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CYTOGENETIC STUDY OF THE FAMILY NABIDAE (INSECTA, HETEROPTERA)

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ABSTRACT. C-banding, silver nitrate staining and base-specific fluorochrome (DAPI and CMA_3) staining were appllied in this study to find out cytogenetic evidence about the directions and mechanisms of the karyotype evolution were applied in this study. Six Nabis species with 2n=16+XY, three species with 2n=32+XY and a species with 2n=26+XY were studied. The chromosomes are holokinetic and male meiosis is achiasmatic. Possible evolutionary mechanisms underlying differences in chromosome number and the taxonomic significance of karyotype variation and the distribution of meiotic patterns in the family are discussed.

KEY WORDS. holokinetic chromosomes, Heteroptera, C-banding, Ag-NOR method, fluorochromes, DNA extraction.

INTRODUCTION

Cytogenetic data on 27 species of Nabidae are now available (UESHIMA 1979; KUZNETSOVA and MARYAŃSKA-NADACHOWSKA 2000 and references cited there; KUZNETSOVA et al. 2003). In this family, a total of four well-documented karyotype patterns are known: 2n = 18 (16 + XY); 2n = 28 (26 + XY); 2n = 34 (32 + XY); 2n = 38 (36 + XY).

Until the present time, all cytogenetic studies of Nabidae, except our papers (GROZEVA and NOKKALA 2003; GROZEVA S. at al. 2004), have been performed on conventionally stained chromosomes. GROZEVA and NOKKALA (2003) have reported the first data on the C-band distribution patterns in the karyotypes of six

species of the genus *Nabis* (Nabinae) with 2n = 18 (16 + XY). In GROZEVA et al. (2004), patterns of C-banding for four nabid species displaying high-chromosome number karyotypes: *Himacerus* (*Himacerus*) *mirmicoides* (O. Costa), *Nabis* (*Aspilaspis*) *indicus* (Stål) and *N.* (*A.*) *viridulus* Spinola with 2n = 34; and *Prostemma guttula* (Fabricius) with 2n = 28 are provided. In addition to C-banding, AgNOR staining and base-specific fluorochrome staining (DAPI and CMA_3) were performed to reveal the nucleolus organizer regions (NORs, i. e. sites of the RNA genes) and AT- and GC-rich chromosome sites in their karyotypes. Data of this kind are provided for the first time regarding the family Nabidae as a whole.

Our object was to obtain more information on the genome organization of the nabid species and to reveal new cytological markers useful for a better insight into the pathways by which the various karyotypes have evolved in Nabidae. The karyotype with 2n = 34 and „touch-and-go pairing“ are considered as plesiomorphic characters in Nabidae. Possible evolutionary mechanisms underlying differences in chromosome number and the taxonomic significance of karyotype variation and the distribution of meiotic patterns in the family are discussed.

MATERIAL AND METHODS

Males of Nabidae were fixed in the field in 3:1 ethanol-acetic acid mixture. Meiotic stages were obtained from young males. Testicular follicles were dissected from abdomen in a drop of 45% acetic acid and squashed under a coverslip. Chromosome preparations were examined by phase-contrast before freezing off the cover slips by dry ice, dehydrating in freshly prepared 3:1 fixative for 20 min and air-drying.

In order to detect the amount and distribution of the constitutive heterochromatin (C-bands) in the chromosomes, the modified procedure of C-banding published by GROZEVA and NOKKALA (2003) was used. Revealing the active nucleolus organizer regions (NORs) followed the method by HOWELL and BLACK (1980). In order to reveal molecular composition of C-heterochromatin, some of the preparations pretreated for C-banding were stained, instead of Giemsa, with GC-specific chromomycin A_3 (CMA_3) and AT-specific 4’-6’-diamidino-2-phenylindole (DAPI) after SCHWEIZER (1976) with minor modifications (GROZEVA and NOKKALA 2002).

Chromosome spreads were analyzed using Olympus BH-2 light microscope with OM-4 camera; fluorochrome labelled slides were studied using the fluorescent microscope Dialux 22 (Leitz, Wetzlar, Germany).

RESULTS and DISCUSSION

In the family Nabidae there are approximately 400 species in 21 genera and 4 subfamilies (KERZHNER 1981; 1996). Presently available data on the nabid karyotypes cover 27 species belonging to four genera of Nabinae (25 species) and to two genera of Prostemmatinae (2 species) (UESHIMA 1979; KUZNETSOVA and MARYAŃSKA-NADACHOWSKA 2000 and references cited there; KUZNETSOVA et al. 2004). These data then represent a small proportion of the

species and genera of Nabidae. The 2n = 28 (26 + XY) karyotype was found to be characteristic of *Pagasa fusca* (Stain) and *Prostemma guttula* from a primitive tribe Prostemmatini (Prostemmatinae). In Nabinae, the species of the genera *Nabis, Himacerus, Lasiomerus* and *Hoplistoscellis* (tribe Nabini) were reported to display the 2n = 18 (in 18 species), 2n = 34 (in 5 species) and 2n = 38 (in one species). Several values in the range of 18 to 40 have been found in different populations of *Himacerus apterus* (Fabricius). Of particular interest is that the 2n = 34 (32 + XY) karyotype shows a precise doubling of the autosome number compared to 2n = 18 (16 + XY). Furthermore, in the genus *Nabis* these karyotypes occur without intermediate values. It evidences for the existence of a peculiar cytogenetic mechanism operating in the karyotype evolution of the Nabinae subfamily (KUZNETSOVA et al. 2004).

Male meiosis in the Nabidae bugs is characterized by a number of significant peculiarities such as the absence of chiasmata; postreduction of sex chromosomes, which separate equationally in AI while segregating reductionally in AII; the „distance pairing“ of sex chromosomes in MII (UESHIMA 1979; NOKKALA and NOKKALA 1984; KUZNETSOVA and MARYAŃSKA-NADACHOWSKA 2000). Unlike the first two characters, sex chromosome „distance pairing“ was thought to be a unique characteristic of Nabidae (NOKKALA and NOKKALA, 1984; KUZNETSOVA and MARYAŃSKA-NADACHOWSKA 2000). Typical of „distance pairing“ is that sex chromosomes do not associate in MII. They are oriented towards opposite poles forming a kind of „distance bivalent“ and segregate in AII. Meiosis in all Nabinae species studied was found to follow a typical for nabids behaviour, including for two *Nabis* species – „distance pairing“ of sex chromosomes. However *P. guttula* appeared to show the orthodox for Heteroptera „touch-and-go“ pairing in MII. Based on the evidence available at the time for the subfamily Nabinae, KUZNETSOVA and MARYAŃSKA-NADACHOWSKA (2000) suggested that „distance pairing“ represents an autapomorphy of Nabidae within the infraorder Cimicomorpha. However, the new data on the subfamily Prostemmatinae indicate that this pattern is probably characteristic only of the subfamily Nabinae.

The Nabidae species are insects with holokinetic chromosome like all Heteroptera. Since holokinetic chromosomes lack centromere as a chromosome marker, conventional technique can reveal only a small proportion of the actual karyotype variation between species. It generally provides information only on chromosome number and sex chromosome system in these insects. GROZEVA and NOKKALA (2003) have reported the first data on the C-band distribution in the Nabidae karyotypes. They studied six species of the genus *Nabis* with 2n = 18 (16 + XY): *Nabis* (*Nabis*) *brevis* Scholtz; *N.* (*N.*) *ericetorum* Scholtz; *N.* (*N.*) *pseudoferrus pseudoferrus* Remane; *N.* (*N.*) *rugosus* (Linnaeus); *N.* (*Dolichonabis*) *limbatus* Dahlbom; *N.* (*Nabicula*) *flavomarginatus* Scholtz. As is often the case in holokinetic chromosomes, these species showed little C-heterochromatin in their karyotypes. Nevertheless, the species were found to differ from each other in both C-heterochromatin amount and distribution. These characteristics, on occasion, varied between different specimens of the same species, however it was difficult to quantify this variation accurately due to the small sample size.

As a part of ongoing cytogenetic studies of nabids (NOKKALA and NOKKALA 1984; KUZNETSOVA and MARYAŃSKA-NADACHOWSKA 2000; GROZEVA and NOKKALA 2003; KUZNETSOVA et al. 2003, GROZEVA at al. 2004), patterns of C-banding were studied in four species displaying high-chromosome number karyotypes: *Nabis* (*Aspilaspis*) *indicus*, *N.* (*A.*) *viridulus* and *Himacerus* (*Himacerus*) *mirmicoides* with 2n = 34 (32 + XY); *Prostemma guttula* with 2n = 28 (26 + XY). Similar to the species with 2n = 18 (GROZEVA and NOKKALA 2003), these species showed little C-heterochromatin in their chromosomes. C-bands were visible at the condensation stage, however, escaped detection later. Using the base-specific fluorochrome DAPI we established this heterochromatin to contain AT-rich DNA in every species. In *H. mirmicoides*, the majority of autosomes displayed C-bands of assorted sizes, mostly interstitial, however we failed to detect their localization with confidence. In *P. guttula*, no conspicuous C-bands were encountered in the autosomes, whereas the Y chromosome was totally heterochromatic. A similar Y chromosome pattern was previously described in *Triatoma infestans* (Heteroptera, Reduviidae) (PEREZ et al. 1997) and three tingid species (Heteroptera, Tingidae) (GROZEVA and NOKKALA 2001). These findings are consistent with the generally accepted idea that the Y chromosome undergoes genetic exhaustion due to accumulation of heterochromatin during evolution (CHARLESWORTH 1996).

N. indicus and *N. viridulus* both showed small but clearly defined C-bands in every autosome. It is remarkable that these species were found to differ in C-band distribution patterns – telomeric in *N. indicus* while interstitial in *N. viridulus*. In Tingidae, another cimicomorphan family, the species sharing the same chromosome number 2n = 14 similarly showed differences in the C-banding patterns evidencing a quite substantial redistribution of heterochromatin within the chromosomes (GROZEVA and NOKKALA 2001).

NOKKALA and NOKKALA (1984) have cytologically studied *Nabis* (*Nabis*) *brevis*, *N.* (*Nabicula*) *flavomarginatus* and *N.* (*Dolichonabis*) *limbatus*, all with 2n = 18. They found that the X and Y chromosomes each carried an unstained gap, or constriction – sub-median in the X and distal in the Y – indicating that nucleolus organizer regions (NORs) are situated on the sex chromosomes. In species studied here, X and Y chromosomes appeared to be also NOR-bearing as revealed by silver nitrate staining.

Data from some insect species have shown NORs labelled with base-specific fluorochrome CMA_3, confirming that the rDNA in these regions is GC-rich (MANICARDI et al. 1996; KUZNETSOVA at al. 2001; KUZNETSOVA et al. 2003; REBAGLIATI et al. 2003; NECHAYEVA et al. 2003). CMA_3 positive signals were revealed on sex chromosomes in every species examined, indicating that their NORs are GC-rich as well (GROZEVA at al. 2004). Notice however that NORs do not always show GC-base richness as evidenced by a bug species *Carlisis wahlbergi* Stål (Heteroptera, Coreidae) (FOSSEY and LIEBENGERG 1995) and an aphid species *Tetraneura akinire* (Homoptera, Pemphigidae) (MANICARDI and LIEBENBERG 1995). In *H. mirmicoides* and *P. guttula*, we were able to locate the NOR position on

both the X and Y chromosomes. As in the *Nabis* species with 2n = 18 (NOKKALA and NOKKALA 1984), in these species NORs showed sub-median location on the X while distal on the Y, indicating that such a pattern is common in Nabidae.

A further common aspect of the sex chromosomes of Heteroptera seems to be that they demonstrate bright fluorescence after both DAPI and CMA_3 during male meiotic prophase (GROZEVA and NOKKALA 2002; REBAGLIATI et al. 2003; present data). It is suggested that this pattern is consistent with the allocycly of these chromosomes during male meiosis and reflects different degrees of sex chromatin condensation rather than differences in base composition (REBAGLIATI et al. 2003).

In summary, the techniques applied had not provided any useful markers for understanding the mechanisms underlying differences in chromosome number in Nabidae. In particular, the problem of *a twofold* difference in autosome number between the karyotypes of 2n = 16 + XY and 2n = 32 + XY, found in some taxa of the subfamily Nabinae, remained obscured. However, these techniques made it clear that taxonomically closely related species with the same chromosome number do not in fact display identical karyotypes due to accumulation of many rearrangements since divergence from the common ancestor.

As indicated above, the karyotypes with 2n = 16 + XY and 2n = 32 + XY occur in the genera *Himacerus* and *Nabis* with no intermediate values of autosome number. At early stage of the chromosome studies in nabids, when the data on the species with 2n = 18 prevailed and were opposed to a few data on the species with 2n = 34, a suggestion was made that the karyotype with 2n = 18 was ancestral and the karyotype with 2n = 34 originated from it as a result of polyploidy (LESTON 1957; THOMAS 1996; KUZNETSOVA and MARYAŃSKA-NADACHOWSKA 2000). LESTON (1957) and particularly THOMAS (1996) invoked true polyploidy as an evolutionary mechanism in Nabidae as well as in some other heteropteran families. To explain why the putative polyploid species each have a single pair of sex chromosomes rather than two pairs, Thomas suggested that sex chromosomes could be prevented from doubling owing to their asynaptic pattern and postreduction in meiosis.

However, the hypothesis that the ancestral nabid karyotype was 2n = 34 and the karyotype with 2n = 18 originated from it as a result of autosomal fusions is in better agreement with the data on related groups and usual mechanisms of karyotype evolution. Chromosome numbers close or even equal to 34 are characteristic of the families closely related to Nabidae: Miridae, Anthocoridae and Cimicidae, including their primitive members (UESHIMA 1979). A character state found both within and outside the group should be considered plesiomorphic unless and until strong contrary evidence appears (RASNITSYN 1996). Although true polyploidy is suggested for some groups of Heteroptera (THOMAS 1996), its mechanisms are unclear and even the fact of such a polyploidy is doubted (JACOBS 2002). Autosomal fusions represent a common mechanism of karyotype evolution and, hence, an easier explanation of the karyotype variability in nabids. If 2n = 34 is the ancestral number of chromosomes in nabids, higher number of chromosomes (2n = 38) in *Himacerus maracandicus*, can be considered a result of fission of 4 autosomes, the reduced number of chromosomes (2n = 28) in Prostemmatinae may be suggested

a result of fusion of 6 autosomes, and the prevailing karyotype with 2n = 18 could be explained by fusion of all autosomes by pairs, i.e. by 16 fusion events.

The fusion hypothesis is new for Nabidae, and further work is indubitably necessary to substantiate it. Applying modern cytogenetic and molecular techniques could prove a useful tool for better understanding the mechanisms of chromosome evolution in Nabidae and especially the phenomenon of „autosomal polyploidy“. It should be noted, however, that our first effort in this direction (using C-banding, Ag-NOR-banding and DNA specific fluorochromes CMA_3 and DAPI) did not give us a deeper insight into the problem (GROZEVA and NOKKALA 2003; GROZEVA et al. 2004).

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