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Sexual isolation between *Drosophila melanogaster* females and *D. simulans* males. Male mating propensities versus success in hybridization

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Received 25 November 1992; accepted 16 March 1993

Abstract. Genetic variation has been found in males of a *D. simulans* population for their eagerness to hybridize with *D. melanogaster* females. In a search for traits involved in this hybridization, males of *D. simulans* were tested for mating speed and sexual vigour. Between-male differences were detected in both sexual traits, but no relationship was noticed between them, nor with the frequency of hybridization. Thus male mating propensities appear to be unrelated to the breakdown of sexual isolation between these sibling species.

Key words. Drosophila; hybridization; male vigour; male mating speed.

Hybridization between *D. simulans* males and *D. melanogaster* females occurs with some frequency in nature[1] and is easily achieved in the laboratory in choice[2] and no-choice experiments[3]. The reciprocal cross is very rare[4] (although some populations show exceptionally high values[5]) and will not be considered in this paper. Genetic variation for the frequency of hybridization has been revealed in intra-[6] and interpopulation analyses[7], and in both of them, male and female genotypes were important in deciding heterospecific mating.

Up to now, the only well-known sexual factor implicated in hybridization success is female receptivity[8,9]. Regarding *D. simulans*, it is not known yet which sexual behaviors are involved in the breakdown of the reproductive isolation. Male vigour has been reported as important for mating performance[10], and mating speed is sometimes mentioned in this respect[11]. This encouraged us to study these two traits in males of *D. simulans* to ascertain their possible influence on the hybridization with *D. melanogaster* females.

The first step was to detect male genotypic variation for hybridization, so twenty isofemale lines (S1–S20) of *D. simulans* established from a single capture were tested against two types of *D. melanogaster* females (M1 and M2). M1 had been selected for high frequency of hybridization[12] and M2 for high homospecific receptivity[13], that is, the readiness of females to accept conspecific mating.

Methods and results

The 'no choice' method was followed: one *D. simulans* male of each of the twenty lines (S1–S20) and one *D. melanogaster* female of the M1 or the M2 lines, all aged 3 h, were kept in a vial with food for 5 d. At the end of the fifth day, the adults were discarded, and 5–6 d later each vial was observed to detect larval progeny, which provided evidence of hybridization. A hundred males from each *D. simulans* line were tested with each female type. The test was conducted at 21 °C, with 12:12 h light:dark cycles.

The number of *D. melanogaster* females out of 100 which produced hybrid offspring with *D. simulans* males appears in table 1. To test for differences between the male lines, homogeneity chi-squares were carried out within each female type. Significant between-male differences in hybridization were found with both the M1 ($\chi^2 = 193.5$; d.f. = 19; $p < 0.001$) and M2 ($\chi^2 = 82.6$; d.f. = 19; $p < 0.001$) females. These results agree with previous work[6,9,14] which showed the occurrence of a

Table 1. Numbers of M1 and M2 *D. melanogaster* females out of 100 hybridizing with each of the twenty male lines of *Drosophila simulans*. In the right-hand column are shown the chi-squares for homogeneity, comparing the hybridization values of each male with both females. The lines marked with a † sign were the lines chosen to test for mating speed and male sexual vigour

Male lines	Female lines M1	 M2	χ^2
S-1†	50	44	0.72
S-2†	45	43	0.08
S-3	63	38	12.63**
S-4	43	29	4.25*
S-5	36	24	3.45
S-6	27	39	3.25
S-7	23	29	0.94
S-8†	28	31	0.21
S-9†	23	27	0.42
S-10†	22	19	0.27
S-11	35	43	1.34
S-12†	71	61	2.22
S-13	40	50	2.02
S-14	60	44	5.12*
S-15	35	24	2.91
S-16	56	39	5.79*
S-17	11	32	13.06**
S-18†	33	39	0.78
S-19†	39	31	1.41
S-20†	35	30	0.57

*$p < 0.05$.
**$p < 0.001$.

wide range of hybridization tendencies in male genotypes of *D. simulans* populations.

The heterogeneity of the percentages did not allow us to sum them into a single mean value. For this reason, the data were transformed by the arcsin function. The mean hybridization values so obtained were 38.19 ± 2.08 with the M1 females and 36.58 ± 1.36 with the M2 females. A Student's t-test indicated no significant differences between them ($t = 0.52$, d.f. $= 38$ n.s.). This is to be expected if we consider that both female lines came from artificial selection processes, one for increased hybridization with *D. simulans*, and the other for increased homospecific receptivity; two highly positively correlated female characteristics.[9,14] The use of these selected lines also explains the high hybridization values found in this experiment.

To examine the behaviour of males with the two females types, chi squares with 1 d.f. were calculated for each pair of values (Table 1, righthand column). Most of the male lines showed a similar hybridization frequency with the two female types, but significant differences were detected in 5 lines, indicating some male-female interaction. This result, which agrees with previous work with the same species[6], is not unexpected if we bear in mind that hybridization is mainly determined by female receptivity[9], a character that usually shows strong male-female interactions when sexual behaviour is being measured.[15]

To check for a relationship between heterospecific mating success and the two homospecific traits in the study we chose nine of the *D. simulans* lines, which represented the widest possible range of hybridization success (high, intermediate and low). Only lines whose males showed similar values with the two female types were taken. These lines were S-1, S-2 and S-12 for high; S-18, S-19 and S-20 for intermediate; and S-8, S-9 and S-10 for low. Two different *D. simulans* isofemale lines (SA and SB), chosen randomly from the same single capture, were used as tester females.

Male mating speed and vigour were evaluated as follows: newly emerged SA (or SB) females were kept in groups of six individuals per vial, and newly emerged males in groups of 15 individuals. Three days later, a single male was aspirated into the vial with the six females. Mating speed was estimated as the time from introduction to first mating. Six hours later, the male was discarded and each female placed in a single vial. This made it possible to find out how many females were inseminated by each male, which constituted the measure of vigour. Several days later the vials were observed to detect larval progeny. Fifty males from each line were tested with SA and SB females. These tests were carried out in the morning, at 21 °C, under constant light.

The mating times in seconds were transformed to a decimal log scale as the best way to get a normal distribution.[16] The mean values appear in table 2. The transformed data were subjected to a two-way ANOVA in which the sources of variation were the 9 male lines and the 2 types of females. There were differences between males ($F_{8,882} = 3.006$; $p < 0.0025$) and between females ($F_{1,882} = 351.971$; $p < 0.001$), and a male-female interaction ($F_{8,882} = 3.28$; $p < 0.001$). Since interaction was present, male differences had to be examined with each female genotype. For this, two one-way ANOVAs were made. With SA females, significant differences for mating speed were found ($F_{8,441} = 29.87$; $p < 0.001$), but no such differences appeared with SB females ($F_{8,441} = 1.61$ n.s.). This means that whereas the mating time is mainly determined by the male genotype when measured with SA females, SB females are somehow responsible for determining the time each male spends in courtship, his genotype being unimportant.

Table 2. Mean values of time to mating (male mating speed) and mean values of inseminated females (male vigour) of the nine male lines of *D. simulans* with two homospecific female lines (SA and SB)

Male lines	Female lines SA	SB		
	Male Mating Speed (sec.)	Male Vigour	Male Mating Speed (sec.)	Male Vigour
S1	277	3.42	788	2.76
S2	439	3.38	759	3.09
S12	476	3.82	540	2.80
S18	842	3.34	698	3.17
S20	170	2.52	640	3.86
S19	337	2.98	744	2.79
S8	345	3.88	552	3.46
S9	273	3.82	512	2.34
S10	268	3.88	514	2.82

The average mating-time of the nine male lines with SA females was 2.54 ± 0.013 (345 seconds) and with SB females was 2.79 ± 0.015 (611 seconds). As mating with SA females was faster we can conclude that SA females are more receptive.

Table 2 also shows the vigour estimates of the 9 lines with the two types of females. A two way ANOVA revealed significant differences between males ($F_{8,882} = 4.82$; $p < 0.001$) and between females ($F_{1,882} = 44.78$; $p < 0.001$), and male-female interaction ($F_{8,882} = 16.67$; $p < 0.001$). As above, the existence of this interaction forced us to test male differences with two one-way ANOVAs. Significant between-male differences were found with both SA females, ($F_{8,441} = 10.63$; $p < 0.001$) and SB females, ($F_{8,441} = 10.89$; $p < 0.001$). The mean number of females inseminated by the male lines was higher with SA (3.45 ± 0.05) and with SB (3.01 ± 0.04) females, which suggests higher receptivity of SA than SB females, result that agrees with the mating speed results.

Conclusions and discussions

In summary, the homospecific study of two sexual traits in *D. simulans* males revealed: 1) differences in mating

Table 3. Spearman's correlations among sexual vigour, mating speed and hybridization percentages of the nine *D. simulans* male lines*

	Vigour SA	Vigour SB	Mating speed SA	Mating speed SB
Mean hybridization	−0.33	−0.12	0.6167	0.6167
Mating speed SA	0.0084	-	-	0.2667
Mating speed SB	-	0.0667	0.2667	-

*Correlations were not significant.

speed with SA females but not with the less receptive SB females, 2) clear differences among lines in sexual vigour and 3) significant male-female interactions.

The occurrence of interaction means that although males differ among themselves, the relative performance of each male depends on the female genotype with which he is faced. This seems to be a common situation in *Drosophila*, making the interpretation of results of studies on sexual behaviour difficult[15,17].

It is worth noting the lack of a relationship between vigour and mating speed. Previous work by Fulker[10] on *D. melanogaster* and Prakash[18] on *D. robusta* indicated that the male that mates faster also fertilizes more females, both traits being important components of fitness.[11] Our results do not support this assertion, but rather they highlight how male sexual behaviour is strongly dependent upon the female genotype, and emphasize the need for careful studies in which male behaviour must be examined with more than one female of the same population.

Finally, the relationships between the mean values of hybridization, mating speed and vigour of the nine male lines were examined. Nonparametric Spearman's tests were used, as the lines were not chosen randomly but based on a hybridization criterion. None of the correlations were significant (table 3), so there is no relationship between sexual vigour, mating speed and hybridization in the *D. simulans* population analyzed. This result means that the main question posed in our work remains unsolved. There is a genetic basis in the *D. simulans* male for its hybridization success with *D. melanogaster*[6,7], so some type of male trait must be involved. Male vigour and mating speed are not implicated in this process, despite their importance in mating. Though it is hard to determine which other male traits could play a role in reproductive isolation, we can presume that the male's persistence in courtship, or his lack of discriminating through hormonal perception, might be among the factors that deserve detailed study.

Acknowledgments. The present work was carried out with aid from the Spanish Ministry of Education and Science (DGICYT Grant No. PS90-0185) and the University of Oviedo, Spain (DF Grant No. 91/21633).

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