Folia biologica (Kraków), vol. **54** (2006), No 1-2

First Evidence of Sex Chromosome Pre-reduction in Male Meiosis in the Miridae Bugs (Heteroptera)

Snejana GROZEVA, Seppo NOKKALA and Nikolay SIMOV

Accepted January 25, 2006

GROZEVA S., NOKKALA S., SIMOV N. 2006. First evidence of sex chromosome pre-reduction in male meiosis in the Miridae bugs (Heteroptera). Folia biol. (Kraków) **54**: 9-12.

The karyotype and male meiosis of *Macrolophus costalis* Fieber (Insecta, Heteroptera, Miridae) were studied using C-banding, AgNOR-banding and DNA sequence specific fluorochrome staining. The chromosome formula of the species is $2n=28(24+X_1X_2X_3Y)$. Male meiotic prophase is characterized by a prominent condensation stage. At this stage, two sex chromosomes, "X" and Y are positively heteropycnotic and always appeared together, while in autosomal bivalents homologous chromosomes were aligned side by side along their entire length, that is, meiosis is achiasmatic. At metaphase I, "X" and Y form a pseudobivalent and orient to the opposite poles. At early anaphase I, the "X" chromosome disintegrates into three separate small chromosomes, X_1, X_2, and X_3. Hence both the autosomes and sex chromosomes segregate reductionally in the first anaphase, and separate equationally in the second anaphase. This is the first evidence of sex chromosome pre-reduction in the family Miridae. Data on C-heterochromatin distribution and its composition in the chromosomes of this species are discussed.

Key words: *Macrolophus costalis*, Miridae, Heteroptera, holokinetic chromosomes, achasmatic male meiosis, sex chromosome pre-reduction.

Snejana GROZEVA, Institute of Zoology, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel, 1000 Sofia, Bulgaria.
E-mail: sgrozeva@yahoo.com
Seppo NOKKALA, Laboratory of Genetics, Department of Biology, University of Turku, FIN-20014 Turku, Finland.
E-mail: seppo.nokkala@utu.fi
Nikolay SIMOV, National Museum of Natural History, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel, 1000 Sofia, Bulgaria.
E-mail: nikolaysimov@yahoo.com, simov@nmnh.bas.bg

The Miridae is the largest family of true bugs (Heteroptera, Cimicomorpha) with approximately 10000 species described (SCHUH 1995). Cytogenetic data are presently available for about 200 species (UESHIMA 1979; NOKKALA & NOKKALA 1986; GROZEVA 2003). In the Miridae species, chromosome numbers vary from 17 to 80 with XY or X_1X_2Y sex chromosomes, but the karyotype of 2n=32+XX/XY clearly predominates. Although many studies on the bugs' chromosomes are presently conducted using different staining techniques, only routine techniques have been so far applied to study of miride karyotypes. As with all other Heteroptera, the Miridae bugs possess holokinetic or holocentric chromosomes (UESHIMA 1979). They are also characterized by an inverted sequence of reductional and equational divisions of the sex chromosomes (post-reduction) in male meiosis (UESHIMA 1979), and the absence of chiasmata in male meiosis, the achiasmatic meiosis being of a peculiar collochore type (NOKKALA & NOKKALA 1986).

In the present study, the first information on the karyotype and male meiosis in *Macrolophus costalis* Fieber, with emphasis on the pre-reduction of the sex chromosomes, was obtained using C-banding, AgNOR-banding and DNA sequence specific fluorochrome staining with DAPI and CMA_3, respectively.

Material and Methods

Males of *Macrolophus costalis* were collected in different parts of Bulgaria by netting in tobacco fields. The bugs were fixed in 3:1 fixative (96% ethanol-glacial acetic mixture). After dissection, the gonads were squashed in a small drop of 45% acetic acid. The cover slips were removed by the dry ice technique. Slides were dehydrated in fresh

fixative (3:1) and air dried. Part of the preparations were stained by the Schiff-Giemsa method of GROZEVA & NOKKALA (1996) in order to study the number and the behaviour of the chromosomes, whereas different methods were applied to the other slides: C-banding (SUMNER 1972 with minor modifications) to reveal the amount of heterochromatin and its distribution in the karyotype; DNA binding fluorochromes, GC-specific chromomycin A_3 (CMA_3) and AT-specific 4'-6' diamino-2-phenylindole (DAPI) were used to reveal molecular organization of the chromatin according to the method of SCHWEIZER (1976) and DONLON & MAFENIS (1983) with minor modifications by KUZNETSOVA *et al*. (2001); and AgNOR-staining by HOWELL & BLACK (1980) to localize the NOR on the chromosomes. The fluorescence images were documented on Kodak 160 ASA slide colour film.

Results

In male meiosis of *Macrolophus costalis*, the condensation stage is most abundant and shows twelve autosomal bivalents and a positively heteropycnotic sex chromosome body (Fig. 1). Size differences between the autosomal bivalents were clearly observed. The complement includes two extremely large bivalents, at least five times the size of the other ten bivalents which are quite similar in size. At this stage, it becomes apparent that the bivalents consist of parallel-aligned homologous chromosomes and chiasmata are absent in male meiosis, i.e. the meiosis is achiasmatic. At metaphase I (MI) the staining of the autosomal bivalents and the sex chromosomes is similar, indicating their equal condensation at this stage. Both autosomal bivalents and the sex chromosomes co-orient with homologous chromosomes facing opposite poles. At this stage the sex chromosomes look like two chromosomes oriented to the poles (Fig. 2). At early anaphase, one of these chromosomes consists of three small chromosomes, referred to X_1, X_2, and X_3 and one is the Y chromosome (Fig. 3). Thus, the chromosome formula of *M. costalis* is 2n=28 ($24+X_1X_2X_3Y$) for males. The sex chromosomes segregate in the first meiotic division and result in two types of daughter cells. Half of the second metaphases have 12 autosomes and three X chromosomes and the other half have 12 autosomes and the large Y (Fig. 4). Male meiosis is hence pre-reductional both for the autosomes and the sex chromosomes.

The C-banded condensation stage demonstrated two pairs of small but conspicuous interstitial bands in two large bivalents, dividing them into three almost equal parts. At this stage, C-blocks on the positively heteropycnotic sex chromatin body were seen (Fig. 5). At MI, the interstitial blocks on the large bivalents could still be seen, the Y is positively heteropycnotic with one telomeric block, while all three X chromosomes are negatively heteropycnotic and do not show any C-bands (Fig. 6).

Staining with DAPI and CMA_3 fluorochromes produces similar staining patterns in the chromosomes, corresponding well to the C-banding. Bright fluorescent bands are observed by both fluorochromes in identical locations both in the large autosomal bivalents and in the sex chromosomes (Figs 7, 8).

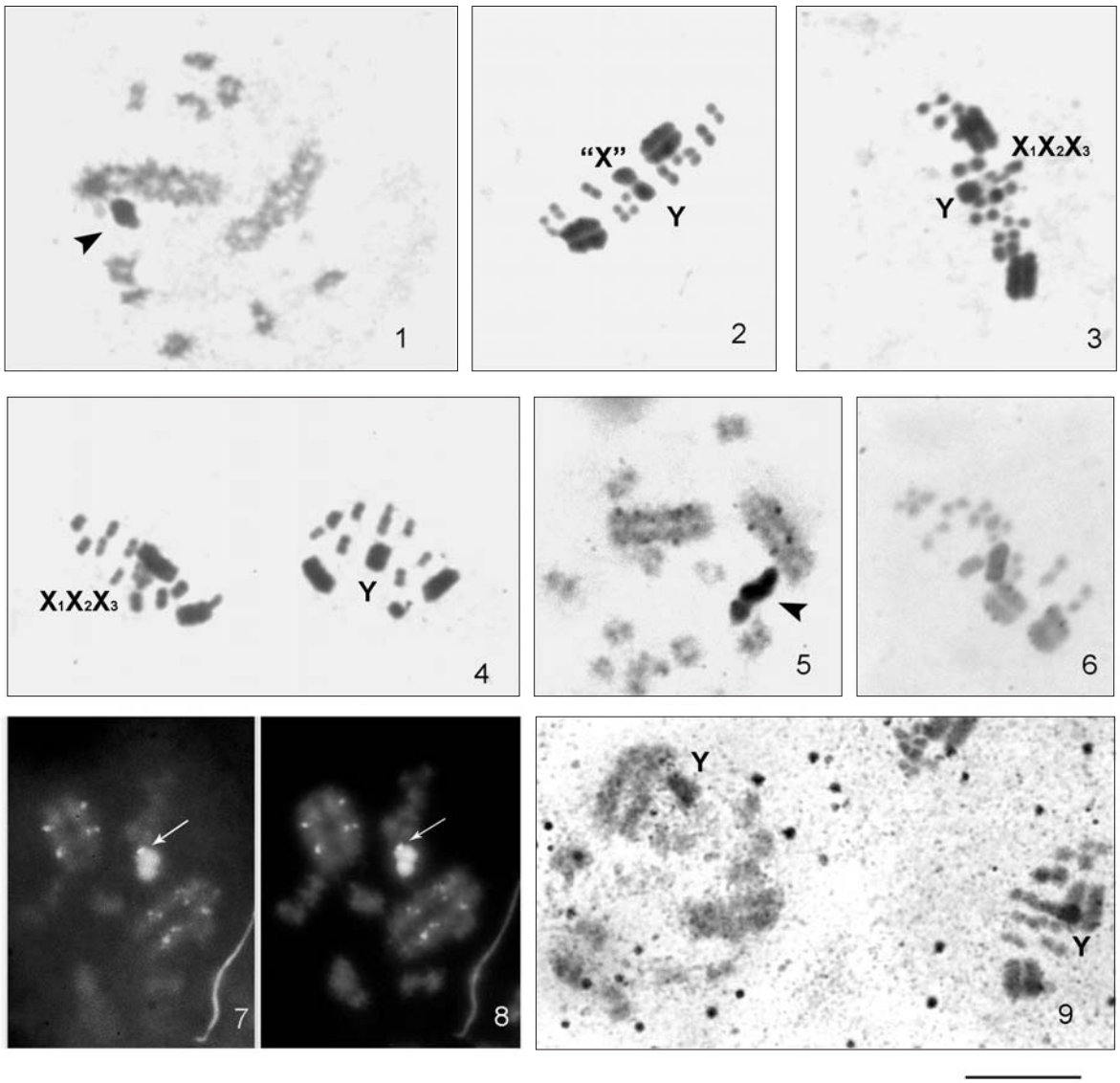
Ag-NOR-staining revealed that NORs are located on the Y chromosome (Fig. 9), as in many other Heteropteran species (GROZEVA *et al*. 2004; unpublished data).

Discussion

Macrolophus costalis is included in the tribe Dicyphini of the subfamily Bryocorinae (Heteroptera, Miridae). In Bryocorinae, besides the modal chromosome number for Miridae (2n=34, XY), some higher (2n=38, XY and $2n=46+XY/X_1X_2Y$) and lower (2n=18-28, XY) chromosome numbers were described (UESHIMA 1979; GROZEVA 2003). The karyotype formula $2n=24+X_1X_2X_3Y$ is reported for the first time in the tribe, and this number of sex chromosomes for the first time in the family.

In the Heteropteran species studied in this aspect, C-heterochromatin, if present, is preferentially located near or on the telomeres (MURAMOTO 1980; CAMACHO *et al*. 1985; PAPESCHI 1988, 1991; PANZERA *et al*. 1992, 1997; PEREZ *et al*. 1992, 1997). Interstitial C-heterochromatin blocks are reported on one autosome pair of *Nezara viridula* (CAMACHO *et al*. 1985) and in *Triatoma patagonica* (PANZERA *et al*. 1997); on all or almost all chromosomes of *Acalypta nigrina* and *Kalama tricornis* (Tingidae), *Nabis* (*Dolichonabis*) *limbatus* and *N.* (*Aspilaspis*) *indicus* (Nabidae), and *Tenagobia* (*Fuscagobia*) *fuscata* (Micronectidae) (GROZEVA & NOKKALA 2001, 2003; GROZEVA *et al*. 2004; ITUARTE & PAPESCHI 2004). In the cases mentioned above, if interstitial blocks are observed then there are no telomeric blocks on the same chromosome. This is also true for the large bivalents of *M. costalis*. At present, there is no apparent explanation for the formation of these peculiar types of heterochromatin distribution.

DAPI and CMA positive signals were observed at the same location as C-bands. This distribution of the fluorescent signals suggests that C-heterochromatin in *M. costalis* karyotype consists of dispersed AT- and GC-rich clusters as previously described in *Ci-*



Figs 1-9. Male meiosis in *Macrolophus costalis* (Heteroptera, Miridae). Fig. 1. Condensation stage after Schiff-Giemsa staining. Fig. 2. Metaphase I after Schiff-Giemsa staining, sex chromosomes look like a pseudobivalent. Fig. 3. Early anaphase I after Schiff-Giemsa staining, all four sex chromosomes can be seen. Fig. 4. Second metaphase daughter cells after Schiff-Giemsa staining with three X-chromosomes, or Y chromosome, respectively. Fig. 5.Condensation stage after C-banding staining, C-blocks on the large bivalents and the sex chromosome body can be seen. Fig. 6. First metaphase after C-banding staining, C-blocks on the large bivalents and the Y chromosome can be seen. Fig. 7. Condensation stage after DAPI staining, positive signals on the same locations as the C-blocks. Fig. 8. Condensation stage after CMA3 staining, positive signals on the same locations as the C-blocks. Fig. 9. Condensation stage after Ag-NOR staining, NOR is located on the Y chromosome. Arrowhead shows the sex chromosome body, while the arrow points to the telomere signal on the Y chromosome. Bar = 10 μm.

mex sp. (Heteroptera, Cimicidae) (GROZEVA & NOKKALA 2002), *Pseudococcus viburni* (Homoptera, Coccinea) (NECHAYEVA *et al*. 2004) and three species of Psocoptera (GOLUB *et al*. 2004).

In spermatogenesis of Heteropteran species, the post-reduction of the sex chromosomes is the rule (UESHIMA 1979). The Tingidae is the only family in the order in which all species share a pre-reduction of the sex chromosomes. There are a number of reports about the sporadic existence of sex chromosome pre-reduction in separate species from different families of Heteroptera: three species of *Anisops* in Notonectidae (JANDE 1961); *Ectrychotes dispar* in Reduviidae (MANNA 1951); and four species from two genera of Coreidae (see UESHIMA 1979). The observation of pre-reduction of the sex chromosomes in *M. costalis* is the first finding in the family Miridae. In addition, the behaviour of the sex chromosomes is highly peculiar. Through the prophase stages and still at metaphase I two separate sex chromosomes, "X" and Y, can be seen. At anaphase I, the "X" chromosome disintegrated into the three separate chromosomes X_1, X_2 and X_3. This kind of behaviour of sex chromosomes, either X or Y, has not been described earlier.

Hitherto, predatory bugs of the genus *Macrolophus* and other Dicyphini species are considered as important natural enemies in suppressing whitefly and aphid populations on vegetables in tomato, cucumber and tobacco greenhouses and successfully used as bio control agents for pest whiteflies (GOULA & ALOMAR 1994; MARGARITOPOULOS *et al*. 2003). The taxonomic status of *Microlophus* species applied as biocontrol agents is still in doubt (PERDIKIS *et al*. 2003), so cytogenetic studies may give some clues for correct determination of these species. Clearly, the highly asymmemtric karyotype with two large autosome pairs, the interstitial distribution of C-heterochromatin in the large autosome pairs, the sequence composition of C-heterochromatin, pre-reduction of sex chromosomes, and peculiar behaviour of the multiple X chromosomes provide excellent cytogenetic markers for karyosystematic studies of *Macrolophus* and closely related species.

Acknowledgements

We thank prof. V. KUZNETSOVA for the valuable comments on the manuscript. This study is partly supported by the Bulgarian Ministry of Education and Science (Grant B-1304), the Bulgarian Academy of Sciences and the Academy of Finland.

References

CAMACHO J. P. M., BELDA J., CABRERO J. 1985. Meiotic behaviour of the holocentric chromosomes of *Nezara viridula* (Insecta, Heteroptera) analysed by C-banding and silver impregnation. Can. J. Genet. Cytol. **27**: 490-497.

DONLON T. A., MAFENIS R. E. 1983. Methyl green is a substitute for distamycin A in the formation of distamycin A/DAPI C-bands. Hum. Genet. **65**: 144-146.

GOLUB N. V., NOKKALA S., KUZNETSOVA V. G. 2004. Holocentric chromosomes of psocids (Insecta, Psocoptera) analysed by C-banding, silver impregnation and sequence specific fluorochromes CMA3 and DAPI. Folia biol. (Kraków) **52**: 143-149.

GOULA M., ALOMAR O. 1994. Miridos (Heteroptera, Miridae) de interes en el control integrado de plagad en el tomate. Guia para su identification. Bol. San. Veg. Plagas **20**: 131-143.

GROZEVA S. 2003. Karyotype of three endemic Mediterranean Miridae species (Heteroptera) from Bulgaria. Acta Zool. Bulg. **55**: 53-59.

GROZEVA S., KUZNETSOVA V., NOKKALA S. 2004. Patterns of chromosome banding in four nabid species (Heteroptera, Cimicomorpha, Nabidae) with high-chromosome number karyotypes. Hereditas **140**: 99-104.

GROZEVA S., NOKKALA S. 1996. Chromosomes and their meiotic behaviour in two families of the primitive infraorder Dipsocoromorpha (Heteroptera). Hereditas **125**: 31-36.

GROZEVA S., NOKKALA S. 2001. Chromosome number, sex chromosomes system and the distribution of constitutive heterochromatin in 13 lacebug species (Heteroptera, Tingidae) Folia biol. (Kraków) **49**: 29-41.

GROZEVA S., NOKKALA S. 2002. Achiasmatic male meiosis in *Cimex* sp. (Heteroptera, Cimicidae). Caryologia **55**: 189-192.

GROZEVA S., NOKKALA S. 2003. C-heterochromatin and extra (B) chromosome distribution in six species of the *Nabis* (Heteroptera, Nabidae) with the modal male genus karyotype 2n=16+XY. Eur. J. Entomol. **51**: 13-21.

HOWELL W. M., BLACK D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a one step method. Experentia **36**: 1014-1015.

ITUARTE S., PAPESCHI A. G. 2004. Achiasmatic male meiosis in *Tenagobia* (*Fuscagobia*) *fuscata* (Stål) (Heteroptera, Corixoidea, Micronectidae). Genetica **122**: 199-206.

JANDE S. S. 1961. Chromosome studies in the family Notonectidae (Cryptocerata-Heteroptera). Chromosoma **12**: 318-326.

KUZNETSOVA V. G., WESTENDORFF M., NOKKALA S. 2001. Patterns of chromosome banding in the sawfly family Tenthredinidae (Hymenoptera, Symphyta). Folia biol. (Kraków) **54**: 227-233.

MANNA G. K. 1951. A study of the chromosomes during meiosis in forty-three species of Indian Heteroptera. Proc. Zool. Soc. (Bengal) **4**: 1-116.

MARGARITOPOULOS J. T., TSITSIPIS J. A., PERDIKIS D. C. 2003. Biological characteristics of the mirids *Macrolophus costalis* and *Macrolophus pygmaeus* prying on the tobacco form of *Myzus persicae* (Hemiptera, Aphididae). Bull. Entomol. Res. **93**: 39-47.

MURAMOTO N. 1980. A study of the C-banded chromosomes in some species of heteropteran insects. Proc. of the Japan Academy, 56, ser. B, **3**: 125-130.

NECHAYEVA G. A., KUZNETSOVA V. G., NOKKALA S. 2004. New data on the karyotype of *Pseudococcus viburni* (Sign.) (Homoptera, Coccinea). Entomol. Obozr. **83**: 23-31.

NOKKALA S., NOKKALA C.1986. Achiasmatic male meiosis of collochore type in the heteropteran family Miridae. Hereditas **105**: 193-197.

PANZERA F., ALVAREZ F., SANCHES-RUFAS J., PEREZ R., SUJI J. A., SCVORTZOFF E., DUJARDIN J. P., ESTRAMIL E., SALVATELLA R. 1992. C-heterochromatin polymorphism in holocentric chromosomes of *Triatoma infestans* (Hemiptera, Reduviidae). Genome **35**: 1068-1074.

PANZERA F., HORHOS S., PEREIRA J., CESTAU R., CANALE D., DIOTAIUTI L., DIJARDIN J. P., PEREZ R. 1997. Genetic variability and geographic differentiation among three species of Triatomine bugs (Hemiptera, Reduviidae). Am. J. Trop. Med. Hyg. **57**: 732-739.

PAPESCHI A. G. 1988. C-banding and DNA content in three species of *Belastoma* (Heteroptera) with large differences in chromosome size and number. Genetica **76**: 43-51.

PAPESCHI A. G. 1991. DNA content and heterochromatin variation in species of *Belastoma* Heteroptera, Belastomidae). Hereditas **115**: 109-114.

PERDIKIS D. C., MARGARITAPOULOS J. T., STAMATIS C., MAMURIS Z., LYKOURESSIS D. P., TSITSIPIS J. A., PEKAS A. 2003. Discrimination of the closely related bio control agents *Macrolophus melanotoma* (Hemiptera: Miridae) and *M. pygmaeus* using mitochondrial analysis. Bull. Entomol. Res. **93**: 507-517.

PEREZ R., PANZERA J., SCAFIEZZO S., MAZZELLA M. C., PANZERA F., DUJARDIN J. P., SCVORTZOFF E. 1992. Cytogenetics as a tool for Triatomine species distribution (Hemiptera, Reduviidae). Mem. Inst. Oswaldo Cruz, Rio de Janeiro **87**: 353-361.

PEREZ R., PANZERA F., PARE J., SUJA J. A., RUFAS J. S. 1997. Meiotic behavior of holocentric chromosomes: orientation and segregation of autosomes in *Triatoma infestans* (Heteroptera). Chromosome Res. **5**: 47-56.

SCHUH R. T. 1995. Plant bugs of the world (Insecta: Heteroptera: Miridae). Systematic Catalog Distributions, Host list, and Bibliography. The New York Entomol. Soc. xii+1329 pp.

SCHWEIZER D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma **58**: 307-324.

SUMNER A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304-306.

UESHIMA N. 1979. Insecta 6. Hemiptera II. Heteroptera, (In: Animal Cytogenetics, B. John, Gebruder Borntraeger, Berlin, Stuttgart): 118.