

Chromosomes in two bug species of *Hebrus* (Hebridae, Heteroptera). The occurrence of neo-XY sex chromosome system in Heteroptera

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Abstract — Chromosomes in two species in the genus *Hebrus* (Hebridae, Heteroptera) were studied. The species *H. ruficeps* (Th.) showed the chromosome number $2n = 22A + XY$ with achiasmate sex chromosomes, which divided in the first meiotic division and separated from one another at the second division. *H. pusillus* (Fn.) displayed $2n=22A + \text{neo-XY}$. The chromosomes of the neo-XY bivalent separated prereductively in the first division. Both species lacked m-chromosomes. It was suggested that although the XY sex chromosome system prevails in Heteroptera, the formation of the neo-XY system has taken place in an XO ancestor after the loss of the Y chromosome.

Key words: Hebridae, Heteroptera, sex chromosomes, neo-XY sex chromosome system.

INTRODUCTION

Quite diverse sex chromosome systems are encountered in Heteroptera. Most common is the XY system characterized by achiasmate sex chromosomes, which divide in the first division and separate from one another at the second division, after a brief "touch and go" pairing (UESHIMA 1979). The XY system is found both in primitive and advanced taxa, and it also appears to be the ancestral sex chromosome system in the order (UESHIMA 1979, GROZEVA and NOKKALA 1996). Fragmentation of the X chromosome has occurred in several taxa, resulting in multiple X chromosome systems (cf. UESHIMA 1979). Also multiple Y chromosomes are known, but only two cases have been recorded so far (GROZEVA and NOKKALA 1996). The loss of the Y chromosome is quite commonly observed. There are many families with the XO system. There are also some genera, where a part of the species possesses a Y chromosome, while another part lacks it (UESHIMA 1979, NOKKALA and NOKKALA 1984).

The quite frequent occurrence of species with the XO sex chromosome system implies the possibility to evolve a derived sex chromosome system in which the univalent X chromosome is translocated to an autosome giving rise to a neo-XY system. In two species in the family Belostomatidae, *Lethocerus sp.* (CHICKERING and BACRON 1933) and *L. indicum* (JANDE 1959) only bivalents have been observed in meiosis, and it has been suggested that both the X and Y have been translocated to one pair of autosomes.

In the present study, we have analyzed the behavior of chromosomes in two Finnish species of the Gerromorphan genus *Hebrus*, *H. ruficeps* (Th.) and *H. pusillus* (Fn.). Our observations provide an unambiguous evidence on the presence of the neo-XY system in one of these species.

MATERIALS AND METHODS

Adult specimens of *Hebrus ruficeps* (Th.) and *H. pusillus* (Fn.) (Hebridae, Heteroptera) were collected from natural populations on the banks of lake Eljarvi, Ylane, 60 km northeast from Turku. Adult males were fixed in a fresh 3:1 mixture of

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ethanol and glacial acetic acid immediately after capture. The material was transferred to laboratory and stored in fixative until preparations were made.

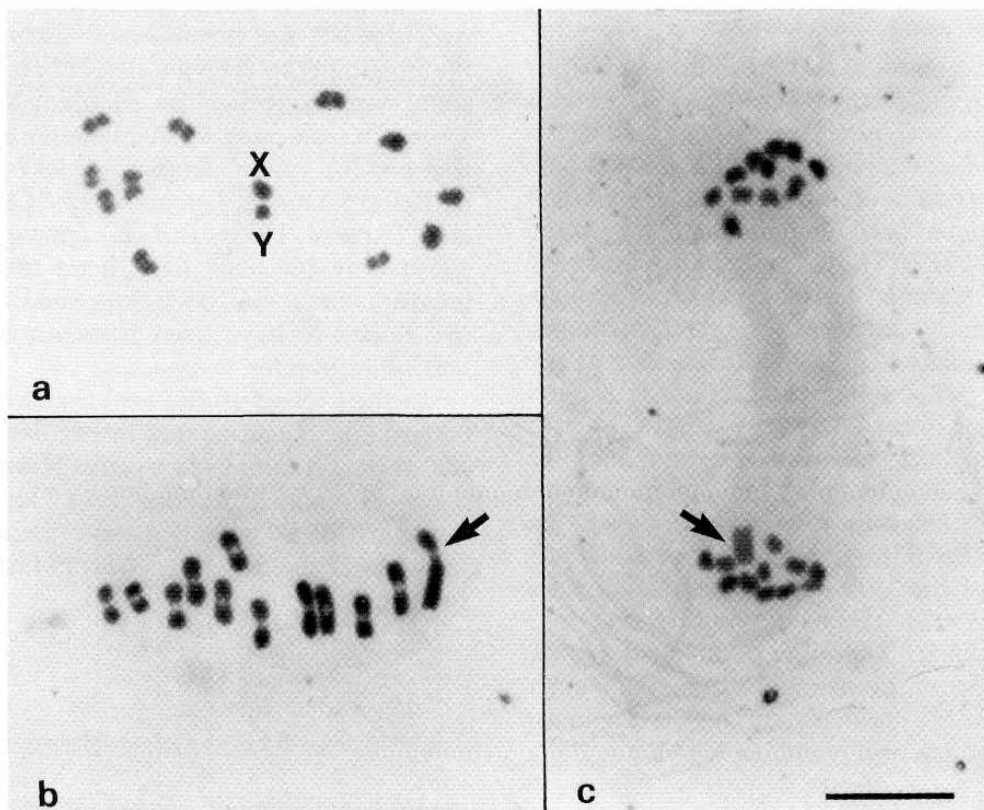
Testes were dissected out and squashed in a drop of 45% acetic acid. After removing cover slips, slides were dehydrated by immersing in fresh 3:1 fixative for 15 minutes, air dried and stored in a dust free place until stained. The Feulgen-Giemsa procedure described by GROZEVA and NOKKALA (1996) was used for staining. Briefly, slides were immersed in 1 N HCl at room temperature for 15-20 min, hydrolyzed in 1 N HCl at 60 °C for 7 min, and stained in Schiff's reagent for 20 min. Slides were rinsed thoroughly with distilled water and finally with SO-rensen's phosphate buffer, pH 6.8 for 5 min, followed by staining with 2% Giemsa in the phosphate buffer for 20 min. After staining, the slides were rinsed briefly with distilled water, air dried, and mounted in Entellan.

RESULTS

Hebrus ruficeps (Th.)

Only cells at metaphase II were available for this species. Metaphase II plates revealed eleven autosomal dyads arranged radially with sex chromosomes, X and Y, forming a pseudobivalent in the center (Fig. 1a). The X chromosome was considerably larger than the Y chromosome. There were no m-chromosomes present. It is evident that the sex chromosomes followed the conventional, post-reductional mode of Heteropteran sex chromosome behavior, i.e., they divided in the first division and segregated in the second.

The chromosome formula of *H. ruficeps* can be expressed as $2n = 22A + XY$, deviating considerably from that reported by COBBEN (1968), viz. $2n = 19(XO)$ for the male of this species.



Figs. 1 a-c. — Meiotic chromosomes in two species of *Hebrus*. a. *H. ruficeps*, M II, showing 11 autosomal dyads and XY pseudobivalent in the center of the plate, b. *H. pusillus*, M I, showing 11 autosomal bivalents and the heteromorphic neo-XY bivalent (arrow), c. *H. pusillus*, A I, the neo-X and neo-Y chromosomes move undivided towards opposite poles. The neo-X chromosome is arrowed. Bar=10 μ m

H. pusillus (Fn.)

In *H. pusillus*, plenty of cells at diakinesis, metaphase I and anaphase I were observed. At diakinesis and metaphase I (Fig. 1b) there were always twelve bivalents with one terminally located chiasma. No univalent sex chromosomes or m-chromosomes were encountered. Among the relatively evenly-sized bivalents, one conspicuous heteromorphic bivalent was observed. At metaphase I, this bivalent oriented similarly as the other bivalents, free telomeres facing poles, indicating co-orientation. Anaphase I cells (Fig. 1c) confirmed that the constituents of the heteromorphic bivalent moved undivided towards opposite poles.

There is no doubt that the heteromorphic bivalent represents a sex chromosome system of this species. The larger element is formed by a fusion between the original X chromosome and an autosome, constituting thus a neo-X chromosome, while the smaller element, a former autosome, forms a neo-Y chromosome.

The chromosome formula of *H. pusillus* can now be expressed as $2n = 22A + \text{neo-X/neo-Y}$.

DISCUSSION

The family Hebridae belongs to the infraorder Gerromorpha. At present information is available on chromosome numbers and sex chromosome systems for five families in this infraorder (UESHIMA 1979, this study). Only the family Gerridae appears to be characterized by an XO sex chromosome system. All other families, Mesovelidae, Hydrometridae, Hebridae, and Velidae, include also species with the XY system in addition to species with the XO system. This data indicates that the XY sex chromosome system is the ancestral one in the infraorder and the XO system is secondary resulting from the loss of the Y chromosome. Evidently, the loss of the Y chromosome has occurred not in one but several occasions in different phases of phylogenetic divergence in this infraorder. The infraorder is further characterized by the absence of m-chromosomes.

The neo-XY sex chromosome system is known in many groups which predominantly show the XO system, e.g. in Orthoptera, Odo-

nata (WHITE 1973), and in the homopteran taxa Psylloidea (KUZNETSOVA *et al.* 1997) and Auchenorrhyncha (KUZNETSOVA 1989; EMELY-ANOV and KIRILLOVA 1989, 1991). The system is also known in Coleoptera where the XY system is prevailing (SMITH and VIRKKI 1978). In Coleoptera most frequent neo-XY formers have been found in taxa with the XO system, indicating that translocation of the X to an autosome has most likely occurred in an XO ancestor. To explain the formation of the neo-XY system in Heteroptera, we like to suggest that also in Heteroptera its formation has taken place in the XO karyotype, i.e. after the loss of the Y. We feel that the translocation of both the X and Y to homologous autosomes (see introduction) is far too complicated process, especially since the liability of Y chromosomes to undergo losses during evolution indicates that the Y has only little if any function in determining the sex.

In *Hebrus* species the free X divides in the first division and separates from the Y at the second division, whereas the X chromosome segment in the neo-X chromosome moves undivided to a pole in the first anaphase. It remains to be seen, if this kind of shift from postreductional to prereductional behavior is typical for neo-XY formation in Heteroptera. In Odonata both postreductional and prereductional behavior of X chromosome segment in the neo-X have been described (WHITE 1973).

REFERENCES

- COBBEN R.H., 1968. — *Evolutionary trends in Heteroptera. Part I. Eggs, architecture of the shell, gross embryology and eclosion.* 475 pp. Center Agric. Publ. & Document, Wageningen.
- EMELYANOV A.F. and KIRILLOVA V. I., 1989. — *Trends and modes of karyotype evolution in the Cicadina (Homoptera). I. Peculiarities and evolutionary changes of the karyotypes in the superfamily Cicadelloidea.* Entomol. Obozr. 68: 587-603 (in Russian).
- , 1991. — *Trends and modes of karyotype evolution in Cicadina. II. Peculiarities and evolutionary changes of the karyotypes in the superfamilies Cercopoidea, Cicadoidea, Fulgoroidea, and in the Cicadina as a whole (Homoptera).* Entomol. Obozr. 70: 796-817 (in Russian).
- GROZEVA S. and NOKKALA S., 1996. — *Chromosomes and their meiotic behavior in two families of the primitive infraorder Dipsocoromorpha (Heteroptera).* Hereditas, 125: 31-36.
- CHICKERING A.M. and BACRON B., 1933. — *Spermatogenesis in the Belostomatidae. IV. Multiple chromosomes in Lethocerus.* Pap. Mich. Acad. Sci., 15: 529-533.

- JANDE S.S., 1959. — *An analysis of the chromosomes in the four species of the family Eelostomatidae (Heteroptera, Cryptocerata)*. Res. BuU. (NS) Panjab Univ., 10: 25-34.
- KUZNETSOVA V.G., 1989. — *The chromosome mechanisms of sex determination in the Insecta (with reference to the Hemiptera)*. In: M. Tonner, T. Soldan, B. Bennettova (Eds), "Regulation of Insect Reproduction. IV". Proc. Symp. 293-301. Praha.
- KUZNETSOVA V.G., NOKKALA S. and MARYANSKA-NADA-CHOWSKA A., 1997. — *Karyotypes, sex chromosome systems, and male meiosis in Finnish Psyllids (Homoptera: Psylloidea)*. Folia biol. (Krakow), 45: 143-152.
- NOKKALA S. and NOKKALA C., 1984. — *Occurrence of the XO sex chromosome system in Dictyonota tricornis (Schr.) (Tmgidae, Hemiptera) and its significance for concepts of sex chromosome evolution in Heteroptera*. Hereditas, 100: 299-301.
- SMITH S.G. and VIRKKI N., 1978. — *Animal Cytogenetics*. Vol. 3: Insecta 5, Coleoptera, 366 pp. Gebruder Borntraeger, Berlin, Stuttgart.
- UESHIMA N., 1979. — *Animal Cytogenetics*. Vol. 3: Insecta 6, Hemiptera II: Heteroptera, 117 pp., Gebruder Borntraeger, Berlin, Stuttgart.
- WHITE M.J.D., 1973. — *Animal Cytology and Evolution*. Cambridge University Press.

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