930; Dobzhansky, 1937; Mayr, 1942, 1959; Muller, 1942). The genetic architecture of male mating propensity in *D. melanogaster* Draveil is not agreeable with the hypothesis that efficient sexual isolation is the result of natural selection against hybridization and the production of unfit hybrids. The hypothesis being that alleles for distinctive signals and mating discrimination would increase in frequency if its bearer had fit, non-hybrid offspring. This process is known as "reinforcement of isolating mechanisms" and consequently results in a population of males and females, who should have lowered propensity and receptivity, respectively, to mate interspecifically. Hence, the genetic architecture suggests that in *D. melanogaster* Draveil males sexual isolation is more an incidental byproduct of genetic divergence between the two sibling species. The same genes that make the neospecies diverge in morphological, physiological, and behavioral traits render them reproductively isolated. Evolutionary forces that may drive genetic and behavioral divergence in different populations include genetic drift and founder events (Mayr, 1954, 1963; Carson, 1968, 1975; Templeton, 1980), local adaptation (Muller, 1940; Paterson, 1981, 1982), and sexual selection (Lande, 1981, 1982; Thornhill and Alcock, 1983; West-Eberhard, 1983; Price et al., 1987; Butlin, 1989; Kaneshiro, 1989).

An interesting and surprising result of the first experiment is that *D. melanogaster* males were more successful in mating with *D. simulans* females than the reciprocals. Although this result is not new (Ronen, 1957; Barker, 1962, 1967; Kamping and van Delden, 1988), the general finding is that matings between *D. melanogaster* females and *D. simulans* males are more frequent in laboratory experiments (Sturtevant, 1929; Biddle, 1932; Manning, 1959; Hadorn, 1961; Parsons, 1972; Watanabe and Kawanishi, 1979; Robertson, 1983; Casares and Carracedo, 1985; Carracedo and Casares, 1985; Carracedo et al., 1989; Kamping and van Delden, 1991) and in collections from the wild (Sperlich, 1962; Ménsua and Pérez, 1977; Inoue et al., 1990).

A major cause of the apparent discrepancy in the findings may lie in the method of measuring the frequency of interspecific mating (Barker, 1967). Many studies were done without direct behavioral observations of interspecific matings. In the laboratory studies, the number of fertile bottles is usually counted after one to seven days of mass matings with unequal sex ratios. In the wild studies, the presence of both females and males in the progeny is considered as an indication for the absence of hybridization between the two species. From the presence of only hybrid male or only hybrid female progeny produced it was inferred which crosses between the two species have occurred in nature. The findings of Barker (1962, 1967) and our study result from experiments with pair matings, where the females were scored individually. The details of the method of observation thus resolves a great deal of the recorded differences.

The causes of variation in sexual isolation between *D. melanogaster* and *D. simulans* must be sought in the specificity of the behavioral interaction between the two sexes. *Drosophila melanogaster* and *D. simulans* are characterized by the same ethogram of courtship behaviors, but there are quantitative and sequential differences between the two species in the performance of these behaviors (e.g., Burnet and Connolly, 1974; Welbergen et al., 1987). Intraspecific courtship of the two species is primarily a selected multivariate and fine-structured interplay between signals and responses of female and male, which both sexes must complete to achieve the act of copulation (Connolly and Cook, 1973; Markow and Hanson, 1981; Cobb et al., 1986; Markow, 1987; Welbergen et al., 1987, 1992; Liimatainen et al., 1992). We think that an interspecific mating between the two sibling species is likely to be the incidental byproduct of appropriate courtship stimulation provided by the allospecific sexes. A female might accept a male of another species if his courtship responses are pretty close to intraspecific courtship responses. The differences in in-

terspecific mating frequencies of outbred and inbred males might then be an effect of their differences in the elaboration of the response profile. To make a reasonable assessment of the relationship between sexual isolation and communication, however, intersexual sequences of behavior should be observed for interspecific situations. We know very little about the behavioral changes that occur when females and males of different *Drosophila* species meet and interact sexually (see for a review Welbergen, 1992).

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