Sexual isolation in *Drosophila*. II. Intraspecific variation in mate recognition systems

Ignacio Marín*

Departamento de Genética y Microbiología, Unidad de Genética, Universidad Autónoma de Barcelona, Bellaterra 08193 (Barcelona), Spain

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Abstract

A simple behavioral model is used to investigate whether differences in the specific-mate-recognition system (SMRS), occur within species of the *Drosophila* genus. This model takes into account, and overcomes, the distorting effect of vigor differences on experimental results. Analysis shows significant deviations from the expected values under the assumption of identical SMRSs in around one fifth of the multiple-choice experiments performed with natural strains of twelve different *Drosophila* species. Different selection procedures raise the number of significant assortative mating results between strains of *D. melanogaster* and *D. pseudoobscura* from 3.0% to 32.8%. Finally, sub- or semispecific taxa show variations in their SMRS even more frequently (74.5%). Differences in male vigor and female receptivity are also found. These results show that a classification of *Drosophila* species based on SMRS stability, as proposed by the "Recognition concept of species", is virtually impossible.

Introduction

The "Biological species concept" (BSC), at the core of the modern neodarwinian theory of speciation, defines species as "groups of interbreeding natural populations, reproductively isolated from other such groups" (Mayr, 1988). Thus, two organisms are classified as belonging to different species if they can not interbreed,

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* Present address: Department of Biological Sciences. Stanford University, Stanford, California 94305. U.S.A.

whatever the factors involved, be they pre- or postzygotic. An alternative view, first stated by Paterson (1978, 1985) is the "Recognition concept of species" (RC). This considers that species are self-defining, each species having a unique "specific-mate-recognition system" (SMRS), that is, a set of characteristic signals that allow an individual to recognize an appropriate sexual partner. The proponents of this theory affirm that it makes no sense to study postzygotic isolation: "postzygotic isolation . . . is not a real biological property that is sufficient to describe a species" (White et al., 1990). This controversy actually arises from the difficulties of applying any species definition to particular taxa (the "expansion" from a nondimensional concept to a multidimensional situation, as expressed by Mayr (1982, 1988)). Under favourable circumstances, this problem can be submitted to experimental testing. Two different classifications, each one based on a species concept, can be developed and compared, to see which is more appropriate according to our current knowledge of the classified taxa.

This study investigates this question, taking as a model the *Drosophila* genus, the only case in which both a large number of experimental studies on reproductive isolation among related species and a well developed theory to interpret their results exist. In the *Drosophila* genus, the delimitation of species taxa has been rigorously investigated. As can be expected for such a complex group of species, their classification does not depend just on the outcome of reproductive isolation experiments. In fact, a large number of *Drosophila* species hybridize under laboratory conditions, exceptionally producing fertile hybrids of both sexes; some cases of hybridization in nature have also been described (Bock, 1984). However, when other characters have been studied to establish the status of specific or subspecific taxa, the different taxonomic criteria have shown to be congruent. For example, genetic distances, as measured by allozymic differentiation, are correlated with reproductive isolation (Ayala, 1975; Coyne and Orr, 1989), and can be used to build meaningful phylogenetic trees (MacIntyre and Collier, 1986). On the other hand, chromosomes have been an indispensable tool for classifying those groups where clusters of morphologically and reproductively very close species are common (Wasserman, 1982). Finally, detailed morphological studies and biogeography have also helped to understand some complex groups of closely related species (Dobzhansky and Powell, 1975; Val, 1977; Fontdevila et al., 1988, 1990). In spite of this multidisciplinary approach, the current classification of *Drosophila* taxa is strictly based on the BSC. When direct evidence of reproductive isolation in nature is not available, as occurs when considering allopatric taxa, efforts have been focused on inferring the potential degree of reproductive isolation under eventual sympatry in the wild. Moreover, the purpose of studying different characters is to obtain indirect estimates of reproductive isolation in nature. Despite the problems found when classifying certain taxa, the final decision had to be consistent with the BSC.

In this study, I consider the problem of determining an RC-based classification, and compare it with the current BSC-based one. To do this, differences in empirically-determinable distinctive features have to be looked for. A main point of difference between the two species concepts concerns the stability of the SMRS. Proposers of the RC state that a species SMRS is stable throughout its range, while

adherents to the BSC quote examples of SMRS variation within species (Coyne et al., 1988). These contradictory predictions are a good target for a theoretical analysis.

In a previous paper (Marín, 1991), simple models for analyzing the results of multiple-choice experiments were developed. They depended on three basic parameters: 1) Male competitive ability (C), a sex-limited mating propensity component, characteristic of each type of male in a given multiple-choice experiment, that determine their probability of being involved in a courtship; 2) Female receptivity (R), the percentage of females prone to mate; and 3) The coefficient of females acceptance (A), the percentage of receptive females that are disposed to mate with a certain type of male. These models rely on the simplest behavioral assumptions, single parameters for intrasex differences as well as for intersex interactions, and can be used to distinguish deviations from random mating that can be explained simply by mating propensity differences from those that have to be attributed to sexual isolation.

In this study, the results from 27 previous investigations of different *Drosophila* species are analyzed using this minimal behavioral model. The main questions are: 1) How often are modifications of behavioral parameters found in different populations of a *Drosophila* species?; 2) Are there any differences when subspecies or semispecies are considered in spite of the absence of changes in intrasubspecific or intrasemispecific populations of the same species?; 3) What parameters are sensitive to selection under laboratory conditions?.

Methods

A full exposition of the models for multiple-choice experiments was presented in Marín (1991). The simplified model assuming discrete preferences (Marín, 1991, p. 274) is used in this study. I have selected from the literature those experiments that fit the conditions of the model as described there. In particular, only experiments with the same number of males and females have been considered, to avoid eventual "rare male" effects. Under these conditions, the formulae of Table 1 are deduced. As previously shown, from the results of just one multiple-choice experiment it is impossible to establish the actual degree of sexual isolation, even under very favourable assumptions (Marín, 1991). For this reason, this paper is devoted to estimate the percentage of cases in which either assortative mating, differences in female receptivities or differences in male vigor exist.

The method used to analyze the results started from the assumption that there was no discrimination ($a = b = 1$, being a and b the coefficients of female acceptance). Expected frequencies for each cross were then calculated (from formulae 2.1 to 2.4 in Tab. 1) and compared with the observed data. Deviations were tested by means of a chi-square test with one degree of freedom at the 95% level of significance. If the deviations were not significant, it was assumed $a = b = 1$. Differences in male vigor could then be tested by determining if they were significant ($p < 0.05$) deviations of C'_X (the maximum likelihood estimate of C_X, the males X vigor parameter) from the 0.5 value, expected if there was no significant difference (Formulae 2.5 and 2.6). The maximum likelihood estimate for R (relative

Table 1. Formulae, derived from the model of discrete preferences (Marín, 1991), used for analyzing the results of multiple-choice experiments.

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(1) (1.1) $R' = (n_1 + n_2)/(n_3 + n_4)$

   (1.2) $V(R') = (R'(R' + 1)^2)/N$

(2) If $a = b = 1$

   (2.1) $E(X, X) = (n_1 + n_2)(n_1 + n_3)/N$

   (2.2) $E(Y, X) = (n_1 + n_2)(n_2 + n_4)/N$

   (2.3) $E(X, Y) = (n_1 + n_3)(n_3 + n_4)/N$

   (2.4) $E(Y, Y) = (n_2 + n_4)(n_3 + n_4)/N$

   (2.5) $C'_x = (n_1 + n_3)/N$

   (2.6) $V(C'_x) = (n_1 + n_3)(n_2 + n_4)/N^3$

R': maximum likelihood estimate for relative female receptivity (R_x/R_y). a, b: coefficients of female acceptance for females X and Y respectively. $a = b = 1$ means that there is no assortative mating. C'_x: maximum likelihood estimate for males X vigor. $E(A, B)$: expected number of matings of males A with females B. n_1, n_2, n_3, n_4: observed number of matings (X, X), (Y, X), (X, Y) and (Y, Y) respectively. N: total number of observed matings.

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female receptivity) was obtained independently from the assumption of $a = b = 1$, as demonstrated in Marín (1991) (Formula 1.1). Deviation of R from the unit, i.e. a significant ($p < 0.05$) difference between the receptivities of the two types of females, was also tested by using formulae 1.1 and 1.2 in Table 1. Because of the significance level chosen, approximately 5% of the tests should be statistically significant due to type 1 errors. The number of true deviations will therefore be smaller than the number of significant differences. This problem, however, does not affect the conclusions.

Assortative mating, here taken as a deviation from the expected result $a = b = 1$, was considered as a demonstration of differences in the SMRS of the strains tested, following implicit assertions by Coyne et al. (1988) and White et al. (1990). To compare results within or between Tables 2–4, the test of equality of percentages (Sokal and Rohlf, 1981) was used.

Interactions between the sexes are not considered in this theoretical model. Although studies such as Cobb and Jallon (1990) have shown that male courtship can be stimulated by chemical signals produced by females, for our purposes, it does not matter whether a male courts more frequently due to an intrinsic property or to an increased stimulation by the females present in the experiment. We will simply asume that deviations from random mating that can be explained by differences in male activity, as occurred when a given type of male pairs more frequently than another with both homo- and hetero-typic females, do not imply changes in the SMRS. Moreover, because results from different experiments are not compared, applying the model does not require that C is an invariant genetically-determined behavioral male component, as Marín (1991) postulated. It might change from experiment to experiment as female stimulation of males varies.

Results

Tables 2 to 4 show the estimations of deviations in the behavioral parameters a, b, R and C from 27 different previously reported *Drosophila* studies. Table 2 corresponds to intraspecific crosses between strains of different geographical origin. Table 3 shows subspecific or semispecific crosses. Finally, Table 4 summarizes the results of those experiments in which laboratory-induced changes have been tested.

Around one fifth of the crosses between natural strains of *Drosophila* species show deviations from the expected values under the assumption of no discrimination by females (Tab. 2). However, results are significantly heterogeneous. In some well-studied species, such as *D. melanogaster* or *D. pseudoobscura*, deviations from $a = b = 1$ are exceptional $(3.0 \pm 2.1\%)$ while in other species, as those of the *willistoni* group, they are frequent $(55.6 \pm 9.7\%)$. *Repleta* group species are intermediate $(23.1 \pm 8.4\%)$. Differentiation is higher in subspecific or semispecific taxons (Tab. 3). In particular, there is a clear tendency towards assortative mating

Table 2. Estimations of deviations of behavioral parameters for strains of different geographical origin in the *Drosophila* genus.

Species (group)		No. of crosses	Deviations from			Refs.
			$a = b = 1$	$R = 1$	$C_x = 0.5$	
D. melanogaster	(*melanogaster*)	38	0/38	6/38	2/38	1
		1	1/1	0/1	–	2
D. pseudoobscura	(*obscura*)	9	0/9	0/9	1/9	3
		8	0/8	2/8	0/8	4
		7	1/7	2/7	3/6	5
		3	0/3	0/3	0/3	6
		1	0/1	0/1	0/1	7
D. willistoni	(*willistoni*)	6	2/6	0/6	2/4	8
D. equinoxialis	(*willistoni*)	6	3/6	1/6	0/3	8
		4	1/4	0/4	3/3	9
D. tropicalis	(*willistoni*)	10	8/10	1/10	0/2	8
D. paulistorum	(*willistoni*)	1	1/1	0/1	–	7
D. mojavensis	(*repleta*)	8	2/8	0/8	0/6	10
D. nigrospriacula	(*repleta*)	3	0/3	0/3	0/3	11
D. mettleri	(*repleta*)	1	0/1	0/1	0/1	11
D. pachea	(*repleta*)	7	1/7	0/7	1/6	11
D. nigricruria	(*repleta*)	3	1/3	1/3	1/2	12
D. mercatorum	(*repleta*)	4	2/4	0/4	2/2	13
Total		120	23/120	13/120	15/97	
Deviations ± s.e. (%)			19.2 ± 3.6	10.8 ± 2.8	15.5 ± 3.7	

References: 1) Henderson and Lambert (1982); 2) Cohet and David (1980); 3) Anderson and Ehrman (1969); 4) Millar and Lambert (1985); 5) Millar and Lambert (1986); 6) Prakash (1972); 7) Ehrman (1966); 8) Ehrman and Petit (1968); 9) Ayala et al. (1974); 10) Zouros and D'Entremont (1980); 11) Markow et al. (1983); 12) Wasserman and Koepfer (1977); 13) Koepfer and Fenster (1991).

Table 3. Estimations of deviations of behavioral parameters in subspecific or semispecific taxons.

Species (group)		No. of crosses	Deviations from			Refs.
			$a = b = 1$	$R = 1$	$C_x = 0.5$	
D. pseudoobscura subsp.	(obscura)	3	0/3	0/3	0/3	1
D. willistoni subsp.	(willistoni)	2	2/2	0/2	–	2
D. equinoxialis subsp.	(willistoni)	7	2/7	0/7	3/5	3
D. paulistorum semisp.	(willistoni)	19	19/19	9/19	–	4
		1	0/1	0/1	0/1	5
D. mojavensis subsp.	(repleta)	11	9/11	3/11	1/2	6
		2	2/2	0/2	–	7
D. mercatorum semisp.	(repleta)	6	4/6	1/6	2/2	8
Total		51	38/51	13/51	6/13	
Deviations ± s.e. (%)			74.5 ± 6.2	25.5 ± 6.2	46.2 ± 14.4	

References: 1) Prakash (1972); 2) Dobzhansky (1975); 3) Ayala et al. (1974); 4) Malogolowkin-Cohen et al. (1965); 5) Dobzhansky and Pavlovsky (1971); 6) Zouros and D'Entremont (1980); 7) Markow et al. (1983); 8) Koepfer and Fenster (1991).

Table 4. Estimations of deviations of behavioral parameters following selection

Species (group)		No. of crosses	Deviations from			Refs.
		$a = b = 1$	$R = 1$	$C_x = 0.5$		
D. melanogaster	(melanogaster)	4	2/4	0/4	0/2	1
		2	1/2	0/2	0/1	2
		3	0/3	0/3	0/3	3a
		1	1/1	0/1	–	3b
		3	0/3	2/3	2/3	4
		2	2/2	1/2	–	5
D. simulans	(melanogaster)	5	1/5	5/5	4/4	6
D. pseudoobscura	(obscura)	38	7/38	4/38	12/31	7
		36	11/36	5/36	12/25	8a
		13	0/13	1/13	1/13	8b
		16	14/16	1/16	0/2	9
		1	1/1	0/1	–	10
Total		124	40/124	19/124	31/84	
Deviations ± s.c (%)			32.3 ± 4.2	15.3 ± 3.2	36.9 ± 5.3	

References and characteristics selected: 1) Markow (1981) (phototaxis, geotaxis); 2) Coyne and Grant (1972) (escape reaction); 3) Grant and Mettler (1969) (escape reaction, a): disruptive, b): directional); 4) McKenzie and Parsons (1971) (escutelar chaeta number); 5) Crossley (1974) (sexual isolation) 6) Ringo et al. (1986) (flush-crash populations); 7) Dodd and Powell (1985) (flush-crash populations); 8) Powell (1978) (a: flush-crash populations, b: inbred populations); 9) Del Solar (1966) (phototaxis, geotaxis); 10) Ehrman (1966) (temperatures).

(74.5 ± 6.2% of the crosses). It is remarkable that, in spite of this tendency, differentiation between the subpsecies of *D. psdudoobscura* has not been found (Prakash, 1972).

All but one of the laboratory experiments designed to induce reproductive isolation recorded in Table 4 were performed with *D. melanogaster* or *D. psdudoobscura*. The percentage of deviations from $a = b = 1$ for stocks of these species (32.8 ± 4.3%) is significantly higher than their natural differentiation (3.0 ± 2.1%) ($p < 0.001$). Male competitive ability differences are also significantly increased after selection. Considering only the data from *D. melanogaster* and *D. pseudoobscura*, it varies from 9.2 ± 3.6% in natural strains to 33.8 ± 5.3% in selected stocks ($p < 0.001$). R deviations for the same species are not affected by selection (14.9 ± 4.4% in nature against 11.8 ± 3.0% after laboratory treatment).

Discussion

This study is a first attempt to apply a model that considers the behavior of *Drosophila* individuals in order to detect whether the outcome of a choice experiment is explained by the presence of assortative mating or by mating propensity differences. It compiles data from studies of intraspecific differentiation in sexual behavior, as detected in multiple-choice experiments, in order to draw as general as possible a picture of the *Drosophila* genus. Generally, the authors of these studies arrived at similar conclusions to those reported here. Nevertheless, because their analysis were based on isolation indices, that can be affected by male and female mating propensity differences (Marín, 1991), our approach is preferable.

Stability of the SMRS

Variability in the SMRS exists within species. Under different selection regimes, it has been possible to alter the behavior of *Drosophila* stocks, inducing a tendency towards homogametic mating (Tab. 4). However it is necessary to explain why the percentage of experiments where assortative mating has been found is not high (31.1%) and why some experiments have produced negative results. These failures could be due to the fact that, except for one experiment (Crossley, 1974), selection was not on reproductive isolation, but on other characters, with testing being carried out after selection in order to see if mate choice had been indirectly affected. The rationale of most of the experiments shown in Table 4 was to test whether modifications in sexual behavior were associated with different populational changes or with selection for other morphological or behavioral characters. When direct selection on courtship characters, such as mating speed, has been attempted, rapid responses have been found (Manning, 1961; Kessler, 1968, 1969; Singh and Chaterjee, 1988). Selection for sexual isolation has also usually been performed indirectly, by using as progenitors for the next generation those individuals that come from homogametic matings (detected because they had a certain phenotype,

such as a visible mutation). Although theoretical models show that this indirect selection pressure is not very effective, depending on factors such as the linkage of assortative mating loci and the loci under selection (Felsenstein, 1981; Sved, 1981a, b), isolation has been obtained (Knight et al., 1956; Crosley, 1974).

A second aspect concerns the stability of SMRS in natural populations. Our analysis confirms the assertions of Coyne et al. (1988), about the variation of SMRS in natural populations, at least within certain species. Table 2 summarizes the analysis os 120 multiple-choice experiments performed with natural strains of twelve species that belong to four different groups of the *Drosophila* genus. 19.2% of the experiments show assortative mating between strains. However, this average masks an extreme heterogeneity in results when different species are considered. This variability cannot be attributed only to random changes in SMRS due to long-term laboratory culture as suggested by White et al. (1990). Some strains that showed significant differences were tested soon after collection (Ehrman and Petit, 1968). In fact, Miller and Lambert's (1986) results, quoted by White et al. (1990) as a proof that long periods of laboratory culture induce changes in SMRS, can be interpreted as being produced by changes in male competitive ability, not as indicative of modifications in the SMRS. In their experiments, only one out of seven crosses shows a significant tendency towards homogametic mating (Tab. 2). Variations of male and female mating propensities can be induced by inbreeding (Ringo et al., 1987 and references therein). The same process probably explains Miller and Lambert's (1986) results. As Tables 2 and 4 show, changes in male competitive ability are frequently found intraspecifically, both in natural and laboratory conditions. Distinguishing them from assortative mating is one of the main interests of the procedure used in this study.

Most *Drosophila* subspecies or semispecies have different SMRSs. There is also a higher number of significant differences in R and C. All these results point to a high genetic differentiation in behavioral patterns when these intraspecific taxa are considered, and they are consistent with the neodarwinian scenario of parallel increases in ethological and postzygotic differentiation eventually leading to species formation (Coyne and Orr, 1989). It is worth emphasizing that the intraspecific taxa considered in this study were classified according to partial postzygotic isolation or (in the case of *D. mojavensis* subspecies) because of their chromosomal and morphological differentiation. They were subsequently chosen for studies of ethological isolation because of their interest as putatively incipient species, but there is not a single case in which there taxa have been exclusively classified according to their sexual isolation.

The subspecies of *D. pseudoobscura*, whose SMRSs seem to be identical, constitute an exception. This is a feature that cannot be explained by a recent origin, because Nei's genetic distance between these subspecies is similar to those found in the other intraspecific taxa (reviewed in Coyne and Orr, 1989). It is significant that the SMRS of strains of *D. melanogaster* (no subspecies or semispecies found in spite of their cosmopolitan distribution) and of *D. pseudoobscura* (only one subspecies known, but without any differentiation in the SMRS) are so invariant. These results reinforce the impression that differences exist between species for stability of their SMRS.

Two different explanations have been advanced to explain SMRS stability. Firstly, the "external" explanation of selective constraints acting against changes in the SMRS which is considered to be adapted to the current environment of the species. A second explanation is that the SMRS is internally stabilized by developmental constraints. This second aspect has been emphasized by some proponents of the RC (White et al., 1990) although others consider both factors to be equally important (Paterson, 1985; Masters et al., 1987). The finding of differences in SMRS stability in related groups of species has to be considered in this context. It is possible to envisage differences in the populational structure of *Drosophila* species, for example greater or lesser fragmentation in small demes, rates of migration among populations, etc., that affect the probability of random fixation of altered behavioral patterns. A number of genes have been found which produce changes in sexual attractivity or courtship behavior (Tompkins, 1986; Belote and Baker, 1987; Markow, 1987; Hall and Rosbash, 1988; Bernstein et al., 1992). Changes in SMRS will depend on the genetic basis of those behavioral modifications which are able to produce assortative mating. If they do not depend on more than a few genes, "dialects" in the mate recognition system would be expected to emerge frequently. After that, with a probability which depends on populational parameters and genetic linkage, they will occasionally become fixed. The neodarwininan view that intraspecific ethological differentiation is dependent on populational structure and increase with time in isolated populations, is consistent with the results of this study. SMRS is not sufficiently conservative within *Drosophila* species to require special explanations for its stability.

Biological species concept versus Recognition concept

Using a strict RC method, the taxonomic status of several of the *Drosophila* species analyzed would have to be reexamined. Because subspecies and semispecies have frequently diverged in their SMRS, they would have to be considered full species. New "species" would have been produced in the laboratory by a variety of selection schemes. Proposers of the RC have tried to avoid these conclusions by considering that experiments have been wrongly performed (using strains which have been cultured in the laboratory for many years, White et al., 1990) or that differences were not in the SMRS but in other "peripheral" characters such as "familiarity, conditioning, environmental influences and size effects" that can induce assortative mating (Masters and Spencer, 1989). The first criticism has been answered above. It is unlikely that this bias exists. The second point is difficult to answer, because every example of assortative mating could be attributed to "peripheral" effects. In the absence of data showing the influence of external factors on experimental outcome, we have to rely on the evidence and interpret it as due to real differences in the mate-recognition system.

It can be controversial if some subspecific taxa deserve full species status, a problem that merits further investigation. However, our analysis has shown that within well-established species changes in SMRS occur both in natural and in

experimental conditions. The conclusion of this study is that the mate-recognition system is so unstable that it is not a useful criteria for defining species, at least in the genus *Drosophila*. RC proposers have overemphasized the importance of prezygotic factors in species definition. A mate recognition system exists, and it would indeed be optimal to define species by an intrinsic characteristic, that is, every species, according to its own SMRS. The question is if SMRS is the "right" character for defining species or if we have to consider several characters, including postzigotic differentiation, in order to meaningfully classify living beings. Because this study shows that a classification based strictly on the recognition concept of species is anarchic, the second option has to be favoured.

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