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Ethological isolation and mating experience in *Drosophila paulistorum*

(aging/mating behavior/reproductive isolation/semispecies/sperm storage)

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ABSTRACT **With few exceptions, previously published experiments documenting ethological isolation between *Drosophila* populations have used young virgin females. We have observed directly females of *Drosophila paulistorum* semispecies in choice experiments with both homogamic and heterogamic males. Aged females' sexual selection did not differ from that of young females. Previous heterogamic copulatory experience did not change the degree of sexual isolation; however, females with homogamic copulatory experience showed a significantly higher preference for homogamic males.**

Behavioral and genetic analyses of sexual isolation in species of the genus *Drosophila* have generally utilized females experiencing their first mating. Because of the recent work of Quinn, Harris, and Benzer (1) on conditioned behavior in *D. melanogaster* and of Pruzan (2) on sexual selection in *D. pseudoobscura*, it seemed appropriate to examine the effects, if any, of previous copulatory experience, and concurrently of aging, on sexual isolation.

The species complex *Drosophila paulistorum* provides rich material for a survey of this sort because it is composed of six semispecies or incipient species among which there exist many degrees of sexual isolation, varying with allo- or sympatricity (3). Females of this superspecies have been shown to mate repeatedly (4).

MATERIALS AND METHODS

The Mesitas, Colombia, Andean semispecies (M) and the Santa Marta, Colombia, Transitional semispecies (SM) are *Drosophila paulistorum* strains that produce sterile male hybrids when crossed (5). The Agua Fria, Costa Rica (C), and Salvador (S) strains belong to the Centroamerican semispecies.

Females were aged either 3 or 9 days; all males were 3 to 4 days old. For copulatory experiences, 3-day-old females were confined with either homogamic or heterogamic males for 1 day or 2 days, respectively, in mass cultures. Each female was then placed alone in a vial containing food for 4–5 days. Larvae indicated that mating had taken place, and only females that produced larvae were used for further tests.

The direct observation method (6) allows the simultaneous scoring of four possible types of matings, two homo- and two heterogamic as itemized in Tables 1 and 2. Therefore, data presented in the comparable sets of rows for the two types of females in these tables, i.e., rows *1* and *5*, *2* and *6*, *3* and *7*, etc., were scored at the same time within the same mating chambers.

H. Levene's (6) coefficient of joint isolation, I, was calculated; it ranges from −1.00 (exclusively heterogamic mat-

Table 1. Number of *Drosophila paulistorum* female matings*

	Matings observed			
	M♀ × M♂	M♀ × SM♂	SM♀ × M♂	SM♀ × SM♂
M Females				
(*1*) 3-day-old virgins	55	5		
(*2*) 9-day-old virgins	53	7		
(*3*) 9-day-old, mated to M ♂♂ when 3 days old	36	0		
(*4*) 9-day-old, mated to SM ♂♂ when 3 days old	4	2		
SM Females				
(*5*) 3-day-old virgins			10	50
(*6*) 9-day-old virgins			8	52
(*7*) 9-day-old, mated to SM ♂♂ when 3 days old			0	35
(*8*) 9-day-old, mated to M ♂♂ when 3 days old			1	11
Joint isolation coefficients				
Rows *1* and *5*, 0.75 ± 0.06				
Rows *2* and *6*, 0.74 ± 0.06				
Rows *3* and *7*, 1.00				
Rows *4* and *8*, 0.67 ± 0.18				

* Females varied according to age and previous mating experience: half were from the Mesitas, Colombia, Andean semispecies (M), and the rest from the Santa Marta, Colombia, Transitional semispecies (SM) strains.

Table 2. Number of *Drosophila paulistorum* female matings*

	Matings observed			
	C♀ × C♂	C♀ × S♂	S♀ × C♂	S♀ × S♂
C Females				
(*1*) 3-day-old virgins	33	27		
(*2*) 9-day-old virgins	31	18		
(*3*) 9-day-old, mated to C ♂♂ when 3 days old	38	12		
(*4*) 9-day-old, mated to S ♂♂ when 3 days old	39	30		
S Females				
(*5*) 3-day-old virgins			22	38
(*6*) 9-day-old virgins			26	33
(*7*) 9-day-old, mated to S ♂♂ when 3 days old			8	18
(*8*) 9-day-old, mated to C ♂♂ when 3 days old			19	30
Joint isolation coefficients				
Rows *1* and *5*, 0.19 ± 0.08				
Rows *2* and *6*, 0.19 ± 0.09				
Rows *3* and *7*, 0.47 ± 0.10				
Rows *4* and *8*, 0.17 ± 0.09				

* Females varied according to age and previous mating experience: half were from the Agua Fria, Costa Rica, Centroamerican semispecies (C), and the rest from the Salvador, Centroamerican semispecies (S) strains.

ings) through zero (random matings) to +1.00 (exclusively homogamic matings).

RESULTS AND DISCUSSION

Comparison of the results reported in rows *1* plus *5* and *2* plus *6* of Tables 1 and 2 indicates that age does not affect the magnitude of sexual isolation in these strains; the coefficients of joint isolation are equivalent when calculated from data acquired with young and with old *Drosophila paulistorum* virgins. In a different species, *Drosophila pseudoobscura*, frequency-dependent sexual selection has been shown to be influenced by both age and previous experience (2, 7). As to the latter, *D. paulistorum* responds similarly: previous mating experience with homogamic males enhances the likelihood of subsequent homogamic matings (rows 3 and 7 of Table 1 and the same rows in Table 2). This is in agreement with the homo- and heterospecific rearing experience study of LeMoli and Mainardi (8). Previous heterogamic experience was the most difficult to manage due to the scarcity of females that accepted alien males; for this reason little data could be obtained (row *4*, Table 1). However, because the C and S strains come from the same semispecies, such were obtainable (row *4*, Table 2). The importance of early experiences and their effects on assortative mating and population genetics has recently been treated mathematically by Matessi and Scudo (9), who showed that these experiences can be an effective source of genetic barriers between geographical races. *Drosophila paulistorum* females have been shown here to alter their sexual preferences as a result of earlier copulatory experiences. The increased tendency to mate with homogamic males can act as a barrier to gene flow between the semispecies and lead to more rapid speciation.

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