Cytological Studies in Heteroptera II

Chromosome complement and meiosis in the males of three pyrrhocorid bugs

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Introduction

In recent years the family Pyrrhocoridae has become very popular with the researchers on account of the interesting cytological results it has yielded. Ray-Chaudhuri and Manna (1952) described for the first time a new type of segregation namely the 'double reduction' of the sex chromosomes, X and Y, in *Dysdercus koenigii*. This results in the absence of the sex elements in fifty per cent. of the sperms, while of the remaining fifty per cent. half carry the X and half the Y. These findings of Ray-Chaudhuri and Manna (1952) have, however, been doubted recently by Battaglia (1956) and Sharma (1956).

Ray-Chaudhuri and Manna (1955), also for the first time, described the Y as a homologue of the 2Xs in *Physopelta schlanbuschi*. According to them, the X_1 and Y, in this species, are the largest and equal in size, while the X_2 is one of the smaller elements in the complement. They are of the opinion that the X_1 and Y, in this species, represent a pair of autosomes which have recently taken up the sex determining function.

Even in the other four species, *Euryphthalmus rufinennis* (Piza 1946), *Largus cinctus*, *L. succintus* and *Pyrrhocoris apterus* (Wilson, 1909), so far investigated cytologically, the autosomes show a good deal of numerical variation, although the sex mechanism in them is uniformly XO.

The present paper furnishes new cytological data on three more pyrrhocorid species, *Antilochus conqueberti*, *Scantius volucris* and *Pyrrhopheplus posthumus*. In addition to the numerical variations in the chromosomes of the three species, the sex chromosomes, XX, in *Pyrrhopheplus posthumus* are interesting inasmuch as the sex univalents come together to finally fuse during anaphase I and this fused mass behaves as such in the second division. *P. posthumus*, therefore, presents the final stage in the fusion of the sex univalents which, perhaps, starts in *Dysdercus cingulatus* (Sharma 1956), and *Dysdercus mendesi* (Piza 1947), where the sex univalents simply come together, the intermediate position being occupied by *Dysdercus koenigii* (Battaglia 1956 and Sharma 1956), where the two fuse to form a single split element which behaves as a single

body in meiosis II.

Material and technique

The three pyrrhocorid species, *Antilochus conqueberti*, *Scantius volucris* and *Pyrrhopeplus posthumus* belong to the sub-family Pyrrhocorinae. The adult males of *Antilochus conqueberti* and *Scantius volucris* were collected locally from *Duranta*, the hedge plant in the college and *Calotropis* respectively. The specimens of *Pyrrhopeplus posthumus* were, however, collected from Land's-End, Nainital (Kumaon hills, 7,000 ft. above Sea level) during an Entomological excursion in August, 1956.

The testes from the adult males of each species were removed in the physiological solution (0.2 c.c. of 10% CaCl_2 in 100 c.c. of 0.7% NaCl solution) and directly transferred to the fixatives. Three types of fixatives viz., Sanfelice, Navashin and Carnoy were used but the observations were mainly made from Sanfelice preparations because of the superior results they yielded. Sections cut varied from $7\frac{1}{2}$ to 15μ in thickness and these were, subsequently, stained with 0.5% Iron haematoxylin.

Observations

Antilochus conqueberti Fabr.

The diploid number of chromosomes in *Antilochus conqueberti*, as revealed by the spermatogonial metaphase in its polar view (Fig. 1a), is twenty-seven and is the highest so far recorded for any species of this family. The gonial chromosomes are typically heteropteran and rounded in form. The single X chromosome which is well marked as the largest may lie at the periphery of the spindle or occupy its centre. In most of the cells, however, it occupies the centre and it is only in 10% of the plates studied that it has been found to lie at the periphery.

The general course of meiosis is also typically heteropteran with the synaptotene marked by the contraction of the autosomes, the presence of the diffused stage with the complete resting condition of the nucleus and the absence of the resting interkinetic stage. The sex chromosome during the contraction stage (Fig. 1b) is represented as a positively heteropycnotic body which presents a vesicular or duplex structure during the diffused stage (Fig. 1c). The diplotene bivalents (Fig. 1d), as they reappear out of the diffused stage, have usually a single non-terminal chiasma each. The chiasma frequency and terminalization coefficient, at this stage, are 13.300 and 0.645 respectively. During diakinesis (Fig. 1e) the bivalents undergo further condensation along with the terminalization of their chiasmata. The mean chiasma frequency and the terminalization coefficient, at diakinesis, are 13.133

and 0.924 respectively. The X chromosome is split longitudinally during this stage.

The first division metaphase reveals fourteen elements in its polar view (Fig. 1f). The metaphase elements have undergone the highest degree of contraction and appear as rounded bodies, the sex chromosome still being the largest. The mean chiasma frequency and terminalization coefficient, at this stage, are 13.067 and 1.000 respectively. The anaphase I (Fig. 1g) typically reveals fourteen elements going to each pole of the spindle. The sex univalents, however, lag behind the autosomes (Fig. 1h). Due to the large size of the X chromosome, its anaphase movement parallel to the spindle fibres is well marked.

The second division metaphase again reveals fourteen elements (Fig. 1i). During anaphase II the autosomes divide equationally (Fig. 1j), while the X chromosome,

lagging behind them, is segregated to only one of the two daughter cells. Some cells reveal the X chromosome near the equator of the spindle even in the late telophase and in such cases it appears to be passively included in one of the daughter cells with only a little anaphase movement. It, however, lies at right angles to the equator of the spindle, parallel to spindle fibres (Fig. 1k) which, by telophase, become elongated with the elongation of the cell itself.

Fig. 1. *Antilochus conquiberti*. a, spermatogonial metaphase (polar view). b, contraction stage. c, diffused stage. d, diplotene. e, diakinesis. f, metaphase I (polar view). g, anaphase I (subpolar view). h, telophase I. i, metaphase II. j, anaphase II (subpolar view). k, telophase II.

Scantius volucris Gerstaeck

The spermatogonial metaphase, in the present species, shows nineteen elements in its polar view (Fig. 2a). The gonial chromosomes are very small and rounded in form. Regarding their size differences, six of the diploid set are larger than the remaining ones. The sex chromosome is indistinguishable from the autosomes at this stage.

Fig. 2. *Scantius volucris*. a, spermatogonial metaphase (polar view). b and c, contraction stages in the growth of the auxocyte. d, metaphase I (polar view). e, metaphase I, with tetrapolar spindle. f, telophase I or prometaphase II. g, metaphase II. h, anaphase II (subpolar view).

that the diplotene bivalents appear. The single X forms a condensed heteropycnotic mass which is entangled in the autosomes during the contraction stage (Fig. 2b). During the later stages of growth, however, it becomes associated with a nucleolus (Fig. 2c). The diplotene and the diakinesis each reveals nine autosomes and a single X element which becomes split in the later stages. During diakinesis each bivalent has a single terminal chiasma. The large bivalents may, however, have two.

The first division metaphase reveals ten elements in its polar view (Fig. 2d). During this division the centrosome at each pole divides precociously at the metaphase or the early anaphase. The resulting daughter centrosomes start repelling each other (Fig. 2e) and by telophase I, as the chromosome elements, nine split and one sex univalent reach the poles and the cell starts dividing they have moved wide apart with a clear spindle area in between (Fig. 2f). The interkinetic period, in the present species, is, therefore,

The general course of meiosis in this species differs from that in *Antilochus conqueberti* and other Heteroptera in that the nucleus in the contraction stage directly enters the growth period during which the autosomes are still represented by a confused reticulum. This, consequently, results in the absence of the regular zygotene and the pachytene. It is, again, out of this confused reticulum

altogether absent. Consequently, the anaphase elements as they reach the poles are directly transferred to the prometaphase II.

The chromosomes during the second division metaphase (Fig. 2g) are comparatively smaller as compared with those in metaphase I. All the ten elements do not show any definite group pattern. During anaphase II (Fig. 2h) the autosomes divide equationally while the sex chromosome is segregated to only one of the daughter cells. The comparison of the size of the chromosomes in the two anaphase groups reveals that the X chromosome, in this species, is one of the smaller elements.

Pyrrhopheplus posthumus Horv.

The present species presents a very interesting behaviour of the sex chromosomes inasmuch as the two Xs which remain quite independent up till metaphase I come together during anaphase and finally fuse to form a single element which behaves as such in the second division.

The diploid complement (Fig. 3a) is represented by twenty-four round chromosomes which do not show any marked size difference among themselves. The sex chromosomes are indistinguishable from the autosomes.

The general course of meiosis in *Pyrrhopheplus*

Fig. 3. *Pyrrhopheplus posthumus*. a, spermatogonial metaphase (polar view). b, contraction stage. c, pachytene. d and e, diffused stages. f, diplotene complement. g, metaphase I (polar view). h, anaphase I (polar view). i, metaphase II. j, anaphase II (polar view).

posthumus resembles that in *Scantius volucris* in that the growth of the auxocyte starts abruptly right at the contraction stage. Consequently the nucleus attains, by the pachytene stage, a great size and the polarized bivalents occupy one side of it (Fig. 3c). The pachytene bivalents have a tendency to come together with the result that the individual elements can hardly be made out. The sex chromosomes during both the contraction (Fig. 3b) and the pachytene (Fig. 3c) are represented by two heteropycnotic rounded bodies which are practically of equal size. During the diffused stage, however, the X elements may be fused (Fig. 3d) or lie separately (Fig. 3e), while the autosomal bivalents lose their staining capacity and are represented by diffused elements.

The diplotene bivalents (Fig. 3f), as they reappear out of the diffused stage, present the lampbrush appearance. The lampbrush fibres projecting out of the chromonemata resemble those of some orthopteroid forms (Srivastava 1954 and 1956) regarding their appearance as well as their fate since they appear to be directly withdrawn into the chromonemata during diakinesis. The heteropycnotic sex mass, at least in some cells, presents a wavy appearance and resembles the diakinetic autosomal figures of Srivastava (1956), but its lampbrush fibres have not been observed at any stage. Sharma and Parshad (1957) correlate the appearance of the lampbrush fibres in Orthoptera with the growth of the auxocyte in which the progressive activity of the bivalents continues without, of course, their complete denucleination.

Of the thirteen elements in the first division metaphase (Fig. 3g) the eleven autosomal bivalents are arranged in a ring enclosing the two Xs in its centre. The congression and orientation of the metaphase elements are typical so that the bivalents undergo co-orientation while the sex chromosomes auto-orientation. This, consequently, results in the regular segregation of the autosomes and the equational division of sex chromosomes during anaphase I (Fig. 3h). The two sex univalents, however, come together and finally fuse to form a single element which behaves as such during the second division.

Metaphase II (Fig. 3i) reveals twelve elements; eleven autosomal diads and a single mass representing the fused sex univalents. The latter is segregated during anaphase II (Fig. 3j) and is finally included in one of the daughter cells. Such a division, therefore, results in fifty per cent. sperms with the fused sex elements and the remaining fifty per cent. without them.

Discussion

The sex chromosomes

The close association or even the fusion of the sex chromosomes to form a single body, during the male meiosis of *Pyrrhopheplus posthumus*, is not a new phenomenon as it has been reported, at least partially, in a number

of animals like *Phylloxera* (Morgan 1909), *Syromastes* (Gross 1904), Man (Guyer 1910), *Ascaris* (Edwards 1910) and even in Heteroptera (Manna 1951, Wilson 1911, Piza 1947 and Sharma 1956).

The association of the two sex chromosomes from anaphase I onwards has also been described earlier in the family Pyrrhocoridae. In *Dysdercus mendesi* (Piza 1947) and *Dysdercus cingulatus* (Sharma 1956) the two sex univalents, though come together, yet remain distinct in the second division. In *Dysdercus koenigii* the two elements though fuse at an early anaphase, the split remains quite distinct during the second division (Battaglia 1956 and Sharma 1956). *Pyrrhopheplus posthumus*, thus, presents the final stage of this association where we have a single sex mass in which the two sex univalents are completely indistinguishable. This is segregated to one of the daughter cells in the second division.

Battaglia (1956) regards the association of the sex univalents from anaphase I onwards, in *Dysdercus koenigii*, as a non-homologous association between the heterochromatin which is similar to the one manifested by the sex elements themselves during diplotene. The interpretation of Battaglia (1956) appears to be quite reasonable since the heterochromatin has the property of mutual attraction (Schultz 1936 and Ray-Chaudhuri and Manna 1950). Prokofieva (1935), however, regards that the heterochromatic regions of the chromosomes are genetically inert. They must, therefore, be homologous and their association is due to the attraction of the homologous inert genes. The absence of any association between the heteropycnotic sex chromosomes in the early prophase of the first division reveals a non-homologous nature of these elements and this appears to be quite true of Heteroptera where the sex elements which are always heterochromatic consist entirely of the differential segments. Darlington (1936) suggested that the fusion of the non-homologous chromosome ends is conditioned by the presence of nucleolar material. The association of the sex chromosomes in the prophase, particularly in the diffused stage, of most of the heteropterans may be correlated with the appearance of the nucleolar material. The association or the fusion of the sex univalents from anaphase I onwards, in Pyrrhocoridae, is however, unaccompanied by the appearance of any nucleolar material.

It, therefore, appears most probable that the nucleoprotein in the heterochromatic sex chromosomes become polymerized and consequently sticky after metaphase I and this, finally, results in their association or fusion as in the present species, *Pyrrhopheplus posthumus*. Such an interpretation would suggest a progressive increase in the amount of heterochromatin or the degree of polymerization in *Dysdercus cingulatus*, *Dysdercus mendesi* and *Dysdercus koenigii* where the sex univalents reveal varying degree of association leading finally to their complete fusion in *Pyrrhopheplus posthumus*.

The sex chromosome in *Antilochus conqueberti* needs a special mention. On account of its largest size it is well marked throughout the male meiosis.

Moreover its anaphase movement, parallel to the spindle fibres and at right angles to the equator of the spindle, reflects its acrocentric nature. In fact it appears to be simply pulled to the pole or otherwise passively included in one of the daughter cells. The movement in the former case is due to a single fibre attachment at its polar end where the kinetochore is localized.

Summary

1. The present investigation furnishes cytological information on three pyrrhocorid bugs, *Antilochus conqueberti*, *Scantius volucris* and *Pyrrhopeplus posthumus* belonging to the sub-family Pyrrhocorinae.

2. The diploid number of chromosomes in the three species, as determined in the spermatogonial metaphase, is as follows: Twenty-seven in *Antilochus conqueberti*, nineteen in *Scantius volucris* and twenty-four in *Pyrrhopeplus posthumus*.

3. *Antilochus conqueberti* and *Scantius volucris* have each an XO type of sex mechanism while *Pyrrhopeplus posthumus* has two Xs which fuse, during anaphase I, to form a single body. This behaves as such during the second division.

4. The general course of meiosis is fairly constant in all the three species. In *Scantius volucris* and *Pyrrhopeplus posthumus* the growth of the auxocyte starts right in the contraction stage. This, consequently, results in the absence of the regular zygotene and pachytene in *Scantius volucris*.

5. The first division is reductional for the autosomes and equational for the sex chromosomes while the reverse is true of the second.

6. The nature of the association of the sex univalents from anaphase I onwards, in *Pyrrhopeplus posthumus*, has been discussed.

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