

# Molecular evidence of ribosomal DNA (rDNA) amplification of a minichromosome derived from *Drosophila arizonae* in *D. mulleri*-*D. arizonae* hybrid males

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## ABSTRACT

We present data supporting cytogenetic observations on nucleolar dominance in hybrids between *Drosophila arizonae* and *D. mulleri*. Our approach was to compare the rDNA restriction patterns between the parental species and their hybrids. Results demonstrated that the minichromosome attached to the nucleolus in hybrid males is derived from *D. arizonae*.

## INTRODUCTION

In members of the Mulleri complex (*Drosophila*), ribosomal DNA (rDNA) is located in the X chromosome and in the minichromosome (Bicudo and Richardson, 1977; Bicudo, 1981).

Nucleolar dominance in hybrids between *D. arizonae* males and *D. mulleri* females (the reciprocal cross is incompatible) was observed in a cytogenetic analysis of polytenic cells (Bicudo and Richardson, 1977; Bicudo, 1981, 1982, 1985). In hybrid females, a single nucleolar organizer region (NOR) is active and is associated with chromosome X from *D. arizonae*. In hybrid males the NOR from *D. mulleri* remains nonfunctional and a minichromosome, probably derived from *D. arizonae*, assumes NOR activities via a 4-fold increase in DNA content (Bicudo and Richardson, 1977). Nevertheless, the identification of this chromosome is based only in its morphological characteristics.

## MATERIAL AND METHODS

### *Drosophila* stocks

The *D. mulleri* stock was derived from a pool of 20 isofemale lines from Guayalejo, Mexico, and the *D. arizonae* stock from a pool of 25 isofemale lines from the same area.

### Single fly DNA extraction

Genomic DNAs were isolated from adult flies according to Jowett (1986).

### Gel electrophoresis and Southern transfer

Both gel electrophoresis and Southern transfer were carried out as indicated in Figure 1.

### DNA labeling and hybridization

The probe pDm238 (containing a complete rDNA repeating unit from *D. melanogaster*, Rohia *et al.*, 1981) was labelled by nick translation, using the BRL Nick Translation Kit, with [ $\alpha$ <sup>32</sup>P]dATP as the label. Hybridization was carried out as described by Sambrook *et al.* (1989).

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## RESULTS AND DISCUSSION

We isolated genomic DNA from single flies of both parental species and F<sub>1</sub> DNA from hybrids (males and females). Genomic DNAs were double digested with *Eco* RI and *Bst* 1107 I restriction endonucleases.

As can be seen in Figure 1, in these flies as well as in other members of the genus *Drosophila*, the rDNA

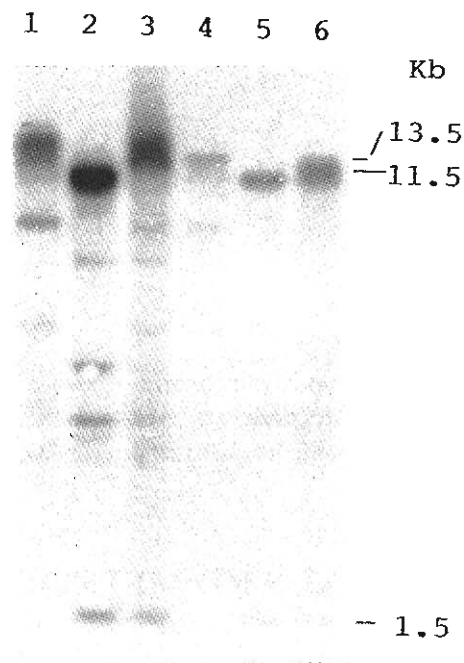


Figure 1 - Autoradiogram of single fly genomic DNAs digested with *Eco* RI/*Bst* 1107 I, electrophoresed in 0.7% agarose gel, transferred to a nylon membrane and hybridized to pDm238 probe. Lanes: 1. *Drosophila arizonae* female; 2. *D. mulleri* female; 3. Hybrid female; 4. *D. arizonae* male; 5. *D. mulleri* male; 6. Hybrid male.

Table I - Lengths (in kb) of *Eco* RI/*Bst* 1107 I rDNA fragments present in Figure 1. *Drosophila arizonae* female (1), *D. mulleri* female (2), hybrid female (3), *D. arizonae* male (4), *D. mulleri* male (5), hybrid male (6).

1	2	3	4	5	6
14		14			
13.5		13.5	13.5		13.5
	11.5	11.5		11.5	11.5
8.0		8.0	8.0		
	6.5	6.5			
5.0		5.0			
	4.3	4.3		4.3	4.3
	3.5	3.5		3.5	3.5
	3.2	3.2			
	1.5	1.5		1.5	1.5

presented a complex restriction pattern. Both *D. arizonae* and *D. mulleri* presented differences at this level (see Table I).

Hybrid females presented the expected juxtaposition of parental bands. The 13.5-11.5 kilobase bands represent complete rDNA units derived from both X chromosomes. The rDNA units without insertion sequences are different in length in both species (*D. arizonae* and *D. mulleri*; unpublished results).

On the other hand, hybrid males also presented two major bands. In this case, males have only one X chromosome derived from *D. mulleri*. One major band corresponds to units present in this chromosome. The other, the upper one, corresponds to *D. arizonae* units. These units must be present only in a minichromosome derived from *D. arizonae*. We do not know if this chromosome also has units with insertion sequences. If so, a replicative selection phenomenon could also be present, as observed in *D. melanogaster*, *D. simulans* and their hybrids (Goodrich-Young and Krider, 1989).

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## RESUMO

Apresentamos aqui dados que confirmam observações citogenéticas de dominância nucleolar em híbridos entre machos de *Drosophila arizonae* e fêmeas de *D. mulleri*. Nós comparamos os padrões de restrição do rDNA das espécies e os seus híbridos. Os resultados demonstraram que o minicromossomo derivado de *D. arizonae* é o associado ao nucléolo nos machos híbridos.

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