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New evidence for nucleolar dominance in hybrids of *Drosophila arizonae* and *Drosophila mulleri*

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ABSTRACT. *Drosophila mulleri* (MU) and *D. arizonae* (AR) are cryptic species of the mulleri complex, *mulleri* subgroup, repleta group. Earlier cytogenetic studies revealed that these species have different regulatory mechanisms of nucleolar organizing activity. In these species, nucleolar organizing regions are found in both the X chromosome and the microchromosome. In the salivary glands of hybrids between MU females and AR males, there is an interspecific dominance of the regulatory system of the *D. arizonae* nucleolar organizer involving, in males, amplification and activation of the nucleolar organizer from the microchromosome. The authors who reported these findings obtained hybrids only in that cross-direction. More recently, hybrids in the opposite direction, i.e., between MU males and AR females, have been obtained. The purpose of the present study was to evaluate, in these hybrids, the association of the nucleoli with the chromosomes inherited from parental species in order to cytogenetically confirm the dominance patterns previously described. Our results support the proposed dominance of the

AR nucleolar organizer activity over that of MU, regardless of cross-direction.

Key words: *Drosophila*, Nucleolus, Nucleolar dominance, Polytene chromosome, Microchromosome

INTRODUCTION

Drosophila mulleri (MU) and *D. arizonae* (AR) are cryptic species of the mulleri complex, *mulleri* subgroup, repleta group. Earlier studies revealed that these species, as well as others of the same complex, have different regulatory mechanisms of nucleolar organizing activity (Bicudo and Richardson, 1977; Bicudo, 1979, 1981a,b, 1982, 1983, 1985), and thus allow an approach to the study of the importance of gene variation in regulatory mechanisms in the evolutionary process. This notion has been previously advocated by other authors, such as Ohno (1969), Wilson et al. (1974), Carson (1976), Avise and Durval (1977) apud Bicudo (1982).

In the species of the mulleri complex, nucleolar organizing regions (NORs) are present in the X chromosome and the microchromosome. The main NOR is located in the proximal region, close to the centromere of the X chromosome, and the microchromosomal NOR operates as a secondary NOR region, which is activated in emergencies to ensure an adequate supply of rRNA for cell activities (Bicudo and Richardson, 1977; Bicudo, 1985).

Different NOR control mechanisms have been detected in cytogenetic studies of interspecific hybrids of these species. This divergence is manifested by an interspecific nucleolar dominance, which is morphologically detected by the preferential association of the chromosome bearing the nucleolar organizer with the nucleolus (Bicudo and Richardson, 1977). Nucleolar dominance is an epigenetic phenomenon that results in the preferential activation of a set of rRNA genes inherited from a dominant parent in an interspecific hybrid, where the non-dominant parent genes are silenced (Chen et al., 1988; Lewis et al., 2004).

In the polytene chromosomes of the salivary glands, the X chromosome is clearly attached to the nucleolus in females and males of both species, while a single X (AR) appears associated with the nucleolus in hybrid females. In hybrid males, one of the elements of the microchromosome pair shows a 4-fold increase in its DNA content. This amplified microchromosome very frequently associates with the nucleolus. Sometimes, a small nucleolus-like body appears attached to the amplified microchromosome when it is not associated with the normal nucleolus. These observations have been interpreted as indicative of a dominance of the regulatory system of the *D. arizonae* nucleolar organizer over that of *D. mulleri*, including the activation of a secondary nucleolar organizer associated with gene amplification (Bicudo and Richardson, 1977). Later, this study was extended to other species of the mulleri complex (Bicudo, 1982). In *Drosophila*, nucleolar dominance was also described in interspecific hybrids of *D. melanogaster* and *D. simulans* in which the suppression of the X chromosome nucleolar constriction in *D. simulans* occurs in both cross-directions (Durica and Krider, 1977, 1978).

Bicudo and Richardson (1977) were able to obtain crosses only between MU females and AR males, where all hybrid males bear the MU X chromosome and females bear X chro-

mosomes of both species. Baffi and Ceron (2002) and Lage (2005) obtained hybrids in the opposite direction, i.e., between MU males and AR females, by crossing lines different from those previously used. The purpose of this study was to evaluate the association between nucleoli and the chromosomes inherited from parental species in the hybrids obtained from this new cross-direction, in order to confirm cytogenetically the dominance patterns previously described.

MATERIAL AND METHODS

Hybrid larvae were produced by mass crosses in 1/4-liter bottles containing an average of 40 to 60 couples (Bicudo and Richardson, 1978). Virgin males and females of both species were kept apart until the age of 5 to 7 days, when crossing took place. Twenty-one mass crosses were performed.

The specimens of *D. arizonae* and *D. mulleri* used were collected in Guayalejo (Mexico) and brought to our laboratory in Brazil in 1976. Hybrids analyzed in this study were obtained by using two lines derived from those stocks, *D. arizonae* EST-5f and *D. mulleri* EST-5s, which are respectively homozygous for "fast"(f) and "slow"(s) 5-esterase, one of the main esterases in adult flies of these species.

Salivary glands of hybrid larvae at the third-late stage were squashed and stained with lacto-acetic orcein for the preparation of slides. These were analyzed under a Jenaval light microscope at the Laboratory of Insect Cytogenetics of the Department of Biology, and photomicrographed with a Zeiss AXIOSKOP 2 light microscope connected to a microcomputer at the Laboratory of Morphology of the Department of Biology - IBILCE, UNESP - São José do Rio Preto, SP, Brazil.

RESULTS AND DISCUSSION

The 21 crosses between AR females and MU males yielded an average of five individuals each, once more demonstrating that, depending on the lines used, it is possible to obtain hybrids of these species in both cross-directions. The presence of unpaired homologue chromosome regions confirmed hybridism, which, could also be detected in this study by the esterase bands obtained by gel electrophoresis.

Good preparations of the salivary glands were obtained for five hybrid males and five females, from which a total of 46 cells (37 from males and 9 from females) were analyzed. Male hybrids (AR X-chromosome) showed 31 cells (84.0%) that displayed the AR X-chromosome associated with the nucleolus. In these cells, microchromosomes were normal and not associated with the nucleolus. In two cells (5.0%), the MU microchromosome alone was associated with the nucleolus and in four cells (11.0%) one microchromosome was amplified but not associated with the nucleolus (Figure 1A-C). In female hybrids (bearing X chromosomes and microchromosomes originating from both species), eight cells showed an association between the AR X-chromosome and the nucleolus (89.0%), and in just one cell this association involved the MU X-chromosome (11.0%). Microchromosomes showed no amplification in females (Figure 2A-C).

The specific origin of the X-chromosome is identified by a marker band characteristic of AR (Figure 2, arrowhead), as described by Bicudo and Richardson (1977). This band is present in the proximal region, at the F2 section of the AR X-chromosome. The origin of the

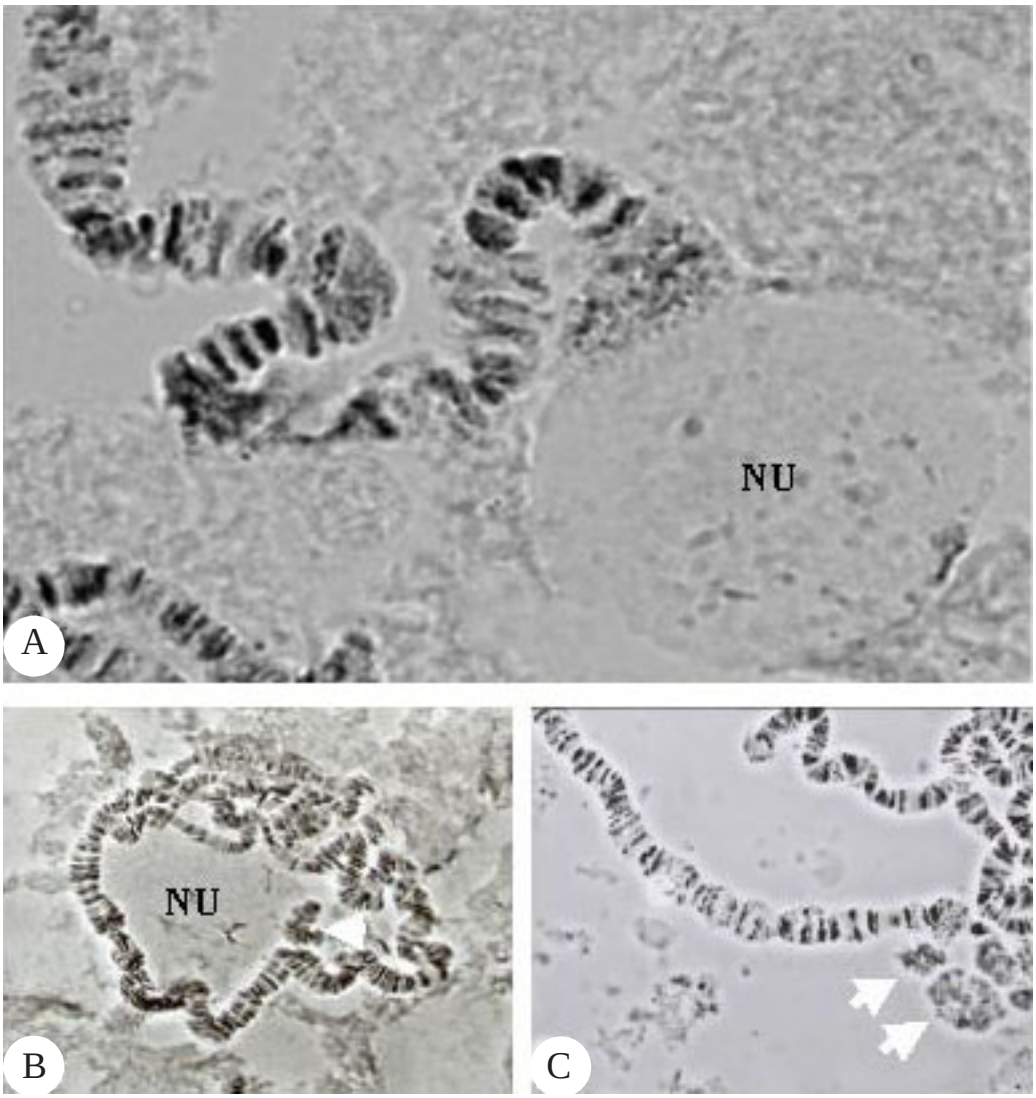

Figure 1. Lacto-acetic orcein-stained salivary gland of male hybrids (*Drosophila arizonae* (AR) females and *D. mulleri* (MU) males) showing an association between AR X-chromosome and the nucleolus (NU) (A), an association between MU microchromosome and the nucleolus (arrowhead) (B), and amplification of one of the microchromosomes (arrows) (C). Magnification: 1000X.

microchromosome can also be determined based on the microchromosome shape, which is longer in AR than in MU, as described by the same authors.

Therefore, in the present study, in male hybrids the AR X-chromosome preferentially associated with the nucleolus without being necessary to activate secondary mechanisms to supply the adequate rRNA amount. Though infrequent, variations in the dominance pattern may be attributed to some functional autonomy among cells with regard to nucleolar synthesis, which has been reported by various authors including Bicudo (1982).

Thus, the results of this study support previous reports of a dominance pattern for the AR NOR as proposed by Bicudo and Richardson (1977) from studies of hybrids between MU

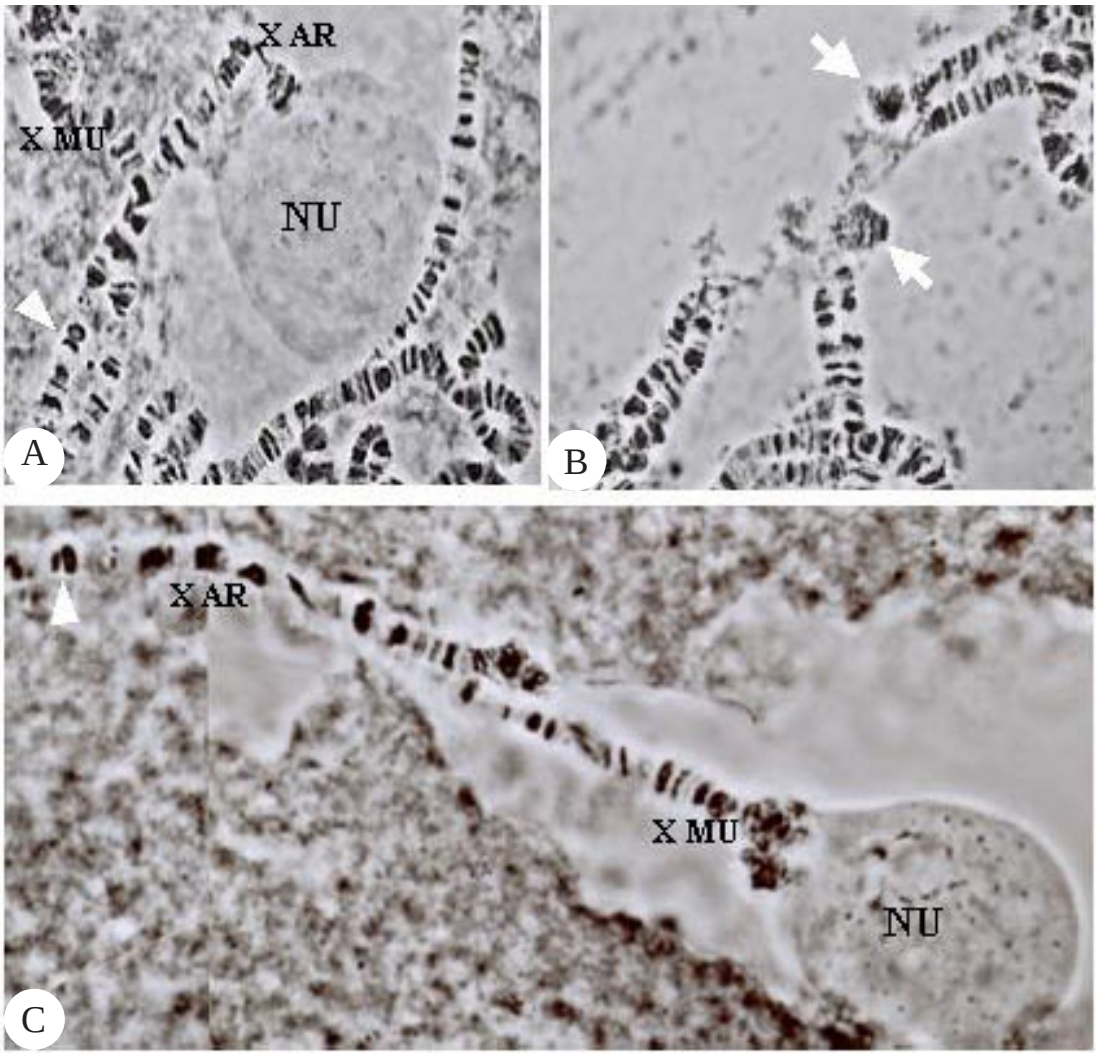

Figure 2. Lacto-acetic orcein-stained salivary glands of female hybrids (*Drosophila arizonae* (AR) females and *D. mulleri* (MU) males) showing an association between AR X-chromosome and the nucleolus (NU), where the arrowhead indicates the marker band of the AR X-chromosome (A). No microchromosome amplification was observed in females (arrows) (B). Association between MU X-chromosome and the nucleolus (C). Magnification: 1000X.

females and AR males. In this case, the suppression must be a relationship that occurs between chromosomes because it apparently remains unchanged in females whether the cytoplasm is from MU or from AR, in both cross-directions.

These observations reflect the differentiation that occurred between *D. mulleri* and *D. arizonae* in the evolutionary process and show the effects of the interaction between their chromosomes when put together in the same cell.

The results of the present study also show that cytogenetics is still a reliable tool for obtaining preliminary data on cell physiology. The dominance described here has also been confirmed molecularly, based on the analysis of rDNA restriction patterns in males and females

of parental species and hybrids, demonstrating that the microchromosome, which attaches to the nucleolus in male hybrids obtained from the cross between AR males and MU females, derives from AR (Leoncini et al., 1996). Furthermore, transcription analysis of the intergenic non-coding region ITS-1 has shown that the rRNA cistrons, present in both the X chromosome and microchromosome of AR, are preferentially transcribed in hybrids (Lage, 2005).

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