

**Cytogenetic analysis of some aradid species (Heteroptera: Aradidae)**

SNEJANA GROZEVA

Institute of Zoology, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel, 1000 Sofia, Bulgaria

**Karyotype, male reproductive system, flat bugs, Aradidae**

**Abstract.** Known sex chromosome mechanisms in Aradidae are XX : XY or compound X( $X_nY$ ). The present paper adds data obtained from studying testes of adults and stage IV and V larvae of four aradid species. The number of chromosomes and their behaviour during spermatogenesis (in Aradinae for the first time) were documented and illustrated: *Aradus cinnamomeus* Panzer –  $2n = 35$  ( $32 + X_1X_2Y$ ); *Aradus corticalis corticalis* (L.) –  $2n = 28$  ( $26 + XY$ ); *Aradus conspiculus* Herrich-Schaeffer  $2n = 28$  ( $26 + XY$ ); *Aneuris avenius* Dufour –  $2n = 27$  ( $24 + X_1X_2Y$ ). Mechanisms which could have played a role in karyotype evolution in the family are discussed in the context of this study and data from literature.

*Introduction*

Within the Aradidae there are about 1,800 known species and 211 genera (Kormilev & Froeschner, 1987). On the basis of select morphological characters, the Aradidae are placed in the infraorder Pentatomomorpha. However, in contrast to all other Pentatomomorpha the Aradidae have no abdominal trichobothria and hold a unique place in the infraorder. The relationships between eight aradid subfamilies (Usinger & Matsuda, 1959) are discussed by different authors (Kumar, 1967; Vasarhelyi, 1986; Grozeva & Kerzhner, 1992).

Karyological investigations in the family are very scarce; the chromosome numbers of only one isodermine species, 9 species of Aneurinae, 3 carventine species and two species of Mezirinae (Table 1) are known. There is no available description of the karyotype, although the spermatogenesis and oogenesis in the Aneurinae were investigated by Jacobs (1986) in a study of the type of sex determination mechanism ( $X_1X_2Y$  or  $XY_1Y_2$ ). The sex chromosome mechanism of studied aradid species is not very uniform; those studied are XX : XY mechanism or compound X ( $X_nY$ ) chromosome mechanism. There are no m-chromosomes in the Aradidae thus far studied.

It is known that spermatogenesis of *Aradus cinnamomeus* is completed during the last larval stage (Southwood & Leston, 1959). It is likely that this is also true for other aradid species and this hypothesis was confirmed in our investigations. Karyological studies of this family are hindered by difficulties in collecting specimens in the stage that is most suitable for chromosome analysis.

*Material and methods*

Material for the present study was collected in Bulgaria (*Aradus conspiculus* Herrich-Schaeffer – 12♂), Estonia [*Aradus corticalis corticalis* (L.) – 18♂ and *Aradus cinnamomeus* Panzer – 15♂] and Ukraine (*Aneuris avenius* Dufour – 13♂).

Only adults of the first two of these mentioned species and only stage IV and V larvae of the latter two species were studied. Specimens were fixed in 2.5 : 1 methanol and propionic acid (after Jacobs, 1986). Spermatogenesis was investigated on squash-preparations of gonads that were stained by lactoacetocein.

*Results*

Behaviour of the chromosomes during male meiosis was studied. All stages that are typical for most Heteroptera were observed. After diplotene the nucleus enters a “diffuse stage”. During this stage, the nucleus returns to an interphase-like state. The first division of meiosis is reductional for the autosomes and equational for the sex chromosomes, as is the case in most Heteroptera (Ueshima, 1979). At metaphase II the pseudo bi- or trivalent of the sex chromosomes lies in the circle of the autosomes.

TABLE 1. Chromosome numbers of aradid species.

Species	2n	MI	References
<b>Aradinae</b>			
<i>Aradus cinnamomeus</i>	35	16 + X <sub>1</sub> X <sub>2</sub> Y	New data
<i>A. corticalis corticalis</i>	28	13 + XY	New data
<i>A. conspiculus</i>	28	13 + XY	New data
<b>Isoderminae</b>			
<i>Isodermus gayi</i>	23	10 + X <sub>1</sub> X <sub>2</sub> Y	Ueshima, 1963
<b>Aneurinae</b>			
<i>Paraneurus ruandae multifarius</i>	32	15 + XY	Jacobs, 1986
<i>P. brincki brincki</i>	27	12 + X <sub>1</sub> X <sub>2</sub> Y	Jacobs, 1986
<i>P. brincki marieps</i>	26	12 + X <sub>1</sub> X <sub>2</sub> Y	Jacobs, 1986
<i>P. nodosus</i>	27	12 + X <sub>1</sub> X <sub>2</sub> Y	Jacobs, 1986
<i>P. congolensis</i>	40	18 + X <sub>1</sub> X <sub>2</sub> X <sub>3</sub> Y	Jacobs, 1986
<i>Breviscutaneurus breviscutatus</i>	16	7 + XY	Jacobs, 1986
<i>B. medioscutatus</i>	24	11 + XY	Jacobs, 1986
<i>B. helenae</i>	22	10 + XY	Jacobs, 1986
<i>Aneurillus foliaceus</i>	24	11 + XY	Jacobs, 1986
<i>Aneurus avenius</i>	27	12 + X <sub>1</sub> X <sub>2</sub> Y	New data
<b>Carventinae</b>			
<i>Adamanotus uncotibialis</i>	16	7 + XY	Jacobs, 1990
<i>Dundocoris nigromaculatus</i>	20	9 + XY	Heiss & Jacobs, 1989
<i>Trichocarventus klapperichi</i>	28	13 + XY	Heiss & Jacobs, 1989
<b>Mezirinae</b>			
<i>Dysodius lunatus</i>	31	14 + X <sub>1</sub> X <sub>2</sub> Y	Schrader, 1947
<i>Mezira pacifica</i>	27	12 + X <sub>1</sub> X <sub>2</sub> Y	Ueshima, 1963

#### Subfamily Aradinae

*Aradus cinnamomeus* Panzer: 2n = 35 (32 + X<sub>1</sub>X<sub>2</sub>Y)

At metaphase I (MI) of spermatogenesis 16 autosomal bivalents and three univalent sex chromosomes are visible (Fig. 1). The autosomal bivalents are all similar in size. At this stage it is difficult to identify the sex chromosomes. Their number was determined by analysing chromosome behaviour during the second meiotic division. At MII autosomes are situated in a circle with the sex chromosomes associated in a pseudo-trivalent at the center (Fig. 2).

*Aradus corticalis corticalis* (L.): 2n = 28 (26 + XY)

At MI of spermatogenesis 13 autosomal bivalents and two univalent sex chromosomes are visible (Fig. 3). Autosomal bivalents with well-visible chromatids decrease gradually in size. The sex chromosomes are nearly the smallest autosomal bivalents.

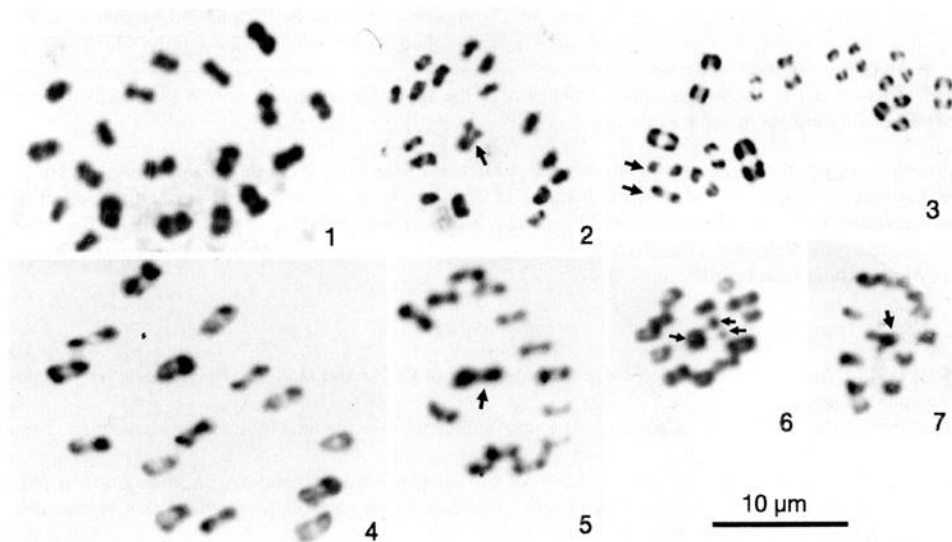
*Aradus conspiculus* Herrich-Schaeffer: 2n = 28 (26 + XY)

The karyotype of this species is similar that of *A. corticalis corticalis* (Fig. 4). At MII, 13 autosomes which form a circle, and the pseudo-bivalent of sex chromosomes, arranged in the center, are visible (Fig. 5).

#### Subfamily Aneurinae

*Aneurus avenius* Dufour: 2n = 27 (24 + X<sub>1</sub>X<sub>2</sub>Y)

At MI, 15 elements are visible: 12 autosomal bivalents and three sex chromosomes (Fig. 6). The sizes of the autosomes decrease gradually. During MII, a pseudo-trivalent of sex chromosomes (X<sub>1</sub>X<sub>2</sub>Y) is visible in the circle formed by the autosomes (Fig. 7).



Figs 1–7. Karyotypes of Aradidae (sex chromosomes marked by arrows). Figs 1–2: *Aradus cinnameus* Panzer –  $2n = 32 + X_1X_2Y$ . 1 – metaphase I; 2 – metaphase II. Fig. 3: *Aradus corticalis corticalis* (L.) –  $2n = 26 + XY$ , metaphase I. Figs 4–5: *Aradus conspiculus* Herrich-Schaeffer –  $2n = 26 + XY$ . 4 – metaphase I; 5 – metaphase II. Figs 6–7: *Aneuris avenius* Dufour –  $2n = 24 + X_1X_2Y$ . 6 – metaphase I; 7 – metaphase II.

#### Discussion

The karyotypes of 18 species within four aradid subfamilies have now been studied (Ueshima, 1979; Jacobs, 1986; Heiss & Jacobs, 1989; Jacobs, 1990). The tendency of related species to possess widely different chromosome numbers has been observed: from  $2n = 16$  (in Aneurinae and Carventinae) to  $2n = 40$  (in Aneurinae) (Table 1). This characteristic is not typical for other Pentatomomorpha (Ueshima, 1979).

It is difficult to say which mechanisms may have played a role in karyotype evolution in the family. Jacobs (1986) asserts that, rather than simple accidental fragmentation, a mechanism like chromatid autonomy (Schrader & Hughes-Schrader, 1956, 1958) may be responsible. The concept of chromosome autonomy has received serious criticism (Nokkala, 1985). No autosome polyploidy has been found in *Banasa* (Pentatomidae) (Thomas & Yonke, 1985) or Nabidae (Kuznetsova, 1993). Fragmentations have surely occurred (the chromosomes of the species with high chromosome numbers are visibly smaller), but this phenomena must be confirmed by further investigations.

Half of examined species show an  $XX : XY$  system and the rest have multiple sex chromosome systems ( $X_nY$ ). The origin of multiple sex chromosomes is somewhat problematical. In insects with monocentric chromosomes, the number of autosomes decreases when the number of sex chromosomes increases as a result of Robertsonian translocations (White, 1973). Troedsson (1944) and Schrader (1947) suggested that simple fragmentation of holokinetic sex chromosomes is the major source of multiple sex chromosomes in Heteroptera. Schrader & Hughes-Schrader (1956) experimentally proved this theory by inducing fragmentation of sex chromosomes in some pentatomids by exposing them to X-rays. I believe that it may be safely assumed that, for the Aradidae, the origin of the multiple X ( $X_n$ ) results from simple fragmentation of sex chromosomes. The comparative sizes and behaviour of sex chromosomes between species with  $XY$  and  $X_nY$  confirm this suggestion. Multiple X are smaller than simple X. At MII, multiple X lie close to each other.

No data regarding oogenesis is available and it is difficult to determine whether oogenesis involves a multiple X or multiple Y sex chromosome system. On the other hand, Jacobs (1986) studied mitoses in male and female embryos of two aneurine species and demonstrated the presence of a  $X_1X_2Y/X_1X_1X_2X_2$  sex chromosome system. Therefore it is assumed, for the species discussed herein, that fragmentation takes place in X, but not in Y chromosomes.

The diversity of chromosome number and sex chromosome systems that is found in examined Aradidae illustrates that intensive processes of karyotype evolution are prevalent in the family. Many species are in the process of microdifferentiation.

In order to clarify the means and mechanisms of the karyotype evolution within the family species from other subfamilies must be examined.

ACKNOWLEDGEMENTS. I express my gratitude to T. Vasarhelyi, Hungarian Natural History Museum, Budapest (*Aneurus avenius*), and M. Josifov, Institute of Zoology, Sofia (other species), for help in collection and determination of the study material. I thank I.M. Kerzhner and V.G. Kuznetsova, Zoological Institute, St. Petersburg for help and valuable advice during this study. This paper was partially supported by the fund "Scientific researches" (Sofia) – B-27.

#### References

- GROZEVA S.M. & KERZHNER I.M. 1992: On the phylogeny of the Aradid families (Heteroptera, Pentatomomorpha). *Acta Zool. Hung.* **38**: 199–205.
- HEISS E. & JACOBS D.H. 1989: Studies on African Aradidae II. New records of apterous Carventinae from South Africa. *Mitt. Münch. Entomol. Ges.* **79**: 47–59.
- JACOBS D.H. 1986: Morphology and taxonomy of sub-saharan *Aneurus* species with notes on their phylogeny, biology and cytogenetics (Heteroptera: Aradidae: Aneurinae). *Entomol. Mem. Dep. Agric. Wat. Supply Repub. S. Afr.* **64**: 1–45.
- JACOBS D.H. 1990: *Adamonotus uncotibialis* a new genus and species of South African Carventinae (Heteroptera, Aradidae). *J. Entomol. Soc. Sth. Afr.* **53**: 81–91.
- KORMILEV N.A. & FROESCHNER R.C. 1987: Flat bugs of the world: a synonymic list (Heteroptera, Aradidae). *Entomography* **5**, 246 pp.
- KUMAR R. 1967: Morphology of the reproductive and alimentary systems of the Aradoidea (Hemiptera), with comments on relationships within the superfamily. *Ann. Entomol. Soc. Am.* **60**: 17–25.
- KUZNETSOVA V.G. 1993: Evolutionary mechanism of autosomal polyploidy in the bugs of the family Nabidae (Heteroptera, Insecta). In: *Evolution '93, Proc. of the 4th Congress of ESEB, Montpellier (France), August 22–28, 1993*. p. 239.
- NOKKALA S. 1985: The structural organization of metaphase chromosomes. *Rep. Dep. Biol. Turku* **12**: 1–32.
- SCHRADER F. 1947: The role of kinetochore in the chromosomal evolution of the Hemiptera and Homoptera. *Evolution* **1**: 134–142.
- SCHRADER F. & HUGHES-SCHRADER S. 1956: Polyploidy and fragmentation in the chromosomal evolution of various species of Thianta (Hemiptera). *Chromosoma* **7**: 469–496.
- SCHRADER F. & HUGHES-SCHRADER S. 1958: Chromatid autonomy in *Banasa* (Hemiptera, Pentatomidae). *Chromosoma* **9**: 193–215.
- SOUTHWOOD T.R.E. & LESTON D. 1959: *Land and Water Bugs of the British Isles*. Frederick Warne, London, 436 pp.
- THOMAS D.B. & YONKE T.R. 1985: Cladistic analysis of zoogeography and polyploid evolution in the stinkbug genus *Banasa* Stal (Hemiptera, Pentatomidae). *Ann. Entomol. Soc. Am.* **78**: 885–862.
- TROEDSSON P.H. 1944: The behaviour of the compound sex chromosomes in females of certain Hemiptera-Heteroptera. *J. Morphol.* **75**: 103–147.
- UESHIMA N. 1963: Chromosome complements of two species of flat bugs (Aradidae: Hemiptera). *Chromosome Inf. Serv.* **4**: 12–14.
- UESHIMA N. 1979: Hemiptera II: Heteroptera. In John B. (ed.): *Animal Cytogenetics 3, Insecta 6*. Gebrüder Borntraeger, Berlin, pp. 1–118.
- USINGER R.L. & MATSUDA R. 1959: *Classification of the Aradidae (Hemiptera, Heteroptera)*. British Museum, London, 410 pp.
- VASARHELTYI T. 1986: On the relationships of the eight Aradid subfamilies (Heteroptera). *Acta Zool. Hung.* **33**: 263–267.
- WHITE M.J.D. 1973: *Animal Cytology and Evolution*. Cambridge Univ. Press, Cambridge, 961 pp.

Received September 16, 1996; accepted January 21, 1997