A NEW TYPE OF SEGREGATION OF THE SEX CHROMOSOMES IN THE MEIOTIC DIVISIONS OF THE COTTON STAINER *DYSDERCUS KOENIGII* (FABR.)

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(With Thirteen Text-figures)

I. Introduction

The centromeres of the sex chromosomes divide, as a rule, synchronously with those of the autosomes during meiosis, and consequently the *X*- and *Y*-chromosomes segregate, in the heterogametic sex, to the opposite poles during the first and divide equationally in the second meiotic division. In most of the Heteroptera and in all Odonata (see White, 1948; Ray-Chaudhuri & Das Gupta, 1949), on the other hand, the sex chromosomes separate equationally in the first and reductionally in the second meiotic division. A quite unorthodox behaviour of the sex chromosomes has been discovered in the males of a pyrrhocorid bug, *Dysdercus koenigii*, where, for the *X*- and *Y*-chromosomes, both meiotic divisions are reductional. As far as we are aware, no parallel case has so far been reported in any animal or plant. A detailed account of this phenomenon is the subject of this paper.

II. Material and methods

The bug investigated belongs to the family Pyrrhocoridae and was collected from the suburbs of Calcutta, India, where it infests the cotton plant, *Gossypium* sp. Very favourable fixation of the chromosomes of the testes was obtained by using San Felice fixative. Heidenhain's haematoxylin was found to be the best stain for the material.

III. Observations

Spermatogonial metaphase chromosomes are illustrated in Fig. 1, which shows sixteen chromosomes. A pair of small chromosomes is always distinguishable in the complement, and a study of the different meiotic stages reveals that they are the sex-chromosome pair. Both of them appear structurally similar, and it is therefore not possible to distinguish the *X*- from the *Y*-chromosome.

The nucleus of the primary spermatocyte, during the diffuse stage, shows a fairly large heteropycnotic body and one or two spherical nucleoli of similar size (Fig. 2). There are, however, a number of small, granular, deeply stained bodies within the nucleus, two of which appear to dip within the substance of the nucleoli. The granules perhaps correspond to the chromomeres, or they may be the heterochromatic segments of the autosomes, and the large pycnotic mass perhaps represents the fused *X*- and *Y*-chromosomes.

During the early diplotene stage (Fig. 3) the nucleus shows eight distinct paired structures. All of them except the smallest are undoubtedly bivalents having either one or very rarely two chiasmata. These are mostly interstitial, and appear to be localized near one end of the bivalent. One cannot be sure about the presence of chiasmata in the

smallest pair, since an interstitial chiasma has never been seen in this pair. Moreover, soon afterwards, the individual components of the smallest pair drift apart. Thus their association is not due to chiasma formation, because even complete terminalization of the chiasmata in a bivalent does not lead to the separation of the chromosomes until after the lapse of terminal affinity which holds them together until anaphase. Very likely the pairing is of the nature of non-homologous association between heterochromatin which manifested itself earlier during the resting stage by fusion of the *X* and *Y* into a hetero-

Fig. 1.

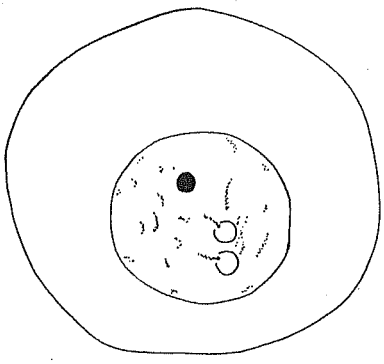
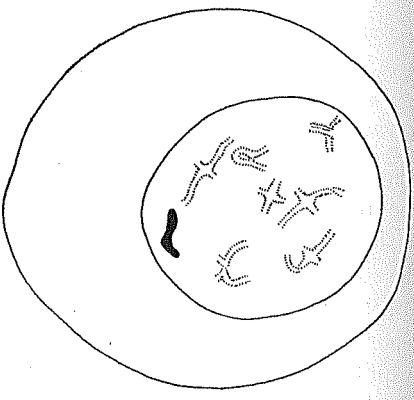


Fig. 2.



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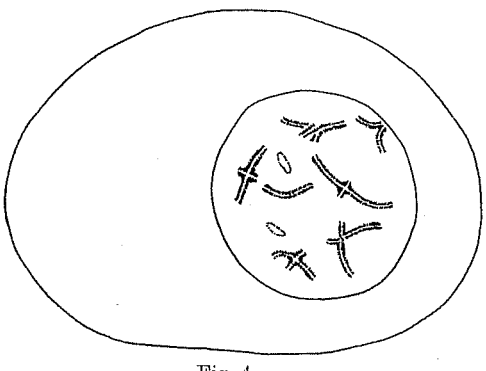

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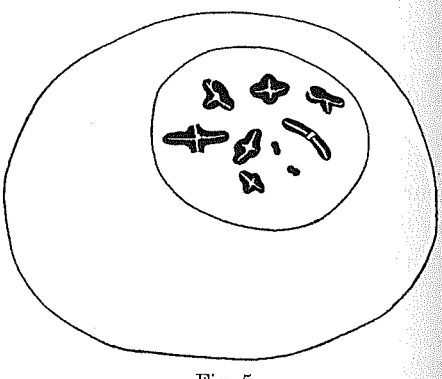

Fig. 5.

Fig. 1. Spermatogonial metaphase chromosomes, $2n = 16$. The smallest pair are the sex chromosomes. (× 3500.)
Fig. 2. Primary spermatocyte; diffuse stage with two nucleoli and a heteropycnotic body. (× 2100.)
Fig. 3. Early diplotene with eight paired bodies. (× 2100.)
Fig. 4. Late diplotene with seven autosomal bivalents and two univalent sex chromosomes. (× 2100.)
Fig. 5. Diakinesis with nine elements. (× 2100.)

pycnotic mass. At late diplotene (Fig. 4) nine elements are found of which seven are distinctly bivalent in nature and the other two small univalents. The latter are sometimes slightly understained at this stage, and are interpreted as the sex-chromosome pair. The chiasmata in the autosomal bivalents remain more or less in the same interstitial position in the diakinesis stage (Fig. 5), and the univalent sex chromosomes lie at random within the nucleus.

The spermatocyte cells of *Dysdercus koenigii* are characterized by having a large amount of cytoplasm in comparison to that of nuclear material (Figs. 2–5). At metaphase the spindle is no bigger than the size of the nucleus in earlier stages (compare Figs. 5 and 6),

and it is therefore likely that the spindle substance is derived mainly from the nucleoplasm, the contribution of the cytoplasm towards its formation being very little.

The arrangement of the chromosomes in metaphase I is typically heteropteran, especially in respect of the sex chromosomes. The latter lie in the centre of the plate, and each of them is bipartite (Fig. 6), as if ready for the equational separation. The interstitial chiasmata of the autosomes, present during diplotene and diakinesis, are mostly terminalized. The relative position of the sex chromosomes in respect of the autosomes

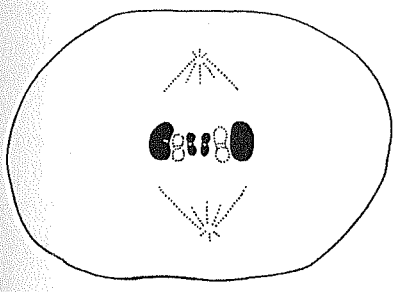

Fig. 6.

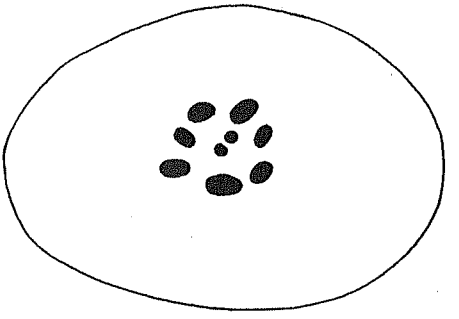

Fig. 7.

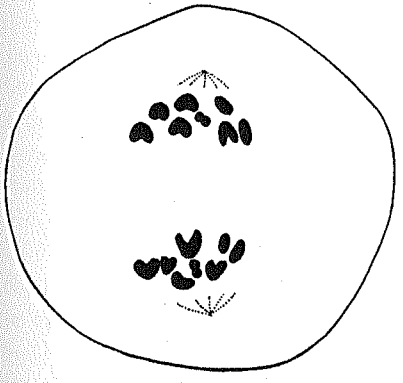

Fig. 8.

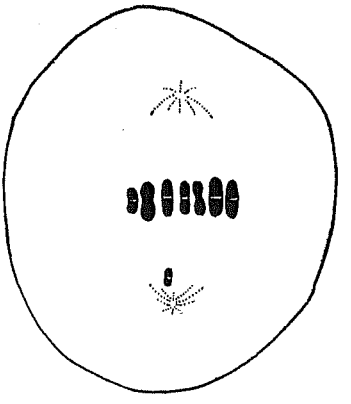

Fig. 9.

Fig. 6. Metaphase I, side view with the bipartite sex chromosomes at the centre of the plate. (× 2100.)
Fig. 7. Polar view, metaphase I. (× 2100.)
Fig. 8. Anaphase I, the X- and Y-chromosomes are near the pole and are bipartite. (× 2100.)
Fig. 9. Metaphase II, side view. One of the sex chromosomes is still bipartite and forms an accessory plate. (× 2625.)

is very clear when the chromosomes appear in polar view (Fig. 7). It is then seen that the two univalent sex chromosomes lie at the centre surrounded by the seven autosomal bivalents.

So far the behaviour of the sex chromosomes is quite normal, but the anomaly reveals itself for the first time during the anaphase of the first meiotic division (Fig. 8), when it is observed that instead of nine elements at each pole (seven autosomes plus the split halves of the X and Y) as expected, we find only eight. The bipartite X and Y, instead of separating equationally, segregate to the opposite poles in all cases, and they can be identified in those positions as the smallest elements still retaining their bipartite condition.

A further unexpected behaviour of the sex chromosomes is observed during the second meiotic division. At the metaphase stage (Fig. 9) of this division seven bipartite elements

are found arranged on the equatorial plane of the spindle, and the smallest element, evidently one of the sex chromosomes, forms an accessory plate near one of the poles of the spindle. We cannot be sure whether in any particular case it is the X or the Y. Presumably in 50% of cases it is the X and the rest the Y. The chromosome forming the accessory plate still retains its bipartite condition observed during the metaphase and anaphase stages of the first division. In the second division metaphase plate when examined from the top (Fig. 10), seven elements are found lying in one plane, and the eighth one in a different plane of the spindle.

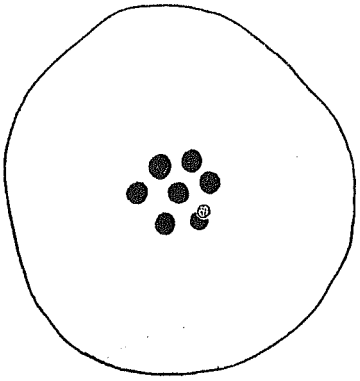

Fig. 10.

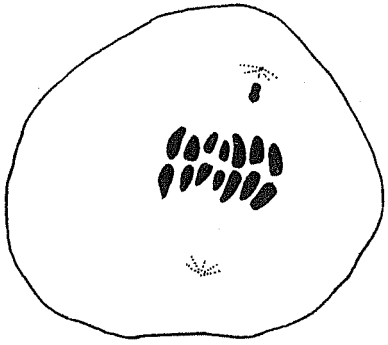

Fig. 11.

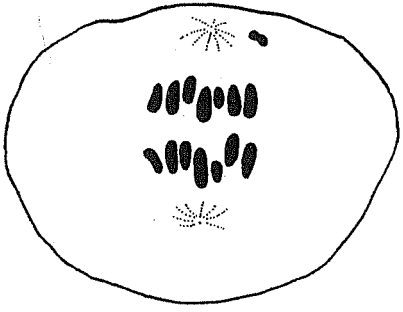

Fig. 12.

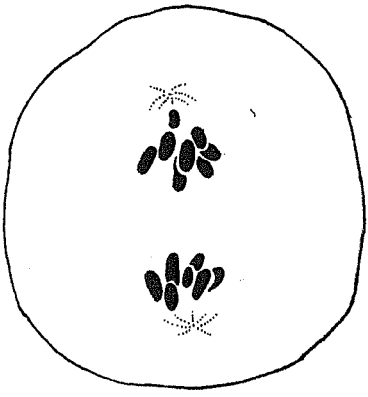

Fig. 13.

Fig. 10. Metaphase II, top view. The sex chromosome lying in a different plane. (×2625.)
Fig. 11. Early anaphase II. The sex chromosome is seen to move to one pole earlier. (×2625.)
Fig. 12. Mid-anaphase II. (×2625.)
Fig. 13. Late anaphase II. (×2625.)

The final and most convincing proof of the segregation of the sex chromosome to one pole of the spindle in the second division of meiosis comes from an examination of the anaphase stages of this division. The early anaphase II (Fig. 11) shows eight chromosomes going to one pole and seven towards the other. The sex chromosome, the bipartite nature of which is clearly visible, travels earlier than the rest of the complement. Mid-anaphase II shows the sex chromosome very near to one pole (Fig. 12), and seven autosomes can be counted easily on each side. Even at a very late anaphase stage (Fig. 13) the number of chromosomes in the two poles can be clearly made out to be eight on one side and seven

on the other. It is clear from the above account that the sex chromosomes move to the pole in the bipartite condition, and the daughter chromatids presumably separate during the first cleavage division of the zygote.

IV. DISCUSSION

An abnormality in the time of division of the centromeres of the sex chromosomes in this species (either diffuse or individualized, cf. Hughes-Schrader & Ris, 1941) is the basis of the unusual event described above. The normal synchronous division of the centromeres of the sex chromosomes and autosomes at the second meiotic division has been replaced during evolution by the precocious division of the former in Heteroptera and Odonata. A few *XO*:*XX* species, however, are exceptional among the Heteroptera (Wilson, 1905*a*, *b*; Schrader, 1932; Manna, 1951) in having the usual behaviour which is probably a reversion. On the other hand, in the present case we find a complete inhibition of the division of the centromeres of the sex chromosomes during meiosis, which ultimately leads to the production of two kinds of sperms; half of them have either the *X* or the *Y* presumably in the ratio of 1:1 and the other half are without any sex chromosome at all. These deficient sperms are not likely to be fully functional, because if properly functional they would have produced, when united in fertilization with a normal egg bearing the *X*-chromosome, an *XO* individual, i.e. an individual which is most probably a male with 15 chromosomes. We have examined a large number of males cytologically, but have never found a single individual with any abnormality in its chromosome number. We have to consider two possibilities to explain the absence of males with 15 chromosomes in the population. Either the sperms without the sex chromosomes degenerate or else they are capable of fertilizing the eggs but the zygotes are lethal. We have not been able to find any obviously degenerating sperms in the testes, and therefore it might be presumed that the deficient zygotes are lethal. The evolution of the precocious type of division of the centromeres of the sex chromosomes from the normal in Hemiptera and the reversion noted in some species of the same group is quite possible because no serious consequences result from these happenings. A mechanism of meiosis resulting in the death of 50% zygotes would be a great handicap for the species, and it is difficult to imagine the evolution and establishment of such a mechanism through natural selection. The other alternative, i.e. the degeneration of 50% sperms, is not a great handicap and is quite possible for a species to tolerate. We are unable just now to decide between the two alternatives without determining the hatchability of the fertilized eggs.

Another interesting and in a sense curious phenomenon is the regular segregation of the *X*- and *Y*-chromosomes to the two poles during anaphase of the first meiotic division. It is therefore not a random affair, and we have to postulate some sort of determinate disjunction by which the movement of the *X* is determined by that of the *Y* or vice versa. The mechanism of disjunction is quite unlike what has been recorded in neuropteran sex chromosomes (Naville & de Beaumont, 1933, and others) or in the multiple sex chromosomes of an undetermined species of Gryllidae belonging to the genus *Euscyrtus* (Ray-Chaudhuri & Manna, 1950). In the above instances the sex chromosomes associate themselves on the spindles in such a way that the two *X*-chromosomes are near one pole and the *Y* near the other. The split *X* and *Y* in *Dysdercus koenigii*, on the other hand, lie on the metaphase plate at the centre but still segregate regularly to the two poles.

Although our record seems to be the first definite case of abnormal segregation in

regular members of the chromosome complement, there are several instances of such a possibility among the supernumerary fragment in certain plants. For example, in *Zea Mays*, Longley (1927) describes the occasional production of $n+2ff$ gametes by $2n+1f$ parents. Darlington (1929) finds that the number of fragments in certain plants of *Tradescantia crassifolia* is sometimes greater than in the parent. This, according to his inference, is due to 'double reduction', i.e. the passing of both the chromatids of a fragment into the same nucleus at the second telophase. Lawrence (1929) also considered the possibility of such a phenomenon in the quadrivalent of a tetraploid *Dahlia*. The same interpretation was again put forward with regard to the behaviour of *B*-chromosomes in *Zea Mays* by Darlington & Upcott (1941). Our definite demonstration of the regular passage of the daughter chromatids of the sex chromosomes in *Dysdercus koenigii* in the second meiotic division provides strong evidence as to the correctness of Darlington's inference (1929) of 'double reduction'.

A claim similar to our findings is made by Wallace (1905). She showed that the sex chromosomes (X_1X_2) of the male tube-weaving spider, *Agalena naevia*, segregate regularly without division in both meiotic divisions. Wallace published her final paper on the spermatogenesis of *A. naevia* in 1909 and reached quite different conclusions, stating that her previous work was erroneous and that the sex chromosomes divide equationally at the second meiotic division. In view of our observation in *Dysdercus koenigii*, there is just a possibility of finding 'double reduction' in the species of spider mentioned above, and therefore it will perhaps be worth while reinvestigating the problem of meiosis in *Agalena naevia*.

V. Summary

1. The chromosome complement of *Dysdercus koenigii* males is $2n=16$, and the species has an $XX:XY$ mechanism of sex determination.

2. The X- and the Y-chromosomes segregate to the opposite poles of the spindle in the first meiotic division, and in the second division either the X or the Y, as the case may be, forms an accessory plate and passes undivided to one pole, resulting, on an average, in the formation of three kinds of sperms, 50 % being without any sex chromosome, 25 % containing the X and 25 % the Y.

3. The deficient sperms either degenerate, or if they are capable of fertilizing normal eggs, probably produce lethal zygotes, since individuals with 15 chromosomes were not found in the natural population.

4. The split sex chromosomes which are incorporated in the sperms presumably separate during the first cleavage division of the zygote.

The authors are indebted to Dr A. P. Kapur, Entomologist, Zoological Survey of India, for the identification of the bug investigated.

[*Note added in proof.*] A paper by Piza, S. de Toledo (*Bragantia*, **7**, 269–71. 1947) not known to us during the preparation of the present paper contains a very brief description of a similar finding in *Dysdercus mendesi*. His earlier account (*Luiz de Queiroz*, **4**, 210–16. 1947) on the meiosis of *Dysdercus* is, however, different.

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