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Morphology, Behaviour and Metrical Studies of the Germinal Chromosomes of Ten Species of Membracidae (Homoptera)

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About 40 species of membracids have been cytologically studied so far by other workers (*vide infra*) to which the chromosome number of ten species has been added by us (Bhattacharya and Manna 1967). Besides these ten species, only eight were reported from India (Banerjee 1958, Menon 1958, Rao 1956 and Sharma *et al.* 1964). The present study deals with the morphology of gonial metaphase chromosomes of both sexes, the behaviour and measurement of spermatocyte chromosomes and the cytotaxonomical evaluation of the family. Nine out of the ten species have been cytologically investigated for the first time. Further, no species belonging to *Cocosterphus* and *Tricentrus* was studied previously.

Material and methods

Several adult male and female individuals of ten species were collected locally from different host plants (Table 1) and their testes and ovaries were fixed in acetic-alcohol for squash preparations. Testes of some individuals were also fixed in Sanfelice for sectioning. Iron-alum haematoxylin and Fuelgen stain were used for the testes while the oogonial complements were studied from temporary acetic-carmine preparations. A metrical study of first spermatocyte metaphase chromosomes was done following the method described elsewhere (Bhattacharya and Manna 1970 and Manna 1951).

Observations

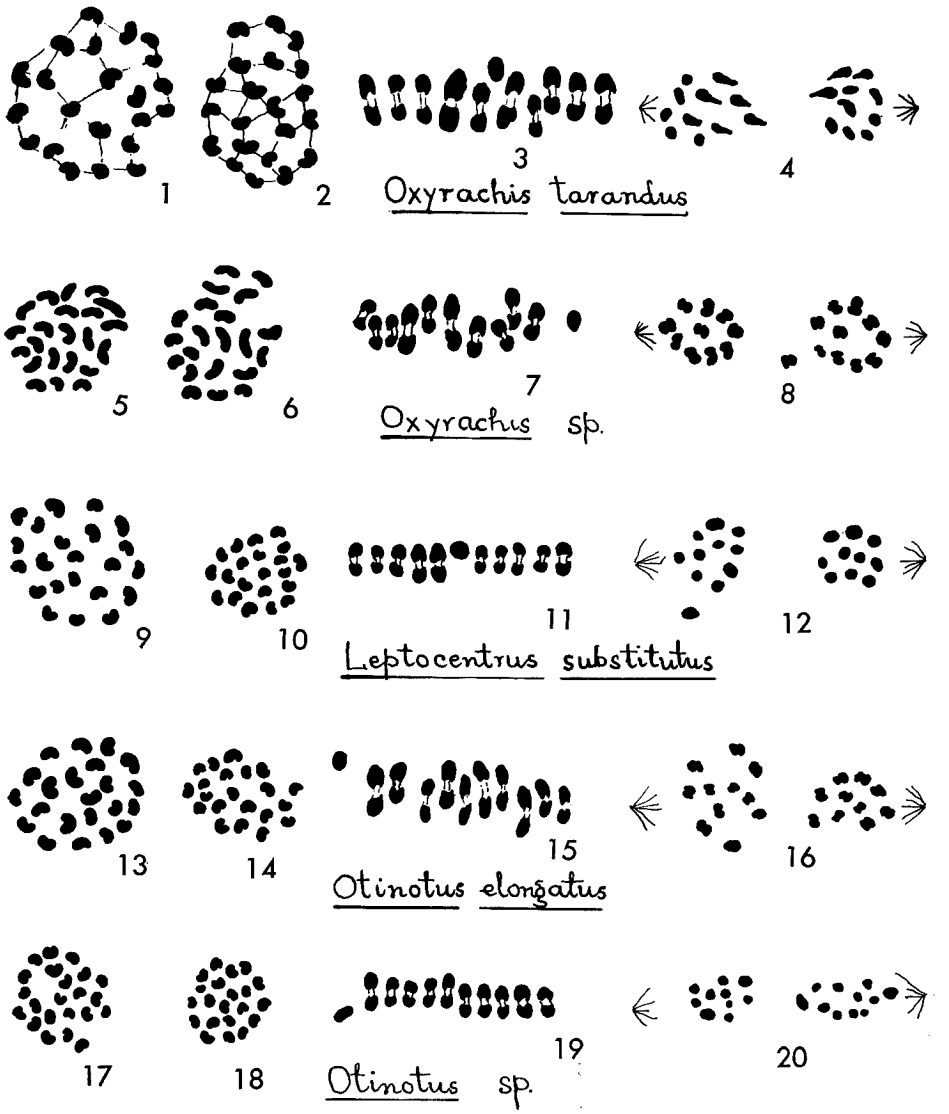
The pattern of male meiosis was more or less the same in different species, but they differed to some extent when the morphology and the behaviour of gonial and meiotic chromosomes were critically analysed. For this reason some accounts of them has been presented one by one.

Oxyrachis tarandus had 22 chromosomes in the oogonial (Fig. 1) and 21 in the spermatogonial metaphase complements (Fig. 2). Chromosomes were gradually seriated bean shaped structures and inter-connected, likepenta tomid bugs in both types of gonial complements. The difference of one chromosome suggested XX: XO sex mechanism but X chromosome was not demarcable by its size or staining

Table 1. Ten species of membracids with their host plants and relative volumes in percentage of the haploid set of metaphase I chromosomes

Subfamily, division and name of species	Host plant	Serial number of autosomes										X chromosome.
		A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_9	A_9	A_{10}	
Subfamily—Membracinae												
1. *Oxyrachis tarandus* (Fabr.)	*Accacia arabica*	11.19	10.47	10.47	9.28	8.09	7.61	7.61	6.66	6.66	6.42	15.47
2. *Oxyrachis* sp.	*Accacia arabica*	14.00	11.80	9.68	9.28	8.21	8.21	7.86	7.50	6.80	5.70	11.07
Subfamily—Centrotinae												
Division—Leptocentrani												
3. *Leptocentrus substitutus* (Walk.)	*Solanum melongena*	12.07	11.37	11.03	8.96	8.62	8.62	8.00	7.58	7.58	7.00	8.62
4. *Otinotus elongatus* (Dist.)	*Cassia fistula*	10.88	10.54	9.86	9.18	8.88	8.88	8.19	7.82	7.48	7.18	11.22
5. *Otinotus* sp.	*Zizyphus jujuba*	10.43	10.43	9.60	8.73	8.73	8.73	8.29	8.29	7.46	7.46	12.66
Division—Centrotusaria												
6. *Tricentrus albomaculatus* (Dist.)	*Cajanus indicus*	19.40	11.56	10.07	9.79	8.95	8.58	7.46	7.01	5.97	—	11.19
7. *Tricentrus* sp.	*Anacardium occidentale*	21.42	11.07	9.64	9.64	8.92	7.86	7.86	7.14	5.71	—	10.71
Division—Gargaria												
8. *Gargara contraria* (Dist.)	*Accacia arabica*	23.61	11.82	10.50	9.03	8.16	7.87	7.58	6.70	5.24	—	9.61
9. *Gargara flavolineatus* (Dist.)	*Cassia fistula*	24.46	11.58	9.87	9.12	8.11	7.72	6.86	6.43	5.57	—	10.30
Division—Cocosterphusaria												
10. *Cocosterphus decoloratus* (Dist.)	*Cicer arietenum*	26.16	10.28	9.34	8.87	8.41	7.74	7.74	6.54	5.60	—	9.34

difference. In male the univalent X could be identified during the primary spermatocyte prophase as a positively heteropycnotic element and at metaphase I (Fig. 3) by its univalent nature and isolated disposition. The metaphase I plate contained ten dumb-bell shaped autosomal bivalents because of the single terminal-



Figs. 1, 5, 9, 13, 17, 21, 25, 29, 33, and 37. Oogonial metaphases.
Figs. 2, 6, 10, 14, 18, 22, 26, 30, 34, and 38. Spermatogonial metaphases.

ised chiasma in each of them and the X, while at diplotene and diakinesis stages some bivalents had interstitial chiasma. The first division anaphase (Fig. 4) was reductional incorporating the X in one of the poles along with 10 autosomes of the two daughter halves. The second division was equational. According to the metrical data (Table 1) chromosomes could be grouped as one large—the X

(15.47) and 10 pairs of medium to small gradually seriated autosomes whose relative volumes ranged between 11.19%–6.42%.

Oxyrachis sp: Like *O. tarandus* this species also had 22 in the oogonial (Fig. 5) and 21 chromosomes in the spermatogonial metaphase (Fig. 6) complements but without inter-chromosomal connections. A comparison of the gonial complements did not reveal the identity of the sex chromosomes but it could easily be followed by its positively heteropycnotic behaviour from the early primary spermatocyte prophase. The diplotene nucleus contained ten autosomal bivalents each with a single chiasma and an univalent positively heteropycnotic X. At metaphase I (Fig. 7) all the 10 dumb-bell shaped bivalents had a single terminalized chiasma and the univalent X was lying in different region of the spindle. The anaphase I was reductional (Fig. 8) which gave rise to two types of second spermatocyte metaphase, one with X and 10 autosomes and the other with 10 autosomes only. The metrical data (Table 1) of this species differed considerably when compared with *O. tarandus*. Autosome No. I was the largest (14.00%) while A_2–A_{10} were gradually seriated between the values of 11.80%–5.70%. The X was third in rank and measured (11.07%) close to A_2 (11.80%).

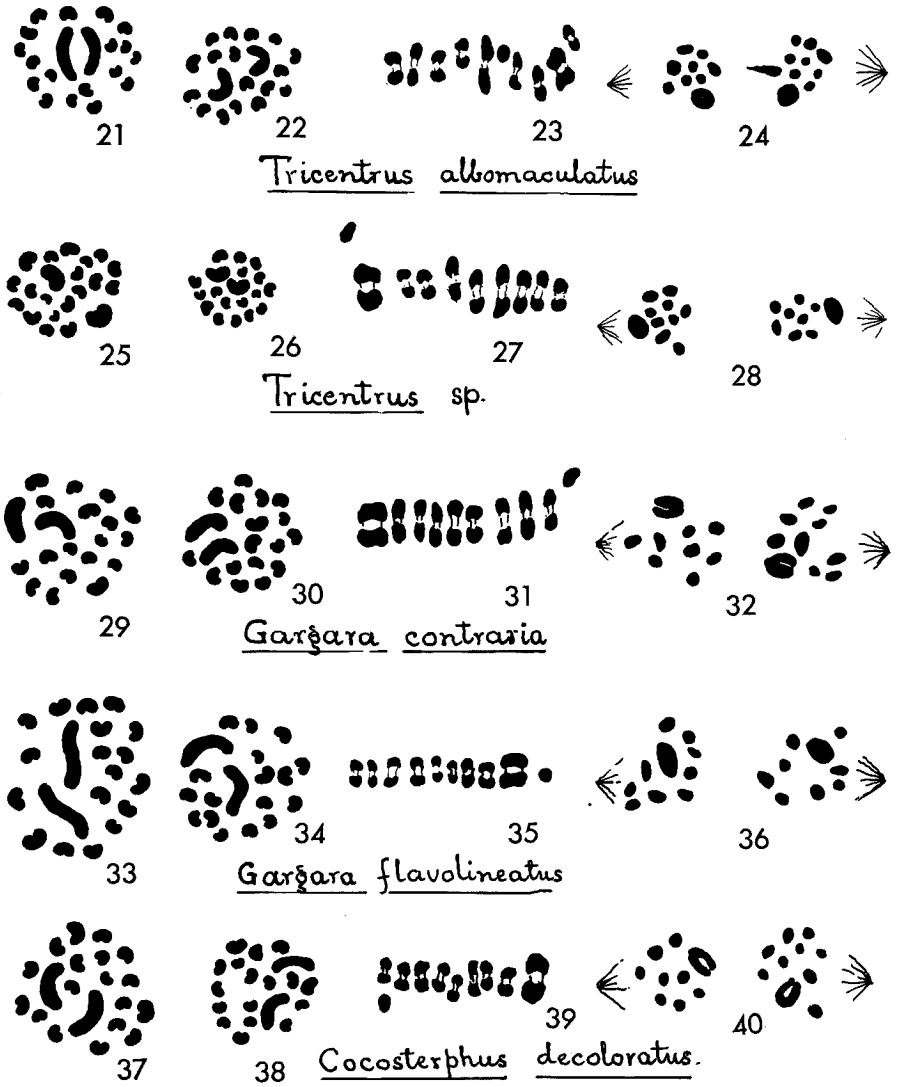
Leptocentrus substitutus had 2n=22 in the female (Fig. 9) and 21 chromosomes in the male (Fig. 10). Identification of sex chromosomes in the gonial complements was not practicable. However, it was easily recognised in the early primary spermatocyte prophase nucleus as it was forming the base of the faint bouquet arrangement. During diplotene and diakinesis it retained its deeper stain. At metaphase I (Fig. 11) it appeared as a spherical element while ten autosomal bivalents were dumb-bell shaped structures. The chiasma frequency was one per bivalent. The X was sometimes asynchronous in its movement but anaphase I (Fig. 12) was reductional for the chromosomes. The second division was equational. The metrical data (Table 1) revealed that all the chromosomes were very gradually seriated as the relative percentage values ranged from 12.08 to 7%. The X measured (8.62%) similar to A_5 or A_6.

Otinotus elongatus: The oogonial metaphase complement of 22 chromosomes (Fig. 13) and the spermatogonial complement of 21 chromosomes (Fig. 14) were also found in this species. Neither the staining behaviour of the gonial chromosomes nor the comparison of the gonial complements made the sex chromosomes discernible. Chromosomes in the gonial complements were bean shaped without much difference in their size. In the primary spermatocyte prophase nucleus, a faint bouquet was observed in which the positively heteropycnotic sex chromatin mass formed the base. The first spermatocyte metaphase I (Fig. 15) had ten autosomal bivalents each with a single chiasma and an univalent X chromosome that formed an accessory plate. During anaphase I (Fig. 16) the X was sometimes seen as a laggard but no maldistribution was encountered. The second division was equational.

The metrical data (Table 1) revealed that the X was the largest member (11.22%), while the autosomes were gradually seriated between relative values of 10.88 and 7.18%.

Otinotus sp. also had 2n=22 in the female (Fig. 17) and 21 chromosomes in

the male (Fig. 18) complements. Gonial chromosomes were bean shaped and gradually seriated. The meiosis in males was of the same pattern as in *O. elongatus*. Metaphase I (Fig. 19) contained 10 dumb-bell shaped autosomal bivalents and an univalent X chromosome. The anaphase I (Fig. 20) was reductional but the



Figs. 3, 7, 11, 15, 19, 23, 27, 31, 35, and 39. Spermatocyte metaphase I.
Figs. 4, 8, 12, 16, 20, 24, 28, 32, 36, and 40. Spermatocyte anaphase I.

second spermatocyte division was equational for the chromosomes. The relative percentage volume (Table 1) revealed the X as the largest element (12.66%) and the autosomes were ranging between 10.43%–7.46%.

Tricentrus albomaculatus had 20 chromosomes in the oogonial (Fig. 21) and 19 in the spermatogonial (Fig. 22) complements. Both the gonial complements

had a conspicuously large pair of autosomes while the rest were small and bean shaped. At the early primary spermatocyte stage a faint bouquet on a heterochromatin mass was found. Nine autosomal bivalents and the heteropycnotic X were found in the diplotene and diakinesis stages. The bivalents had one chiasma each. One of the bivalents was very large. Metaphase I (Fig. 23) also had nine bivalents and an univalent spherical X chromosome. The first division anaphase was reductional (Fig. 24) and the second was equational for the chromosomes. Metrical data (Table 1) revealed that autosome No. 1 was very large (19.40%) while the rest including the X had relative percentage values ranging between 11.56% and 5.97%. The X measured 11.19% which was close to A_2.

Tricentrus sp. also contained the same diploid number of 20 in the female (Fig. 25) and 19 chromosomes in the male (Fig. 26) like *T. albomaculatus*. A pair of autosomes was very large but all the chromosomes were relatively small than the other species. In the early spermatocytic prophase a bouquet formation was seen while the diplotene nucleus contained nine bivalents and the univalent X. The large bivalent had two while the remaining eight had one chiasma each. Metaphase I (Fig. 27) contained nine rod-like bivalents and the deeply condensed X. The first division anaphase (Fig. 28) was reductional and second division was equational for the chromosomes. The large autosome was demarcable in all these stages of meiosis. Metrical data (Table 1) revealed the relative percentage value of the large autosomes as 21.42% and the rest were gradually seriated. The X measured 10.71% which was close to A_2.

Gargara contraria, like the species of *Tricentrus*, had 20 chromosomes in the oogonial (Fig. 29) and 19 in the spermatogonial (Fig. 30) complements which included a pair of very large chromosome and the rest were small and bean shaped. The X chromosome was not identifiable. During primary spermatocyte prophase no bouquet was seen. The late spermatocyte prophase contained nine bivalents including a very large one and a rod-like positively heteropycnotic X chromosome. At metaphase I (Fig. 31) the large bivalent with eight other were dumb-bell shaped in appearance while the X was condensed. As usual the first division was reductional (Fig. 32) but the second was equational for the chromosomes. The metrical data (Table 1) revealed that the relative percentage value of the large chromosome was 23.61 while the remaining chromosomes, inclusive of the X, were in between 11.82–5.24%. The size of X is as in between A_3 and A_4 as it measured 9.61.

Gargara flavolineatus had 20 in the oogonial (Fig. 33) and 19 chromosomes in the spermatogonial (Fig. 34) complements with a large pair of autosomes. Other chromosomes were small. The number of elements present in the primary spermatocyte prophase was followed from the diplotene stage. At this stage there were nine autosomal bivalents, including the large one and a rod-shaped X chromosome. The early prophase had a faint bouquet arrangement. The large bivalent had two but others only one chiasma each. Metaphase I plate (Fig. 35) had also nine autosomal bivalents including the large one and the X was over-condensed chromosome. The anaphase separation (Fig. 36) incorporated the X in one of the poles. The second division was equational. According to the metrical data (Table 1) the relative percentage value of the large chromosome was 24.46%. The

rest were small and gradually seriated 11.58%–5.57%. The X was in between A_2 and A_3 measuring 10.30%.

Cocosterphus decoloratus also had 2n=20 in female (Fig. 37) and 19 in male (Fig. 38) gonial complements with an outstandingly large pair of chromosomes. Sex chromosomes could not be identified in the gonial complements. It belonged to the smaller group of chromosomes. The early primary spermatocyte prophase contained no bouquet arrangement. The diplotene nucleus of the primary spermatocyte had ten elements—one large bivalent, 8 small bivalents and an univalent X. The metaphase I (Fig. 39) contained the large bivalent, 8 small bivalents and the univalent X with its conventional disposition. The X moved to one of the poles at anaphase I (Fig. 40) and the second division was equational. According to the metrical data (Table 1) chromosomes could be grouped as one large (26.16%) and the rest small 10.28%–5.60%. The measurement of the X and A_3 was the same (9.34%).

Discussion

No positive cytological characterisation of the family Membracidae is yet available. Broadly speaking a well defined bouquet as seen in Cercopidae (Bhattacharya and Manna 1970) was not generally found in this family. It sometime appeared in traces and not in all the species. The chromosome number in different subfamilies ranged between a narrow limit of 17 and 21 in males with always an XO/XX sex-mechanism except the controversial record of XY/XX in *Enchenopa binotata* (Kornhauser 1914). Boring (1907) and Halkka and Heinonen (1964) have claimed XO/XX. Both the reports XO/XX and XY/XX might be correct since males with two different sex-chromosome constitutions have been found in some other species of Homoptera, e.g., *Dicranotropis hamata* and *Raphopleyx preyssleri* by Halkka (1959) and *Parabolocratus albomaculatus* by Bhattacharya and Manna (1969).

The spermatogonial numbers as reported or compiled by different authors like Halkka, (1959), Halkka and Heinonen (1964), Rao (1956), Menon (1958), Whitten (1965), Medler (Personal communication) and Bhattacharya and Manna (1967) are—subfamily Darninae 2n=19—*Hebecoides confusus*; 2n=21 *Stictopelta acutula*; subfamily Membracinae 2n=19, 20 *Campylanchia latipes, C* (=*Enchenopa*) *binotata*; subfamily Smiliinae—2n=17—*Lallemandia nodosa, Vanduzea arcuata, V. laeta*; 2n=21—*Amastris elevata, A. maculata, Atymna castanea, Ceresa bupulus, C. diceros, C. taurina, Entylia sinuata, Eufairmairia fraternus, Telengana cousorbrina, Sextius virescens*; subfamily Centrotinae—2n=19—*Centrotus cernutus, Cocosterphus decoloratus, Gargara contraria, G. flavolineatus, G. mixta, Tricentrus* sp., *T. albomaculatus*; 2n=21—*Leptocentrus* sp., *L. taurus, L. substitutus, Otinotus* sp., *O. elongatus, O. lignicola, Oxyrachis* sp., *O. tarundus, O. rufescens, Micrutalis calva, Pogon bostigmatus, Tolania obtusa.* In the subfamily Darninae two species of two genera having 19 and 21 chromosomes would represent heterogeneity while it may not be so for Membracinae because two species investigated in this subfamily have 19 (18A+X) chomosomes each.

The data are very meagre and the characterisation is very tentative. The situation is a little better in Smiliinae since relatively more species are cytologically known. The spermatogonial number in different species varied between 17 and 21 but with an XO: XX type of sex determination. However 21 chromosomes seem to be the modal number because seven out of nine genera, cytologically investigated, possess this number. The subfamily Centrotinae is also well represented cytologically. Two types of spermatogenial numbers 19 and 21 have been recorded. 19 chromosomes have been found in 7 species belonging to 5 genera while eleven species of 5 genera have 21 chromosomes. The sex determining mechanism is XO: XX in all the species. It is rather difficult, at present, to assign a modal number to this subfamily since the two numbers are equally represented.

It appears from the above list of cytologically worked out species that 21 chromosomes would be the modal number in the family Membracidae because of its presence in large majority of the species. Some of the species have the lower diploid number of 17 and 19. The origin of lower number could have been due to fusion mechanism. Metrical data of the ten species (Table 1) scored in this investigation might throw light on this assumption. The relative percentage values of the autosome No. 1 are in the range of 10.43% to 14% in 21 chromosome bearing species while in 19 chromosome group between 19.40% and 26.16%. The values in the remaining autosomes in these two groups were less variable while A_{10} is missing in the 19 group. The increase or decrease in the value of autosome No. 1, as the case may be, is perhaps due to fusion or fission, resulting in the absence of autosome No. 10 in 19 chromosome group or its presence in the 21 chromosome group. The value of X chromosome varies in between 8.62% and 15.47% in different species and had no correlation in the two groups. Besides the metrical data, a comparison of the gonial complements of two groups would clearly indicate that species with 19 chromosomes in males have a pair of autosomes as large as four smaller ones of the 21 chromosome species. In the classification of this family Distant (1910) has included genus *Oxyrachis* in the subfamily Membracinae but Prof. Medler of Wisconsin University (Personal communication) in his compilation has brought it under Centrotinae. Other genera of the present study have been put by both of them under the subfamily Centrotinae. The inclusion of *Oxyrachis* in Centrotinae seems to be justified as it has similarity in the chromosome morphology, their values, and the meiotic pattern with other genera of the present study.

Summary

The karyotype analysis of ten species of Membracidae (Homoptera) showed the oogonial and spermatogonial chromosomes as bean shaped and gradually seriated elements. Sex chromosomes were two in the female and only one in the male but these did not exhibit any staining difference compared to the autosomes. The diploid number in female and male complements were 22 and 21 respectively in *Oxyrachis tarandus, Oxyrachis* sp., *Leptocentrus substitutus, Otinotus elongatus, Otinotus* sp. and 20 and 19 in *Tricentrus albomaculatus, Tricentrus* sp.,

Gargara contraria, G. flavolineatus, Cocosterphus decoloratus.

The pattern of meiosis in males of the species investigated was very conservative type. There was a faint "bouquet" at the early primary prophase. The first division was reductional while the second equational. The sex determining mechanism was XX: XO in all of them. The 19 chromosome species were characterised by a huge autosomal bivalent. The relative percentage volumes of the chromosomes was determined from primary spermatocyte metaphase plates. In the light of the data published by earlier workers and the results obtained in this investigation, the probable mode of evolution in the group has been discussed.

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References

Banerjee, M. R. 1958. A study of the chromosomes during meiosis in 28 species of Hemiptera (Heteroptera and Homoptera). Proc. Zool. Soc. **11**: 19–36.

Bhattacharya, A. K. and Manna, G. K. 1967. A study of germinal chromosomes during meiosis in 50 species of Homoptera (Hemiptera). Proc. 54th Ind. Sci. Congr. Pt. **3**: 618–619.

— and — 1969. Males with different sex chromosome constitution in a homopteran species, *Parabolocratus albomcaculatus*. Proc. 56th Ind. Sci. Congr. Pt. **3**: 445.

— and — 1970. Structure, behaviour and metrical studies of germinal chromosomes in four species of spittle bugs. Proc. Nat. Acad. Sci., India **40**(B): 129–135.

Boring, A. M. 1907. A study of the spermatogenesis of 22 species of Membracidae, Jassidae, Cercopidae and Fulgoridae with special reference to the behaviour of odd chromosomes. J. Exp. Zool. **4**: 469–514.

Distant, W. L. 1902 and 1910. Rhynchota in Fauna of British India, **4** and **7**.

Halkka, O. 1959. Chromosome studies on the Hemiptera, Homoptera, Auchenorrhyncha. Ann. Acad. Scien. Sr. A, IV Biologica, **43**: 1–72.

— 1962. The chromosomes of the Membracidae. Hereditas **48**: 215–219.

— and Heinonen, L. 1964. The chromosomes of seventeen Nearctic Homoptera. Hereditas **52**: 77–80.

Kornhauser, S. I. 1914. A comparative study of the chromosomes in the spermatogenesis of *Enchenopa binotata* (Say) and *Enchenopa* (*Campylenchia*- Stal) *curvata* (Fabr.). Arch. Zellf. **12**: 241–298.

Manna, G. K. 1951. A study of chromosomes during meiosis in forty-three species of Indian Heteroptera. Proc. Zool. Soc. Bengal **4**: 1–116.

Menon, P. S. 1958. Studies on the chromosome behaviour in three species of Indian Homoptera, Caryologia **11**: 57–67.

Rao, S. R. V. 1956. Studies on the spermatogenesis of some Indian Homoptera III. A study of chromosomes in two members of the family Membracidae. Caryologia **8**: 309–315.

Sharma, T., Pandha, S. K., Kakati, S., Moitra, B. J. and Patnaik, J. K. 1964. On the study of chromosomes of eight Indian species of Homoptera (Auchenorrhyncha). Proc. 50th Ind. Sci. Cong. Pt. **3**: 480.

White, M. J. D. 1954. Animal Cytology and Evolution. 2nd Edition, Cambridge University Press.

Whitten, M. J. 1965. Chromosome numbers in some Australian leafhoppers (Homoptera, Auchenorrhyncha). Proc. Linn. Soc. New South Wales **90**: 78–86.