



Sexual Isolation in *Drosophila*. III. Estimating Isolation Using Male-choice Experiments

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It has been generally assumed that “choice experiments” are useful to measure sexual isolation between *Drosophila* strains or species. Theoretical models have demonstrated however that the results obtained using one of these designs, namely multiple-choice experiments, are insufficient to determine the degree of isolation, even under very favorable assumptions. In this work, a simple behavioral model is developed to test whether male-choice experiments can be used to measure sexual isolation in *Drosophila*. This model shows that, although the outcome of male-choice experiments is affected by differences in female receptivities, a procedure to estimate the minimum degree of isolation using this experimental design can be established. The application of the methods derived from the theoretical model to previously reported experimental data demonstrates that a substantial degree of isolation frequently exists intraspecifically, while isolation is far from complete interspecifically. These results have important implications for discussions based on the comparative analysis of *Drosophila* behavior, both intra- and interspecifically. Most especially, they are in contradiction with the expectations of the Recognition concept of species.

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Introduction

A number of discussions in modern evolutionary theory are based on the results of studies where the degree of sexual isolation among strains or species is measured and compared. Some examples are the debates about the importance of reinforcement in the production of new species (Dobzhansky, 1937; Paterson, 1978), about the likelihood of speciation through changes in courtship elements after a founder effect (Kaneshiro, 1976; Giddings & Templeton, 1983) or about the existence of intraspecific sexual isolation, critical for establishing the validity of the “Recognition concept of species” (Coyne *et al.*, 1988; Masters & Spencer, 1989; White *et al.*, 1990). Studies on *Drosophila* species have strongly influenced these discussions, because this genus is among the best studied from an ethological point of view. Sexual isolation in *Drosophila* has been traditionally measured in the laboratory using choice experiments.

The two designs most commonly used are known as “male-choice experiments”, when one type of males is simultaneously confined with females of the same strain and with females of a different strain, and “multiple-choice experiments”, when both males and females of the two strains are put together. The number of homogamic and heterogamic matings is then determined by dissection of the females (in male-choice designs) or direct observation (applicable to both designs).

A general problem of these experimental designs is that differences in vigor between the strains can be easily mistaken as reproductive isolation. This difficulty was recognized a long time ago and several authors tried to settle the problem by proposing different “isolation indices”, whose goal was to distinguish isolation and mating propensity [reviewed in Spieth & Ringo (1983)]. However, it has been demonstrated that isolation indices do not solve this question. First, Gilbert & Starmer (1985) showed, using simulations, that differences in sexual drive in the absence of assortative mating can affect the value

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of these indices. To demonstrate that this difficulty is rooted in the experimental design, and that therefore will affect any index that can be defined, requires a different approach. The first study that considered this problem was based on the idea of developing very simplified models of *Drosophila* behavior, and tested whether the results of multiple-choice experiments can be used to measure isolation under these favorable circumstances. This analysis showed that, with a multiple-choice experiment, it is possible to establish whether isolation exists, but its degree cannot be measured because there is no way to discriminate between the effects of isolation and those of differences in male vigor (Marín, 1991). In order to solve this difficulty, I have undertaken the analysis of how suitable male-choice experiments are to measure sexual isolation. Male-choice experiments are an interesting alternative, because problems due to differences in male vigor are eliminated in this design, where only one type of male is used. The results of this study suggest that male-choice experiments have clear advantages over multiple-choice experiments and that it is possible to use them to obtain a minimum estimate of the degree of isolation.

A Minimal Behavioral Model for Male-choice Experiments

In this section, a general behavioral model useful for analysing male-choice experiments is developed. It has basically the same postulates as the models defined previously for multiple-choice experiments (Marín, 1991). The two main differences are the absence of a parameter for male vigor, because only one type of male is present in these experiments, and the fact that the number of receptive females that will pair during the experiment is considered to be dependent on the number of courtships. In the models that I described previously, it was supposed that all receptive females paired during the experiment. The more open structure model that I describe below allows the development of a continuum of formulae with the number of courtships as a variable. The models named as “discrete” and “continuous” preferences in Marín (1991) are the extreme cases of this continuum, with a number of courtships equal to one and infinity respectively.

The model assumes the following conditions:

(a) A certain number of males of one strain is put together with females of both their own and a different strains. For simplicity, it will be considered that the number of females of both strains is the same.

(b) Males do not discriminate. They court any females present at the same rate.

(c) Females of both strains may differ in their receptivity (R), defined as the percentage of females that are physiologically prone to pair under the conditions of the experiment.

(d) Females of the two strains may have differences in their degree of acceptance of the males present in the experiments, that is, they can exert discrimination. The coefficient of female acceptance (A) is defined as the probability that a female mates after being courted by one of the males. This coefficient is considered constant during the experiment.

Now, calling the two strains X and Y respectively, we can define:

Relative female receptivity: $R = R_X/R_Y$, where R_X , R_Y are the receptivities of females of the strains X and Y respectively. This is a measure of relative female mating propensity under the particular conditions of the experiment.

Relative assortative mating coefficient for the strain X : $a = A_{Y,X}/A_{X,X}$, where $A_{X,X}$ and $A_{Y,X}$ are the coefficients of acceptance for females of the strain X regarding their matings with males X and Y respectively.

Relative assortative mating coefficient for the strain Y : $b = A_{X,Y}/A_{Y,Y}$, where $A_{X,Y}$ and $A_{Y,Y}$ are the coefficients of acceptance for females of the strain Y with respect, in each case, to males X and Y .

For simplicity, we will also consider that females do not discriminate against their own males, so $A_{X,X} = A_{Y,Y} = 1$. Therefore, $a = A_{Y,X}$ and $b = A_{X,Y}$. a and b are the isolation parameters that we want to estimate. These parameters establish quantitatively the degree of discrimination in a particular cross. I showed before that it is not possible to determine the values of these two parameters using multiple-choice experiments. The information obtained from those experiments confounds female discrimination and male vigor (Marín, 1991). The objective of this work is to determine whether a and b can be established using male-choice designs.

According to these assumptions and definitions, it is easy to demonstrate that the expected relative frequencies of each type of mating, after every female has been courted once are:

Experiments with males X :

$$f_i(X, X) = R/(R + b) \quad (1)$$

$$f_i(X, Y) = b/(R + b) \quad (2)$$

Experiments with males Y :

$$f_i(Y, X) = Ra/(Ra + 1) \quad (3)$$

$$f_1(Y, Y) = 1/(Ra + 1) \quad (4)$$

Assuming that females discriminate in an all or nothing fashion, this will be the final outcome of the experiment (after the first courtship, all the receptive non-discriminating females would pair. The rest will not pair, no matter how many courtships are performed). Therefore, these formulae are equivalent to those obtained for the simplified model of discrete preferences applied to multiple-choice experiments (Marin, 1991, Table 3). According to our definitions of a and b (probability of acceptance after every courtship), we can consider now what would happen if females are courted several times and they discriminate in each courtship with a certain probability. Because by definition females do not discriminate against their own males, all the homogamic matings will happen after the first courtship. However, concerning heterogamic pairs, the probability of mating for any female will increase with the number of courtships. For example, considering the experiment with males X , the probability that a receptive Y female pairs (β) after a certain number (c) of courtships would be:

$$\begin{aligned} c = 1 \quad \beta_1 &= b \\ c = 2 \quad \beta_2 &= b + b(1 - b) = 2b - b^2 \\ c = 3 \quad \beta_3 &= 2b - b^2 + b(1 - 2b + b^2) \\ &= 3b - 3b^2 + b^3 \\ &\dots \\ c = k \quad \beta_k &= \beta_{k-1} + b(1 - \beta_{k-1}) \end{aligned} \quad (5)$$

β increases with the number of courtships and its equal or larger than b . It is immediate that if $b \ll 1$, then $\beta_n \approx nb$. It is also easy to see that when $c = \infty$, $\beta = 1$, that is, all the receptive females will pair after infinite courtships. The same conclusions apply to the parameter a . These results have interesting implications. According to this model, the outcome of a male-choice experiment when females discriminate with a given probability for each courtship of the alien males will be determined to a great extent by the number of receptive females, especially when the number of courtships is high and the value of the acceptance coefficient is also high (i.e. when females accept males of the alien strain frequently). For example, if $b = 0.5$, 94% of the receptive females will pair after only four courtships. The first conclusion of this work is that the outcome of male-choice experiments is greatly dependent on female receptivities. A second conclusion is that, except in those cases where females discriminate in an

all/none fashion, it is impossible to estimate the values of a and b without knowing the number of courtships per female. This has some subtle consequences. For example, at first glance it would appear possible to combine the results of the two reciprocal male-choice experiments to estimate the value of R , for example using formulae (1) to (4) or similar formulae derived from (5). However, this is not possible unless the number of courtships is assumed to be identical in both reciprocal crosses. In the absence of experimental data, this supposition is equivalent to assume that the vigor of both types of males is the same. I demonstrated before that this assumption is violated in a large number of cases (Marin, 1994).

Extensions of the Models: Strategies to Estimate the Degree of Isolation

In spite of these problems, several strategies can be devised to obtain estimates of the degree of isolation. Let us start by considering the values of the expected relative frequencies after a certain number, c , of courtships. By analogy with formulae (1) to (4), they are:

Experiments with males X :

$$f_c(X, X) = R/(R + \beta) \quad (6)$$

$$f_c(X, Y) = \beta/(R + \beta) \quad (7)$$

Experiments with males Y :

$$f_c(Y, X) = R\alpha/(R\alpha + 1) \quad (8)$$

$$f_c(Y, Y) = 1/(R\alpha + 1) \quad (9)$$

where α , β are the probability of a female X or Y , respectively, of mating with an alien male after c courtships. According to the procedures detailed in Li (1978), the maximum likelihood estimates of α and β are:

$$\alpha' = n_2/(n_4 R) \quad (10)$$

$$V(\alpha') = \alpha'(R\alpha' + 1)^2/N_a R \quad (11)$$

$$\beta' = n_3 R/n_1 \quad (12)$$

$$V(\beta') = \beta'(R + \beta')^2/N_b R \quad (13)$$

where n_1 , n_2 , n_3 and n_4 are respectively the number of crosses (X, X), (Y, X), (X, Y) and (Y, Y) observed, and $N_a = n_2 + n_4$, $N_b = n_1 + n_3$ are the total number of crosses for each of the reciprocal male-choice

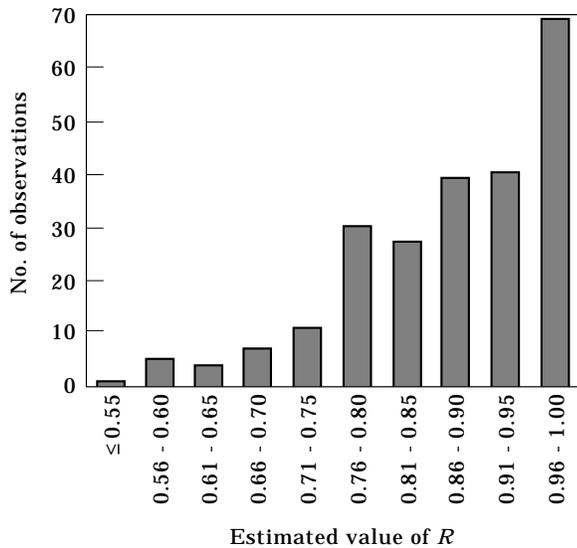


FIG. 1. Distribution of the parameter R , estimated according to Marín (1991), in crosses between strains of different geographical origin. Data obtained from Ehrman (1966, 1970); Ehrman & Petit (1968); Dobzhansky *et al.* (1968); Anderson & Ehrman (1969); Prakash (1972); Ayala & Tracey (1973); Ayala *et al.* (1974); Petit *et al.* (1976); Wasserman & Koepfer (1977a); Ehrman & Parsons (1980); Zouros & D'Entremont (1980); Cohet & David (1980); Henderson & Lambert (1982); Markow *et al.* (1983); Millar & Lambert (1985); Koepfer & Fenster (1991); Singh & Chatterjee (1991b, 1992).

experiments. As we saw in the previous section, α' and β' are underestimates (that is values closer to 1) of a and b respectively. Therefore, formulae (10) and (12) show that a minimum estimate of the degree of isolation can be obtained once we know the value of R .

We are now in a similar situation to when we were studying multiple-choice experiments (Marín, 1991), that is, the number of parameters is larger than the number of equations. However, the situation is much more favorable here, because only two, instead of three, parameters are involved (male vigor is not relevant in male-choice designs). The objective is thus to find a way to determine the value of R or to avoid the effects of this parameter in our conclusions. There are three different procedures that can be used:

(1) I have shown before (Marín, 1991) that it is possible to estimate the value of R , irrespective of the number of courtships, when a multiple-choice design is used:

$$R = [f(X, X) + f(Y, X)]/[f(X, Y) + f(Y, Y)] \quad (14)$$

The standard deviation of R was given by Marín (1991). This means that a combination of a male-choice and a multiple-choice experiment provides, under the assumptions of the models developed in Marín (1991) and in this study, all the information

needed to estimate α and β . The first strategy consists therefore in the combination of different experimental designs to obtain all the required information.

(2) Although the previous strategy is optimal, it is not always necessary. If we are interested only in a conservative estimate of a and b , a different strategy can be used, namely to determine a minimum value of R and to use this value in formula (10) [or its inverse in formula (12)]. To establish what would be a reasonable minimum value of R , I have analysed all the available literature where multiple-choice experiments are used in order to determine the distribution of this parameter [estimated according to formula (14)] in intraspecific, sub- and semispecific and interspecific crosses respectively. In Figs 1–3, the summary of 24 previous studies is shown. For calculating the values that appear in these figures, X and Y were arbitrarily assigned, and the smaller value between R and $1/R$ was chosen. Figure 1 includes all the experiments that analysed crosses between wild type strains of different geographical origin. Figure 2 shows the range of values of R for subspecies and semispecies. Finally, Fig. 3 considers only interspecific crosses. The conclusions of this analysis are: (1) Intraspecifically, R is not very variable. The average value for wild type strains ($n = 233$) is $R = 0.87 \pm 0.01$ and for semi- or subspecies ($n = 60$) is $R = 0.85 \pm 0.02$. These results are compatible with those described in Marín (1994), where I showed that, for 89% of the crosses between strains and for 74% of the studies using subspecies or semispecies, the

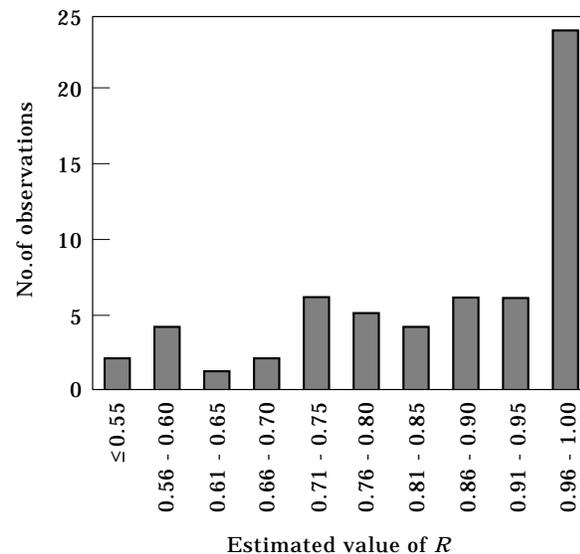


FIG. 2. Distribution of R . Subspecies and semispecies. References: Malogolowkin-Cohen *et al.* (1965); Dobzhansky & Pavlovsky (1971); Prakash (1972); Ayala & Tracey (1973); Ayala *et al.* (1974); Dobzhansky (1975); Zouros & D'Entremont (1980); Markow *et al.* (1983); Koepfer & Fenster (1991); Kim *et al.* (1992).

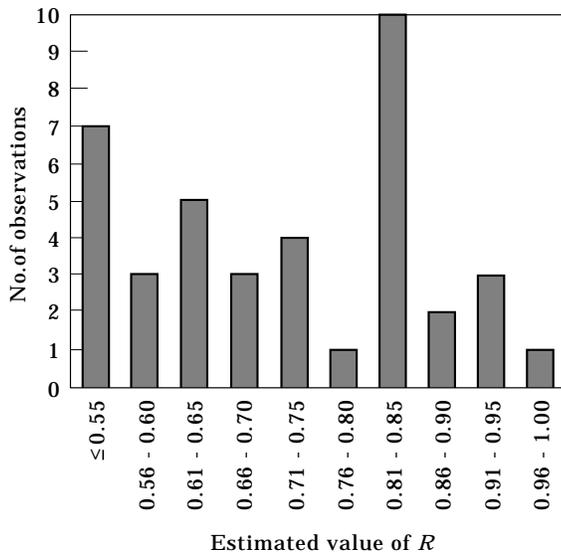


FIG. 3. Distribution of R in interspecific crosses. References: Kessler (1966); Dobzhansky *et al.* (1968).

value of R is not significantly different from 1. However, 10% of the values are under $R = 0.72$ for strains and under $R = 0.62$ for semi- or subspecies. (2) Interspecifically, R varies substantially ($n = 40$, average $R = 0.71 \pm 0.03$). See Fig. 3). The 10% limit is $R = 0.40$. The 10% limit values can be used as minimum estimates of R in the absence of any other data: because the minimum value of the pair R and $1/R$ was selected to obtain Figs 1–3, it means that about 95% of the experiments yield values of R greater than these limits. This procedure will be used in the next section to establish the minimum degree of isolation that exists intra- and interspecifically.

(3) Finally, we can think of another strategy if we are interested only in establishing whether general patterns (as an excess of homogamic or heterogamic matings) exist when studying a large number of experiments. Rearranging formulae (10) and (12) we find that, after a certain number of courtships, the ratio of number of heterogamic matings (Ht)/number of homogamic matings (Hm) has values:

$$(Ht/Hm)_X = \beta/R \quad (15)$$

$$(Ht/Hm)_Y = R\alpha \quad (16)$$

for experiments with males X and Y respectively. As shown above, R in interspecific crosses is generally close to one, and, therefore, deviations from $Ht/Hm = 1$ in this type of crosses can be indeed interpreted as meaning that certain degree of isolation exists. Moreover, even without assuming that $R = 1$, we can obtain very significant information when reciprocal crosses are considered: because R has

opposite effects in formulae (15) and (16), the only possible explanation if $Ht/Hm < 1$ in both reciprocal crosses is positive assortative mating in at least one of them. This opens the possibility to perform analyses to establish whether systematic biases exist (for example, excess of homogamic matings intraspecifically). This type of analysis will be presented elsewhere.

Estimating the Minimum Degree of Intra- and Interspecific Isolation

Many authors have used male-choice experiments to investigate ethological differentiation in the *Drosophila* genus. As was shown in the previous section, this information can be used to estimate a minimum value of the degree of isolation. Tables 1 and 2 summarize the available results for crosses between strains of different geographical origin and between different species respectively. In these tables, I have included all the experimental findings noted in the literature with the following features: (a) at least one class of matings (homo- or heterogamic) was observed 25 times or more; (b) when less than 100 total matings were observed, the number of females tested was identical in the homo- and heterogamic classes or, if the number was different, the difference was ≤ 5 females. For calculating α' in those cases where the number of females tested is different (included in this analysis because the difference is ≤ 5 females or the total number of matings is larger than 100), the percentages of mated females have been used instead of the observed frequencies. Tables 1 and 2 include: (a) the name of the species used and the groups and subgenus to which they belong; (b) the number (N) of different male-choice experiments performed; (c) the average values of α' obtained using $R = 0.7$ for intraspecific experiments and $R = 0.4$ for interspecific one (these are the 95% limits established in the review of the multiple-choice experiments presented in the previous section). I have supposed that, when the value of α' is higher than 1.00, there is no isolation (most of the results with $\alpha' > 1.00$ will be due to the conservative estimate of R being used), so in these cases the value obtained has been substituted by $\alpha' = 1.00$.

The intraspecific value of α' is 0.91 ± 0.02 and values of $\alpha' \geq 1.00$ are found in only four of the sixteen species, demonstrating that a certain degree of intraspecific isolation exists in species of the *Drosophila* genus (Table 1). It is likely that this value substantially underestimates the degree of isolation per courtship. Considering that the behavior of these organisms is probably very similar and largely

compatible, the assumption of discrimination of the all/none type is very unlikely. Then, assuming that the number of courtship per female is large in this type of experiment, the value of a , that is the probability of discrimination after each courtship against an alien male, will be substantially smaller than α , the probability of discrimination after n courtships, as explained previously [see the discussion on eqn (5)]. From Table 2, we conclude that, as expected, interspecific isolation is strong between *Drosophila* species. However, the number of interspecific matings is quite large (the average for the 64 crosses is $\alpha' = 0.31 \pm 0.04$). Even assuming that the number of courtships per female is large and females accept an alien male with a certain probability after each courtship, this probability has to be substantial, because the number of interspecific matings is on average 13% of the number of intraspecific ones. Because an all/none discrimination is much more likely interspecifically than it is inside a species, this value of 13% may be close to the actual probability of acceptance per courtship for males of a different species.

Discussion

In this study I have shown that, to measure isolation, male-choice experiments have clear advan-

tages over multiple-choice experiments. This is due to the fact that male vigor differences do not affect male-choice designs. Consequently, I have shown that, under the assumptions of a simple behavioral model, a procedure can be developed to estimate the minimum degree of isolation between two strains or species. However, as we have seen, neither the results of a multiple-choice nor a male-choice experiment allow the exact determination of the degree of isolation (Marín, 1991 and this study). Further refinements are necessary. There are two critical parameters that complicate the direct use of the outcome of a male-choice experiment to estimate the degree of isolation. The first one is the number of courtships per female. To determine this value should not be a problem under well-planned experimental conditions. The second is the estimation of the parameter R , the relative female receptivity. As I suggested above, a possible solution is to perform parallel male-choice and multiple-choice experiments. According to the models developed by Marín (1991), the value of R can be estimated from the results of the multiple-choice design and this estimate then combined with the male-choice data.

When the models are applied to analyse the available literature concerning male-choice designs, two conclusions are obtained: (1) a low degree of

TABLE 1
Intraspecific isolation in the Drosophila genus

Species	Group (Subgenus)	N	α'	Reference
<i>D. melanogaster</i>	<i>melanogaster</i> (<i>Sophophora</i>)	2	0.87	1
<i>D. ananassae</i>	<i>melanogaster</i> (<i>Sophophora</i>)	43	0.87	2,3
<i>D. prosaltans</i>	<i>saltans</i> (<i>Sophophora</i>)	37	0.72	4
<i>D. sturtevantii</i>	<i>saltans</i> (<i>Sophophora</i>)	19	0.88	5
<i>D. miranda</i>	<i>pseudoobscura</i> (<i>Sophophora</i>)	8	1.00	6
<i>D. paulistorum</i> ^a	<i>willistoni</i> (<i>Sophophora</i>)	39	0.88	7
<i>D. willistoni</i> ^a	<i>willistoni</i> (<i>Sophophora</i>)	17	1.00	8
<i>D. nebulosa</i>	<i>willistoni</i> (<i>Sophophora</i>)	2	0.88	5
<i>D. virilis</i>	<i>virilis</i> (<i>Drosophila</i>)	6	0.84	9
<i>D. texana</i>	<i>virilis</i> (<i>Drosophila</i>)	6	0.94	9
<i>D. montana</i>	<i>virilis</i> (<i>Drosophila</i>)	6	0.91	9
<i>D. americana</i>	<i>virilis</i> (<i>Drosophila</i>)	12	1.00	10
<i>D. arizonae</i>	<i>repleta</i> (<i>Drosophila</i>)	12	0.99	11
<i>D. mojavensis</i> ^a	<i>repleta</i> (<i>Drosophila</i>)	4	0.94	11
<i>D. peninsularis</i>	<i>repleta</i> (<i>Drosophila</i>)	2	0.88	12
<i>D. pegasa</i>	<i>repleta</i> (<i>Drosophila</i>)	2	1.00	13
<i>D. gasci</i>	<i>mesophragmatica</i> (<i>Drosophila</i>)	7	0.88	14
Total		224	0.91 \pm 0.02	

N : number of crosses analysed. α' : average values of the maximum likelihood estimator of α [see eqn (10) and text].

References: (1) Cohet & David 1980; (2) Singh & Chatterjee, 1985a; (3) Singh & Chatterjee, 1985b; (4) Dobzhansky & Streisinger, 1944; (5) Dobzhansky, 1944; (6) Dobzhansky & Koller, 1938; (7) Carmody *et al.*, 1962; (8) Dobzhansky & Mayr, 1944; (9) Patterson *et al.*, 1947; (10) Stalker, 1942; (11) Wasserman & Koepfer, 1977b; (12) Patterson & Wheeler, 1947; (13) Wasserman & Zweig, 1991; (14) Brncic & Koref-Santibañez, 1965.

^a In these cases, where some kind of subspecific differentiation has been described, only the crosses between strains of the same race, subspecies or semispecies have been included.

TABLE 2
Interspecific isolation in the Drosophila genus

Species 1 (males, females)	Species 2 (females)	Group	<i>N</i>	α'	References
<i>Sophophora</i> subgenus					
<i>D. bipectinata</i>	<i>D. malerkotliana</i>	<i>melanogaster</i>	10	0.57	1,2
<i>D. malerkotliana</i>	<i>D. bipectinata</i>	<i>melanogaster</i>	10	0.41	1,2
<i>D. bipectinata</i>	<i>D. parabiepectinata</i>	<i>melanogaster</i>	1	0.10	1
<i>D. parabiepectinata</i>	<i>D. bipectinata</i>	<i>melanogaster</i>	1	1.00	1
<i>D. parabiepectinata</i>	<i>D. malerkotliana</i>	<i>melanogaster</i>	1	0.72	1
<i>D. malerkotliana</i>	<i>D. parabiepectinata</i>	<i>melanogaster</i>	1	0.52	1
<i>D. takahashii</i>	<i>D. pseudotakahashii</i>	<i>melanogaster</i>	1	1.00	3
<i>D. pseudotakahashii</i>	<i>D. takahashii</i>	<i>melanogaster</i>	1	0.59	3
<i>D. pseudoobscura</i>	<i>D. miranda</i>	<i>obscura</i>	6	0.22	4
<i>D. pseudoobscura</i>	<i>D. persimilis</i>	<i>obscura</i>	1	0.27	5
<i>D. persimilis</i>	<i>D. pseudoobscura</i>	<i>obscura</i>	1	0.89	5
<i>D. athabasca</i>	<i>D. azteca</i>	<i>obscura</i>	1	0.63	4
<i>D. insularis</i>	<i>D. paulistorum</i>	<i>willistoni</i>	1	0.03	6
<i>D. insularis</i>	<i>D. equinoxialis</i>	<i>willistoni</i>	1	0.17	6
<i>D. insularis</i>	<i>D. tropicalis</i>	<i>willistoni</i>	1	0.12	6
<i>D. insularis</i>	<i>D. willistoni</i>	<i>willistoni</i>	2	0.19	6
<i>D. paulistorum</i>	<i>D. insularis</i>	<i>willistoni</i>	1	0.13	6
<i>D. equinoxialis</i>	<i>D. insularis</i>	<i>willistoni</i>	1	0.21	6
<i>D. tropicalis</i>	<i>D. insularis</i>	<i>willistoni</i>	1	0.22	6
<i>D. willistoni</i>	<i>D. insularis</i>	<i>willistoni</i>	2	0.19	6
<i>Drosophila</i> subgenus					
<i>D. virilis</i>	<i>D. americana</i>	<i>virilis</i>	5	0.17	7,8
<i>D. americana</i>	<i>D. virilis</i>	<i>virilis</i>	5	0.82	7,8
<i>D. virilis</i>	<i>D. texana</i>	<i>virilis</i>	1	0.10	8
<i>D. texana</i>	<i>D. virilis</i>	<i>virilis</i>	1	1.00	8
<i>D. virilis</i>	<i>D. montana</i>	<i>virilis</i>	1	0.06	8
<i>D. montana</i>	<i>D. virilis</i>	<i>virilis</i>	1	0.63	8
<i>D. virilis</i>	<i>D. laticola</i>	<i>virilis</i>	1	0.03	8
<i>D. laticola</i>	<i>D. virilis</i>	<i>virilis</i>	1	1.00	8
<i>D. americana</i>	<i>D. texana</i>	<i>virilis</i>	1	0.03	8
<i>D. texana</i>	<i>D. americana</i>	<i>virilis</i>	1	0.04	8
<i>D. americana</i>	<i>D. montana</i>	<i>virilis</i>	1	0.00	8
<i>D. montana</i>	<i>D. americana</i>	<i>virilis</i>	1	0.03	8
<i>D. texana</i>	<i>D. montana</i>	<i>virilis</i>	1	0.03	8
<i>D. montana</i>	<i>D. texana</i>	<i>virilis</i>	1	0.04	8
<i>D. americana</i>	<i>D. laticola</i>	<i>virilis</i>	1	0.00	8
<i>D. laticola</i>	<i>D. americana</i>	<i>virilis</i>	1	0.00	8
<i>D. texana</i>	<i>D. laticola</i>	<i>virilis</i>	1	0.00	8
<i>D. laticola</i>	<i>D. texana</i>	<i>virilis</i>	1	0.10	8
<i>D. montana</i>	<i>D. laticola</i>	<i>virilis</i>	1	0.00	8
<i>D. laticola</i>	<i>D. montana</i>	<i>virilis</i>	1	0.22	8
<i>D. arizonae</i>	<i>D. mojavensis</i>	<i>repleta</i>	17	0.52	9,10
<i>D. mojavensis</i>	<i>D. arizonae</i>	<i>repleta</i>	17	0.21	9,10
<i>D. aldrichi</i>	<i>D. mulleri</i>	<i>repleta</i>	1	0.19	10
<i>D. mulleri</i>	<i>D. aldrichi</i>	<i>repleta</i>	1	0.16	10
<i>D. arizonae</i>	<i>D. mulleri</i>	<i>repleta</i>	1	0.20	10
<i>D. buzzatii</i>	<i>D. mulleri</i>	<i>repleta</i>	1	0.00	10
<i>D. hamatofila</i>	<i>D. mulleri</i>	<i>repleta</i>	1	0.10	10
<i>D. mojavensis</i>	<i>D. mulleri</i>	<i>repleta</i>	1	0.37	10
<i>D. arizonae</i>	<i>D. aldrichi</i>	<i>repleta</i>	1	0.49	10
<i>D. hamatofila</i>	<i>D. aldrichi</i>	<i>repleta</i>	1	0.10	10
<i>D. mojavensis</i>	<i>D. aldrichi</i>	<i>repleta</i>	1	0.60	10
<i>D. aldrichi</i>	<i>D. mojavensis</i>	<i>repleta</i>	1	0.31	10
<i>D. buzzatii</i>	<i>D. arizonae</i>	<i>repleta</i>	1	0.00	10
<i>D. arizonae</i>	<i>D. buzzatii</i>	<i>repleta</i>	1	1.00	10
<i>D. hamatofila</i>	<i>D. arizonae</i>	<i>repleta</i>	1	0.24	10
<i>D. arizonae</i>	<i>D. hamatofila</i>	<i>repleta</i>	1	0.07	10
<i>D. arizonae</i>	<i>D. peninsularis</i>	<i>repleta</i>	1	0.38	10
<i>D. hamatofila</i>	<i>D. buzzatii</i>	<i>repleta</i>	1	1.00	10
<i>D. mojavensis</i>	<i>D. buzzatii</i>	<i>repleta</i>	1	0.19	10
<i>D. buzzatii</i>	<i>D. peninsularis</i>	<i>repleta</i>	1	0.00	10

TABLE 2—Continued

Species 1 (males, females)	Species 2 (females)	Group	N	α'	Reference
<i>D. mojavensis</i>	<i>D. hamatofila</i>	<i>repleta</i>	1	0.16	10
<i>D. hamatofila</i>	<i>D. mojavensis</i>	<i>repleta</i>	1	0.51	10
<i>D. hamatofila</i>	<i>D. peninsularis</i>	<i>repleta</i>	1	0.31	10
<i>D. hamatofila</i>	<i>D. ritae</i>	<i>repleta</i>	1	0.00	10
Total			129	0.31 ± 0.04	

References: (1) Singh *et al.*, 1981; (2) Singh & Chatterjee, 1991a; (3) Dwivedi *et al.*, 1982; (4) Dobzhansky & Koller, 1938; (5) Mayr & Dobzhansky, 1945; (6) Dobzhansky *et al.*, 1957; (7) Stalker, 1942; (8) Patterson *et al.*, 1947; (9) Wasserman & Koepfer, 1977b; (10) Patterson, 1947.

intraspecific isolation is very often found intraspecifically when strains of different geographical origin are studied. (2) Isolation is intense but far from complete between close species. In Marín (1991), I discussed extensively the limitations of this type of model. The most important limitation is the possibility of interspecific male discrimination, which has been demonstrated in some cases (Wood & Ringo, 1980). However, although this problem may affect the values of α' estimated for interspecific crosses (Table 2), it does not affect the basic conclusion obtained for those crosses, i.e. that isolation is largely incomplete between species. The conclusions obtained are significant for discussions of the stability of the specific-mate recognition system (SMRS). Adherents of the Recognition concept of species (RC; Paterson, 1980, 1985), have argued that all the organisms of a species are characterized by having the same SMRS, defined as the system of signals used to recognize appropriate sexual partners (Paterson, 1989; White *et al.*, 1995). However, demonstrating that there is intraspecific isolation and that interspecific isolation is often incomplete argues against the possibility of using behavioral isolation as the way to define whether two organisms are conspecific. According to strict RC, some of the species as defined today should be split into several taxa (Table 1), while most of the species pairs considered in Table 2 should be lumped into single taxa. There are two alternatives to explain these contradictory conclusions. The first explanation is that the analysis is distorted by the fact that what we have been calling populations and species have been defined according to the biological species concept [as discussed in Marín (1994)], so this classification is based not only on pre- but also often on postzygotic isolation [and also on indirect evidences of isolation, as morphology, chromosomal or genetic differentiation, etc., see Coyne *et al.* (1988) and Marín (1994)] and can be largely in correct. This in fact has been suggested by White *et al.* (1990, p. 405). I will not discuss this unlikely possibility extensively. The congruency among the current classification and tests independent of any measure of

isolation, such as genetic distances obtained through the analysis of electrophoretic data [reviewed in Ayala (1975); MacIntyre & Collier (1986)], is well known. It is an open possibility for RC adherents to build a new classification of the genus, able to accommodate the results presented above, and show its advantages over the one currently used. The second and more acceptable explanation is the old wisdom that ethological differentiation is progressive, small between populations, larger but not necessarily complete among species. If this is the case, neither of the two following postulates are true: (i) "all the organisms of a species share the same SMRS" and (ii) "all the organisms of different species have different SMRS". Once the biunivocal correspondence between species and SMRS is falsified, it is impossible to apply the RC consistently.

An obvious criticism of the conclusions of this work is that they are based on laboratory experiments and it is not known whether they apply to natural conditions. However, to conclude that the results obtained are artifactual, it has to be hypothesized that the experimental situation produces behavioral changes which (1) cause positive assortative mating among strains at the same time and (2) eliminate the complete assortative mating that exists among species. I discussed previously the weakness of the evidence quoted as demonstrating that isolation appears artificially in the laboratory (Marín, 1994). The second possibility is, however, more reasonable; certainly laboratory conditions favor interspecific crosses. However, this can be due to two different causes. It is possible that an artificial breakdown of the isolation between species is produced because behavioral patterns suffer alterations under laboratory conditions. However, the alternative is that the experimental procedures just expose the basic "slippage" of the recognition system by increasing the number of opportunities for an error to occur. That a certain degree of slippage exists in the *Drosophila* genus is demonstrated by the fact that interspecific hybridization in natural conditions has been described for several pairs of species [reviewed in Bock,

(1984) and Kaneshiro (1990)]. Whether the flies indeed discriminate differently in the laboratory or that females are just exposed in the experiments to a courtship intensity by alien males that is hundreds of times stronger than that found in nature is an open question.

A general conclusion of this and my previous studies is that considerable difficulties exist in measuring the degree of reproductive isolation in *Drosophila*, even making simplified assumptions about how these organisms behave. As I cited in the introduction of this work, several classical discussions in evolutionary biology have been based on the results of *Drosophila* studies, without properly considering these difficulties. A good example is Coyne & Orr's study (1989), where the authors used a single index $[1 - (Ht/Hm)]$ to measure isolation in both multiple-choice and male-choice experiments, in order to analyse such basic problems as determining the rhythm of acquisition of behavioral isolation or whether prezygotic and postzygotic isolation appear in parallel. However, as can be easily deduced comparing Marín (1991) and this study, this index is not measuring the same parameters in both experimental designs unless very restrictive conditions are fulfilled. This follows logically from the fact that male-male competition may occur in multiple-choice but not in male-choice experiments. The conclusions of Coyne & Orr's (1989) and many other studies depend thus on particular assumptions, never stated by the authors, and intrinsic to the experimental designs classically used in *Drosophila*. My work establishes the need for a thorough re-evaluation of those conclusions.

REFERENCES

- ANDERSON, W. W. & EHRMAN, L. (1969). Mating choice in crosses between geographic populations of *Drosophila pseudoobscura*. *Amer. Midl. Natur.* **81**, 47–53.
- AYALA, F. J. (1975). Genetic differentiation during the speciation process. *Evol. Biol.* **8**, 1–78.
- AYALA, F. J. & TRACEY, M. L. (1973). Enzyme variability in the *Drosophila willistoni* group. VIII. Genetic differentiation and reproductive isolation between two subspecies. *J. Hered.* **64**, 120–124.
- AYALA, F. J., TRACEY, M. L., BARR, L. G. & EHRENFELD, J. G. (1974). Genetic and reproductive differentiation of the subspecies, *Drosophila equinoxialis caribbensis*. *Evolution* **28**, 24–41.
- BOCK, I. R. (1984). Interspecific hybridization in the genus *Drosophila*. *Evol. Biol.* **18**, 41–70.
- BRNCIC, D. & KOREF-SANTIBAÑEZ, S. (1965). Geographical variation of chromosomal structure in *Drosophila gasci*. *Chromosoma*, **16**, 47–57.
- CARMODY, G., DIAZ COLLAZO, A., DOBZHANSKY, TH., EHRMAN, L., JAFFREY, I. S., KIMBALL, S., *et al.* (1962). Mating preferences and sexual isolation within and between the incipient species of *Drosophila paulistorum*. *Amer. Midl. Natur.* **68**, 67–82.
- COHET, Y. & DAVID, J. R. (1980). Geographic divergence and sexual behaviour: comparison of mating systems in French and Afrotropical populations of *Drosophila melanogaster*. *Genetica*, **54**, 161–165.
- COYNE, J. A. & ORR, H. A. (1989). Patterns of speciation in *Drosophila*. *Evolution* **43**, 362–381.
- COYNE, J. A., ORR, H. A. & FUTUYMA, D. J. (1988). Do we need a new species concept? *Syst. Zool.* **37**, 190–200.
- DOBZHANSKY, TH. (1937). *Genetics and the Origin of Species*. New York: Columbia University Press.
- DOBZHANSKY, TH. (1944). Experiments on sexual isolation in *Drosophila*. III. Geographic strains of *Drosophila sturtevantii*. *Proc. Natl. Acad. Sci. U.S.A.* **30**, 335–339.
- DOBZHANSKY, TH. (1975). Analysis of incipient reproductive isolation within a species of *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3638–3641.
- DOBZHANSKY, TH. & KOLLER, P. CH. (1938). An experimental study of sexual isolation in *Drosophila*. *Biol. Zentralb.* **58**, 589–607.
- DOBZHANSKY, TH. & MAYR, E. (1944). Experiments on sexual isolation in *Drosophila*. I. Geographic strains of *Drosophila willistoni*. *Proc. Natl. Acad. Sci. U.S.A.* **30**, 238–244.
- DOBZHANSKY, TH. & PAVLOVSKY, O. (1971). Experimentally created incipient species of *Drosophila*. *Nature* **230**, 289–292.
- DOBZHANSKY, TH. & STREISINGER, G. (1944). Experiments on sexual isolation in *Drosophila*. II. Geographic strains of *Drosophila prosaltans*. *Proc. Natl. Acad. Sci. U.S.A.* **30**, 340–345.
- DOBZHANSKY, TH., EHRMAN, L. & KASTRITSIS, P. A. (1968). Ethological isolation between sympatric and allopatric species of the obscura group of *Drosophila*. *Anim. Behav.* **16**, 79–87.
- DOBZHANSKY, TH., EHRMAN, L. & PAVLOVSKY, O. (1957). *Drosophila insularis*, a new sibling species of the *willistoni* group. *Univ. Texas Publ.* **5721**, 39–47.
- DWIVEDI, Y. N., SINGH, B. N. & GUPTA, J. P. (1982). One-sided sexual isolation between *Drosophila takahashii* and *Drosophila pseudotakahashii*. *Experientia* **38**, 318.
- EHRMAN, L. (1966). Mating success and genotype frequency in *Drosophila*. *Anim. Behav.* **14**, 332–339.
- EHRMAN, L. (1970). Sexual isolation versus mating advantage of rare *Drosophila* males. *Behav. Genet.* **18**, 111–118.
- EHRMAN, L. & PARSONS, P. A. (1980). Sexual isolation among widely distributed populations of *Drosophila immigrans*. *Behav. Genet.* **10**, 401–407.
- EHRMAN, L. & PETIT, C. (1968). Genotype frequency and mating success in the *Willistoni* species group of *Drosophila*. *Evolution* **22**, 649–658.
- GIDDINGS, L. V. & TEMPLETON, A. R. (1983). Behavioral phylogenies and the direction of evolution. *Science* **220**, 372–377.
- GILBERT, D. G. & STARMER, W. T. (1985). Statistics of sexual isolation. *Evolution* **39**, 1380–1383.
- HENDERSON, N. R. & LAMBERT, D. M. (1982). No significant deviation from random mating of worldwide populations of *Drosophila melanogaster*. *Nature* **300**, 437–440.
- KANESHIRO, K. (1976). Ethological isolation and phylogeny in the planitibia subgroup of Hawaiian *Drosophila*. *Evolution* **30**, 740–745.
- KANESHIRO, K. (1990). Natural hybridization in *Drosophila*, with special reference to species from Hawaii. *Can. J. Zool.* **68**, 1800–1805.
- KESSLER, S. (1966). Selection for and against ethological isolation between *Drosophila pseudoobscura* and *Drosophila persimilis*. *Evolution* **20**, 634–645.
- KIM, Y.-K., EHRMAN, L. & KOEPFER, H. R. (1992). Developmental isolation and subsequent adult behavior of *Drosophila paulistorum*. I. Survey of the six semispecies. *Behav. Genet.* **22**, 545–556.
- KOEPFER, H. R. & FENSTER, E. J. (1991). Asymmetrical mating patterns between geographic strains of *Drosophila mercatorum*: a test of the Kaneshiro hypothesis. *Evolution* **45**, 455–458.
- LI, C. C. (1978). *First Course in Population Genetics*. Pacific Grove, California: The Boxwood Press.

- MACINTYRE, R. J. & COLLIER, G. E. (1986). Protein evolution in the genus *Drosophila*. In: *The Genetics and Biology of Drosophila*. Vol. 3e. (Ashburner, M., Carson, H. L. & Thompson, J. N., Jr, eds) pp. 39–146. London: Academic Press.
- MALOGOLOWKIN-COHEN, CH., SOLIMA SIMMONS, A. & LEVENE, H. (1965). A study of sexual isolation between certain strains of *Drosophila paulistorum*. *Evolution* **19**, 95–103.
- MARÍN, I. (1991). Sexual isolation in *Drosophila*. I. Theoretical models for multiple-choice experiments. *J. theor. Biol.* **152**, 271–284.
- MARÍN, I. (1994). Sexual isolation in *Drosophila*. II. Intraspecific variation in mate recognition systems. *J. Evol. Biol.* **7**, 303–314.
- MARKOW, T. A., FOGLEMAN, J. C. & HEED, W. B. (1983). Reproductive isolation in sonoran desert *Drosophila*. *Evolution* **37**, 649–652.
- MASTERS, J. C. & SPENCER, H. G. (1989). Why we need a new genetic species concept. *Syst. Zool.* **38**, 270–279.
- MAYR, E. & DOBZHANSKY, TH. (1945). Experiments on sexual isolation in *Drosophila*. IV. Modification of the degree of isolation between *Drosophila pseudoobscura* and *Drosophila persimilis* and of sexual preferences in *Drosophila prosaltans*. *Proc. Natl. Acad. Sci. U.S.A.* **31**, 75–82.
- MILLAR, C. D. & LAMBERT, D. M. (1985). The mating behavior of individuals of *Drosophila pseudoobscura* from New Zealand. *Experientia* **41**, 950–952.
- PATERSON, H. E. H. (1978). More evidence against speciation by reinforcement. *S. Afr. J. Sci.* **74**, 369–371.
- PATERSON, H. E. H. (1980). A comment on “mate recognition systems”. *Evolution* **34**, 330–331.
- PATERSON, H. E. H. (1985). The recognition concept of species. In: *Species and Speciation* (Vrba, E. S., ed.) pp. 21–29. Pretoria: Transvaal Museum.
- PATERSON, H. E. H. (1989). A view of species. In: *Dynamic Structures in Biology* (Goodwin, B., Sibatani, A. & Webster, G., eds) pp. 77–88. Edinburgh: Edinburgh University Press.
- PATTERSON, J. T. (1947). Sexual isolation in the *mulleri* subgroup. *Univ. Texas Publ.* **4720**, 32–40.
- PATTERSON, J. T. & WHEELER, M. R. (1947). Two strains of *Drosophila peninsularis* with incipient reproductive isolation. *Univ. Texas Publ.* **4720**, 116–125.
- PATTERSON, J. T., WHARTON, L. & STONE, W. S. (1947). Sexual isolation between members of the *virilis* group of species. *Univ. Texas Publ.* **4720**, 7–31.
- PETIT, C., KITAGAWA, O. & TAKAMURA, T. (1976). Mating system between Japanese and French geographic strains of *Drosophila melanogaster*. *Jap. J. Genet.* **51**, 99–108.
- PRAKASH, S. (1972). Origin of reproductive isolation in the absence of apparent genic differentiation in a geographical isolate of *Drosophila pseudoobscura*. *Genetics* **72**, 143–155.
- SINGH, B. N. & CHATTERJEE, S. (1985a). A study of sexual isolation among natural populations of *Drosophila ananassae*. *Rev. Brasil. Genet.* **8**, 457–463.
- SINGH, B. N. & CHATTERJEE, S. (1985b). Symmetrical and asymmetrical sexual isolation among laboratory strains of *Drosophila ananassae*. *Can. J. Genet. Cytol.* **27**, 405–409.
- SINGH, B. N. & CHATTERJEE, S. (1991a). No character displacement for reproductive isolation between *Drosophila bipectinata* and *Drosophila malerkotliana*. *Genome* **34**, 849–852.
- SINGH, B. N. & CHATTERJEE, S. (1991b). Evidence for incipient sexual isolation within *Drosophila bipectinata*. *Evolución Biológica* **5**, 105–113.
- SINGH, B. N. & CHATTERJEE, S. (1992). Intraspecific sexual isolation in *Drosophila*. *Ind. J. Exp. Biol.* **30**, 260–263.
- SINGH, B. N., DWIVEDI, Y. N. & GUPTA, J. P. (1981). Sexual isolation among three species of the *Drosophila bipectinata* species complex. *Ind. J. Exp. Biol.* **19**, 898–900.
- SPIETH, H. T. & RINGO, J. M. (1983). Mating behavior and sexual isolation in *Drosophila*. In: *The Genetics and Biology of Drosophila*. Vol. 3c. (Ashburner, M., Carson, H. L. & Thompson, J. N., Jr, eds) pp. 223–284. London: Academic Press.
- STALKER, H. D. (1942). Sexual isolation studies in the species complex *Drosophila virilis*. *Genetics* **27**, 238–257.
- WASSERMAN, M. & KOEPFER, H. R. (1977a). Cytological differentiation and sexual isolation between populations of *Drosophila nigricuria*. *J. Hered.* **68**, 100–104.
- WASSERMAN, M. & KOEPFER, H. R. (1977b). Character displacement for sexual isolation between *Drosophila mojavnensis* and *Drosophila arizonensis*. *Evolution* **31**, 812–823.
- WASSERMAN, M. & ZWEIG, H. (1991). Sexual preference for females reared on cactus media by *Drosophila pegasa* males. *Evolution* **45**, 433–435.
- WHITE, C. S., MICHAUX, B. & LAMBERT, D. M. (1990). Species and Neo-Darwinism. *Syst. Zool.* **39**, 399–413.
- WHITE, C. S., LAMBERT, D. M. & FOSTER, S. P. (1995). Chemical signals and the recognition concept. In: *Speciation and the Recognition Concept. Theory and Application* (Lambert, D. M. & Spencer, H. G., eds) pp. 301–326. Baltimore: The Johns Hopkins University Press.
- WOOD, D. & RINGO, J. M. (1980). Male mating discrimination in *Drosophila melanogaster*, *D. simulans* and their hybrids. *Evolution* **34**, 320–329.
- ZOUROS, E. & D'ENTREMONT, C. J. (1980). Sexual isolation among populations of *Drosophila mojavnensis*: response to pressure from a related species. *Evolution* **34**, 421–430.