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Role of sexual behaviours in sexual isolation in *Drosophila*

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ABSTRACT. *Role of sexual behaviours in sexual isolation in Drosophila.-* A question of interest to evolucionists addresses the role of sexual behaviour in speciation. A review of the literature on sexual isolation between closely related species of *Drosophila* shows that changes in sexual behaviour are minor and quantitative rather than qualitative. These changes diminish but without excluding interespecific crosses, suggesting that the basic organization of courtship is similar in related species. The conclusion arrived at is that courtship behaviour is very stable from an evolutionary point of view, and it is difficult to accept that changes in this trait can propitiate speciation in *Drosophila.*

KEY WORDS. Sexual behaviour, Sexual isolation, Hybridization, *Drosophila*

Introduction

Species and speciation are two central points in evolutionary biology. Many biologist have long been engaged in a rich debate about the concept of species, and the controversy is far from finished (Cracraft, 1989; Templeton, 1989). Although there are many definitions of species, it is widely recognized that the use of a particular species concept depends on the organisms studied, and on the evolutionary questions with which researchers are concerned. We are interested in the mechanisms and processes that originate and maintain isolation between species, that is, those directly related with reproductive aspects. In this context, there are today two different and widely accepted species definitions: The biological species concept (BSC) (Mayr, 1963; Dobzhansky, 1970) and the recognition species concept (RSC) (Paterson, 1985).

The BSC defines the species in terms of the pre- and postmating isolation mechanisms that act to prevent gene flow between populations. Accordingly, the isolating mechanisms are fundamental characteristics of a species, in such a way that all processes related with their origin and maintenance are important aspects of the speciation process. In contrast, the RSC focuses upon all normal functions and activities that facilitate mating and reproduction among the members of a population. The species have specific recognition mating systems that promote syngamy among conspecific individuals. Modifications of these systems may make reproduction difficult among individuals of different populations, giving rise to premating isolation barriers. Therefore, the isolation barriers are not active parts of the speciation process; rather, they arise as byproducts of the evolution of other functions (Paterson, 1980, 1985). The RSC places emphasis on the causality of speciation and provides a good conceptual framework from which we will proceed to discuss the evolution of sexual behaviours and their role in species isolation. In this respect, we will center on the following crucial question:

Can sexual behaviour develop into an isolating mechanism, that is to say, are modifications of sexual behaviour, arising from small cumulative steps, sufficient to ensure reproductive isolation?

Drosophila is a good organism for behavioural studies that can help us to answer this question. The more interesting characteristics of the genus *Drosophila* are:

-It comprises more than 800 species.

-Many species can successfully be reared in laboratory conditions, thus facilitating quantitative and qualitative analyses.

-The courtship behaviour of numerous species has been observed and its basic elements characterized (Sturtevant, 1915, 1921, 1942; Bastock & Manning, 1955; Brown, 1964, 1966; Welbergen et al., 1987, etc.).

-Reproductive isolation has been studied in many species.

-There are many morphological and behavioural mutants which facilitate genetic and behavioural research.

The Hawaiian drosophila fauna, which comprises around 350 *Drosophila* species, is not considered here. Owing to the particular geological, environmental and biotic variables of the Hawaiian archipelago, some specific evolutionary agents, infrequent in the continental fauna, have favoured the occurrence of a fast and prolific speciation, with species showing bizarre sexual behaviours that are worthy of a separate study.

Sexual behaviour

Mating, in the genus *Drosophila*, is preceded by a sequential display of stereotyped activities performed by both sexes in a continuos interplay that constitutes the so-called sexual courtship. Although each species has a particular repertoire of signals, all the drosophilids present common patterns of courtship, whose most relevant features appear in table I, based on Spieth (1974), Spieth and Ringo (1983) and Cobb et al. (1985).

During courtship, as a rule, males supply females with a battery of stimuli: auditory, based on wing vibration, chemical, through the release of pheromones, and perhaps mechanical, from the different kinds of contact that occur during courtship. Females supply males with visual acceptance or rejection signals, and secrete different cuticular pheromones that the male receives by olfaction and contact. The final outcome of courtship depends upon the physiological state of the female. A female needs a given amount of courtship before accepting mating. If she is receptive, the summation of the male courtship stimuli lowers her threshold acceptance and mating is performed (Manning, 1966).

Detailed studies of the patterns and sequence of courtship in *D. melanogaster* show that transitions between different male and female postures and displays can occur in varied ways, indicating that the *D. melanogaster* male's courtship, although it follows a characteristic pattern, is flexible in its manifestations (Sustare, 1978, Wood et al., 1980; Welbergen et al., 1987). These studies and direct observations of courtship also show that although males usually display all their major courtship elements, a given male does not need to perform all these to achieve mating. The same conclusion is provided by laboratory experiments using different mutant strains. The ebony, white, vermilion and brown mutations reduce the male ability to orient

TABLE I. Major courtship elements in *Drosophila*.
[Elementos principales del cortejo en *Drosophila*.]

	MALES
Orientation:	The male takes up and maintains a position near the female with his body axis oriented directly towards her.
Wing vibration:	The oriented male extends the wing nearest to the female and vibrates it for brief periods.
Tapping:	The male touches some part of the female's body with the tarsus, usually with one of the first pairs of legs.
Licking:	The male extends his proboscis and licks the female's genitalia.
Attempted copulation:	The male grasps the female's abdomen with his foretarsi. The female's abdomen is raised up by the licking movement and the male's abdomen is curled downward and forward and attempts introduction.
Copulation:	Male mounting female. The copulation time is about 20 minutes.
	FEMALES
ACCEPTANCE BEHAVIOR	
Wing spreading:	Female spreads both wings and holds them extended until copulation.
Genital spreading:	Female drops her abdomen, extrudes genitalia and spreads ovipositors apart.
REPELLING BEHAVIOR	
Decamping:	Female responds to courting male by running, jumping or flying.
Kicking:	Female kicks vigorously backwards at the male with the hind legs.
Abdomen elevation or abdomen depression:	Female elevates or depresses her abdomen and inhibits male courtship actions.
Extrusion:	Extension and elongation tip of abdomen. The extended member is thought to be the ovipositor, but on some occasions, she can extrudes and retracts an egg.

upon and maintain contact with females and determine inappropriate wing vibration (Reed & Reed, 1950; Rendel, 1951; Bastock, 1956; Jacobs, 1961; Geer & Green, 1962; Connolly et al., 1969; Grossfield, 1972; Burnett & Connolly, 1973); vestigial mutants do not perform wing vibration (Rendel, 1951), aristal mutants do not maintain orientation towards females (Burnet et al., 1971), olfaction-deficient (olfD) mutants show diminished responses to a variety of compounds which otherwise act as attractants or repellents to normal flies (Gailey et al., 1986). In all these cases, males do not provide females with fully adequate stimuli or do not respond adequately to female signals; however, given sufficient time, mating occurs. Then a first statement, to which we will return later, is that the pattern of courtship is very flexible, as a way to ensure reproduction, even if only deficient courting males are disposable.

In accordance to the previous comments, there are specific patterns of male courtship, but at the same time, a perfect male's courtship display is not necessary to achieve mating. These considerations suggest the following question: Can sexual behaviours be genetically modified by selective forces in an appreciable and quick fashion? A first consideration centers on the genetic bases underlying sexual behaviour. Many laboratory experiments have been carried out in several *Drosophila* species demonstrating the occurrence of between- and within-species genetic variability in mating characteristics, such as copula duration, male vigour or mating speed. Biometrics studies have evidenced an additive genetic component for several aspects of courtship and, in close agreement, selection experiments have been effective in changing major male courtship signals, such as wing vibration or frequency of licking (table II). These results clearly suggest that both natural and sexual selection, as well as other evolutionary agents, are able to modify

TABLE II. Experimental manipulation and genetic analyses of courtship elements.
[Manipulación experimental y análisis genético de elementos del cortejo.]

Character	Species	Method	Author (year)
Mating speed	*melanogaster*	Artificial selection	Manning (1961;1963)
	pseudoobscura	F1, F2, backcrosses	Kessler (1968;1969)
	melanogaster	Diallel crosses	Parsons (1964;1965)
Male mating speed	*simulans*	Artificial selection	Manning (1968)
	melanogaster	Diallel crosses	Fulker (1966)
	melanogaster	Diallel crosses	Casares et al. (1993)
Female mating speed	*melanogaster*	Diallel crosses	Casares et al. (1992)
	melanogaster	Artificial selection	Piñeiro et al. (1993)
Wing display	*melanogaster*	Recording	McDonald (1979)
	simulans	Recording	Wood & Ringo (1982)
Courtship song	*pseudoobscura*	Song recording	Ewing (1969)
	persimilis	Song recording	
	melanogaster	Song recording	Kawanishi & Watanabe (1981)
	simulans	Song recording	
Wing vibration, licking, attempted copula, copulation, orientation	*melanogaster*	Diallel and Cavalli crosses	Collins & Hewitt (1984)

some elements of courtship, at least in a quantitative way.

If mating is amenable to genetic change, the next step is to examine the courtship patterns of evolutionary related species. Studies of this kind have been done in the *melanogaster* group of species (Cowling & Burnet, 1981; Cobb et al., 1985; Welbergen et al., 1987), in the *obscura* group (Brown, 1964, 1966), semispecies of *D. paulistorum* (Koref-Santibañez, 1972a, b), in the *mesophragmatica* group (Koref-Santibañez & del Solar, 1961; Koref-Santibañez, 1963), in the subgroup *nasuta* of the *immigrans* group (Spieth, 1969), in the *virilis* group (Hoikkala, 1986), etc. As a rule, all four aspects of male courtship, speed, frequency, sequence and form or emphasis, rarely differ dramatically between closely related species and differences are quantitative rather than qualitative (Spieth, 1958). For example, the male *melanogaster* and the male *simulans* display the same 16 different courtship elements, although they differ in the intensity or in the frequency of manifestation of several of them (Welbergen et al., 1987). All the above suggests that the courtship behaviour of each species evolves by gradual modification of pre-existing behaviour rather than by the sudden appearance of new behavioural elements (Spieth & Ringo, 1983).

So, are the quantitatively modified patterns of courtship sufficient to guarantee sexual isolation between related species?

Sexual isolation

Sexual isolation is a premating isolating mechanism that occurs when individuals of different taxa meet but do not mate. Sexual isolation between remote species is generally intense but it is usually partial between related species, semispecies or races, mainly in laboratory conditions.

Sexual isolation can be ascertained in a variety of ways:

-In nature. If two species occur sympatrically and their hybrids are viable, females can be caught in the wild and examined in the laboratory for hybrid progeny production. Studies of this kind are scarce due to the difficulty of identifying hybrids which, generally, have little viability. When it has been possible, it has been found that the frequency of natural hybridization is low to extremely low and concerns closely related species (Bock, 1984).

-In the laboratory. There are two different basic methodologies. (1) Males and females from two species are put together so that they can make some sort of "choice". Many studies have been done using this method. For example, Mayr & Dobzhansky (1945) when studying isolation between *D. pseudoobscura* and *D. persimilis*; Patterson et al. (1947) and Patterson & Stone (1949) between five species of the *virilis* group; Merrell (1954) in the pair *D. pseudoobscura* and *D. persimilis*; Dobzhansky et al. (1968) testing isolation between five species of the *obscura* group; Wasserman & Koepher (1977) with *D. mojavensis* and *D. arizonensis*; Watanabe & Kawanishi (1979) between *D. melanogaster*, *D. simulans* and *D. mauritiana*; Kurokawa et al. (1982) between four sibling species of *D. auraria* complex. Results show that, in general, most matings are homospecific indicating a high grade of sexual isolation between related species. (2) "Non-choice" tests, in which males from a species are confined with females from another species. This methodology was first used by Sturtevant (1920) with *D. melanogaster* and *D. simulans*, and since then, many studies have been published in the species groups *repleta*, *virilis*, *melanica*, *robusta*, *funebris*, *guarani*, *mesophragmatica*, *tripunctata*, *cardini*, *quinaria*, *immigrans*, *melanogaster*, *obscura*, *willistoni* and *saltans* (reviewed by Bock, 1984). The summary of these studies is that heterospecific crosses between related species occur with relative ease, and that the lack of a full sexual isolation can be considered more the rule than the exception.

As we have just seen, there are contrasting views between what can be concluded about species sexual isolation from choice and non-choice studies. Which of the two tests is more adequate is largely a matter of opinion. We think that the laboratory non-choice test yields a good image of normal encounters between different species in the field, and is useful for defining sexual isolation: Individuals meet but do not mate (or mate). In consequence, we are more inclined to agree with the conclusion that full sexual isolation between related species has by no means been achieved yet.

The fact that related species can hybridize in the laboratory is not surprising if we consider the resemblance in their male courtship. It is true that any modification of the normal sexual behaviour can increase courtship duration and diminish the probability of mating; but it is also true that if a female is intensely courted by a male from a closely related species, the probability she hybridizes is high. This is to say that sexual isolation is not guaranteed by modifications of sexual behaviours so slight as those found in related species. On the other hand, we must also consider the previously mentioned great flexibility of the female to accept mating. This is particularly evident in the pair *D. melanogaster* and *D. affinis* (Mc Robert & Tompkins, 1986), and in *D. albomicans* and *D. nasuta* (Chang & Ayala, 1989), in which despite the very different courtship exhibited by their males, hybridization occurs easily.

Nonrandon mating

For evolutionists, a more attractive question than estimating the degree of sexual isolation between species is determining how isolation can develop from populations of the same species. A first problem for researchers in this subject is the way of measuring incipient sexual isolation between conspecific individuals. The classic methodology is to use one or several of the male, female or multiple-choice tests, from which the numbers of homo and heteropopulational matings are recorded and used to calculate isolation indices. There are many studies in which sexual isolation has been measured in this manner using very different materials, a review of which, although not exhaustive, appears in table III.

The use of choice tests to evaluate sexual isolation between strains or populations of the same species, although widely used, has two important associated problems. The first deals with the desirability of the choice tests for measuring isolation. In these tests we obtain a "relative" measure of the tendency of different sexes and genotypes to mate between them. But if we search for an "absolute" measure of sexual isolation we must use the no-choice test, i.e., males and females from different source put together. It is certain that if we apply this test to conspecific individuals we will not find any evidence of sexual isolation between them, even for those cases showing signification in the choice tests. The second problem involves the analysis of the choice test results. It has long been recognized that mating propensities and mate discrimination influence the outcome of these tests (Bateman, 1949; Schaeffer, 1968; Gilbert & Starmer, 1985), but there is no statistical analysis for obtaining separate measurements of the two traits. This is a conceptually important problem, since discrimination between individuals of two populations implies some type of incipient isolation between them, whereas the finding of different mate propensities is a simple proof of the expected genetic variation among populations of the same species (Bateman, 1949; Merrell, 1950, 1960; Bryant, 1979; Van den Berg et al., 1984). Thus, the

TABLE III. A review of nonrandom mating tests carried out in some *Drosophila* species from different sources. [Revisión de test de apareamientos no al azar llevados a cabo en algunas especies de *Drosophila.*]

Drosophila species	References
Between populations with different geographic origin	
melanogaster	Petit et al., 1976; Cohet & David, 1980; Henderson & Lambert, 1982; Lambert & Harper, 1985; Lambert & Henderson, 1986
simulan	Ringo & Wood, 1980; Carracedo et al., 1993
pseudoobscura	Anderson & Ehrman, 1969
equinoxialis	Ayala et al., 1974; Ehrman & Petit, 1968
immigrans	Ehrman & Parsons, 1980
tropicalis	Ehrman & Petit, 1968
willistoni	Ehrman & Petit, 1968
Between lines selected for non-sexual characters	
melanogaster	Mather & Harrison, 1949; Koref-Santibañez & Waddington, 1958; Parsons, 1965; Burnet & Connolly, 1974; Van Dijken & Scharloo 1979; Kilias et al., 1980; Markow, 1981; Kilias & Alahiotis, 1982
pseudoobscura	Ehrman, 1964; del Solar 1966; Dood & Powell 1985; Dood, 1989
Between inbreeding lines	
melanogaster	Merrell, 1949; Koref-Santibañez & Waddington, 1958; Hoenigsberg & Koref-Santibañez, 1960; Averhoff & Richardson, 1974
pseudoobscura	Powell & Morton, 1979
simulans	Ringo et al., 1986; Ringo et al., 1987
Between different mutants	
melanogaster	Merrell, 1949; Koref-Santibañez & Waddington, 1958; Bosiger, 1962; Geer & Green, 1962
suboscura	Rendel, 1945
pseudoobscura	Tan, 1946

finding of a significant isolation index should be taken with caution and complemented with other sorts of tests before concluding that it is evidence of incipient isolation. Despite these problems, in many studies the finding of significant choice tests has been taken as evidence of sexual isolation instead of differential mating activities of the strains being tested.

In summary of the above, the following points may be highlighted: (i) The courtship patterns of *Drosophila* species are very flexible to accommodate a wide norm of reaction; (ii) The same basic courtship elements are present in related species, and species differences in courtship are quantitative more than qualitative; (iii) Hybridization between related species is the rule rather than the exception; and (iv) Differences in courtship within populations are scarce and do not cause sexual isolation.

What about the role of the sexes in sexual isolation? It is generally agreed that in insects and especially in drosophilids, females are more important than males in deciding mating. As for their role in sexual isolation, there are conflicting views. Some authors hypothesized that females are important in determining the degree of sexual isolation between closely related species of *Drosophila* (Merrell, 1949, 1954; Bateman, 1949); others, that males are the primordial agents of sexual isolation (Spieth, 1952, 1974), while others suggest that the relative importance of each sex varies from case to case (Schilcher & Dow, 1977; Wood & Ringo, 1980).

As previously mentioned, a female needs a given amount of male courtship before mating, and this amount is directly dependent on her receptivity (Manning, 1962). When a female encounters a male of another species and receives courtship that in some aspects differs from the normal conspecific courtship, the probability that she hybridizes is high if her receptivity is high but low if this is low. Thus it can be considered that low receptive females are more discriminating than high receptive ones and have less risk of producing non-viable offspring (Bateman, 1948, 1949; Merrell, 1950, etc.). This is a "passive" sort of female discrimination in which the female does not make an active choice. It follows from this that the level of female receptivity could be directly involved in the degree of sexual isolation, and we have obtained results that illustrate this point in the pair of sibling species *Drosophila melanogaster* and *D. simulans*.

We have for several years worked on the genetics of reproductive isolation between these species and, particularly, on the possible relationship between male and female homospecific sexual traits and sexual isolation. *Drosophila melanogaster* and *D. simulans* are sympatric species, morphologically very similar, between which there exists a total reproductive isolation since hybrids have little viability and are sterile. Sexual isolation is not total. Hybridization occurs more often in the cross between *D. melanogaster* females and *D. simulans* males than in the reciprocal case, and it is rare in nature but relatively easy in the laboratory.

Hybridization

We measure hybridization between *D. melanogaster* females and *D. simulans* males using the no-choice method as follows: Individuals of each sex and species, freshly emerged, are introduced into a vial with food for five days and then, each female is placed into a vial to see if she leaves hybrid progeny. We have examined several populations (fig. 1) from which the more important conclusions are: (i) There are notable interpopulation differences in hybridization values; (ii) There is notable intrapopulation genetic variation for hybridization in both species; (iii) The females contribute more to genetic variation than the males (Carracedo & Casares (1985); Carracedo et al. (1987, 1991).

The finding of genetic variation for hybridization, a trait supposedly subjected to disappearance by the action of natural selection, suggested to us that some characteristic of the normal sexual behaviour could also be involved in hybridization success. We focused our attention on one of the most important sexual traits of the female: her receptivity, defined as the proclivity of sexually mature females to accept conspecific males.

We evaluated female receptivity as the time elapsed from the introduction of individuals of both sexes (virgin of 3 days old) into a vial, until copulation. As a first approximation, we took eight isofemale lines of *D. melanogaster* from two different populations, lines characterized by their high or low hybridization values, and we examined their receptivity (Carracedo et al., 1987). The Spearman's ranks correlation estimated between hybridization and female receptivity was $r_s = 0.82$ ($p < 0.01$) indicating that the *D. melanogaster* female lines with higher receptivity were those hybridizing more

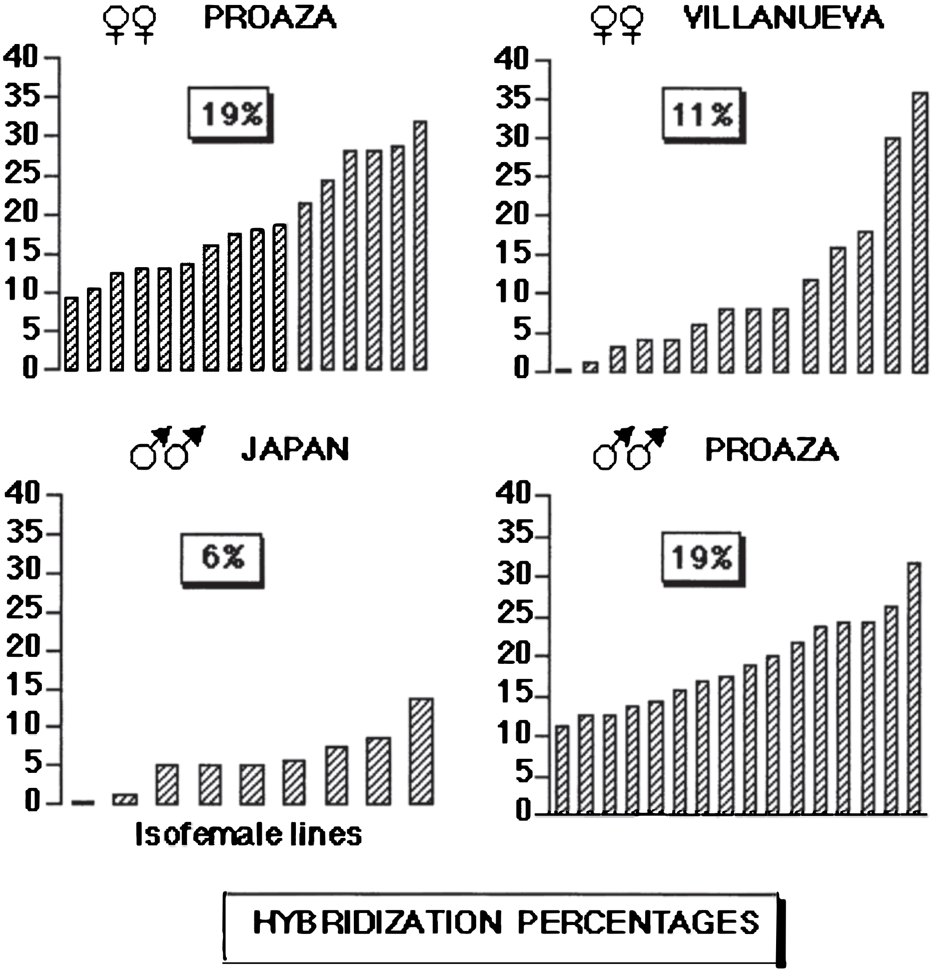

FIGURE 1. Between and within population differences in the frequency of hybridization of *Drosophila melanogaster* females with *D. simulans* males.

[Diferencias entre y dentro de poblaciones en la frecuencia de hibridación de hembras de *Drosophila melanogaster* con machos de *D. simulans.*]

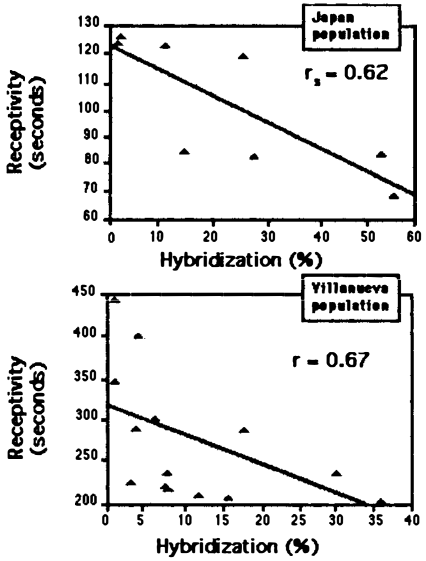

FIGURE 2. Spearman's (r_s) and Pearson's (r) correlations between female receptivity and frecuency of hybridization in isofemale lines of two populations.

[Correlaciones de Spearman (r_s) y de Pearson (r) entre receptividad de hembras y frecuencia de hibridación en líneas de hembras iguales de dos poblaciones.]

TABLE IV. Hayman's analysis of variance of female receptivity in a 5x5 diallel cross.

[Análisis de varianza de Hayman de la receptividad de la hembra en un cruce diadélico 5x5.]

Component	DF	MS[a]	F
a	4	179.70	44.64 *
b	10	13.91	3.45 *
b1	1	3.77	0.94
b2	4	7.03	1.74
b3	5	21.44	5.33 *
c	4	4.18	1.04
d	6	4.57	1.14
ERROR	325	4.02	

[a]multiplied by 10^{-3} * $P < 0.001$

frequently with *D. simulans* males (top of fig. 2). To see if this important result was general or only particular to our selected lines, we analyzed hybridization and receptivity of 14 isofemale lines taken at random from a recently caught population. We found an important correlation between both traits (r=0.67 $P<0.008$) (the graphic representation is at bottom of fig. 2) (Carracedo et al., 1991). Thus, we can generalize that hybridization between *D. melanogaster* females and *D. simulans* males is mostly determined by the level of female receptivity.

With this conclusion in mind, it is now easy to imagine that in the sites in which both species coexist and use the same substrates to eat, court and ovoposit, natural selection acting against the more receptive *melanogaster* females (therefore highly likely to hybridize) could be counteracted by natural selection for the same females favouring fast mating between conspecific individuals. There should be a balance between these two opposite selective forces acting in the population, one in the intraspecific level, for sexual selection, and the other in the interspecific level, for sexual isolation. So perhaps for this reason it has been possible and logical to

TABLE V. Mean values of female receptivity and hybridization in the selected lines and in the control.

[Valores medios de la receptividad de hembras e hibridación en las líneas seleccionadas y en el

Line	Receptivity (seconds)	Hybridization (%)
High receptivity (H1)	255	35
High receptivity (H2)	343	43
Low receptivity	2307	2
High hybridization	165	79
CONTROL	836	10

TABLE VI. Analysis of variance of chromosome contribution for receptivity and hybridization. In parenthesis, the degrees of freedom of hybridization.

[Análisis de varianza de la contribución del cromosoma para la receptividad e hibridación.]

		Receptivity	Hybridization
Source of variation	d f	MS	MS
X chromosome	1	0.8014	212.65 **
Chromosome II	1	0.0150	2071.39 ***
Chromosome III	1	8.4190 ***	1457.90 ***
X chromosome * chromosome II	1	0.4920	8.66
X chromosome * chromosome III	1	0.1985	39.53
Chromosome II * chromosome III	1	0.1290	660.87 ***
Three-way interaction	1	0.0011	12.58
Error	792 (8)	0.2575	4.72

* $P< 0.05$; ** $P<0.01$; *** $P< 0.001$

find intrapopulation genetic variation for hybridization, a variation that can simply reflect genetic variation in female receptivity for *D. melanogaster*.

Natural selection can increase or decrease female receptivity only if this trait has genetic variation, mainly of the additive type. In a diallel analysis of female receptivity we found that a large part of the observed genetic variation was additive (table IV) indicating that receptivity is amenable to genetic change by natural selection in the examined population. This result encouraged a deeper analysis of the relationship between receptivity and sexual isolation by carrying out two programs of artificial selection in the same population used in the later work: one, for increasing hybridization between *melanogaster* females and *simulans* males, which was successful since hybridization increased from 10 to 79% (Izquierdo et al., 1992) in only 12 generations; the other, for increasing and decreasing receptivity, was carried out for 16 generations (Piñeiro et al., 1993). The selection was successful and we obtained two lines with high (4'15"; 5'43") and one line with low female receptivity (38'27"). The mean time of the base population was 13'56".

At the end of the two selection processes, the selected lines were tested for receptivity and hybridization (table V). Again, we found a clear association between the two traits (Spearman's correlation=-0.9), indicating that manipulation of their genetic systems did not modify their close relationship. Our finding also suggests that both traits have the same genetic system or that most of the genes involved in the two traits are common. To learn about it, we carried out a chromosomal study to find the location of the genes involved in female receptivity and hybridization. We accomplished a chromosome substitution analysis in two of the lines selected for high and low levels of receptivity and hybridization. From crosses of these lines with a laboratory stock marked whit several mutations and inversions, we constructed eight substitution lines, each with a different combination of the X, II and III chromosomes (analyses of variance, to determine the influence of each chromosome in both traits, appear in table VI). Genes for female receptivity are located in the chromosome III, and for hybridization in the three chromosomes, mainly in the II and III. The analysis indicates that the genetic systems determining both traits are different, although it is possible that the genes located in the chromosome III are common. This result does not invalidate the fact that receptivity of *melanogaster* females is of paramount importance in maintaining sexual isolation with males of *D. simulans*.

It does not escape our minds that the conclusions we have found in the pair *D. melanogaster* and *D. simulans* could be applied to other sympatric species between which hybridization is relatively frequent. If hybridization is, at least in part, a measure of the female propensity to mate, and if males of related species court their own and foreign females with similar intensity, it follows that female receptivity could be an important element in determining sexual isolation. In this context, we hypothesize that natural selection could act against highly receptive females to avoid the undesirable consequences of producing inviable or sterile hybrids. From this, we also venture the working hypothesis that sympatric populations of newly formed species must have lower receptivity levels than allopatric ones.

Summary

It has been said that mating behaviour is an adaptive trait of fundamental significance, that has evolved to facilitate fertilization, and comprises a set of elements that constitute a Specific Mate Recognition System (Paterson, 1980). Adaptive changes in mating behaviour can only occur in small steps with coadaptation between male and females being re-established at each step. Therefore, mating evolves slowly, and differences between related species are minor and mainly of a quantitative nature, as we have seen previously. Mating shows a great phenotipic flexibility, that is, individuals have a broad norm of reaction which facilitates mating under an ample diversity of conditions and between individuals that do not exhibit all elements of the specific courtship behaviour.

Answering the question posed at the beginning of this dissertation, as to whether sexual behaviour can develop into an isolation barrier, we have seen that sexual isolation between related species is far from being complete, since the occurrence of interspecific crosses is a quite common event. Therefore, sexual isolation between evolutionarily close species is not guaranteed by the appearance of small, cumulative changes in courtship elements.

We have demonstrated that highly receptive *D. melanogaster* females have a great probability of accepting foreign *D. simulans* males. This fact suggests that although the males of the two species display somewhat different courtship elements, both are recognized and accepted by the *D. melanogaster* females, despite the large time elapsed since the divergence of these species. The basic elements of the Mate Recognition System are present in males of both species, which is evidence of its stability, and supports the general agreement that premating mechanisms appear later in the evolution of *Drosophila*.

Our results also suggest that a way to minimize the negative effects of hybridization is through natural selection for scarcely receptive females.

Resumen

Papel de los comportamientos sexuales en el aislamiento sexual en Drosophila

Se ha dicho que el comportamiento de apareamiento es un rasgo adaptativo de significado fundamental, que se ha desarrollado para facilitar la

fertilización, y comprende un conjunto de elementos que constituyen un Sistema de Reconocimiento Específico de Pareja (Paterson, 1980). Los cambios adaptativos en el comportamiento de apareamiento pueden ocurrir solamente en pequeñas pasos, reestableciéndose la coadaptación entre macho y hembra en cada paso. Por tanto, el apareamiento evoluciona lentamente, y las diferencias entre especies relacionadas son pequeñas y principalmente de tipo cuantitativo, como se ha visto previamente. El apareamiento muestra una gran plasticidad fenotípica, es decir, los individuos tienen una amplia norma de reacción que facilita el apareamiento bajo una amplia diversidad de condiciones y entre individuos que no presentan todos los elementos del comportamiento específico de cortejo.

Para responder a la cuestión planteada al principio de esta conferencia, de si el comportamiento sexual puedellegar a ser una barrera de aislamiento, hemos visto que el aislamiento sexual entre especies relacionadas está muy lejos de ser completo, ya que la aparición de cruzamientos interespecíficos es un suceso bastante común. Por tanto, el aislamiento sexual entre especies evolutivamente cercanas no se garantiza por la aparición de pequeños cambios acumulativos en los elementos del cortejo.

Hemos demostrado que hembras altamente receptivas de *D. melanogaster* tienen una gran probabilidad de aceptar machos de *D. simulans*. Este hecho sugiere que aunque los machos de las dos especies realizan algunos elementos del cortejo diferentes, ambos son reconocidos y aceptados por las hembras de *D. melanogaster*, a pesar del largo tiempo transcurrido desde la divergencia de estas especies. Los elementos básicos del Sistema de Reconocimiento de Pareja estan presentes en los machos de ambas especies, lo que evidencia su estabilidad, y apoya el acuerdo general de que los mecanismos de preapareamiento aparecen mas tarde en la evolución de *Drosophila*.

Nuestros resultados también sugieren que una forma de minimizar los efectos negativos de la hibridación es a través de la selección natural para hembras escasamente receptivas.

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