



## Differential transcription of ribosomal cistrons denoting nucleolar dominance in hybrids of *Drosophila mulleri* and *Drosophila navojoa* (*mulleri* complex, Repleta group)

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### Abstract

The fruit flies *Drosophila mulleri* and *Drosophila navojoa* are included in the *mulleri* complex of the *mulleri* subgroup and Repleta group. Although there is no demonstration that interspecific crosses between them occur in nature, they intercross in the laboratory in both cross directions. Previous data have shown the occurrence of nucleolar dominance in interspecific hybrids of some species in the *mulleri* complex. We investigated nucleolar dominance in *D. mulleri*/*D. navojoa* hybrids using the transcription profiles of the rDNA internal transcribed spacer (ITS-1) region. The results showed that the ribosomal cistrons present in the X chromosome and in the microchromosome of *D. navojoa* are exclusively or preferentially transcribed in these hybrids depending on the cross direction, denoting the complete or partial nucleolar dominance of this species over *D. mulleri*.

**Key words:** *Drosophila mulleri*, *D. navojoa*, interspecific hybrids, nucleolar dominance, rDNA.

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In animals and plants the rRNA gene family consists of tandemly arrayed repeats of the 18S, 5.8S, and 28S structural genes (Long and Dawid 1980). Each transcription unit is composed of an external transcribed spacer (ETS) leader promoter region, an 18S rRNA coding region, a noncoding internal transcribed spacer (ITS-1), a 5.8S rRNA coding region, a second noncoding ITS region (ITS-2), a 28S rRNA coding region, and, finally, a nontranscribed intergenic spacer segment (IGS) (Hillis and Dixon, 1991, Polanco *et al.*, 1998). Regulatory regions are located in the intergenic spacer (IGS) region and typically include a promoter and arrays of internal sub-repeats thought to act as transcription enhancers (Busby and Reeder, 1983, Pikaard, 2000a). In addition to regulatory studies, the IGS, ITS1 and ITS2 regions have been used for species characterization in taxonomy studies (Mateos and Markow, 2005, Djadid *et al.*, 2006, Mateos *et al.*, 2006).

Studies on interspecific hybrids have shown dominance of the nucleolar organizing activity of one parental species over another, this process having been documented in both animals (Honjo and Reeder, 1973, Bicudo and

Richardson, 1977) and plants (Wallace and Landridge, 1971, Pikaard, 2000b). Nucleolar dominance occurs when one parental set of ribosomal RNA (rRNA) genes is transcribed but the rRNA genes inherited from the other parent are silent (Grummt and Pikaard, 2003). In *Drosophila*, nucleolar dominance has been observed in interspecific hybrids from crosses between *Drosophila melanogaster* and *Drosophila simulans* (Durica and Krider, 1978) and in hybrids of species from the Repleta group *mulleri* complex (Bicudo and Richardson, 1977, Bicudo, 1981, Leoncini *et al.*, 1996).

In species from the Repleta group *mulleri* complex, the nucleolus organizer region (NOR) is present in the X chromosome and in the microchromosome (Bicudo and Richardson, 1977; 1981). The microchromosome is pair 6 in the metaphase plate of repleta group species, corresponding to Muller's element F (González *et al.*, 2002). Bicudo and Richardson (1981) considered the occurrence of rDNA cistrons in the microchromosome of *mulleri* complex species an example of genetic homology, supporting the view of a common phylogenetic origin for the X chromosome and the microchromosome in *Drosophila*. In hybrids involving *Drosophila mulleri* and *Drosophila arizonae* (also a *mulleri* complex species), cytogenetic data have suggested that the ribosomal cistrons present in the X chromosome and microchromosomes of *D. arizonae* are dominant

over those from *D. mulleri* (Bicudo and Richardson, 1977; Bicudo, 1981; Oliveira *et al.*, 2006). The confirmation of this fact came from molecular studies (Leoncini *et al.*, 1996; Baffi and Ceron, 2002). In addition, cytogenetic studies involving hybrids of *D. mulleri* and other species in the *mulleri* complex have indicated different classes of nucleolar dominance, with *D. mulleri* NORs being dominant over those of *Drosophila wheeleri* and *Drosophila aldrichi* but recessive when associated with NORs from *Drosophila navojoa* or *Drosophila mojavensis* in hybrids (Bicudo, 1981).

We used differential transcription of ITS-1 rRNA genes to investigate nucleolar dominance in hybrids between *D. mulleri* (from Guayalejo Tamazunchale, México) and *D. navojoa* (from Tehuantepec, México), *D. mulleri* belonging to the *mulleri* cluster of the *mulleri* complex whereas *D. navojoa* is included in the *mojavensis* cluster of the same complex (Vilela, 1983, Wasserman, 1992). Bicudo (1981) obtained laboratory hybrid males and females of these species only in the direction *D. mulleri* females and *D. navojoa* males, although in our study reciprocal crosses also yielded males and females. The flies used were verified as hybrids by analysis of the EST-5 esterase band before RNA extraction, this being possible because the *D. mulleri* we used was homologous for the slow form of this esterase while the *D. navojoa* was homologous for the fast form of the enzyme so that hybrid flies showed the two bands simultaneously in polyacrylamide gels.

The Trizol method (GIBCO BRL Technologies, U.S.A.) was used to extract total RNA from single parental and hybrid male and female flies frozen at -70 °C. After treatment with RQ 1 RNase-Free DNase (Promega, U.S.A.) the RNA was submitted to reverse transcription polymerase chain reaction (RT-PCR) amplification to obtain the cDNA single stranded fragment corresponding to rRNA ITS-1 utilizing the 5'GCTGCGTTCCTTCATCGA C3' oligonucleotide as a reverse primer. The thermocycling profile consisted of 5 min at 25 °C for annealing the reverse primer, plus 60 min at 42 °C for extension of the chain, followed by incubation at 70 °C for 15 min for enzyme inactivation. A second PCR amplification reaction utilizing the 5' CCTAACAAGGTTTCCGTACC3' oligonucleotide allowed us to obtain a double-stranded amplified cDNA fragment corresponding to the entire ITS-1 sequence of the parental species and hybrids. In this step the thermocycling profile consisted of incubation at 95 °C for 2 min, followed by 35 cycles of 1 min at 95 °C, 1 min at 50 °C and 1 min at 72 °C plus a final extension for 2 min at 72 °C.

A small number of hybrids was produced in the two directions of crosses involving *D. mulleri* and *D. navojoa*. We tried to analyze all of them, but the RNA extracted from hybrids was not good enough to detect the transcription pattern in every fly. Thus, in the *D. mulleri* female X *D. navojoa* male crosses 10 hybrid males obtained but only four could be analyzed, while none of the three hybrid fe-

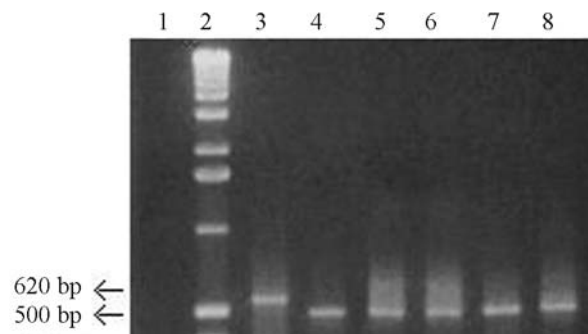
males showed good results. In the reciprocal crosses (*D. navojoa* females X *D. mulleri* males) only two of eight hybrid males and four of 9 hybrid females showed transcription patterns that could be analyzed.

The ITS-1 cDNA amplified fragment from *D. mulleri* was about 620 bp long while in *D. navojoa* this fragment was about 500 bp long (Figure 1). In hybrid males from crosses involving *D. mulleri* females and *D. navojoa* males (hybrids bearing an X chromosome from *D. mulleri* and microchromosomes from both species), the cDNA amplified fragment corresponding to the ITS-1 region displayed a length of 500 bp, indicating that, in this case, transcribed RNA comes from the *D. navojoa* genome. As mentioned, females were not analyzed because none of them showed good results in this cross direction.

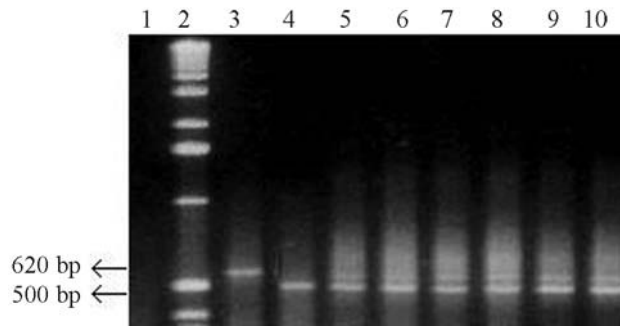
In the reciprocal crosses involving *D. mulleri* males and *D. navojoa* females (Figure 2), hybrid males (bearing an X chromosome from *D. navojoa* and microchromosomes from both species) and females (bearing X chromosome and microchromosomes from both species) presented two fragments of 500 and 620 bp, showing that in this direction of crosses rRNA genes of both species are transcribed. However, rRNA genes from *D. navojoa* seem to have been transcribed at a higher level, as indicated by the degree of fluorescence of the gel bands.

The results obtained in this study indicate that there is complete nucleolar dominance of *D. navojoa* in *D. mulleri* female/*D. navojoa* male hybrids. In this case, the 500 bp fragment corresponds to the amplification of the rRNA ITS-1 region located in the microchromosome from the parental *D. navojoa* male which is then activated in the hybrid males while the NOR in the *D. mulleri* X chromosome remains suppressed. Cytogenetical studies by Bicudo (1981) in this cross direction had already suggested dominance of *D. navojoa* NOR over *D. mulleri* NOR.

As mentioned above, in females obtained in reciprocal crosses (*D. mulleri* males and *D. navojoa* females), tran-



**Figure 1** - Amplified cDNA internal transcribed spacer (ITS-1) intergenic spacer (IGS) fragments obtained from hybrids of crosses between *Drosophila mulleri* females and *Drosophila navojoa* males using the reverse transcriptase polymerase chain reaction (RT-PCR) and visualized in 2.5% (w/v) agarose gel. Lanes are as follows: (1) water; (2) 1 kb DNA ladder marker; (3) *D. mulleri* parental female; (4) *D. navojoa* parental male; (5), (6), (7) and (8) hybrid males.



**Figure 2** - Amplified cDNA internal transcribed spacer (ITS-1) intergenic spacer (IGS) fragments obtained from hybrids of crosses between *Drosophila mulleri* males and *Drosophila navojoa* females using the reverse transcriptase polymerase chain reaction (RT-PCR) and visualized in 2.5% (w/v) agarose gel. Lanes are as follows: (1) water; (2) 1 kb DNA ladder marker; (3) *D. mulleri* parental male; (4) *D. navojoa* parental female; (5) and (6) hybrid males; (7) (8), (9) and (10) = hybrid females.

scription of the ribosome cistrons from both species was observed but at apparently different levels, suggesting that in these hybrid females the transcription of the ribosome cistrons from *D. navojoa* X chromosome partially suppressed the transcription of the NOR from the *D. mulleri* X chromosome. In the hybrid males obtained in the same cross direction both the NOR from the X chromosome of *D. navojoa* (the only X present) and the NOR of *D. mulleri* microchromosome are operating. In the next step of this research we plan to use quantitative PCR to produce more accurate information regarding the degree nucleolar dominance.

On the basis of these data we cannot say if the NORs of both microchromosomes are also active in females or if, in males, the *D. navojoa* microchromosome NOR is also being transcribed. However, data from *D. mulleri*/*D. arizonae* hybrids indicated that the dominant microchromosome NOR is activated only when the X chromosome NOR from the same species is absent, thus acting as a secondary NOR (Bicudo and Richardson, 1977). If the situation is the same for *mulleri/navojoa* hybrids, the microchromosome NORs from both parents should not operate in females (where both X-NORs are present) and the *D. navojoa* microchromosome NOR is also not expected to operate in males because the *D. navojoa* X-NOR is present.

The complete dominance observed in males from the cross direction involving *D. mulleri* females and *D. navojoa* males and the partial dominance operating in the cross direction involving *D. navojoa* females and *D. mulleri* males differs from the apparently complete dominance observed in hybrids from both cross directions involving *D. mulleri* and *D. arizonae* (Bicudo and Richardson, 1977). Such findings suggest regulatory differentiation between *D. arizonae* and *D. navojoa* in the evolutionary process which produced these species, reinforcing once more the concept that modifications in the regulatory

mechanisms have had a very important role in speciation (Ohno, 1969, Carson, 1976).

A mechanism frequently considered to work in NORs dominance in hybrids is the “enhancer imbalance” model in which differences in the sequence or number of regulatory elements in the ITS regions results in a greater binding affinity for transcription factors to the dominant genes (Honjo and Reeder, 1973, Flavell, 1986). However in the marine copepod *Tigriopus californicus*, data obtained by Flowers and Burton (2006) showed that repetitive elements in the IGS are not required for the uniparental gene silencing of rRNA transcription. The continuation of studies on *D. mulleri*/*D. navojoa* hybrids seems to be a promising way to focus on resolving this ambiguity.

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