Correspondence between C-banding and Ag-NOR in the Sex Chromosomes of *Belostoma oxyurum* (Belostomatidae, Heteroptera)

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Most reports of C-banding in Heteroptera describe the presence of positive bands located generally at telomeric or subtelomeric positions (Papeschi 1991); C-bands at interstitial regions have seldom been described (Camacho *et al.* 1985, Dey and Wangdi 1990, Panzera *et al.* 1992). While in most cases bands are described as C positive, in *Nezara viridula* (Pentatomidae) autosomal bands are light C (Camacho *et al.* 1985). Among insects, the presence of light C-bands has been previously reported in the X chromosome of some species of Orthoptera (Cardoso and Dutra 1979).

Silver staining techniques are customarily applied to reveal those NORs which contain active rRNA genes; according to Miller *et al.* (1976a, b) silver impregnation selectively stains those NORs that were active in the previous interphase. A close correspondence between nucleolus organizing regions and constitutive heterochromatin (as revealed by C-banding) has been frequently reported (García de la Vega *et al.* 1982, Rufas *et al.* 1983). In Heteroptera, Ag-NOR banding has been only applied to *Nezara viridula* (Camacho *et al.* 1985); in this species the active NOR is located in the secondary constriction of the longest autosome and is also associated with an interstitial C positive band.

In *Belostoma oxyurum* (2n = 6 + XY, male) the sex chromosome X and Y appear always associated to a single nucleolus during both mitosis and meiosis (Papeschi and Bidau 1985, Papeschi 1992). Previous results of C-banding have revealed that at diplotene the X chromosome presents a positive C band in one telomeric region and a light C one in the other, while the Y chromosome is completely light C banded (Papeschi 1988). In order to establish the relationship between the intensity of C-bands and the nucleolus organizing activity in the sex chromosomes of *B. oxyurum*, the results of C-banding in mitotic and meiotic cells are analyzed in conjunction with the results of Ag-NOR banding.

Materials and methods

Adult males and male and female embryos of *B. oxyrum* were used in the present study. Testes were removed and fixed in 3:1 methanol:glacial acetic acid. Mitotic cells were obtained from embryos. As is characteristic of the Belostomatinae, in *B. oxyrum* eggs are invariably attached to the back of the males in a pod of mucilaginous cement (Lauck and Menke 1961). Embryos were dissected from the eggs, treated 15 min with an hypotonic solution of sodium citrate 1% and then fixed in 3:1 methanol:glacial acetic acid during at least an hour.

The fixed material was disrupted and squashed in 45% acetic acid; the coverslip was then removed by the dry-ice method, and slides were air dried. In order to guarantee a high number of mitotic cells three embryos were included in each slide.

C-banding was performed as previously described (Papeschi 1988) and the Ag-NOR
banding technique of Rufas et al. (1982) was applied to mitotic and meiotic cells.

Results

C-banding

At mitotic prometaphases the X chromosomes of female (Fig. 1a) and male (Fig. 1b, c) cells show positive C bands at both telomeric regions; the Y chromosome, on the other hand, is completely C positive (Fig. 1b, c).

During male meiosis, as it has previously been described, the three autosomal pairs show...
positive C bands at telomeric regions. The largest pair has a very small C band at only one end (Fig. 1e–h), and it is frequently difficult to detect; the second sized autosomal pair has positive C-bands at both telomeric regions, and the smallest bivalent present conspicuous telomeric bands at both ends (Fig. 1e–h). On the other hand, the intensity of the C-bands of the sex chromosomes is variable. At late diplotene, diffuse stage (Fig. 1d) and early diakinesis (Fig. 1e, f) both the Y chromosome and one telomeric region of the X present a light C-band (Fig. 1d–f), while the other telomeric region of the X chromosome is C positive. At late diakinesis the light C bands of the X and the whole of the Y chromosome are C-positive (Fig. 1g, h).

**Ag-NOR banding**

A single nucleolus is present at mitosis and at early meiosis. At mitotic prometaphases a positive Ag-NOR is present at one telomeric region of the X chromosome. A total number of 86 mitotic banded cells have been analyzed (17 male and 69 female cells). Among female cells 62% show active NORs in both X’s (Fig. 2a, b), and in the other 26 cells one of the X’s presents scarce or null NOR activity (Fig. 2c). All the analyzed male cells show a positive Ag-NOR in the X chromosome, while no band is present in the Y chromosome (Fig. 2d).

During meiosis, both the X and Y chromosomes are associated to the nucleolus from synizesis up to the diffuse stage. At early diakinesis, the nucleolus separates from the sex chromosomes and disorganizes, but no positive Ag-NOR region has been detected in the sex chromosomes.

**Discussion**

The sex chromosomes of *Belostoma oxyurum* are the only chromosomes always associated to the nucleolus during both mitosis and meiosis (Papeschi and Bidau 1985, Papeschi 1992). This observation strongly suggests that both sex chromosomes are involved in the nucleolus organization. The results of Ag-NOR banding at mitosis reveal in fact the presence of a
positive Ag-NOR at one telomeric region of the X chromosome; however, the Y chromosome is Ag-NOR negative. This fact can be due to a difference between the nucleolar synthetic activity in both sex chromosomes; the Y could have a lower number of ribosomal genes, or else could become inactivated earlier during mitosis. It can be argued that the Y chromosome does not bear a NOR, but then it should be explained its constant not specific association to the nucleolus. It is noticeable that in some mitotic female cells only one of the X chromosomes shows a positive Ag-NOR. Since three embryos were included in each slide, it is not possible to ascertain whether the presence of one or two active NORs reflects an inter- or intraindividual variation. Many instances of variation in the number of active NOR's both within and between individuals have been reported (Gosalvez et al. 1986, Czaker 1978, Miller et al. 1976a). In Belostoma oxyurum it is not possible to give a precise explanation, but it is evidently another example of variation in the expression of nucleolar organizing regions.

During meiosis no positive Ag-NOR has been detected. This result agrees with other reports in insects (Rufas et al. 1985), and according to these authors it would be the consequence of NOR inactivation at early meiosis.

In B. oxyurum, in contrast to the autosomal C bands which are always positive, the intensity of the C bands of the sex chromosomes varies during meiosis. The light banding of one telomeric region of the X and the whole of the Y chromosome from diplotene up to early diakinesis develops into positive C bands by late diakinesis. Cardoso and Dutra (1979) made similar observations in the meiotic sex chromosomes of three species of Orthoptera, in which the light C banding changed to a positive banding at diplotene. According to these authors, the weakly positive staining would be the result of differences in chromatin packing, more than the consequence of the presence of low repetitive DNA as has been previously suggested (Bianchi and Ayres 1971). In B. oxyurum, however, the light C-bands of the sex chromosomes are very probably related to their nucleolus organizing function, since both chromosomes continue associated through these regions to the nucleolus until the end of the diffuse stage. In this species, then, both the DNA composition (repetitive DNA, rRNA genes) and its packaging (later condensation) would be responsible of the intermediate staining.

Summary

The mitotic and meiotic sex chromosomes of Belostoma oxyurum have been analyzed by C-banding and Ag-NOR staining. In mitotic prometaphases the Y chromosome and both telomeric regions of the X are C positive. However, at meiosis one telomeric region of the X chromosome presents always a C positive band, while the other telomeric region of this chromosome and the whole of the Y chromosome are light C banded during diplotene, the diffuse stage and early diakinesis. All these bands appear C positive by late diakinesis. In mitotic prometaphases, a positive Ag-NOR is revealed at one telomeric region of the X chromosome, but not in the Y chromosome. Besides, some intra and/or interindividual variation in NOR expression in the X chromosome is encountered. No positive Ag-NOR band is observed at meiosis.

A comparison of the results obtained in mitotic and meiotic cells after C- and Ag-NOR banding lead to the conclusion that in Belostoma oxyurum the intermediate staining of the C-bands of the sex chromosomes at meiosis is related to both the DNA composition (repetitive DNA, rRNA genes) and its packaging (cycle of condensation).

Key words: Heteroptera, holokinetic chromosomes, C-banding, Ag-NOR banding, sex chromosomes.
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