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Absence of recombination in males of *Drosophila mediopunctata*.

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Abstract

A century ago Morgan reported for the first time that *Drosophila* males do not show recombination. Since then, geneticists have taken advantage of this property to carry out genetic analysis. However, males of a few species of the genus show some recombination. Here, we report the results of an experiment to assess the recombination frequency in *D. mediopunctata* males. We did not find a single recombinant among 1522 flies from the backcross of double heterozygote males for two distant second chromosome mutations. Thus, recombination in males is either absent or very rare (at most 0.2%, upper 95% CI).

Introduction

For the last 25 years we have been working with *Drosophila mediopunctata* to transform this species into a model organism for evolutionary and population genetics (Klaczko, 2006). We have repeatedly obtained scattered data showing that, as in many other *Drosophila* species, there is no recombination in males, as first reported for *D. melanogaster* by Morgan (1912). Assuming this premise, we have carried out experiments of genetic (chromosomal) analysis (*e.g.*, Carvalho and Klaczko, 1993; Hatadani *et al.*, 2004). There are, however, a few species of *Drosophila* showing male recombination, such as *D. ananassae* (Kikkawa, 1938; Hinton, 1970), and *D. willistoni* (Franca *et al.*, 1968). Thus, in this paper we try to assess the recombination rate in *D. mediopunctata* males.

Materials and Methods

Two sets of double heterozygote mutant (**DHM)** lines of flies were obtained through appropriate crossings (not shown) among strains **CR26A, CR32C,** and **AT34M4** (Hatadani *et al.*, 2004). The flies in **both sets** carry the dominant mutation *Delta* (*Δ*) and the recessive mutation *merlot* (*mt*) in the second chromosome and in the homologous chromosome the wild allele for both loci (*Δ mt* / + +). The first line (**DHM-1**) is homozygous (*DV-PC0/DV-PC0*) for a second chromosome haplotype (Ananina *et al.*, 2002). The flies from the other line (**DHM-2**) are heterozygous (*DV/DS*) in the distal portion of the second chromosome, where *Delta* is located, but are homozygous for the proximal inversion *PC0.* Thus, the karyotype is *DV-PC0/DS-PC0* for these flies (Figure 1).

Males and females of both **DHM-1** and **DHM-2** lines were crossed separately with females or males, respectively, of **CR26A**, a strain which is *DV-PC0/DV-PC0* and homozygous for *merlot* (+ *mt* / + *mt***)**. The analysis of the flies of the progeny allowed us to detect in each cross the frequencies of recombinants (phenotypes Delta or merlot) and parental types (phenotypes Delta–merlot or wild) (see Figure 1).

All crosses were carried out at 18°C in half pint bottles using trimeveledon culture medium (Carvalho *et al.*, 1989). Flies were transferred to new bottles every other day.

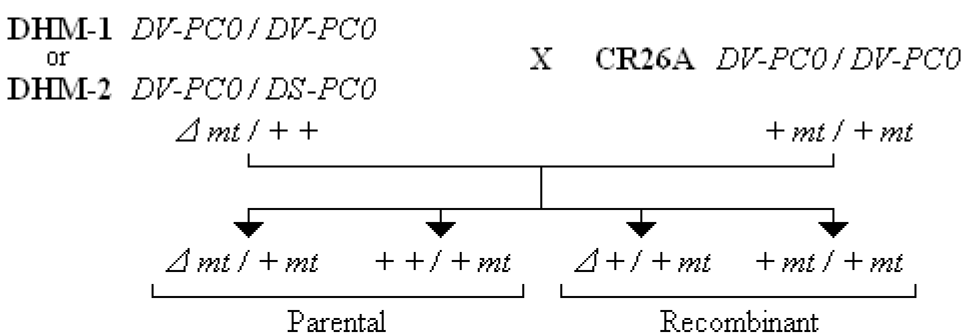

Figure 1. Crosses to assess recombination frequency in *D. mediopunctata*. Females and males of double heterozygote mutant (**DHM)** lines for visible mutations *Delta* and *merlot* crossed, respectively, to males and females of homozygous *merlot* strain **CR26A**.

Results and Discussion

Table 1 shows the results of the recombination tests. The two mutant markers showed independent segregation in the homokaryotypic females (**DHM-1:** *DV-PC0/DV-PC0*) (r = 47%; 95% confidence interval: 38-56%). In the heterokaryotypic females (**DHM-2:** *DV-PC0/DS-PC0*) recombination was not suppressed, but significantly reduced to r = 31% (95% CI: 28-35%).

No recombinant was found among the 1522 flies in the progenies of the double heterozygote males, both homo- and heterokaryotypic (**DHM-1** and **DHM-2**). This leads to an estimate of r = 0% with a joint upper 95% confidence limit of 0.2%. This result is consistent with the findings of Patterson and Suche (1934), who found only a single recombinant among 8329 flies analyzed carrying eight recessive mutations in *D. melanogaster*; as well as the results of Kikkawa (1935), who encountered only three recombinants among 38,598 flies examined in *D. virilis*.

Our results show that there is no recombination in *D. mediopunctata* males or that the frequency is extremely low (at most two recombinants in a thousand individuals from a double heterozygous progeny of genes widely separated). Thus, we can be confident on the results of genetic analysis that assume no male recombination, such as those previously reported (Carvalho and Klaczko, 1993; Hatadani *et al.*, 2004). Moreover, the absence of recombination in males is important to the effective recombination rate in nature, which may be relevant to explain the linkage disequilibrium found between distal and proximal inversions of the second chromosome (Peixoto and Klaczko, 1991).

Table 1. Recombination frequencies in females and males homo- (*DV/DV*) and heterokaryotypic (*DV/DS*) for an inversion in the distal region of the second chromosome.

Sex DHM *Genotype*	Line *Distal Inversion*	Genotype of the Progeny			
		Parental		Recombinant	
		Δ mt / + mt	*+ + / + mt*	*Δ + / + mt*	*+ mt / + mt*
Female *Δ mt / + +*	DHM-1 *DV / DV* *n = 129*	25 (19.4%)	43 (33.3%)	30 (23.3%)	31 (24.0%)
		68 (52.7%) [43.7-62.6%]		61 (47.3%) [38.4-56.3%]	
	DHM-2 *DV / DS* *n = 683*	157 (23.0%)	312 (45.7%)	105 (15.4%)	109 (15.9%)
		469 (68.7%) [65.0-72.1%]		214 (31.3%) [27.9-35.0%]	
Male *Δ mt / + +*	DHM-1 *DV / DV* *n = 774*	323 (41.7%)	451 (58.3%)	–	–
		774 (100%) [99.5-100%]		0 (0.0%) [0.0-0.5%]	
	DHM-2 *DV / DS* *n = 748*	272 (36.4%)	476 (63.6%)	–	–
		748 (100%) [99.5-100%]		0 (0.0%) [0.0-0.5%]	

In the last four columns, the number of observed flies per genotype is shown, followed by the percentage (inside parenthesis) of the total number of animals analyzed for each cross (*n*) given in the second column. The total observed numbers and percentages (inside parenthesis) are shown under each respective pair of parental and recombinant genotypes; and the 95% confidence intervals for their frequencies are shown [inside brackets] below. See text.

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