Chromosomal substitution analysis of receptivity and sexual isolation in *Drosophila melanogaster* females

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A chromosome substitution analysis was carried out in *Drosophila melanogaster* to elucidate the contribution of the X, II and III chromosomes to female receptivity, and to sexual isolation with its sibling species *D. simulans*. Two lines from the same base population with extreme phenotypes, the one showing high receptivity and weak isolation and the other showing low receptivity and strong isolation, were used. Genes involved in receptivity were located in chromosome III whereas those affecting sexual isolation were located in all the three chromosomes. These results indicate that the direct relationship between female receptivity and sexual isolation previously detected in *D. melanogaster* could be because of the occurrence of genes common to the two traits in the chromosome III.

Keywords: chromosomal analysis, *Drosophila melanogaster*, *Drosophila simulans*, female receptivity, sexual isolation.

Introduction

Drosophila melanogaster and *D. simulans* are sibling species with reproductive isolation based on ethological differences that make mating difficult, and on partial inviability and total sterility of their hybrid progenies. However, despite these premating and postmating isolation mechanisms, both species hybridize in nature and in the laboratory, mainly in the *D. melanogaster* female $\times$ *D. simulans* male direction (Sturtevant, 1920, 1929; Manning, 1959; Sperlich, 1962; Carracedo & Casares, 1985).

The determination of the genetic bases of characters involved in premating isolation mechanisms and the understanding of their evolution from homospesic behaviours are two basic aspects of speciation studies. In this respect, several studies have demonstrated that the break-up of sexual isolation in these species is influenced by the level of female receptivity in such a way that the faster females in homospesic mating are those that hybridize more frequently with *D. simulans* males (Carracedo *et al.*, 1987, 1991). To examine the nature of this relationship in depth it is necessary to know if the correlation between hybridization and receptivity has a genetic component.

It has been shown that hybridization and female sexual receptivity have interpopulational and intrapopulational variation (Parsons, 1972; Watanabe *et al.*, 1977; Carracedo & Casares, 1985; Carracedo *et al.*, 1991). Genes involved in hybridization appear to be polygenic with additive effects, as is shown by both the response to artificial selection (Eoff, 1975, 1977; Izquierdo *et al.*, 1992) and the results of crosses between lines with high and low hybridization values (Carracedo *et al.*, 1989). Female receptivity also has an important genetic determination characterized by additive genes and some dominance for high levels of expression, as has been found in a diallel study (Casares *et al.*, 1992), and in a successful artificial selection programme (Piñeiro *et al.*, 1993).

That the above correlation between female receptivity and hybridization is in part genetic has been demonstrated by Carracedo *et al.* (1991) in a populational study using isofemale lines. This genetic correlation can appear if: (i) both traits are pleiotropic effects of the same genetic system, and (ii) the genes underlying the two traits are located in the same chromosome and there is between them linkage disequilibrium maintained by selection (Lande, 1980; Falconer, 1989). To investigate these alternatives, it would be of great interest to know the number and location of genes affecting both traits.

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The chromosome substitution analysis allows the estimation of the relative effects of genes on each chromosome on a particular character (Mather, 1942; Mather & Harrison, 1949; Robertson, 1954; Mather & Jinks, 1982). It is often the first step in analysing the biological basis of *Drosophila melanogaster* phenotypes, mainly those involved in quantitative and/or behavioural traits (Kearsey & Kojima, 1967; Mac Bean *et al.*, 1971; Pyle, 1978; Sokolowski, 1980; Bauer & Sokolowski, 1985; Ricker & Hirsch, 1985, 1988; Fukui & Gromko, 1991; Piñeiro, 1992; Ruiz-Dubreuil & Köhler, 1994).

In this paper we report a chromosome substitution analysis from two *D. melanogaster* lines showing extreme phenotypes for receptivity and hybridization. Both lines came from the same base population and were achieved through independent artificial selection programmes.

Materials and methods

Strains

Selection lines were obtained from a *D. melanogaster* base population having 10 per cent hybridization frequency and a mean time to mating (the standard measure of receptivity) of 11 min. Hybridization and receptivity were estimated as detailed below.

Selection for increasing hybridization between *D. melanogaster* females and *D. simulans* males was successful (Izquierdo *et al.*, 1992) giving rise to a *D.*

melanogaster line, called S, with 80 per cent hybridization. This line showed a receptivity mean time of 4 min 23 s at the end of the selection process.

Selection for decreasing homospecific receptivity was also successful (Piñeiro *et al.*, 1993) and the resulting U line showed a receptivity mean time of 38 min 27 s and 2 per cent of hybridization frequency at the end of the selection.

The substitution lines were obtained using a *D. melanogaster* stock with the chromosome balancers Binscy (In(1) *sc^{SIL} sc^{8R} + dl 49, y^{c4} sc⁸ sc^{s1} B*), SM5 (In(2LR) SM5, *al² Cy lt^v sn² sp²*) and TM3 (In(3LR) TM3, *y⁺ ri p^p sep Sb bx^{34e} e^s*) in chromosomes X, II and III, respectively.

Substitution lines

Following the scheme used by Kearsey & Kojima (1967), the cross of the S and U selection lines with the multiple inversion stock produced eight substitution lines, each with a different combination of the X, II and III chromosomes from the S and U lines (Fig. 1). The IV chromosome was ignored in this analysis.

Other specific lines are described in the corresponding tests.

Receptivity test

One virgin female from one chromosome substitution line and two tester males were aspirated into a

Fig. 1 Mating scheme employed to obtain eight chromosome substitution lines consisting of every possible homozygous combination of the X, II and III chromosomes from the lines selected for low female receptivity (U line) and for high female hybridization (S line).

vial with food and the time to mating in seconds was registered as a measure of receptivity. All the individuals were 3 days old. The tester males came from a *D. melanogaster* iosfemale line, named M-33, that was also used in the selection process for female receptivity (Piñeiro *et al.*, 1993). A total of 100 replicates for each chromosome substitution line were made.

Flies were observed for a maximum of 30 min. The raw data (in seconds) were log-transformed to obtain a normal distribution (Dow, 1976). Because not all flies mated in the observation period, a procedure outlined by Manning (1961) was followed that permits the extrapolation of mean and variances from truncated data, that is, from the observed mating times only. The cumulative percentages of females mated during the test were transformed into probit values and plotted against the corresponding log-transformed mating times in seconds. This procedure gives a good straight-line plot from which we estimate the mean mating time (Piñeiro *et al.*, 1993).

Measurements were performed in the morning in a 21°C thermoregulated room.

Hybridization test

Ten *D. melanogaster* females from one chromosome substitution line and 10 *D. simulans* males, all newly emerged, were placed in a vial with food. Males came from a line selected for increased hybridization success with *D. melanogaster* females (Izquierdo *et al.*, 1992). Five days later, males were discarded and females transferred individually to fresh food vials. The presence of larvae in these vials was taken as evidence of hybridization. The measurement cri-

terion provides the same results as dissection of mated females (M. C. Carracedo unpublished data). Three hundred females per chromosomal substitution line were tested in two sets of 150 females each.

Results

Receptivity

The means and standard errors of the log-transformed mating times are shown in Table 1. The effect for each of the three chromosomes was evaluated by a $2 \times 2 \times 2$ factorial ANOVA (Table 2). Only chromosome III had a highly significant effect and none of the interactions among chromosomes was significant at the 0.05 level. In accordance with these results, genes involved in female receptivity are located in chromosome III.

Hybridization

The hybridization percentages of the two replicates, each based on 150 females, are shown in Table 1. The hybridization values of both replicates were compared by a test of paired data on the arcsine scale. No differences were detected ($t_7 = 0.015$, $P = 0.99$). The effect of each chromosome on hybridization was estimated from the data of the two replicates after transforming percentages to angles. The factorial ANOVA (Table 2) shows significant effects of the three chromosomes and interactions between chromosomes X–III and II–III. These results indicate that the genes involved in female sexual isolation are located in all the major chromosomes, the X chromosome being the least important.

Table 1 Means and standard errors in log of seconds of the mating times (female receptivity) and percentages of hybridization found in each of two replicates ($N = 150$) and in the pooled data ($N = 300$), for each chromosome substitution line

Lines	Female receptivity	Hybridization		
	Mean $\pm$ SE	Replicate I	Replicate II	Pooled data
SSS	2.77 $\pm$ 0.05	77.33	72.00	74.66
SSU	3.29 $\pm$ 0.08	74.66	70.66	72.66
SUS	2.78 $\pm$ 0.06	64.00	64.00	64.00
SUU	3.33 $\pm$ 0.07	10.00	12.66	11.33
USS	2.78 $\pm$ 0.04	66.66	70.66	68.66
USU	3.51 $\pm$ 0.11	52.00	47.33	49.66
UUS	3.01 $\pm$ 0.06	51.33	51.33	51.33
UUU	3.61 $\pm$ 0.10	5.33	4.00	4.66

Table 2 Three-way factorial ANOVAS of the mating times in log of seconds (female receptivity) and of the arcsin transformed percentages of hybridization

Source of variation	Female receptivity		Hybridization	
	d.f.	MS	d.f.	MS
X	1	0.80	1	212.57***
II	1	0.01	1	2071.16***
III	1	8.42***	1	1458.10***
X × II	1	0.49	1	8.65
X × III	1	0.20	1	39.49*
II × III	1	0.13	1	660.87***
X × II × III	1	0.00	1	12.58
Error	792	0.26	8	4.52

* $P < 0.05$, *** $P < 0.001$.

Correlation

The existence of a relation between female hybridization and receptivity is an important aspect on which this study is based. To estimate the correlation between the mean values of receptivity and hybridization we used a nonparametric test as substitution lines were not a random sample of genotypes. The Spearman rank test (Siegel & Castellan, 1988) was significant ($r_s = -0.83$, $P = 0.028$) indicating that the most receptive females (i.e. with shorter mating times) were those that hybridized more frequently. Thus, a clear and direct relationship between these two female behaviours was again found.

Discussion

The strength of our analysis is limited as we performed the chromosomal substitution from selected lines that belong to the same base population (Carracedo *et al.*, 1991). Analyses from other populations or strains will be necessary to generalize our conclusions. However, some aspects about the genetic correlation between female hybridization and receptivity have been sufficiently clarified, mainly the demonstration of the existence of different genetic systems for both traits.

The analysis of chromosome substitution lines shows that large-effect genes for mating speed are located in chromosome III, whereas hybridization genes are in the three major chromosomes, the interaction between chromosomes II and III being very important. From these results it is clear that different genetic systems are involved in receptivity and sexual isolation, although the important correlation between both sexual traits found here and in

other studies using different lines and populations (Carracedo *et al.*, 1987, 1991) strongly suggests that genes in chromosome III could be common to the two characters. This should not be too surprising because males from *D. melanogaster* and *D. simulans* display quite similar courtship behaviours (Cowling & Burnet, 1981; Cobb *et al.*, 1985; Welbergen *et al.*, 1987) and highly receptive *D. melanogaster* females mate with both types of males. In fact, it is supposed that females are faced with a compromise between two opposite aspects of the mating behaviour: sexual selection and conspecific recognition on one side and the need for species discrimination on the other. In this sense, low receptivity and high species discrimination is one of the ends in the female mating behaviour spectrum whereas high receptivity and low discrimination is the other end (Carracedo *et al.*, 1987).

As mentioned in the Introduction, genes affecting sexual isolation between *D. melanogaster* and *D. simulans* seem to be mostly polygenic with additive effects and our results suggest that these genes have accumulated in autosomes more than in the X chromosome. A similar location of premating isolation genes in autosomes has been reported by Coyne (1989, 1992) in females of the *simulans* complex (*D. simulans*, *D. sechellia* and *D. mauritiana*). These data, together with our results, contrast strongly with *Drosophila* postmating isolation studies in which, as a rule, the involvement of the X chromosome is much greater than that of the autosomes; in fact, the different locations of pre- and postmating isolation genes are the expression of their different evolutionary pressures and patterns (Charlesworth *et al.*, 1987; Coyne & Orr, 1989; Orr, 1989; Orr & Coyne, 1989; Coyne, 1991; Cabot *et al.*, 1994).

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