Studies of Gryllid Chromosomes. II. Chromosomal polymorphisms in *Pteronemobius taprobanensis* (Walk.), and chromosome morphology of *Loxoblemmus* sp.*

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In comparison to the long- and short-horned grasshoppers, gryllids amongst orthopteroid insects are cytologically less known. The cytological survey in this group was carried out mostly by the Japanese workers until 1935 (see Makino 1956). Since then a few papers has been published from time to time. However, it would be apparent from the different published accounts that cytologically gryllids are quite an interesting group of insects because of the fact that chromosomal polymorphism with respect to variation of the chromosome shape and number seems to be fairly common.

Cytological investigations on Indian gryllids, in spite of the richness of fauna, are very limited (Dutt 1949, Ray-Chaudhuri and Manna 1950, Manna and Bhattacharjee 1962, 1963, Sharma 1963, Manna and Ray-Chaudhuri 1964). The peculiar chromosome behaviour reported first from India in *Euscyrtus* sp. (Ray-Chaudhuri and Manna 1950), has drawn attention of other workers (Smith 1953, Ohmachi and Ueshima 1957) but the claims made by different workers are not in complete agreement with one another for which the problem is under re-investigation (Manna and Ray-Chaudhuri 1964). In another species of gryllid, *Gryllulus confirmatus*, the phenomenon of chromosomal polymorphism has been recorded from Bengal by Manna and Bhattacharjee (1962), while Sharma (1963) examined cytologically three species including *G. confirmatus* from North Indian populations and found the orthodox chromosome behaviour in all of them.

With a view to the above facts, a cytological survey of gryllids has been initiated and in the present paper the behaviour of the chromosomes during meiosis in a chromosomally polymorphic species, *Pteronemobius taprobanensis* and in another monomorphic unidentified species, *Loxoblemmus* sp. has been reported.

Ohmachi (1935) made the chromosome study of *Nemobius mikado* (Shiraki) which according to him is synonymous of *P. taprobanensis* (Walk.).

* Dedicated to Professor Sajiro Makino of Hokkaido University on the happy occation of his 60th birthday, June 21, 1966, as mark of kindest regards for his outstanding contribution in Chromosome Cytology for long many years.

However, our observations on *P. taprobanensis* revealed some cytological differences in comparison to those of *N. mikado* studied by Ohmachi (1935).

Materials and methods

Two species, *Pteronemobius taprobanensis* (Walk.) and *Loxoblemmus* sp. belonging to the family Gryllidae, were collected from a bushy area in a banana field at Behala, Southern Suburb of Calcutta. The authors are very much thankful to Mr. K. S. Pradhan, Zoological Survey of India, Calcutta for the identifications of the species.

Under the morning dewy grass, particularly during the rainy season, *P. taprobanensis* were easily available. Testes of about 600 males of *P. taprobanensis* were dissected out and fixed separately in acetic-alcohol mixture. Fixed testes of each individual were squashed either following acetic-carmine method or Smith's technique (Smith 1943). Squashed slides were stained mostly in Heidenhain's haematoxylin and a few in Feulgen stain. Out of 600 individuals fixed, 417 males had suitable divisional stages and the records have been made only from them.

Testes of only two male individuals of *Loxoblemmus* sp. constituted the materials for the study.

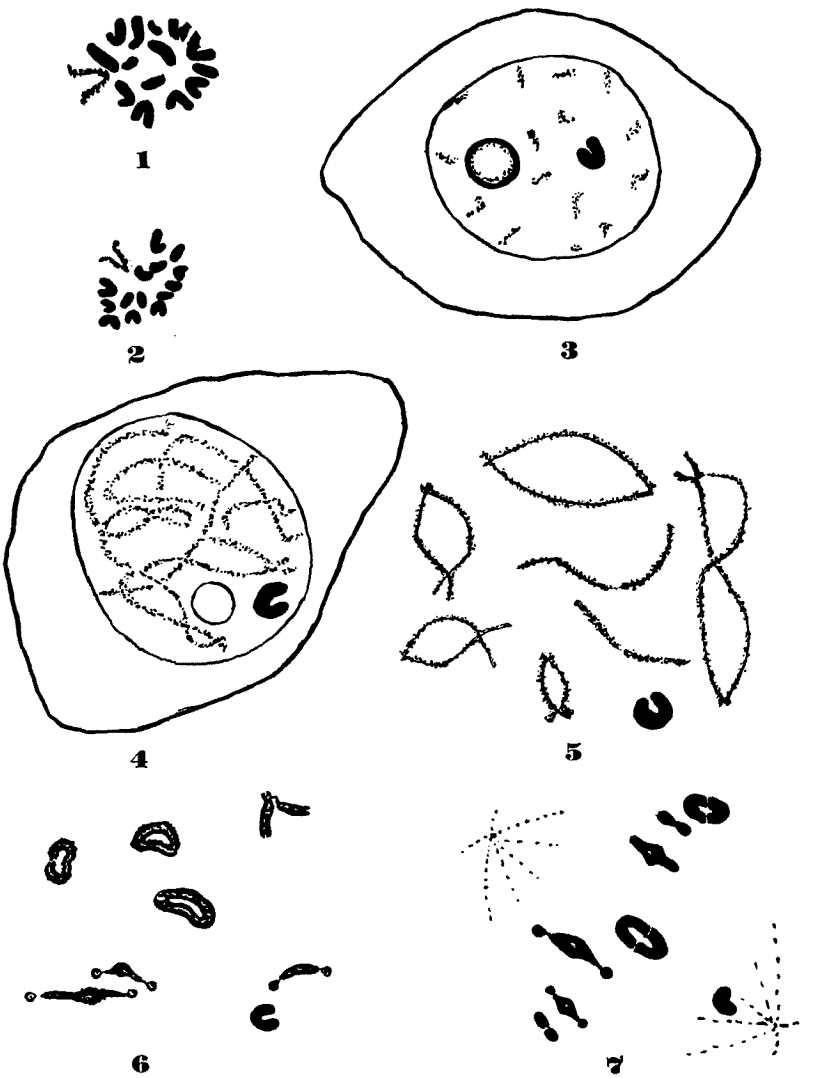
Observations

Pteronemobius taprobanensis (Walk.)

A. *Meiosis in homozygous individuals*: The spermatogonial complement shows 15 chromosomes (Figs. 1 and 2). The X-chromosome stands out distinct as the largest unpaired metacentric chromosome in the whole complement. It appears to be negatively heteropycnotic and thinner than the autosomes. The 14 autosomes can not be accurately put to different size classes—firstly, the seriation is fairly gradual, and secondly, the number of V-shaped elements seems to be variable. However, a rough classification of the spermatogonial complement shows that there are 8 metacentric, 2 sub-metacentric, 4 acrocentric autosomes, and the largest metacentric X-chromosome. The distinction between the metacentric and the sub-metacentric chromosomes is not rigid and so also for sub-metacentric and acrocentric chromosomes. The spermatogonial complement of *N. mikado* (Ohmachi 1935) consists of five pairs of Vs of various sizes, two pairs of rods, and the unpaired largest metacentric X-chromosome. Thus the chromosome number of *P. taprobanensis* and of *N. mikado* is the same but there is some difference in the detailed morphology of the chromosomes. Besides, *P. taprobanensis* shows marked chromosomal polymorphism (*vide infra*) which has not been observed in *N. mikado*.

In *P. taprobanensis*, the chromosomes at the early primary prophase stage, during the period of growth remain in a diffuse state. At this time, besides a positively heteropycnotic body which represents the sex-chromosome

and a concentrated spherical nucleolus, the cell is almost optically empty (Fig. 3). The X-chromosome maintains its V-shaped appearance even at this stage although the arms are more flexible. The autosomes become stainable



Figs. 1-7. Chromosomes of homozygous *P. taprobanensis*. Camera Lucida drawings. ×ca. 1350. 1 and 2, spermatogonial metaphase with 15 chromosomes. 3, zygotene stage showing deeply stained X-chromosome and the spherical nucleolus. 4, pachytene stage. 5, diplotene stage, bivalents showing lamp-brush effect. 6, diakinesis stage, some bivalents showing constrictions at the centromeric regions. 7, metaphase I, X-chromosomes very near to the pole.

at pachytene stage (Fig. 4) when they assume a reticular appearance with the homologous chromosomes in pairs. The study of chiasmata can be made from diplotene stage onwards (Figs. 5-7). On an average from 25 nuclei

studied of each stage, there is a loss of about one chiasma as the stage advances from diplotene to diakinesis since at diplotene the mean value is 10 chiasmata per nucleus which drops down to nine when the diakinesis stage is reached. There is no marked loss of chiasmata between the diakinesis and the metaphase stage since at metaphase I the mean number is nine. A good deal of contraction of the chromosomes is observed from diplotene to metaphase stage (Figs. 5-7). At metaphase, the X-chromosome forms an accessory plate and it may remain at any place in the spindle. In most cases it is situated near to one of the poles (Fig. 7).

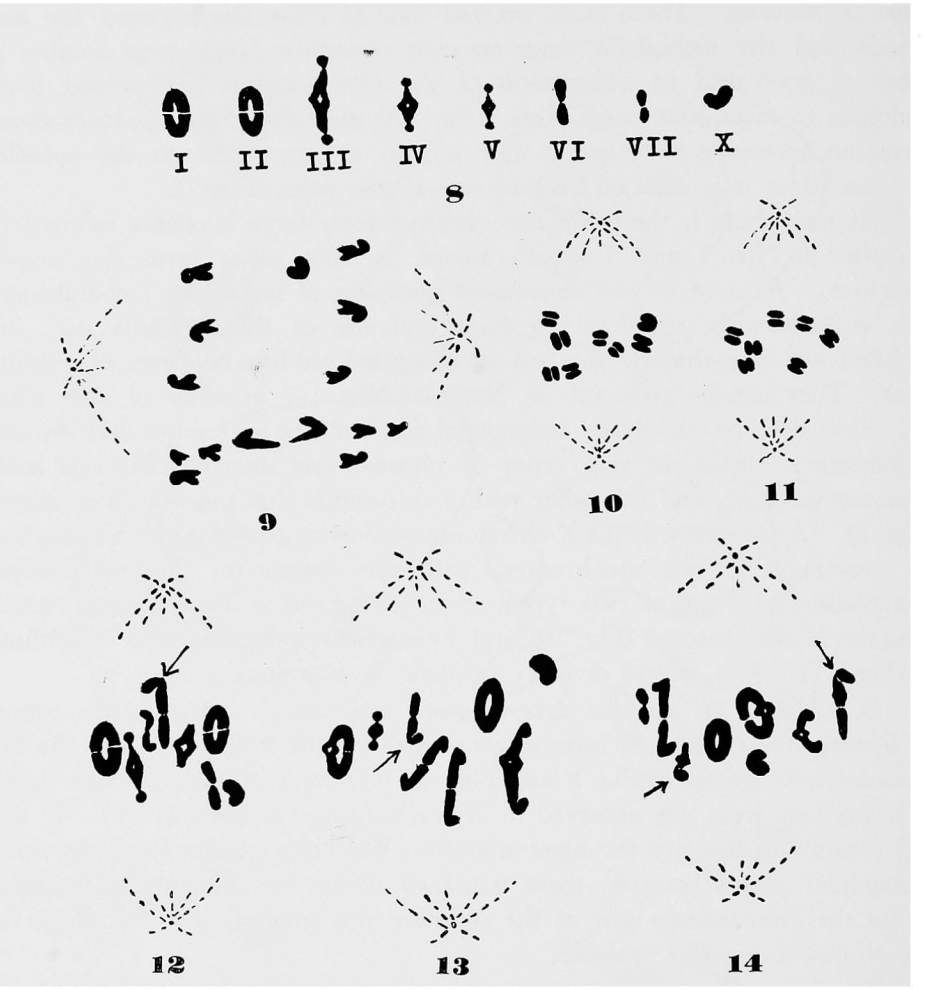
At metaphase I, there are two comparatively large bivalents referred to hereafter as Nos. 1 and 2 which almost in 90% cases form ring-shaped structure. A more or less convenient grouping of metaphase I complement can be made with regard to the shape and size of the bivalents *e.g.*, 1st and 2nd are ring-shaped, the rest are elongated rod-like bivalents of varying sizes. They can be arranged as bivalents Nos. 1-7 in order of sizes (Fig. 8). First division anaphase, reductional for both the autosomes and the sex chromosome, leads to two types of chromosome distributions, one with 7 autosomes only, and the other with 7 autosomes plus the sex chromosome (Fig. 9). At anaphase I, the X-chromosome shows variable position indicating its movement is not synchronous with the autosomes. Second division metaphase plates are of two types, eight chromosome bearing plate containing the X-chromosome (Fig. 10) and 7 chromosome bearing one is without it (Fig. 11). The second division anaphase is equational.

B. *Meiosis in natural heterozygous individuals*: During the course of investigations, natural heterozygotes with regard to the 4th and the 6th chromosomes (compare Fig. 8 and Figs. 12-14) were observed. Such types of individuals were not observed in *N. mikado* by Ohmachi (1935). It was not possible to identify the heteromorphic chromosome pairs from the spermatogonial plates because, there remained always the difficulty of homologizing the chromosome pair as the seriation was gradual and the shape of the chromosomes was variable.

The heteromorphic chromosomes in the natural heterozygotes could not also be followed upto diakinesis stage as their identification was rather difficult because of the flexible condition of the arms of the bivalent as well as inconspicuous centromeric and chiasma regions. However, in certain favourable cases their identity could be followed at diakinesis stage. Heterozygotes possessing the heteromorphic bivalent of either 4th (Fig. 12) or 6th (Fig. 13) or both (Fig. 14) could be best demonstrated from the side view of metaphase I by their hook-shaped appearance. The hook-shaped heteromorphic pair at metaphase I was formed of a J-shaped and a rod-shaped chromosome.

As a result of heteromorphism of this kind either with the 4th or the 6th chromosomes for each case the chromosome complements of different

individuals of this species ought to be of three kinds *viz.*, (a) Homozygous for two Js (b) Homozygous for two rods, and (c) Heterozygous for one rod and one J (Fig. 14) and these three types in short can be symbolized as AA,



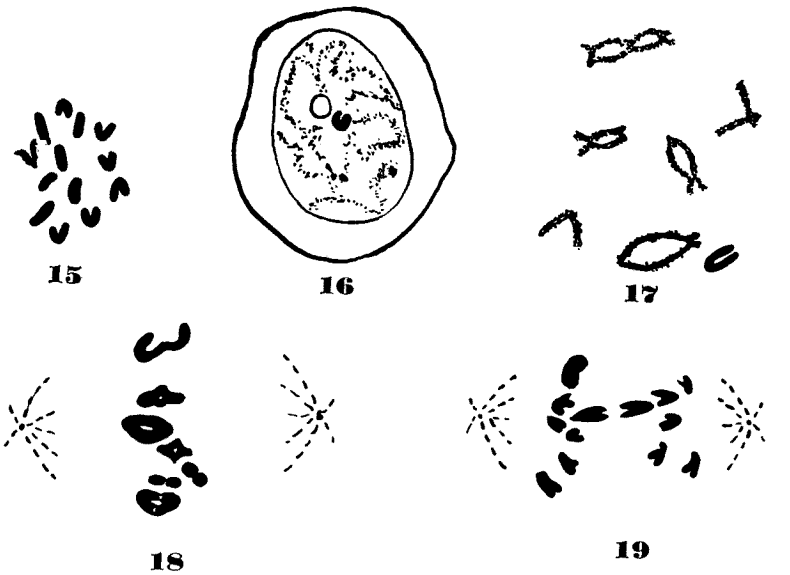
Figs. 8-14. Chromosomes of *P. taprobanensis*. Camera Lucida drawings. ×ca. 1350. 8, metaphase I, bivalents arranged in serial order and numbered I—VII and the sex-chromosome (X). 9, anaphase I showing the reduction division of the X-chromosome. 10 and 11, metaphase II with 8 and 7 chromosomes respectively. 12-14, metaphase I side view of heterozygous individuals. 12, bivalent 4 heteromorphic. 13, bivalent 6 heteromorphic. 14, bivalents 4 and 6 heteromorphic.

Table 1. Frequency distribution of individuals with heteromorphic 4th and 6th chromosomes in *P. taprobanensis*

Total no. of indiv.	4th heteromorphic	6th heteromorphic	Both 4th and 6th heteromorphic
417	162	69	78

aa and Aa respectively. Thus, since there was heterozygosity for two different chromosome pairs, with random mating, there could be theoretically as many as nine types of combinations possible although all of them would have the same number of chromosomes ($2n=15$). However, out of the nine types expected, the frequency of only three types having the heteromorphic bivalent could be detected with surety (Table 1). About the rest *e.g.* the homozygous-heterozygous combinations and homozygous for both the types, because of the difficulty of determination, the frequency could not be determined.

Since the frequency of the two types of homozygotes for a particular chromosome was undeterminable, the frequency of J or rod of the 4th and



Figs. 15-19. Chromosomes of *Loxomblemmus* sp. Camera Lucida drawings. ×ca. 1350. 15, spermatogonial metaphase with 13 chromosomes. 16, zygotene-pachytene stage. 17, diakinesis. 18, metaphase I, X conspicuously large. 19, anaphase I showing the reduction division of the X-chromosome.

the 6th chromosome could not be determined. However, as the frequency of 4th chromosome heterozygotes is about 57% it may be speculated that the frequency of homozygous J and rod is nearly equal but one must be a little more than the other. The frequency of 6th chromosome heterozygotes, on the other hand, is about 35%. It is thus difficult to speculate the frequency of homozygous J or rod of 6th chromosome and at least it is not equal and one must be quite higher than the other. The separation of the anaphase chromosomes of heterozygotes was normal and no disturbance in the meiotic process was observed either in homozygous or in heterozygous individuals.

Loxoblemmus sp.

Meiosis in monomorphic 13 chromosome species: The spermatogonial

cells of *Loxoblemmus* sp. show, at the metaphase plate, 13 chromosomes (Fig. 15). Unlike many gryllids, the X-chromosome is not conspicuously large and easily distinguishable metacentric chromosome. Out of the 13 chromosomes, six rod-like and the rest are comparatively large V-shaped chromosomes. No marked size difference exists among the different members of the acrocentric class, as well as the metacentric class. Further, the shape of the autosomes as well as of the sex-chromosome is to some extent variable.

The chromosomes in early primary spermatocyte prophase stage remain in diffuse condition. The chromosomal reticulum becomes visible at the pachytene stage (Fig. 16). The sex-chromosome is positively heteropycnotic at this stage but relatively small in size. Its shape changes from cell to cell. Sometimes it is rod like, while at other it is rounded or V-shaped or beaded. Bivalents at diplotene and diakinesis may have more than one chiasma (Fig. 17).

At the first spermatocyte metaphase stage, there are six autosomal bivalents and the unpaired X-chromosome (Fig. 18). At anaphase I, the chromosomes of six autosomal bivalents divide and move to opposite poles while the sex chromosome passes undivided to one of the poles (Fig. 19). Thus half of the daughter cells receive six autosomes plus the sex chromosome.

Discussion

The published records of chromosomal polymorphism in gryllids are not many, although the occurrence of such a phenomenon may not be very uncommon. In gryllids, the chromosomal polymorphisms recorded are either due to the variable number of chromosomes in different individuals of a species (Honda 1926, Suzuki 1932, 1933, Ohmachi 1935, Ohmachi and Ueshima 1957) or due to the variation of the chromosome structure (Ohmachi 1935, Manna and Bhattacharjee 1962, 1963). The chromosomal polymorphism of *P. taprobanensis* is of the latter type. Ohmachi (1935) carefully analysing the caryotypes from the spermatogonial chromosomes of some species of gryllids such as *Nemobius nigrofasciatus*, *Anaxipha pallidula* etc., concluded that each of these species has some heteromorphic homologous pair of chromosomes. However, the earlier records would show that the behaviour of this heteromorphic pair during meiosis and their frequency in the natural population were not studied. Manna and Bhattacherjee (1962) made some study on the heteromorphic chromosomes during meiosis in *Gryllulus confirmatus*, but the frequency data was inadequate. *P. taprobanensis* is peculiar in having two heteromorphic chromosome pairs which is believed to be the first case on record and they have been determined from a large number of individuals examined cytologically.

In order to determine the nature of the two heteromorphic bivalents of *P. taprobanensis*, one is confronted with many difficulties. It seems very

likely that the heteromorphic pairs are unequal bivalents rather than asymmetrical bivalents, the reason being the marked difference in the sizes of the two components of each heteromorphic pair of chromosomes. If it was a case of asymmetrical bivalent, there should have been no difference in size between the two components except for the position of the centromeres. The heteromorphism in that case would have been originated due to some intrachromosomal change in the form of peri- or paracentric inversion depending on the morphological structure of the original chromosomes. Since the possibility of asymmetrical bivalent is nullified, the origin of the unequal nature of the bivalents is likely to be due to some interchromosomal rearrangements. There could be a number of possibilities for its origin and the probable one could be due to some duplication or deficiency of chromosomal parts of the heteromorphic pair. One fact stands in the way of determining the exact nature of the change and that is, we are not sure about the condition in the original chromosome complement. If *N. mikado* (Ohmachi 1935) is considered to be synonymous of *P. taprobanensis*, the spermatogonial complement of the Japanese population may be considered as the original chromosome constitution of the species but the difference in the caryotypes of the two when homozygous is not very appreciable. It appears from the caryotype analysis of the homozygous individuals of *P. taprobanensis* that chromosomes 4 are two J-shaped and the 6 are two rod-shaped ones. With such chromosome structures, a deletion in one of the 4th chromosome pair and a duplication in one of the 6th chromosome pair in each case will lead to the origin of the heteromorphic 4th and 6th chromosome pairs. However, such a simplified origin of the heteromorphism may not have occurred and there will always be some lacunae in whatever way the origin is speculated and the reasons are obvious (White 1954).

The behaviour of the unequal bivalent prior to metaphase I could not be followed in all cases. At diakinesis stage of the heterozygous individual, the heteromorphic bivalents were found to be with a single chiasma mostly near the terminal region. The pairing takes place between the long arms leaving the unpaired part beyond the centromere free. Thus, as a result of this kind of behaviour, first spermatocyte division is always reductional for these heteromorphic pair as shown by White (White 1954, page 144).

The occurrence of unequal bivalents has been recorded in widely different groups of animals of which the examples are more in invertebrate than in vertebrate animals. Amongst insects the most well-known group in this respect is short-horned grasshoppers (Carothers 1913, 1931, Wenrich 1916, Ch'en 1942, Hearne and Huskins 1935, Darlington 1936, White 1949, 1951 and White and Nickerson 1951). It has also been reported in Phasmids by Hughes-Schrader (1947), in mole-cricket by Payne (1912, 1916), in grouse locust by Robertson (1915), in mantids by White (1941), in beetles by Manna and Smith (1959) and in crickets by Ohmachi (1935) and by Manna and

Bhattacharjee (1962). To explain the significance White (1954, page 143) speculated that the unequal bivalents are merely a special aspect of the general phenomenon of variation in the extent of the heterochromatic material within the population. According to him presumably the physiological effect of an extra heterochromatic segment in one of the autosomes is similar to that of a supernumerary chromosome but unlike them follow a strictly regular behaviour. The assumption made by White that unequal bivalents have been resulted in most cases due to change in the heterochromatic material is untenable at least in gryllids since in *P. taprobanensis* and in *Gryllulus confirmatus* no evidence of the heterochromatic nature of the unpaired portion of the unequal bivalent was observed.

Out of 417 males examined 309 individuals are heterozygous either for one or both the chromosomes 4 and 6. This fact of about 74% heterozygosity in a population is an indication that the species in the said population is genetically not fixed and therefore, this is perhaps enjoying a selective advantage. A statistical analysis of the frequency of individuals heterozygous for both the chromosomes 4 and 6 indicates that there is no significant difference between the observed and expected number (not significant at 5% level of significance, $\chi^2=3.841$ with 1d. f., observed $\chi^2=1.87$). It seems, therefore, that heterozygosity for both chromosomes when present in an individual does not have any lethal effect.

Cytologically the genus *Loxoblemmus* has become interesting because of the fact that chromosomal polymorphism exists in *L. arietulus* which has not been suspected by earlier workers. The different populations studied by different Japanese workers from time to time showed not only a variation of diploid chromosomes number between 13 to 17 (Honda 1926, Suzuki 1932, 1933, Ohmachi and Ueshima 1955) but also in the number of chromosome arms. The occurrence of a heteromorphic pair of chromosomes like that of 4 th chromosome pair of *P. taprobanensis* has also been reported by Ohmachi and Ueshima (1955) in one individuals of *Loxoblemmus* "mori-okame" collected from Tsu in Japan. Further, Ohmachi and Ueshima (1955) claim that there are three nearly related species which have been cytologically worked out under the common name, *L. arietulus* by many workers before them. Thus the situation becomes quite complicated because firstly three incipient species named as "tambo-okame" "mori-okame" and "hara-okame" by Ohmachi and Ueshima (1955) are included in the species *L. arietulus* which have 13, 15, and 15 and 17 spermatogonial chromosome numbers respectively, secondly there are good deal of variations in chromosome structure in different populations. However, the undetermined species under present study has some peculiarity. Although the diploid number in *Loxoblemmus* sp. is 13 but the structure of the sex-chromosome being small is strikingly different from that of *L. arietulus*. The number of autosome arms is 18 as there are six rods and six V-shaped chromosomes and if this

is compared with *L. arietulus*, there is a general agreement in the total number of autosomal arms of 13 chromosome species.

Summary

1. Cytological examinations of 417 males of *Pteronemobius taprobanensis* and two males of *Loxoblemmus* sp. have been made.

2. The spermatogonial chromosome number in all the individuals of *P. taprobanensis* is 15 although this species is chromosomally polymorphic with respect to chromosomes No. 4 and 6 in order of size (*vide infra*).

3. The behaviour of the chromosomes in homozygous individuals of *P. taprobanensis* is regular and of orthodox pattern. There is some loss of chiasmata from diplotene to diakinesis stage. First meiotic metaphase chromosomes consist of 7 bivalents which could be numbered as 1–7 in order of shape and size, and the univalent X-chromosome. First two bivalents almost always are ring-shaped while bivalents No. 4 and 6 appear to be heteromorphic in heterozygous individuals.

4. In heterozygous individuals of *P. taprobanensis*, at metaphase I, bivalents No. 4 and 6 appear as hook-shaped structure formed of a J-shaped and a rod-shaped chromosomes. The behaviour of the heteromorphic bivalents before metaphase I could be followed occasionally. Out of 417 males examined, 162 individuals are heterozygous for chromosome 4, 69 for chromosome 6 and 78 both for the chromosomes 4 and 6.

5. The origin of the chromosomal polymorphism of *P. taprobanensis* has been discussed. Further, since 309 out of 417 males examined are heterozygous (about 74%), the heterozygotes are likely to enjoy a selective advantage and the species is under genetical fixation.

6. The diploid chromosome number of *Loxoblemmus* sp. is 13. A comparison of the caryotypes of this species with that of *L. arietulus* has been made. The chromosomes of *Loxoblemmus* sp. is different from other species firstly for the relatively small size of the X-chromosome and secondly for the number of V- and rod-shaped autosomes.

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