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RESEARCH ARTICLE

**MALE MEIOSIS IN TWO SPECIES OF PENTATOMID BUGS (ORDER: HEMIPTERA:
SUB-ORDER: HETEROPTERA) FROM JAMMU REGION**

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ABSTRACT

The diploid number of chromosomes in two species of family pentatomidae *Halys dentatus* and *Coridius janus* is 14 ($2n=14=12A+XY$), with XY- male sex determining mechanism in both of the species. Autosomes show gradation in size, X is of medium size while Y is the smallest element. Both X and Y are positively heteropycnotic in diffused stage but become isopycnotic in diplotene/diakinesis stage and divide equationally during meiosis I, At metaphase II, X and Y get associated, the association being strong forming a pseudobivalent XY in *Coridius janus* while weak in *Halys dentatus*. A definite pattern of arrangement of the chromosomes is seen at Metaphase II where in autosomes formed almost a circle and the sex chromosomes forming a pseudobivalent, were observed in the centre of this circle.

Key words:

Pentatomidae, diffuse stage,
Heteropycnotic, Isopycnotic,
Pseudobivalent.

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INTRODUCTION

The family Pentatomidae, one of the major families of Heteroptera, includes shield-shaped bugs, commonly known as “stink bugs” due to presence of stink glands. Pentatomids are important pests of many crops feeding mostly on seeds, vegetables and immature fruits and suck plant juices. Some of the pentatomid bugs are predatory feeding on other insect larvae and some are highly beneficial being predators of other pest insects. Family Pentatomidae is noted for its extreme karyotypic conservation. This family is karyotypically and cytogenetically explored largely by Dasgupta (1950), Manna (1951,1962, 1984), Rao (1954), Dutt (1953), Schrader and Hughes-Schrader (1956,1958), Hughes-Schrader and Schrader (1957), Manna and Deb-Mallick (1981), Naumah (1982), Satapathy and Patnaik (1988,1989), Satapathy et al., (1990), Gonzalez Garcia et al., (1996), Rebagliati (2009), Rebagliati et al., (2001, 2002, 2003), Kerzhner et al., (2004), Papeschi and Bressa (2006). This paper includes the study of diploid complement and behavior of autosomes and sex chromosomes during meiotic cycle in two North Indian heteropteran species from Himalayan sub-region belonging to family Pentatomidae.

MATERIAL AND METHODS

Specimens of *Halys dentatus* and *Coridius janus* belonging to family Pentatomidae, were collected from Jammu region (J & K). Male bugs of each species were dissected to take out the

testis which were fixed in Ethanol: Glacial Acetic acid (3:1) and air-dried chromosomal preparations (slides prepared using Dabbing Technique) were stained in Giemsa stain (2%).

RESULTS

Halys dentatus

The spermatogonial metaphase plate of *Halys dentatus* reveals a diploid complement of 14 chromosome with $2n=12A+XY$ (fig. 1). Autosomes are rod-shaped with gradation in size. Two pairs are large; one pair is slightly smaller while 3 pairs are distinctly small. The sex chromosome, X is small rod-shaped and is equal to the size of the smallest autosomal pair and is darkly stained. Y- Chromosome is dot-shaped and is the smallest and the lightest element. At metaphase, chromosomes appear thick and condensed. Photokaryotype of male *H.dentatus* (fig. 2) possessed six autosomal pairs and sex chromosomes (X and Y). Histogram (fig. 3) was prepared on the basis of decreasing value of RL% from the chromosomal pair no. 1 to 6 and sex chromosomes in the last (Table 1.).

During Pachytene, (fig. 4) chromosomes were condensed, showing crossing-over. The sex chromosomes were darkly stained and formed XY - heteropycnotic body. At diffuse Stage also (fig. 5), 2 Heteropycnotic bodies, one being larger than the other are seen in some cells while in others, 2 are fused to form a single heteropycnotic body, representing X and Y

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chromosomes. At diplotene (fig. 6), 6 Autosomal Bivalents and closely associated X and Y chromosomes are observed.

Table 1 Morphometric data of karyotype of *Halys dentatus* (male–testis) 2n=14.

Chromosome Pair Number	Mean Total Length (μ)	Total Complement Length Percentage (TCL %)	Relative Length Percentage (RL %)
1	10.0	23.529	100
2	7.8	18.353	78
3	6.8	16.0	68
4	5.0	11.765	50
5	4.2	9.882	42
6	3.5	8.235	35
X	3.2	7.529	32
Y	2.0	4.706	20

Table 2 Morphometric data of karyotype of *Coridius janus* (male–testis) 2n=14.

Chromosome pair Number	Mean Total Length (μ)	Total Complement Length Percentage (TCL %)	Relative Length Percentage (RL %)
1	12.2	18.155	100
2	11.5	17.113	94.26
3	10.8	16.071	88.52
4	8.5	12.649	69.67
5	7.0	10.417	57.38
6	6.5	9.673	53.28
X	6.2	9.226	50.82
Y	4.5	6.696	36.89

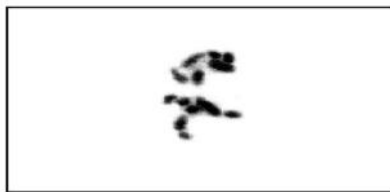


Fig. 1

Fig. 1 Spermatogonial metaphase complement of male *Halys dentatus* showing 14 chromosomes.

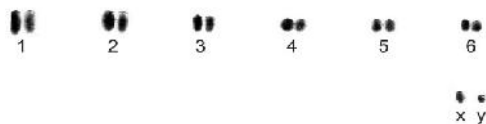


Fig. 2

Fig. 2 Photokaryotype of spermatogonial metaphase complement of *H. dentatus* (2n=14, 12A+XY)

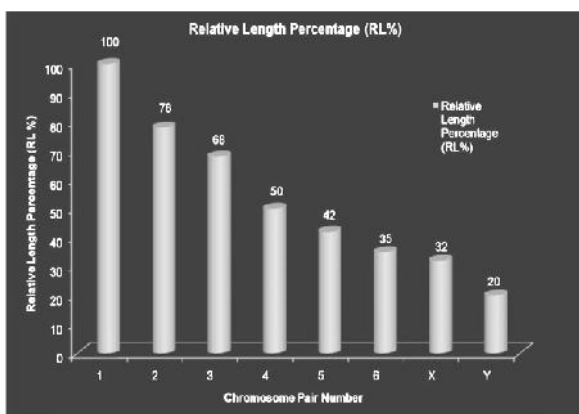


Fig 3 Histogram of Male *Halys dentatus*

Fig. 4-Fig 11 Meiotic stages of *Halys dentatus*

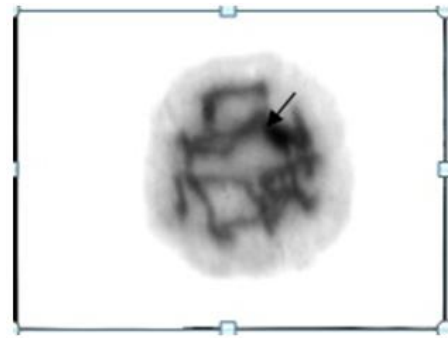


Fig. 4 Pachytene (->= XY-Mass)

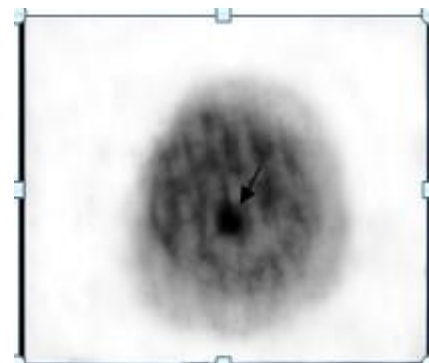


Fig. 5 Diffuse stage (->=XY-mass)

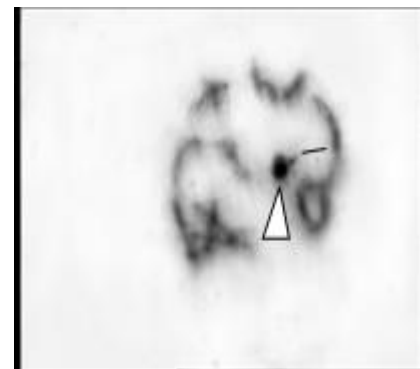


Fig. 6 Diplotene (>=X-chromosome, -=Y-chromosome)

At diakinesis (fig. 7), the sex chromosomes have separated. Bivalents have become more condensed. At metaphase I (fig. 8 and fig 9), the 6 autosomal bivalents are seen to be arranged in a circle and the sex chromosomes are lying in the periphery (polar view) and show close association.

In the side View, sex chromosomes orient in such a manner that X is facing one pole and Y facing the other pole. All chromosomes are arranged in line with the sex chromosomes in between them. A definite pattern of arrangement of bivalents and sex chromosomes is lacking at Metaphase I. At Anaphase I (fig. 8), the sex chromosomes segregated equatorially and are present at the center of the autosomal circles at 2 poles. All Metaphase II plates (fig. 9) show a total of 7 elements each. In polar view, the autosomes are arranged in a circle and the XY-pseudobivalent is lying in the center of the circle. The Meta-II stage reveals a regular pattern of arrangement.

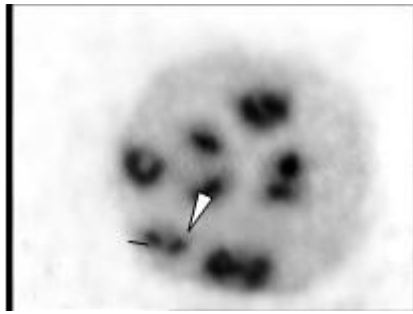


Fig. 7 Diakinesis (\geq X-chromosome, \neq Y-chromosome)

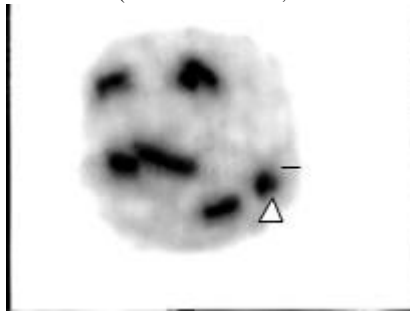


Fig. 8 Metaphase-I (Polar view, \geq X-chromosome, \neq Y-chromosome)

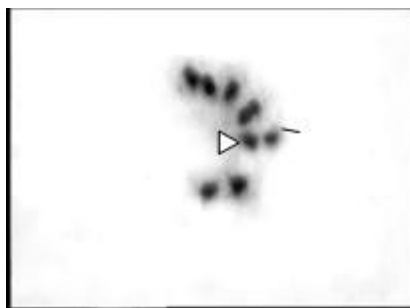


Fig. 9 Metaphase-I (side view, \geq X-chromosome; \neq Y-chromosome)

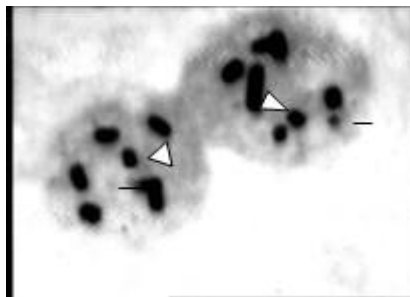


Fig. 10 Anaphase-I (\geq X-chromosome \neq Y-chromosome)

Coridius janus

The spermatogonial metaphase (fig. 10) represents a complement of 14 elements which include 12 autosomes and 2 sex chromosomes (X and Y). Of all the chromosomes, Y chromosome is the smallest. All the chromosomes including sex chromosomes are rod-shaped. Photokaryotype of male *C.janus* (fig. 11) reveals that chromosomes are arranged in decreasing order of their length, showing gradual gradation. Histogram is prepared on the basis of their decreasing value of relative length % age from chromosome pair no. 1 to 6 and sex chromosomes in the last (fig. 12) (Table 2.). The sex-chromosome X is rod-shaped and is equal to the size of the smallest autosomal pair; Y-chromosome is the smallest rod. At

their metaphase stage, chromosomes appear thick, elongated, darkly stained and well spread.

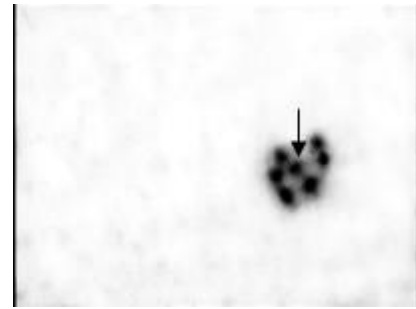


Fig. 11 Metaphase-II (Polar view, \rightarrow =XY-Pseudobivalent)

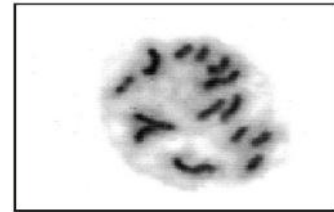


Fig. 12

Fig.12 Spermatogonial metaphase complement of male *Coridius janus* showing 14 chromosomes.



Fig. 13

Fig. 13 Photokaryotype of spermatogonial metaphase complement of *C. janus* ($2n=14, 12A+XY$)

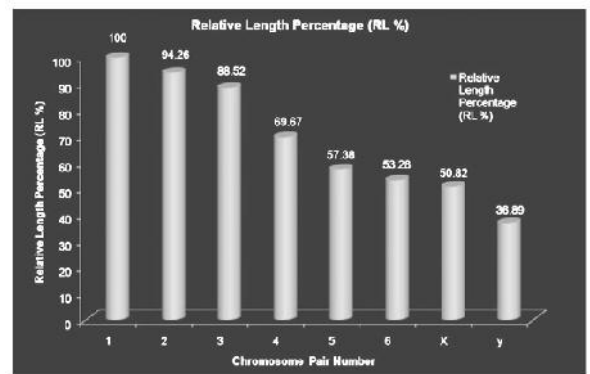


Fig. 14 Histogram of male *Coridius janus*.

Fig. 15-Fig 22 Meiotic stages of *Coridius janus*

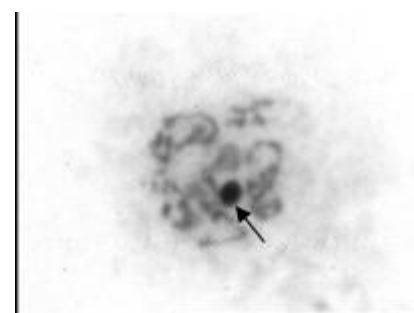


Fig. 15 Pachytene (\rightarrow = XY- mass)

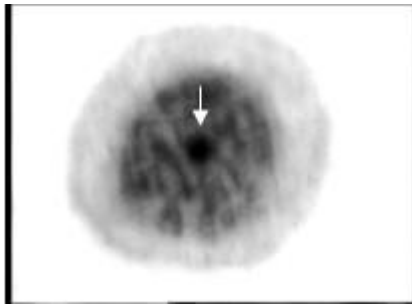


Fig. 16 Diffuse stage (-> = XY- mass)

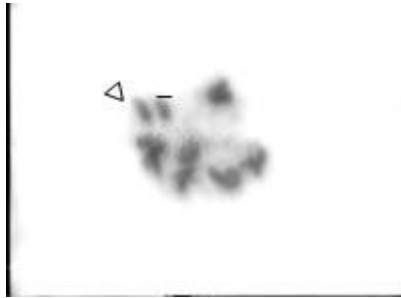


Fig. 17 Diplotene (>= X- chromosome, -=Y-chromosome)

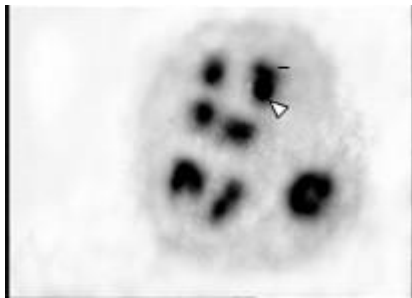


Fig. 18 Diakinesis (>= X-chromosome, -= Y- chromosome)



Fig. 19 Metaphase-I(>= X- chromosome, -=Y-chromosome)

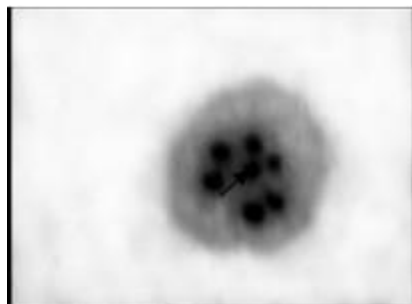


Fig. 20 Metaphase-II (->= XY-Pseudobivalent)

During Pachytene (fig. 13), the chromosomes appear distinct with varying degree of crossing over. The sex chromosomes

are observed as darkly stained positively heteropycnotic (XY) body. At diffuse stage (fig. 14), chromosomes observed are partially decondensed and in the centre of the nucleus, is observed the sex chromosome mass, formed by close association of X and Y chromosomes which is darkly stained. The diplotene stage shows the presence of six autosomal bivalents and 2 separated sex chromosomes (fig. 14). Y chromosome is the smallest. One ring bivalent is also observed. At diakinesis (fig. 15), the sex chromosomes X and Y show slight association. At metaphase- I (fig. 16), a total of 8 elements are observed.

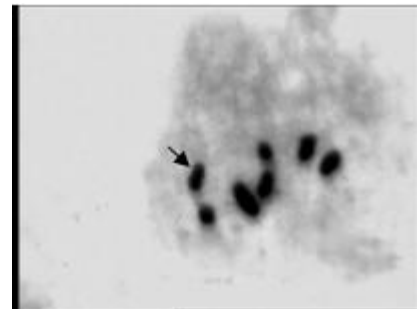


Fig.21. Metaphase II (side view, -> = XY-Pseudobivalent)

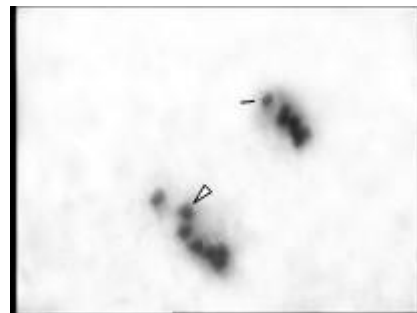


Fig 22 Anaphase-I (>= X- Chromosome, -= Y- chromosome)

The autosomal bivalents form a circle and the sex chromosomes are lying separated. In most of the cells, Y chromosome is observed to be associated with one of the autosomes. Chromosomes are thick and deeply stained. A total of seven elements are observed in metaphase- II. The autosomes form a circle in the centre of which the XY-pseudobivalent is observed (polar view) (fig. 17). In the side view (fig. 18), all chromosomes are seen to be in an alignment. At Anaphase-II (fig. 19), chromosomes at two poles are dot-like and no bivalents are observed. The sex chromosomes show reductional division, that is, one migrates towards one pole and the other migrates towards the opposite pole. At each pole, seven elements are visible.

DISCUSSION

Both the species, *Halys dentatus* and *Coridius janus* showed $2n = 14 = 12A + Y$. The Family Pentatomidae is noted for its extreme karyotypic conservation, in this family, the cytogenetic studies in over 300 species has been done. A diploid number of 14 chromosomes with a sex mechanism of XY is found invariably and has been universally suggested to be the type number/modal number (Parshad 1957a, b; Manna 1962, Ueshima 1979, Naumah 1982). This modal chromosome number has been recorded in more than 85% of the species

investigated (Ueshima 1979, Muramoto 1981, Naumah 1982, Papeschi *et al.*, 2003; Rebagliati *et al.*, 2001; 2005; Lanzone and Souza 2006; Souza *et al.*, 2007; Rebagliati 2009). The karyotypes of family Pentatomidae have been explored largely by Schrader (1960), Manna (1951, 1962, 1984), Schrader and Hughes-Schrader (1956, 1958), Parshad (1957 a,b,c,d), Jande (1959), Takenouchi and Muramoto (1964,1967,1969, 1970a,b, 1971a, b, 1972a,b, 1973), Manna and Deb-Mallick (1981), Naumah (1982), Dey and Wangdi (1985,1988); Satapathy and Patnaik (1989, 1990, 1991); Rebagliati *et al.*, (2001,2002,2003); Kour and Semahagn (2010) and Rebagliati and Mola (2010).

Pentatomidae karyotypes typically lack microchromosomes. (Papeschi *et al.*, 2003, Rebagliati *et al.*, 2005; Lanzone and Souza, 2006; Souza *et al.*, 2007; Rebagliati, 2009; Rebagliati and Mola, 2010).

Chromosomal information on *Halys dentatus* (Fabricus) has previously been given by Makino (1951), Manna (1951, 1958), Rao (1954), Leston (1958) and Srivastava (1965). The studies during the present investigation showed $2n=14(12A+XY)$ in male complement which is in conformation with earlier works. Chromosomal information on seven species of the genus *Coridius* is available (Satapathy and Patnaik, 1988; Naumah, 1982; Satapathy and Patnaik, 1991). The species investigated in the present studies, *Coridius Janus* (Fabricus) was previously studied by Manna (1951, 1958), Makino (1951), Leston (1958) and Satapathy and Patnaik (1991) reporting $2n=14$ with XY type of sex mechanism in males. The present study of *C. janus* with diploid count of 14 chromosomes and XY sex mechanism are in conformity with the earlier workers.

Among the principal mechanisms of karyotype evolution in pentatomids, autosomal fusion and sex chromosome fragmentations are involved (Papeschi 1994, 1996; Kaur and Semahagn, 2010). Since the chromosomes are of Holocentric type, so after fragmentation each and every chromosomal fragment retain the centrometric activity and thus can perpetuate independently so that simple fragmentation easily leads to an increase in chromosome number. Fragmentations indeed have played an important role in the evolution of organism with holocentric chromosomes. Pentatomids have been classified as higher Pentatomids and as advanced type due to addition of one pair of Autosomes to the ancestral type number ($2n=12$) (Leston, 1958). This holds true on Morphological, Anatomical and Biological grounds also.

The diploid chromosome number of both these species has been found to be 14 in conformity with the earlier findings (Rao, 1954; Manna, 1958; Sharma and Parshad, 1956; Hughes-Schrader and Schrader, 1957; Leston 1958; Manna and Deb-Mallick, 1981; Camacho *et al.*, 1985; Satapathy and Patnaik, 1988; 1991; Papeschi *et al.*, 2003). Chromosomes appeared as rods or dots at metaphase stage without displaying any constriction.

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