CHAPTER II STUDY OF THE CHROMOSOME BANDING IN EIGHTEEN SPECIES OF HETEROPTERA

INTRODUCTION

In certain groups of animals and plants, the chromosomes do not seem to have any localized centromeres or primary constrictions which are present in the monocentric organisms (see White 1973; Battaglia & Boyes 1955; John & Lewis 1965). The diffuse nature of heteropteran chromosomes has been established from light microscopic, experimental and ultrastructural studies. From light microscopic observations, Schrader (1935, 1940, 1947) suggested that the heteropteran chromosomes were holokinetic during mitosis, while they behaved like telokinetic chromosomes during meiosis and he proposed the following behavioural characteristics of heteropteran chromosomes in support of the claim. 1. Absence of primary constriction during spermatogonial and somatic mitosis 2. Separating chromatids remain parallel to each other during mitotic anaphase. 3. The chromosomal fibers are organized along the entire length of each chromatid. 4. Meiotic metaphase bivalents orient with their long axes parallel to the interpolar axis of the spindle, and the kinetochore activities are restricted to the terminal ends of each bivalents. These criteria as well as diffuse kinetochore activity received active support from later workers (Heizer 1950; Halkka 1956; Lewis & Scudder 1958), while others proposed localized kinetochore (Mendes 1949; Dutt 1955; Parshad 1957a; 1958), dikinetic with localized kinetochores at each end (Piza 1958) and telocentric (Ruthmann & Dahlberg 1976) for heteropteran chromosomes.

Heteropteran chromosomes were also interpreted as holocentric by Hughes-Schrader & Schrader (1961) who found that the X-ray induced fragments perpetuate themselves during spermatogonial mitosis in three pentatomid bugs of the genus, *Euschistus*. La Chance & Degrugillier (1969) also reported the transmission of fragments through three generatins in *Oncopeltus* and supported the Schrader's view. Other workers, however, did not support this inference regarding heteropteran kinetochore. Desai (1969) irradiated the chromosomes of *Ranatra* (Nepidae) with high doses of X-rays and genaralized that the heteropteran chromosomes were monocentric. Further, the study of fragments in the X-rayed bugs, *Physopelta schlanbuschi* (Manna & Dey 1983, 1986; Dey & Manna 1984) and *Lygaeus hospes* (Barik 1979) did not support the holocentric nature of chromosomes in these two species.

There has been relatively little investigation of the fine structure of diffuse kinetochore in Heteroptera. Buck (1967) studied the mitotic and meiotic chromosomes of *Rhodnius prolixus* (Reduviidae), and found a diffuse kinetochore spread along the whole leading edge of the mitotic chromosomes, while there was no analogous structure in the meiotic chromosomes. Single plate like kinetochore, covering most of the chromosome length, was reported for the mitotic chromosomes of *Oncopeltus* and such plates were found to be absent during meiosis (Comings & Okada 1972). On the other hand, Ruthmann & Permantier (1973) observed localized kinetochore in *Dysdercus* and had suggested monocentric organization of the chromosome. Moreover, the study of the determination of base composition, buoyant density, thermal stability, reassociation kinetics, renaturation of DNA etc. in the chromosomes of *Oncopeltus* revealed that the repeated sequences of DNA were primarily short and scattered throughout the genome in contrast to the tandem repeats of DNAs near the centromere of the organisms with localized kinetochore (Lagowsky et al. 1973).

In recent years the chromosome banding techniques have provided cytogeneticists with some powerful new tools to investigate the chromosome organization. These include the use of C-banding to stain constitutive heterochromatin and Q- and G- banding to produce differential staining in the chromosome arms (Caspersson et al. 1969; Seabright 1971; Sumner 1972; Pardue & Gall 1970; Arrighi & Hsu 1971). However, their applications in the study of holocentric chromosomes are limited. Few studies have been carried out in Heteroptera (Maudlin 1974; Muramoto 1975, 1976, 1978, 1980, 1985; Solari 1979; Camacho et al. 1985), Homoptera (Pijnacker & Ferwerda 1976), Lepidoptera (Bigger 1975; Bedo 1984), *Parascaris* (Goday et al. 1985) and in the plants of the genus, *Luzula* (Ray & Venkateswaran 1978).

MATERIALS AND METHODS

MATERIALS

Eighteen species of Heteroptera belonging to five families were used for banding study. List of the species is given in the Table 9. The normal karyotypes of these species have been described in Chapter I of this thesis.

Table 9. List of the species used for C and G banding studies.

Taxon	Diploid count	C-/G-bands
Pentatomidae		
1. *Nezara icterica*	2n=14(12+XY)	G
2. *Cahara jugotoria*	2n=14(12+XY)	G
3. *Compastes bhutanicus*	2n=14(12+XY)	G
4. *Chrysocoris stollii*	2n=12(10+XY)	C & G
Coreidae		
5. *Cletus* sp.	2n=18(14+2m+X_1X_2O)	G
6. *Dalader acuticosta*	2n=21(18+2m+XO)	G
7. *Petillia patullicollis*	2n=28(24+2m+X_1X_2O)	C & G
8. *Ochrochira granulipes*	2n=21(18+2m+XO)	C & G
9. *Anoplocnemis phasiana*	2n=15(14+XO)	G
10. *Acanthocoris* sp.	2n=24(22+X_1X_2O)	G
Alydidae		
11. *Leptocorisa acuta*	2n=17(14+2m+XO)	C & G
12. *Riptortus pedestris*	2n=13(10+2m+XO)	G
Largidae		
13. *Lohita grandis*	2n=15(12+2m+XO)	G
14. *Iphita limbata*	2n=15(12+2m+XO)	G
15. *Physopelta gutta*	2n=17(12+2m+X_1X_2Y)	G
16. *P. quadrigutta*	2n=17(12+2m+X_1X_2Y)	G
17. *P. schlanbuschi*	2n=17(12+2m+X_1X_2Y)	G
Lygaeidae		
18. *Spilostethus hospes*	2n=14(10+2m+XY)	G

METHODS

Among the eighteen species, chromosomes of four species were studied by both C- and G-banding techniques, while those of rest of the species were studied by G-banding only. C-banding could not be attempted in all the species due to the paucity of materials.

Chromosome banding techniques

For C-banding, BSG method described by Sumner (1972) was followed with minor modifications. The testes cells were squashed by the conventional squash method.The slides were then kept in a hot plate at 60°C for about a week. After preheating, the slides were hydrolysed in 0.2% N HCL for 30 min at room temperature, rinsed in distilled water and placed in freshly prepared 5% aqueous solution of $Ba(OH)_2$ at 50-55°C for about 15 min. After thorough rinsing in three changes of distilled water, the slides were incubated for 1½ hr. at 60-65°C in 2 X SSC (0.3 M NaCl and 0.03 M tri sodium citrate) solution. They were then rinsed in distilled water several times and stained for about 30 min in 10% Giemsa (BDH, India) adjusted to pH 6.8 by using phosphate buffer. Finally, the slides were washed in distilled water, blotted, allowed to dry thoroughly, soaked in xylene and mounted in DPX.

For G-banding, trypsin-Giemsa banding method of Seabright (1971) was followed with minor modification. 0.25% of trypsin (dissolved in isotonic saline solution) or 0.25% aqueous solution of trypsin were used. 3-4 days old squashed slides were placed in the trypsin solution 40 sec to 1 min and then kept in isotonic saline for 8 to 10 min. The slides were stained in 10% Giemsa solution (BDH, India) for 20 to 25 min (adjusted to pH 6.8 with phosphase buffer). The slides were then washed in distilled water, blotted, allowed to dry thoroughly, rinsed in xylene and mounted in DPX.

The pairing of the chromosomes in the karyotype was done by matching bands as far as practicable. Diagrammatic representations of the banded chromosomes in haploid series are also presented. The deep and light bands are indicated by complete black and oblique lines respectively. The white areas represent negative staining zones.

PLATE 50

Explanation of Figures

Fig.1. G-banded kayotype of *Nezara icterica.*

Fig.1a. Diagrammatic representation of the banded chromosomes in haploid series.

Fig.2. G-banded karyotype of *Cahara jugotoria.*

Fig.2a. Diagrammatic representation of the banded chromosomes.

Fig.3. G-banded karyotype of *Compastes bhutanicus.*

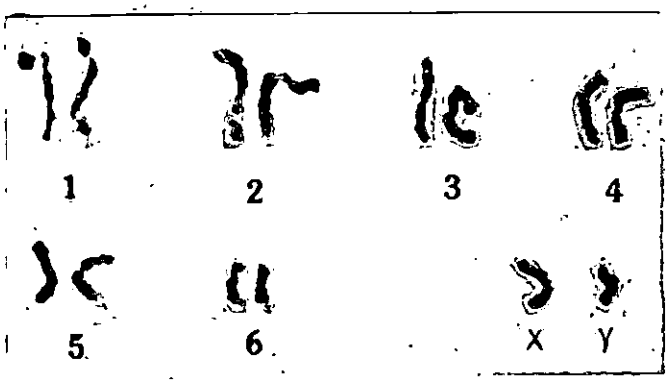
Fig.3a. Diagrammatic representation of the bands.

Fig.4. G-banded chromosomes of diakinesis.

Fig.4a. Diagrammatic representation of the bands.

Fig.5. G-banded chromosomes of diakinesis.

Fig.5a. Diagrammatic representation of the bands.



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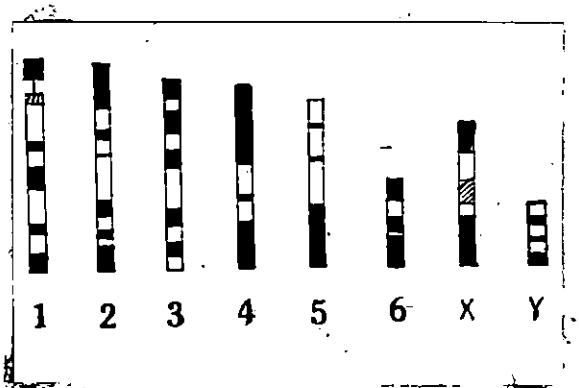

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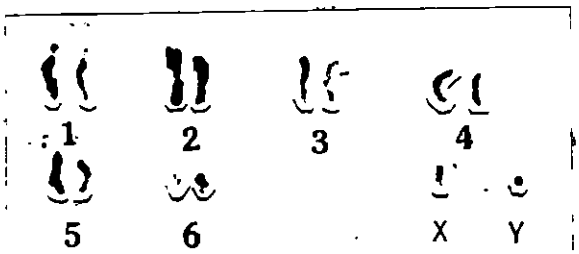

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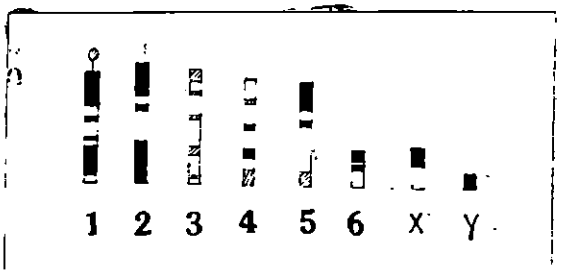

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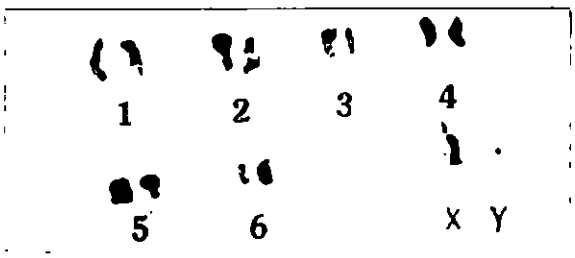

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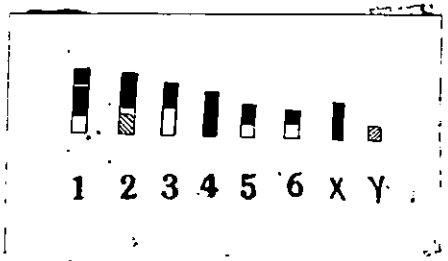

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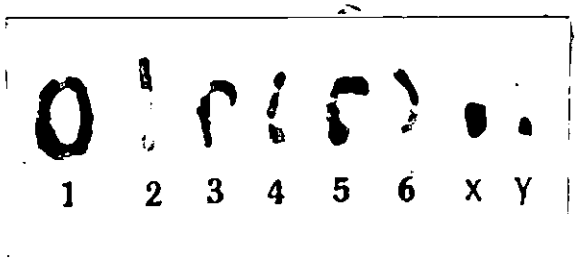

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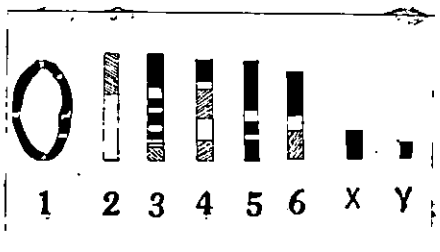

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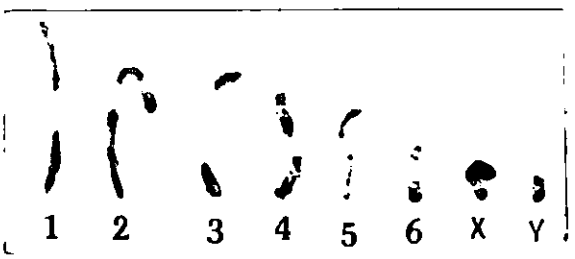

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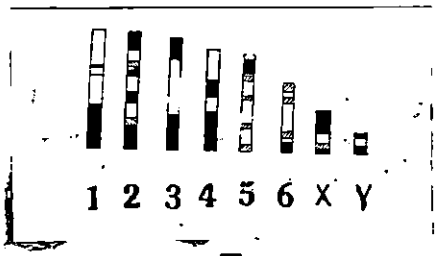


5a

RESULTS

Family **Pentatomidae**

1. *Nezara icterica*
(Plate 50, Figs. 1, 1a)

In *Nezara icterica*, the diploid chromosome number is 14 with XY sex chromosome system in the male. G-bands were observed in the spermatogonial metaphase chromosomes. Fig.1 shows G-banded karyotype, while diagrammatic representation is given in Fig.1a. Chromosome No.1 reveals a satellite like structure with dark band by which it can be demarcated from other members of the complement. Besides this constriction, four deep and one light bands are present in the same chromosome. In the chromosome No.2, six dark bands of various widths are visible, while chromosome No.3 shows six clear dark bands of equal widths. Chromosome No.4 has two large dark bands, covering three fourths of the length of the chromosome and a narrow dark band in between them. Chromosome No.5 exhibits a large dark bands and two narrow bands, whereas chromosome No.6 has three dark bands. The heteromorphic X and Y have their characteristic banding patterns. The X chromosome shows two dark bands of different widths separated by a light band, while two dark bands of equal widths are separated by a comparatively narrow dark band in the Y chromosome.

2. *Cahara jugotoria*
(Plate 50, Figs. 2, 2a)

In *Cahara jugotoria*, G-banded chromosomes were studied from spermatogonial metaphases. This species also has typical diploid count of 14 chromosomes and XY sex chromosome system in the male. G-banded karyotype and diagrammatic representation of the bands are shown in Figs. 2 and 2a respectively. Chromosome No.1 has a satellite like extra element with a light band and two large dark bands which are separated by two narrow dark bands. Chromosome No.2, however, has two large bands and a narrow dark band present in between them, while chromosome No.3 shows five light bands only. Chromosome No.4

has two light and two dark bands, whereas chromosome No.5 exhibits two dark and a light bands of which dark telomeric band is very conspicuous. One member of the chromosome pair No. 6 reveals two dark bands, while another is faintly stained. The X and Y chromosomes are heteromorphic. The X chromosome shows one dark telomeric band, whereas Y chromosome is uniformly darkly stained without apparently distinguishable bands.

3. *Compastes bhutanicus*
(Plate 50, Figs. 3, 3a, 4, 4a, 5, 5a)

In *Compastes bhutanicus*, G-bands were studied in spermatogonial and diakinesis stages. This species shows typical pentatomid count of 14 chromosomes with XY sex chromosome system in the male. G-banded karyotype and diagrammatic representation are given in Figs. 3 and 3a respectively. Chromosome No. 1 shows two dark bands, covering about three fourths of its lengths. Chromosome No.2 is characterized by the presence of a heavily stained terminal heterochromatic block, while chromosome Nos. 3 & 6 have single dark band each at one end only. Chromosome No. 4 is uniformly darkly stained without apparently distinguishable bands. One member of the chromosome pair No. 5 has a terminal heterochromatic block, while another member is uniformly darkly stained. The X and Y chromosomes are heteromorphic. While the X chromosome is entirely heterochromatic, the Y chromosome is lightly stained and did not reveal any bands.

G-bands were also found consistently in the diakinesis stages. G-banded bivalents and sex univalents and their diagrammatic representations are presented in the Figs. 4, 4a, 5, 5a. All the autosomal bivalents show well differentiated G-banding patterns. The X and Y chromosomes are, however, entirely heterochromatic in some plates (Figs. 4, 4a), while they show characteristic bands in other plates. The X chromosome is characterized by a large terminal heterochromatic block and a small dark band, whereas the Y chromosome has two narrow dark bands of unequal widths. (Figs. 5, 5a).

4. *Chrysocoris stollii*
(Plate 51, Figs. 6, 6a, 7, 7a, 8, 8a)

In *Chrysocoris stollii*, the diploid count is 12 with XY sex chromosome system in the male. Both C- and G-banded chromosomes were encountered

PLATE 51

Explanation of Figures

Fig.6. C-banded karyotype of *Chrysocoris stollii*.

Fig.6a. Diagrammatic representations of the bands.

Fig.7. G-banded karyotype of *C. stollii*.

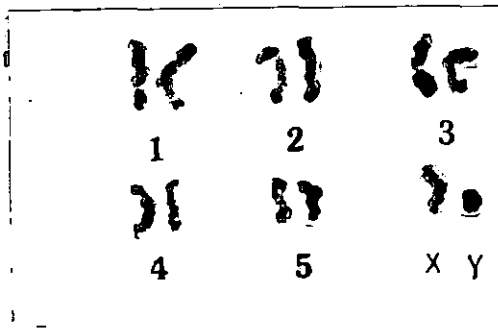
Fig.7a. Diagrammatic representation of the bands.

Fig.8. G-banded chromosomes of early prophase stage.

Fig.8a. Diagrammatic representation of the bands.

Fig.9. G-banded karyotype of *Cletus* sp.

Fig.9a. Diagrammatic representation of the bands in diploid set of chromosomes.



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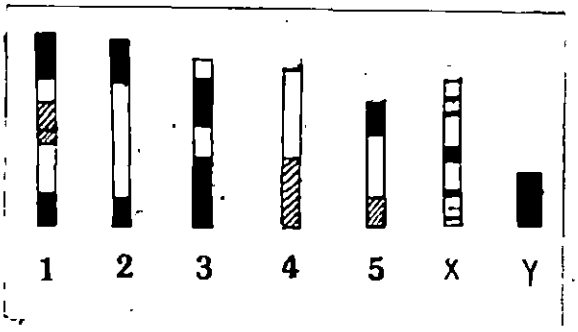

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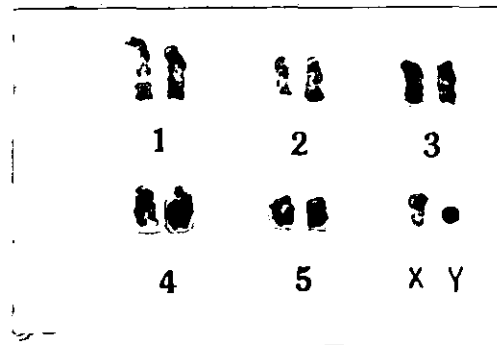

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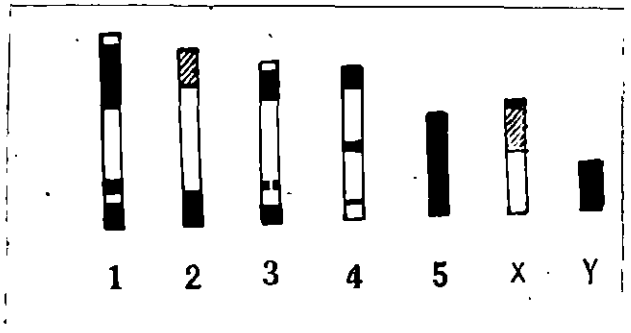

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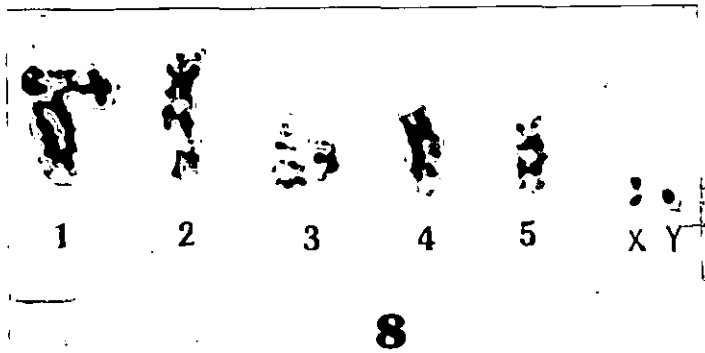

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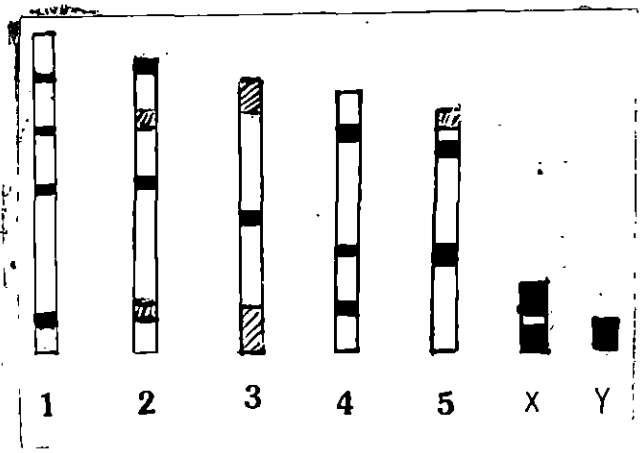

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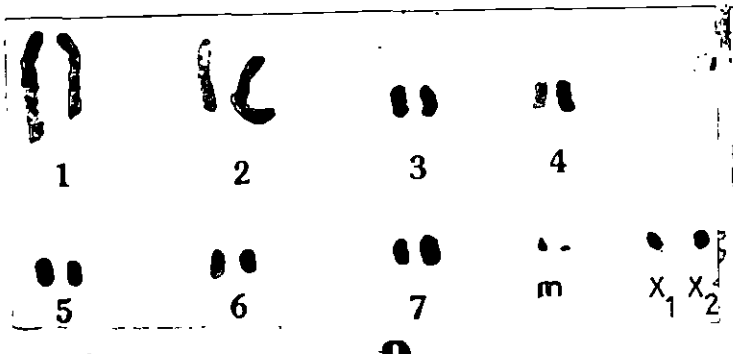

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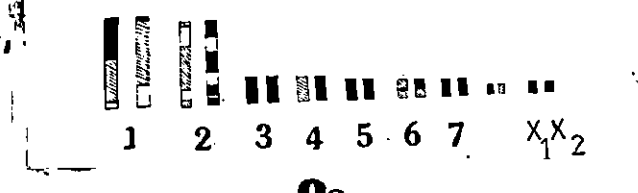


9a

in this species. Figs. 6 & 6a show C-banded karyotype and diagrammatic representation respectively. Chromosome No.1 is characterized by two dark telomeric bands and an interstitial light band, while chromosome No. 2 has two dark telomeric bands only. On the other hand, C-heterochromatin blocks are present in the terminal and subterminal regions of the chromosome No.3. Only a faint band is present in the chromosome No.4, while chromosome No.5 is characterized by one dark and a light bands of equal widths; one in each end of the chromosome. The X chromosome reveals five dark bands of different widths, while the Y chromosome is entirely heterochromatic.

G-banded karyotype and diagrammatic representation are presented in the Figs. 7 & 7a respectively. Chromosome No.1 is characterized by three dark bands of which one is larger than other two, while chromosome No. 2 has one dark and one light telomeric bands. Chromosome Nos. 3 & 4 have three dark bands each, of which one broken band is present in the chromosome No.3. Chromosome No. 5 is uniformly darkly stained. In contrast to the five bands found in the C-banded X chromosome, only two bands : a dark and a light bands are encountered in the G-banded X chromosome. However, the Y chromosome is found to be entirely heterochromatic as in C-banded plate.

G-banded chromosomes were also found in the early prophase stages. G-banded chromosomes and their diagrammatic representation are presented in the Figs. 8 & 8a respectively. Autosomal bivalents are characterized by well differentiated G-bands. The X chromosome has two dark bands, while the Y chromosome is entirely heterochromatic.

Family **Coreidae**

5. *Cletus* sp.
(Plate 51, Figs. 9, 9a)

Cletus sp. had diploid count of 18 chromosomes, including a pair of m-chromosomes and an X_1X_2O sex chromosome system in the male. G-banded chromosomes were studied from spermatogonial metaphase stages. Banded

PLATE 52

Fig.10. G-banded chromosomes of diakinesis stage in *Dalader acuticosta.*

Fig.10a. Diagrammatic representation of the banded chromosomes.

Fig.11. G-banded chromosomes of diakinesis stage in *D. acuticosta.*

Fig.11a. Diagrammatic representation of the banded chromosomes.

Fig.12. C-banded karyotype of *Petillia patullicollis.*

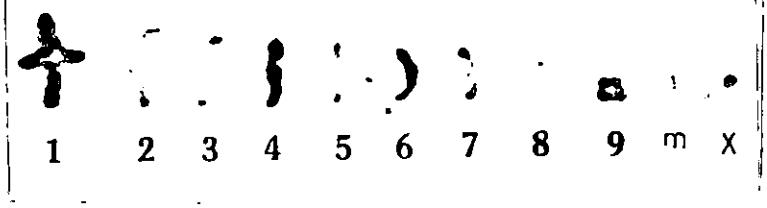
Fig.12a. Diagrammatic representation of the banded chromosomes.

Fig.13. G-banded karyotype of *P. patullicollis.*

Fig.13a. Diagrammatic representation of the banded chromosomes.

Fig.14. C-banded chromosomes of diakinesis stage in *P. patullicollis.*

Fig.14a. Diagrammatic representation of the banded chromosomes.



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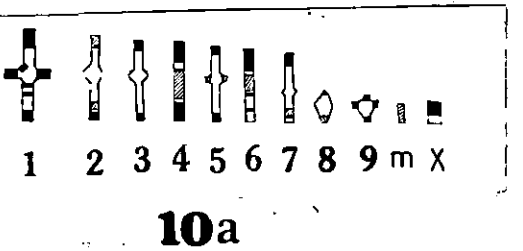

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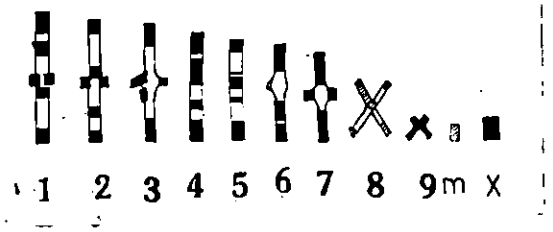

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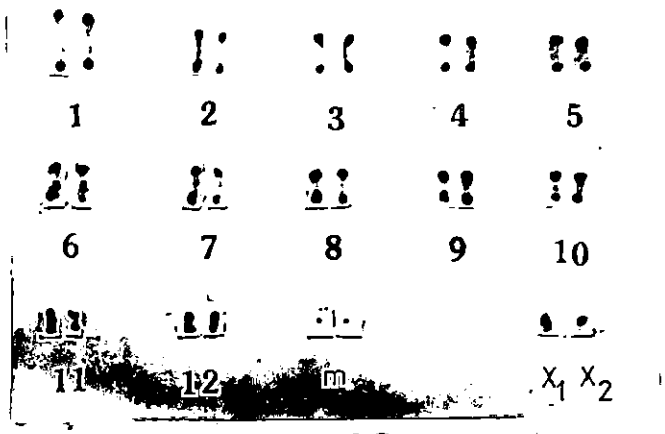

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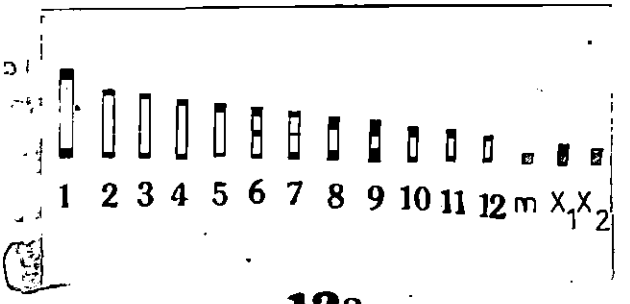

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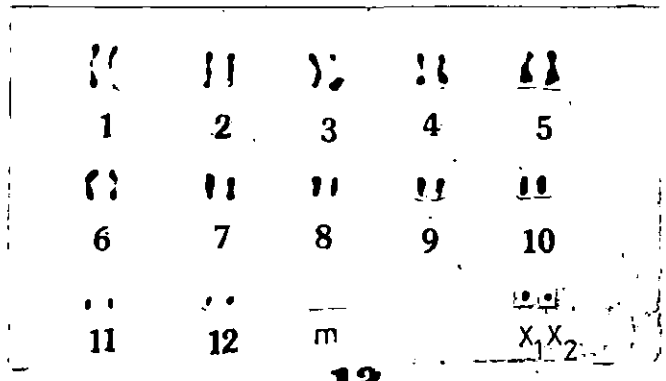

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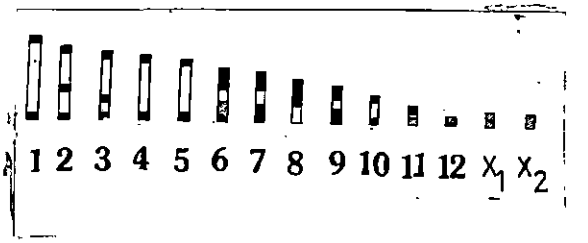

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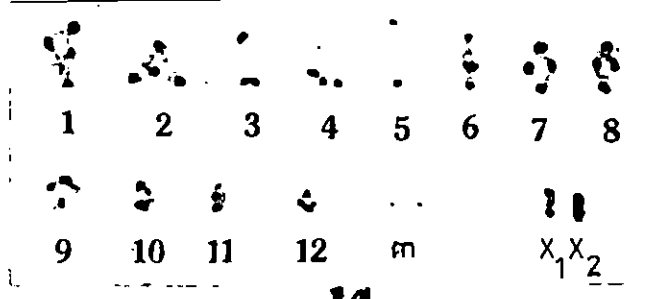

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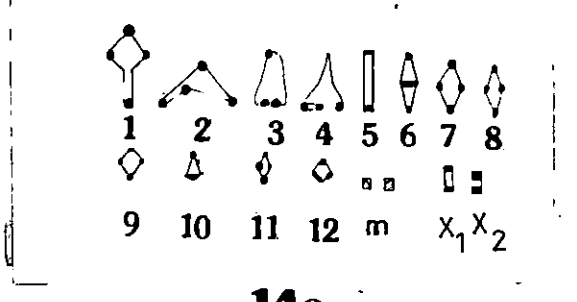


14a

karyotype and their diagrammatic representations are presented in the Figs. 9 & 9a respectively. The analysis of karyotype shows that the members of pairs No. 1, 2 and 6 differ in G-banding patterns. They have homologous as well as non homologous regions. On the other hand, pairs No. 3, 5 and 7 are uniformly darkly stained without apparently distinguishable bands. One member of the chromosome pair No. 4 is also uniformly darkly stained, while another member is lightly stained. Similar situation has been encountered in the m-pair also. The X_1 and X_2 chromosomes did not reveal any bands and were uniformly darkly stained.

6. *Dalader acuticosta*
(Plate 52, Figs. 10, 10a, 11, 11a)

In *Dalader acuticosta*, the diploid chromosome number is 21(18+2m+XO). In this species, only G-banded diakinesis stages were available for study. Bended bivalents are presented in the Figs. 10, & 10a and their diagrammatic representations are shown in the Figs. 11, 11a. The autosomal bivalents are characterized by the well differentiated G-bands. They are usually present in the terminal ends of rod or cross-shaped bivalents. Interstitial bands are also present in the most of the bivalents. Heterochromatic blocks are present in the chiasmatic regions and terminal ends of bivalents No. 1, 2, 3, 4, 5 & 7 (Fig.11). The m-pair did not reveal any bands, while the X chromosome found to have a dark band in some plates (Figs. 10, 10a) and is uniformly darkly stained in others (Figs. 11, 11a).

7. *Petillia patullicollis*
(Plate 52, Figs. 12, 12a, 13, 13a, 14, 14a)

In *Petillia patullicollis*, diploid chromosome number is 28(24+2m+X_1X_2O) Both C- and G-banded chromosomes were studied in this species. C-banded karyotype and diagrammatic representation are presented in the Figs. 12 & 12a respectively. Two telomeric C-bands are present in each of the 12 pairs of autosomes, while the m-chromsomes are faintly stained and did not reveal any bands. Besides telomeric bands, interstitial bands are also found in the chromosome nos. 6 and 7. Of the two sex chromosomes, the X_1 is heterochromatic, while X_2 is faintly stained.

G-banded karyotype and idiogram are given in the Figs. 13 & 13a respectively. Two telomeric bands are present in each of the 1st, 4th, 5th and 10th chromosomes, whereas each of the 6th, 7th and 9th chromosomes have two comparatively large telomeric bands. On the other hand, an interstitial band along with two telomeric bands are present in each of the 2nd and 3rd chromosomes, while chromosome No.8 has a large dark band extending from the telomeric end to three fourths of the length of the chromosome. Chromosome No. 11 and m-chromosomes did not reveal any clear cut bands. The X_1 and X_2 chromosomes are uniformly stained without apparently distinguishable banding patterns.

C-banded chromosomes were also encountered in the diakinesis stages (Figs 14 & 14a). Four C-bands are present in each of the 1st, 2nd, 7th, 8th, 9th and 11th bivalents, where chiasmata are not completely terminalized, while only two terminal bands are found in the rod shaped 5th bivalent. Three C-bands are observed in each of the 3rd, 4th, 6th, 10th and 12th bivalents. The m-chromosomes did not reveal any bands. Two telomeric bands are, however, present in each of the X_1 and X_2 chromosomes.

8. *Ochrochira granulipes*
(Plate 53, Figs. 15, 15a, 16, 16a & 17)

Ochrochira granulipes shows typical coreid count of 21(18+2m+XO) chromosomes. In this species, both C- and G-banded spermatogonial metaphase chromosomes were studied in the testis preparation. C-banded karyotype and diagrammatic representation are presented in the Figs. 15 and 15a respectively. Each of the nine pairs of autosomes shows C-bands at two telomeric ends. The m-chromosome, also have two telomeric C-bands. The X chromosome, on the other hand, is characterized by the presence of two telomeric and an interstitial C-bands.

Figs. 16&17 show G-banded karyotypes and diagrammatic representation is presented in the Fig. 16a. All the chromosomes reveal considerably well differentiated G-banding pattern. Of the autosomes, chromosome No.1 shows five bands of which two bands are in the telomeric regions and two

PLATE 53

Explanation of Figures

Fig.15. C-banded karyotype of *Ochrochira granulipes.*

Fig.15a. Diagrammatic representation of the banded chromosomes.

Figs.16&17. G-banded karyotype of *O. granulipes.*

Fig.16a. Diagrammatic representation of the banded chromosomes.

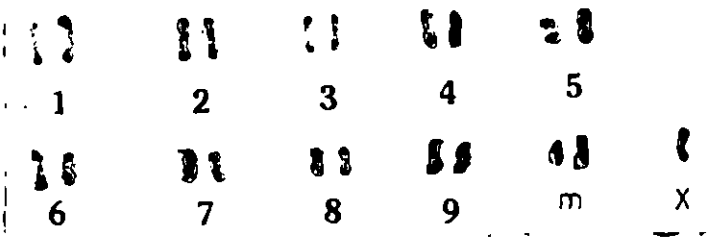
Fig.18. G-banded karyotype of *Anoplocnemis phasiana.*

Fig.18a. Diagrammatic representation of the banded chromosomes.

Fig.19. G-banded karyotype of *Acanthocoris* sp.

Fig.19a. Diagrammatic representation of the banded chromosomes.

15



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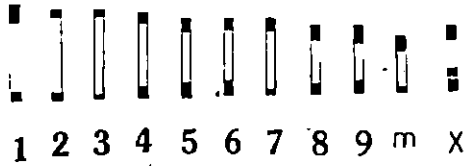

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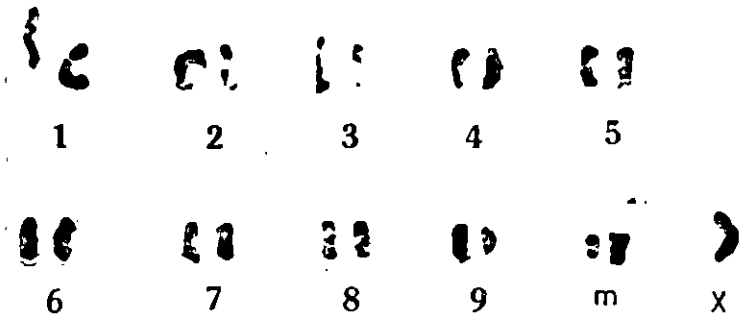

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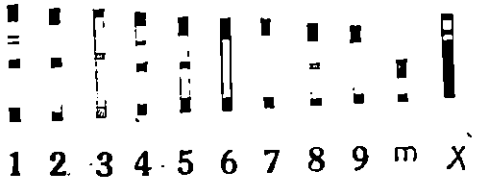

16a

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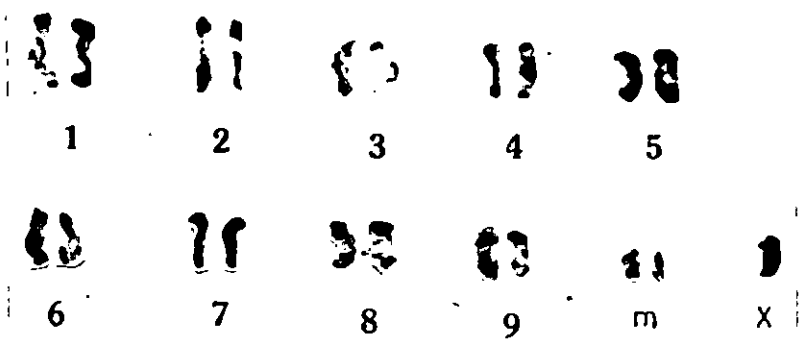

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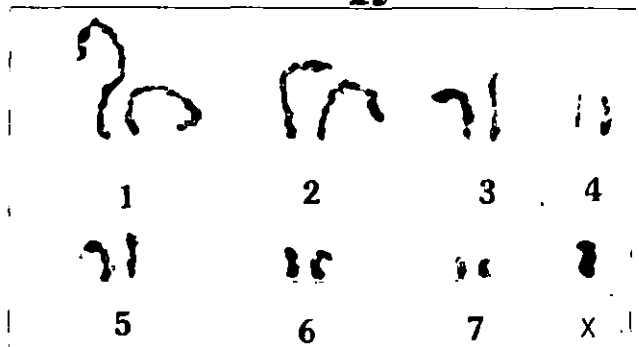
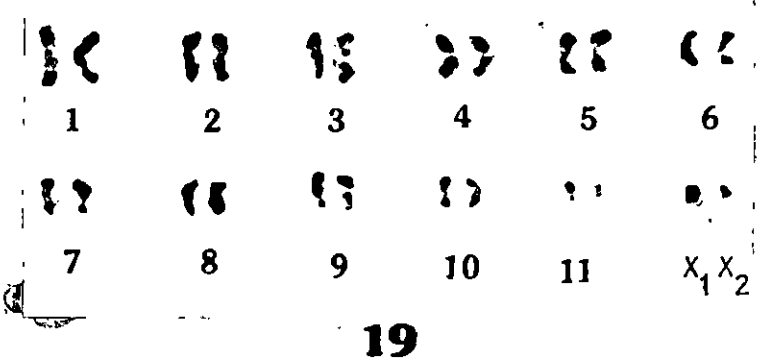


18

18a

19



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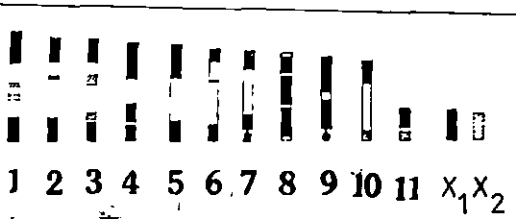


19a

fine bands are present in between a telomeric and an interstitial bands. While chromosome Nos. 2 and 5 have three bands each, the width of the interstitial band is comparatively large in the former chromosome. One dark telomeric band and two inconspicuous light bands are present in the 3rd chromosome, whereas two dark telomeric bands are present in each of the 6th, 7th & 9th chromosomes. The 8th chromosome has three bands of which one faint interstitial band is present in between two telomeric bands. The m-chromosome also shows two dark telomeric bands. The X chromosome, however, has one large and two narrow bands in contrast to the three bands of equal widths present in the C-banded X chromosome.

9. *Anoplocnemis phasiana*
(Plate 53, Figs. 18, 18a)

In *Anoplocnemis phasiana*, the diploid chromosome number is 15(14+XO). Fig. 18 presents a G-banded karyotype and Fig. 18a shows the diagrammatic representation of the bands. Of the autosomes, chromosome No. 1 is characterized by the presence of a heterochromatic block in one end of the chromosome and by ten bands of different widths and intensities, while chromosome No. 2 has five bands of which two prominent dark bands are located in the telomeric ends. Chromosome No. 3 is characterized by a secondary constriction like structure, two dark telomeric bands and an interstitial band. In the 4th chromosome, only two inconspicuous bands are present, while two telomeric and an interstitial dark bands are found in the 5th chromosome. Two large dark bands are present in the chromosome No. 6, covering almost whole length of the chromosome, whereas chromosome No. 7 and the X chromosome are uniformly darkly stained without apparently distinguishable bands.

10. *Acanthocoris*
(Plate 53, Figs. 19, 19a)

Acanthocoris sp. has diploid count of 24(22+X_1 X_2 O) chromosomes. Figure 19 shows G-banded karyotype and Fig. 19a presents diagrammatic representation of bands. Among the autosomes, each of the chromosome Nos. 1 and 3 shows two dark telomeric and two inconspicuous interstitial bands.

Chromosome Nos. 2, 4 and 5 are characterized by the presence of heterochromatic blocks. While chromosome No. 6 has two telomeric and an interstitial bands, only two telomeric bands are present in the chromosome No.10. Three large dark bands are found in chromosome No.8.A satellite-like structure along with two telomeric bands are present in each of the chromosome Nos. 7 and 9. The telomeric bands are very large, covering almost whole length of the latter numbered chromosome. The m-chromosome is characterized by a dark and a light bands. While the X_1 is uniformly darkly stained, the X_2 is lightly stained.

Family **Alydidae**

11. *Leptocorisa acuta*
(Plate 54, Figs. 20, 20a, 21, 21a)

In *Leptocorisa acuta*, the diploid chromosome number is 17(14+2m+XO). In this species, both C-and G-banded chromosomes were studied in the spermatogonial stages of the testes preparation. Fig. 20 depicts C-banded karyotype. Fig. 20a shows diagrammatic representation of the bands. The m-chromosomes were very faintly stained in the chromosome preparation so they were excluded from the karyotype. Chromosome No. 1 reveals a C-heterochromatin block in the interstitial region in addition to other bands. While two telomeric C-bands of different widths are present in each of the chromosome nos. 3, 4, 5 and 6, three inconspicuous C-bands are found in chromosome no.2. On the other hand, chromosome no. 7 has three dot-shaped as well as a telomeric C-bands. The X chromosome also shows inconspicuous C-bands.

G-banded karyotype is presented in the Figure 21, while Figure 21a shows diagrammatic representation of G-bands. The faintly stained m-chromosomes were not incorporated in the karyotype. All the chromosomes are characterized by well differentiated G-bands. Among the autosomes, chromosome no. 1 has a satellite like structure and five dark bands of which two bands are located in the telomeric regions. Chromosome No. 2 has five dark and three light bands, whereas five dark bands are present in each of the chromosome nos. 3 and 5. Chromosome no. 4 exhibits three dark bands, while four

PLATE 54

Explanation of Figures

Fig.20. C-banded karyotype of *Leptocorisa acuta*

Fig.20a. Diagrammatic representation of the banded chromosomes.

Fig.21. G-banded karyotype of *L. acuta*.

Fig.21a. Diagrammatic representation of the banded chromosomes.

Fig.22. G-banded karyotype of *Riptortus pedestris*.

Fig.22a. Diagrammatic representation of the banded chromosomes.

Fig.23. G-banded karyotype of *Lohita grandis*.

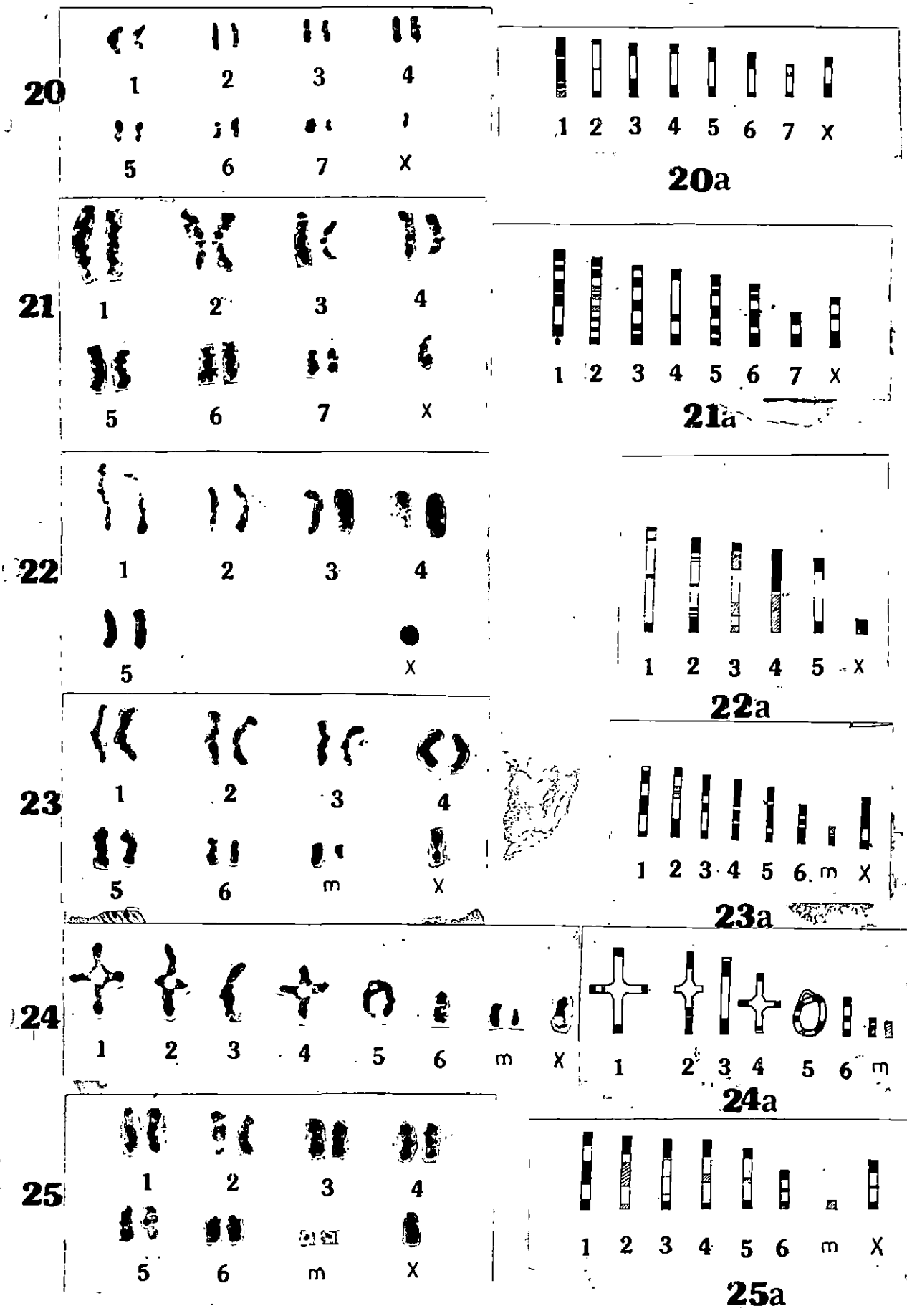
Fig.23a. Diagrammatic representation of the banded chromosomes.

Fig.24. G-banded chromosomes of diakinesis stage in *L. grandis*.

Fig.24a. Diagrammatic representation of the banded chromosomes.

Fig.25. G-banded karyotype of *Iphita limbata*.

Fig.25a. Diagrammatic representation of the banded chromosomes.


20
1 2 3 4
5 6 7 X
20a
1 2 3 4 5 6 7 X
21
1 2 3 4
5 6 7 X
21a
1 2 3 4 5 6 7 X
22
1 2 3 4
5 X
22a
1 2 3 4 5 X
23
1 2 3 4
5 6 m X
23a
1 2 3 4 5 6 m X
24
1 2 3 4 5 6 m X
24a
1 2 3 4 5 6 m X
25
1 2 3 4
5 6 m X
25a
1 2 3 4 5 6 m X

bands of different widths are present in chromosome no. 6. Further, each of the chromosome Nos. 2-6 has two telomeric bands. Two heterochromatic blocks are located in the telomeric ends of chromosome no. 7 and the X chromosome is characterized by the presence of three bands of which two are located in the telomeric regions.

12. *Riptortus pedestris*
(Plate 54, Figs. 22, 22a)

Riptortus pedestris shows diploid count of 13(10+2m+XO) chromosomes. In this species, G-banded spermatogonial metaphase chromosomes were studied in the testis preparation. Figure 22 exhibits G-banded karyotype. Figure 22a shows diagrammatic representation of the G-bands. Faintly stained m-chromosomes were not included in the karyotype. Of the autosomes, chromosome no.1 is characterized by four dark bands, while chromosome no. 2 shows seven bands. Chromosome No. 3 has one dark and three light bands. Chromosome no. 4 is characterized by a large dark band, whereas two dark bands of equal widths are present in the terminal ends of chromosome no.5. The X chromosome is uniformly darkly stained without apparently distinguishable bands.

Family **Largidae**

13. *Lohita grandis*
(Plate 54, Figs. 23, 23a, 24, 24a)

In *Lohita grandis*, the diploid chromosome number is 15(12+2m+XO). In this species, G-banded chromosomes were studied in the spermatogonial and diakinesis stages. Figure 23 depicts G-banded karyotype. Figure 23a shows diagrammatic representation of the bands. Among the autosomes, three dark bands of different widths are present in the chromosomes nos. 1, 3, 4, 5 and 6, while chromosome no. 2 is characterized by two dark and two light bands. The m-chromosome shows a dark and a light bands. Two dark bands, of which one is very large, are present in the X chromosome.

The meiotic bivalents also found to have well differentiated G-banding patterns. Figure 24 shows G-banded chromosomes of diakinesis stage. Their diagrammatic representations are given in Figure 24a. Four intense G-bands are usually found in the four ends of cross-shaped bivalents (nos. 1 & 4), while two (no. 3) or three (no. 6) bands are observed in rod shaped bivalents. A ring bivalent (no. 5) has four dark and a light bands. Two separate m-chromosomes have different banding patterns, one of them shows two dark bands while another is lightly stained. The X chromosome is characterized by four narrow bands in contrast to the one large and one narrow bands shown by the X at spermatogonial metaphase.

14. *Iphita limbata*
(Plate 55, Figs. 25, 25a).

The diploid count of *Iphita limbata* is 15(12+2m+XO). In this species, all the chromosomes of spermatogonial metaphase stage show well differentiated G-banding patterns. Figure 25 depicts G-banded karyotype. Figure 25a shows diagrammatic representation of the banded chromosomes. Three dark bands are present in each of the chromosome nos. 1 and 6, while chromosome no. 2 has two light and a prominent dark bands. On the other hand, chromosome no. 3 has two dark and three inconspicuous dotted bands. While chromosome nos. 4 and 5 have two dark and a light bands each, the m-chromosome did not reveal any bands. The X chromosome is characterized by three dark bands. Further, it has been observed that bands are preferentially located in one or both ends of the chromosomes.

15. *Physopelta gutta*
(Plate 55, Figs.26, 26a)

Physopelta gutta contains 17(12+2m+X_1 X_2Y) chromosomes. In this species, G-banded chromosomes were encountered in the spermatogonial metaphase stage. G-banded karyotype is presented in the Figure 26. Banded chromosomes are represented diagrammatically in the Figure 26a. The faintly stained m-chromosomes were not incorporated in the karyotype. Among the autosomes, single terminal heterochromatic blocks are present in each of the chromosome nos. 1, 2 and 6, while chromosome no 3 shows two terminal G-bands. Chromosome nos. 4 and 5 have two large dark bands each. The sex chromosome,

PLATE 55

Explanation of Figures

Fig.26. G-banded karyotype of *Physopelta gutta*.

Fig.26a. Diagrammatic representation of the banded chromosomes.

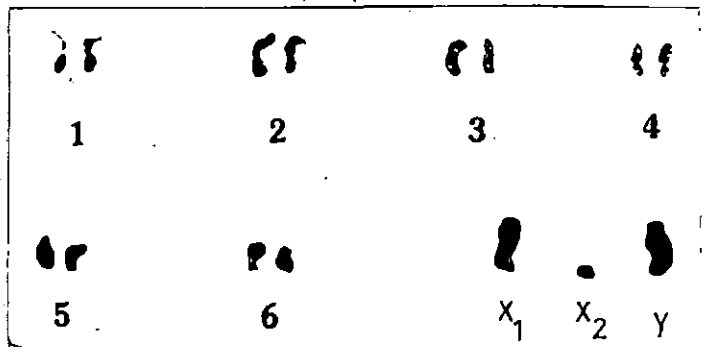
Fig.27. G-banded chromosomes of diakinesis stage in *Physopelta quadrigutta*.

Fig.27a. Diagrammatic representation of the banded chromosomes.

Fig.28. G-banded chromosomes of diakinesis stage in *Physopelta schlanbuschi*.

Fig.29. G-banded sex chromosomes in *P. gutta*, *P. quadrigutta* and *P. schlanbuschi*.

Fig.29a. Diagrammatic representation of the sex chromosomes in *P. gutta*, *P. quadrigutta* and *P. schlanbuschi*.



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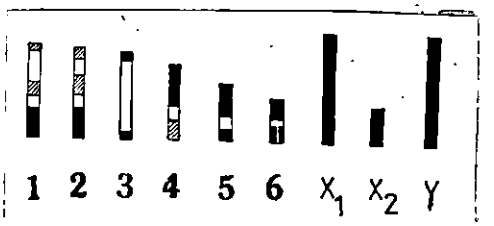
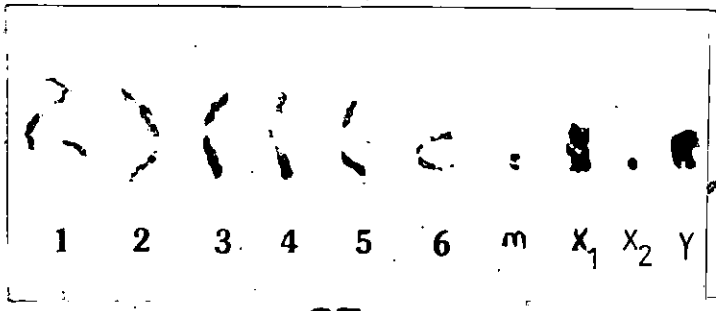
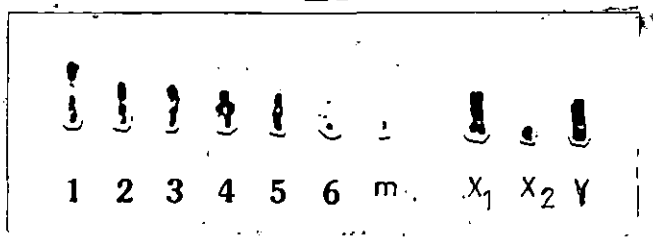


26a



27

27a



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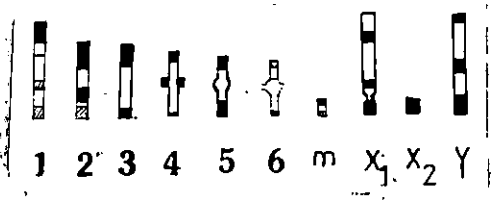
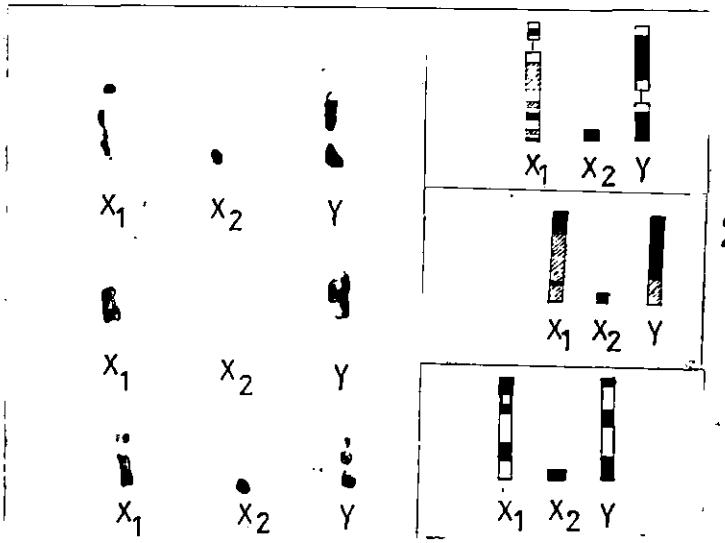


28a

29a



29

29a

X_1X_2Y, are heavily stained throughout their length and did not reveal any bands.

16. *Physopelta quadrigutta*
(Plate 55, Figs. 27, 27a)

In *Physopelta quadrigutta*, the diploid chromosome number is 17 (12+2m X_1X_2Y). In this species, G-banded chromosomes were observed in diakinesis stage. Figure 27 shows G-banded bivalents and univalents sex chromosomes and their diagrammatic representations are presented in Figure 27a. Three dark and a light bands are present in bivalent no.1, whereas single dark and inconspicuous light bands are found in each of the 2nd, 4th, 5th and 6th bivalents. 3rd bivalent shows five dark bands, while two terminal dark bands are also present in the small m-chromosome pair. The X_1 and X_2 are uniformly darkly stained, but two large dark bands are present in the Y chromosome.

17. *Physopelta schlanbuschi*
(Plate 55, Figs. 28, 28a, 29, 29a)

Physopelta schlanbuschi shows diploid count of 17(12+2m+X_1 X_2 Y) chromosomes. As in previous species, well differentiated G-banded chromosomes were encountered in diakinesis stage only (Figs. 28, 28a). Among the autosomal bivalents, besides other bands, single heterochromatic blocks are present in the terminal ends of each of the 1st, 2nd and 3rd bivalents. Single, comparatively smaller, interstitial heterochromatic block is also found in the 2nd bivalent. Bivalent No. 4 shows three dark bands, while two dark bands are located in the terminal ends of the 5th bivalent. Inconspicuous G-bands are present in the 6th bivalent and in m-pair. The sex chromosomes, X_1, X_2 and Y, exhibit well differentiated G-bands. The X_1 has three dark bands of which two are present in the interstitial regions and one in the terminal region. the Y chromosome reveals three bands: one interstitial and two telomeric bands. The X_2 is, however, uniformly darkly stained. Thus, on the basis of G-banding patterns, it is possible to demarcate two identical sized marker sex chromosomes.

PLATE 56

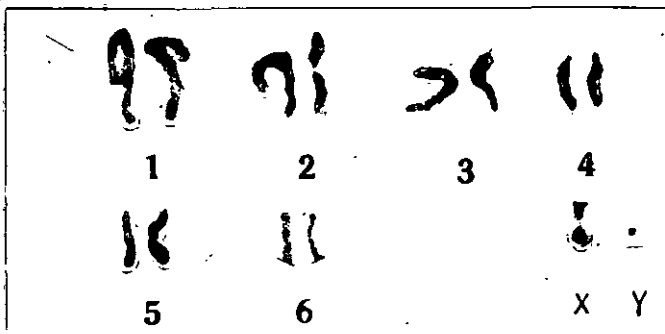
Explanation of Figures

Fig.30. G-banded karyotype of *Spilostethus hospes*.

Fig.30a. Diagrammatic representation of the banded chromosomes.

Figs.31.32. 33 G-banded chromosomes of diakinesis stage in *S. hospes*.

Figs. 31a, 32a & 33a. Diagrammatic representation of the banded chromosomes.



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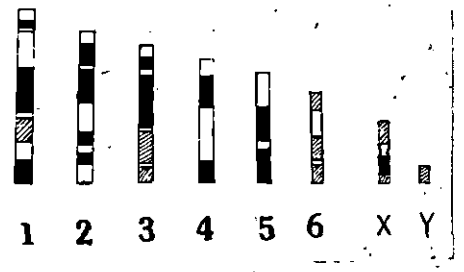
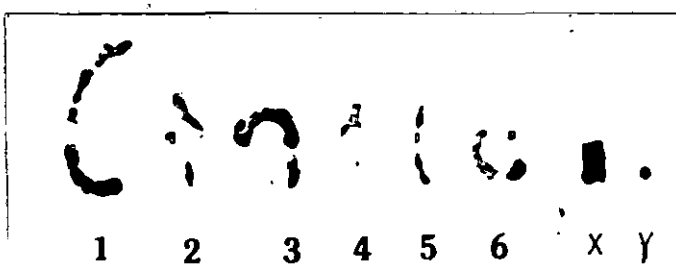


30a



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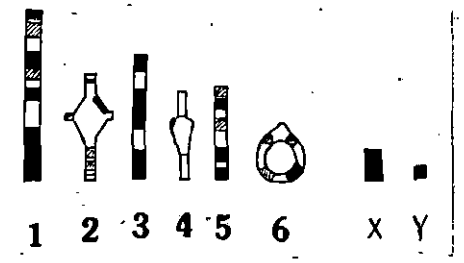

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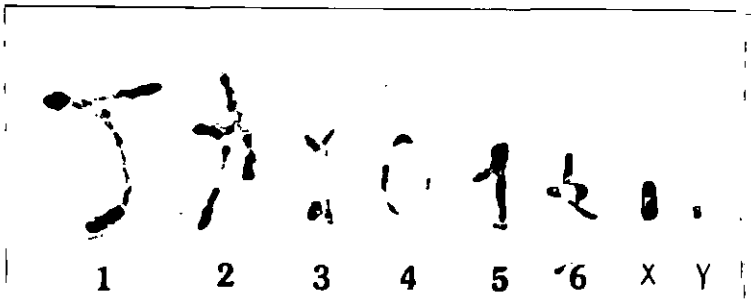

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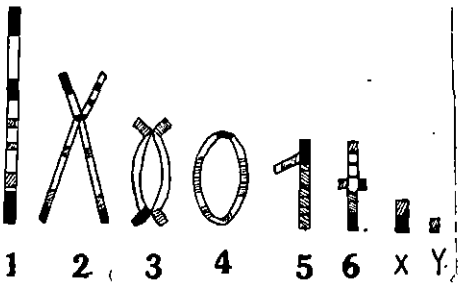

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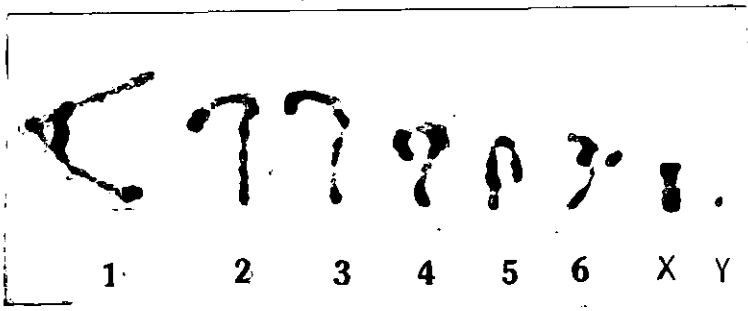

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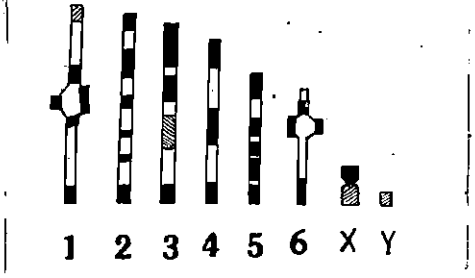

33a

Comparative study of the G-banded sex chromosomes in three species of *Physopelta* viz., *P.gutta, P.quadrigutta* and *P.schlanbuschi* shows that the X_1X_2 and Y chromosomes in each of the three species have characteristic banding patterns. Figure 29 depicts G-banded X_1 X_2 Y chromosomes of *P. gutta, P. quadrigutta* and *P. schlanbuschi.* Figure 29a shows diagrammatic representation of the banded chromosomes. In *P. gutta,* the X_1 exhibits a-satellite-like structure with a narrow dark band, while two dark and three light bands are present in the remaining part of the chromosome. On the other hand, the Y chromosome reveals a constriction in its mid region and two large dark bands which cover almost whole length of the chromosome. In *P. quadrigutta,* the X_1 is characterized by two inconspicuous dark bands, while a large dark band is present in the Y chromosome. In *P. schlanbuschi,* both X_1 and Y have three dark bands each. While X_1 exhibits a dark band in the constricted telomeric end and two other bands in the rest of the chromosome, the Y chromosome shows two telomeric and an interstitial bands. In all these three species the X_2 chromosome is uniformly darkly stained.

Family **Lygaeidae**

18. *Spilostethus hospes*
(Plate 56, Figs. 30, 30a, 31, 32, 33, 31a, 32a, 33a)

In *spilostethus hospes,* the diploid chromosome number is 14(10+2m+ XY). In this species, both G-banded spermatogonial metaphase chromosome and meiotic chromosomes of diakinesis stages were studied. Figure 30 presents G-banded karyotype. Diagrammatic representation of the banded chromosomes is given in the Figure 30a. Chromosome no.1 shows three dark and a light bands of which one dark band is present in the terminal region, while five dark bands are found in the 2nd chromosome. Two dark and two light bands are present in the chromosome no.3. On the other hand, of the two dark bands found in the 4th chromosome, one band is present in the terminal region. In the chromosome no.5, besides an interstitial dark band, single heterochromatic block is present in the terminal

region. Chromosome no. 6 shows inconspicuous light bands. While a dark and two light bands are present in the X chromosome, the Y is uniformly lightly stained.

G-banded meiotic chromosomes were also studied in the diakinesis stages. Figures 31, 32, 33 and 31a, 32a, 33a depict G-banded chromosomes and diagrammatic representations of the banded chromosomes respectively. All the bivalents and the univalent sex chromosomes show well differentiated G-banding patterns. Single large heterochromatic block is present in the terminal region of the bivalent no. 1 (Fig. 31), while two terminal and single interstitial heterochromatic blocks are found in bivalent no.1(Fig.32) of another plate. However, a small terminal heterochromatic block is present in bivalent no. 1 (Fig.33) of yet another plate. Heterochromatic blocks are also found in bivalent nos. 2, 3, 5 & 6 (Fig. 32), bivalent nos. 2, 4, 5(Fig. 33). Each of the ring bivalents, however, exhibits single dark band only (Figs. 31, 32). The X and Y chromosomes are found to be uniformly darkly stained in some plates (Fig.31), while the X chromosome shows an inconspicuous dark bands and the Y is faintly stained in other plates (Fig.32). Yet, in some plates (Fig.33), the X is characterized by a constriction with a dark band, whereas the Y chromosome is lightly stained.

DISCUSSION

Previous attempts to show C- and G-bands in heteropteran chromosomes have met with but limited success. Muramoto (1980) demonstrated C-bands in one or both ends of the spermatogonial chromosomes and in meiotic bivalents of some heteropteran species namely, *Dolycoris baccarum* (Pentatomidae), *Molypteryx fuliginosa* (Coreidae) and *Spilostethus hospes* (Lygaeidae). The same author produced G-bands in mitotic and meiotic chromosomes of four pentatomid bugs (Muramoto 1975, 1978), while he failed to show C-bands in other species of Heteroptera (Muramoto 1985). Further, G-bands were also demonstrated in somatic chromosomes of triatominae bugs, *Rhodnius prolixus* and *Triatoma infestans* by Maudlin (1974). Later, Solari (1979) reported terminal C-heterochromatin blocks in the largest autosomes of the latter species. Recently, Camacho et al. (1985) have produced light C-bands in one end of each autosomes in *Nezara viridula*, and have suggested that the centromeric activity is preferentially located in the telomeric ends of the chromosomes in this species. However, Bigger (1975) and Bedo (1984) failed to induce C- or G-bands in some lepidopteran species, while C-bands are found to be localized at the ends of the chromosomes in the earwig, *Labidura truncata* (G.C. Webb, personal communication to Bedo, ref. Bedo 1984). Further, terminal and interstitial C-heterochromatic blocks are reported in *Parascaris* (Nematoda) (Goday et al. 1985). In the present investigation, we have successfully demonstrated C-bands in the spermatogonial chromosomes of four heteropteran species viz., *Petillia patullicollis*, *Ochrochira granulipes*, *Leptocorisa acuta* and *Chrysocoris stollii*. Terminal C-bands are very prominent in first and second-named species, while C-heterochromatin blocks have been encountered in addition to the terminal C-bands in *L. acuta* and *C. stollii*. Moreover, interstitial C-bands are also found in some of the chromosomes in these species. The Y chromosome is found to be entirely heterochromatic in *C. stollii*, while Muramoto (1980) failed to demonstrate C-bands in the Y-chromosome of *D. baccarum*. The Y chromosome, however, shows a large heterochromatic block in *T. infestans* (Solari 1979). While the X chromosome of *C. stollii* has several interstitial C-bands, no detectable bands were found in the X chromosomes of other two species. Our findings suggest that the terminal ends of the chromosomes are rich in centromeric heterochromatin or constitutive heterochromatin

since C-banding is generally accepted as a marker of this type of heterochromatin (Sumner 1972; Hsu 1973). It occurs not only at the centromeric regions, but also at other sites of the chromosomes (Sumner et al. 1971; Gropp & Natarajan 1972). However, what role the centromeric heterochromatin plays in the movement of holocentric chromosomes is yet to be ascertained though it is kinetically active in the monocentric chromosome. Therefore, it is pertinent to ask whether any relationship exists between C-banding and centromeric activity in the holocentric chromosomes which have uniform appearance under light microscope and display a variety of different structures in the electron microscope. Kinetochore occupies the entire length of the chromosome in *Rhodnius* (Buck 1967), while it occupies 75% of the length of the chromosome in *Oncopeltus* (Comings & Okada 1972). These are in striking contrast to the Ruthmann & Permantier's (1973) observation of localized kinetochore, covering only 4.2% of the chromosome length in *Dysdercus*. Kinetochore plates are, however, absent in *Tetranychus urticae* (Homoptera) (Tempelaar & Drenth-Diephuis 1983) and Pijnacker & Ferwerda (1976) failed to produce C-bands in this species. On the other hand, *Luzula* chromosomes were found by Braselton (1971) and Lambert (1971) to have discrete kinetochore regions spaced along the chromosomes. If each of these had associated heterochromatin the entire chromosome should show an even C-band reaction. However, C-bands in *Luzula* are localized with bands spaced along the whole chromosome length or near one or both ends of the chromosome (Ray & Venketeswaran 1978). Banding studies in *Oncopeltus*, *Rhodnius* and *Dysdercus* are also needed to clarify the status of centromeres in these species because C-bands are found to be localized in the ends of the chromosomes of those species where it has been attempted to date.

In contrast to mitosis, the kinetic organization meiotic chromosome seems to very different. Kinetochore plate are absent in *Oncopeltus* (Comings and Okada 1972) and *Dysdercus* (Ruthmann and Permantier 1973), although in the latter spindle microtubules appear to end singly in dense spots of kinetochore material. The lack of kinetochores has been explained by assuming sparse distribution of kinetic DNA along the axial system of the chromosome, whereas dense distribution of kinetic DNA on a small area results in the formation of kinetochore plate (Nokkala and Nokkala 1985). However, the induction of C-bands in the meiotic bivalents of *Petillia patullicollis*

(present report) and in other species of Heteroptera (Solari 1979; Muramoto 1980) suggest the presence of localized kinetochore. The disproportionate activities shown by the chromosome ends (Piza 1958) or restriction of the kinetochore activities in the terminal regions (Schrader 1935; Hughes-Schrader & Schrader 1961; Geitler 1937) of the meiotic chromosomes of heteropteran species further support our findings. On the basis of experimental and observational evidences, different models have been proposed for heteropteran chromosomes namely, diffuse (Hughes-Schrader & Schrader 1961), telocentric (Ruthmann Dahlberg 1976), diakinetic (Piza 1958), potentially diakinetic (Nokkala & Nokkala, 1985) and monocentric (Manna 1984). But, all these models lack sufficient supportive evidences and therefore open for further consideration.

G-banding studies have been carried out in all the eighteen species (Table 9). Both spermatogonial and meiotic chromosomes show well differentiated banding patterns and the problem of individual chromosome recognition has been solved in these species to some extent. Positive G-bands are also found to be localized preferentially in the ends of the chromosomes and some of the chromosomes have bulk bands or heterochromatic blocks. Furthermore, satellite-like structures, which were not detected by ordinary staining methods, are found to be attached to the ends of some of the G-banded chromosomes. It seems that G-banding treatments facilitate the detection of such structures. However, satellite-like structures were reported earlier in *Brachyplatys subaeneus* (Plataspidae) (Manna 1951) and *Myrmus miriformis* (Corizidae) (Nokkala 1985) by ordinary staining methods.

It is therefore evident from the present study that both C- and G-bands are localized preferentially in one or both ends of the holokinetic chromosomes of the heteropteran species.

SUMMARY

1. The chromosome banding studies have been carried out in eighteen species of Heteroptera belonging to five families. Results are summarized in the Table 9.

2. C-bands are found in one or both ends of the spermatogonial metaphase chromosomes, while well differentiated G-bands are also observed in the spermatogonial and meiotic chromosomes.

3. Besides terminal C-bands, C-heterochromatin blocks, interstitial C-bands are also encountered in some of the C-banded chromosomes.

4. Each chromosome has its characteristic G-banding patterns. Positive G-bands or bulk G-bands are usually located near the ends of the mitotic as well as meiotic chromosomes. Satellite-like structures are also encountered in some of the G-banded chromosomes.

5. Results suggest that the bands are localized preferentially near one or both ends of the chromosomes.