**Studies on the Spermatogenesis of Some
Indian Homoptera
Part IV. The heterochromosome of a Cercopid—
Poophilus sp.**

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Received April 25, 1957

Introduction

It is very recently that the author became interested in the study of the chromosomes during the spermatogenesis of Indian Homoptera Auchenorrhyncha and very interesting observations are made. In this paper it is attempted to study the behaviour of the heterochromosome (sex chromosome) in a cercopid bug, *Poophilus* sp. So far, the author is aware, this is the first account of the chromosomes of Indian Cercopidae.

Our knowledge of the chromosomes of Cercopidae centers round a few publications by Boring (1907, 1913), Boring and Fogler (1917), Misra (1937) and Kurakawa (1953). The American workers (Boring, 1907, 1913; Boring and Fogler 1915) showed in all the species studied, an XO type of sex chromosome mechanism in males, while Misra (1937) and Kurakawa (1953) who worked on the Japanese spittle insects, showed in the majority of males an XY sex chromosome mechanism. It is interesting to know that all the species of the American genus *Aphrophora* show XO males while those indigenous to Japan belong to XY category.

The author has already shown the occurrence of tetraploid spermatocytes in this insect, which is described in detail, elsewhere (MS, sent for publication). In this paper he has shown an XO type of sex chromosome mechanism in males of *Poophilus*. The sex chromosome, typical of the group Auchenorrhyncha (White 1954; Rao 1955, 1956) segregates in the first meiotic division. What is especially interesting is its behaviour in the heterokinetic division and the author has ventured to explain such a behaviour.

Material and methods

The material used in the present study was collected during summer of 1956 at Bangalore. Some were also collected from Pilani and surroundings. The testes in the adults are situated laterally, in the posterior extremity of

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the abdomen. They are round and white in colour.

Some testes were fixed in Allen's Bouin and Carnoy's fluids. They were cut 10 micra thickness. While most of the slides were stained with Heidenhain's Iron Hematoxylin (counterstained with eosin) some were also stained with Feulgen and Newton's modifications of gentian violet.

Observations

Diploid number: There are 25 chromosomes in the diploid complement (fig. 15). All the chromosomes appear round and are uniformly stained. The sex chromosome as such is indistinguishable from the rest of the autosomes. The spermatogonial divisions are quite normal.

Spermatocyte divisions: With the onset of meiosis, however, the sex chromosome appears as round, darkly stained body, with a smooth contour (figs. 1, 2, 3). It is always seen near the periphery of the nuclear membrane, being surrounded by a clear space. This clear space is noticed until first metaphase (in hematoxylin preparations) (figs. 4, 5). The autosomes show the heteropycnotic ends (in Feulgen preparations) in the prophase of meiosis.

In the majority of cases, the sex chromosome is a single body (figs. 3, 4, 5, 6) and this singleness is carried on up to the late telophase I. But in a few cases the sex chromosome appears to be divided -split- (figs. 1, 2, 9, 13). This split condition may be retained throughout the first meiotic divisions. Whatever its condition, be it is split or single, it acts as a single unit during division cycle.

The autosomes when paired, remain parallel and the pairing seems to be a very quick event (figs. 1, 2, 3). There is one chiasma per bivalent which terminalises soon. By the time diakinesis is reached all the chiasmata are terminalised.

The metaphase plate of the primary spermatocytes shows 12 autosome bivalents and X chromosome (fig. 4). The latter always remains eccentric (figs. 4, 6). Certain cases are also noticed where the sex chromosome remains nearer one pole (precession) at first metaphase (fig. 5). In other words it may lie anywhere in the cell. When the sex chromosome is a dumbbell (split), the long axis of the dumbbell may be anywhere, only when the anaphase and telophase forces are at work, then, the long axis of the dumbbell is poleward (figs. 9, 13).

The autosomes as well as the sex chromosome segregate in the first meiotic division, in the usual way, characteristic of Auchenorrhyncha. In anaphase I, while, the autosomes synchronise their movements, the sex chromosome alone shows lagging (succession) irrespective of whether it is nearer the pole or equator, at first metaphase. Only in the late anaphase or telophase it reaches the pole joining the rest of the autosomes (figs. 7,

Figs. 1-18. All figures drawn to the same magnification, $\times 3104$. Except fig. 14 (Feul-

8, 9, 10, 11, 13). It is highly interesting to note that in one of the tetraploid spermatocytes the two X chromosomes lag in the anaphase I as in the diploid relatives (fig. 14).

Immediately after the first division the second division follows, there being no interphase.

Two kinds of secondary spermatocytes are produced—one containing 12 autosomes plus X chromosome and the other containing 12 autosomes alone (fig. 12). The polar view of metaphase II (fig. 16a, b) shows all the chromosomes round while the side view reveals the dumbbell nature indicating the split between the chromosomes (chromatid split) (fig. 17). The sex chromosome since it is distributed along with the rest of the autosomes cannot be identified. The interesting behaviour which was so evident in the first division (lagging at anaphase) is altogether replaced by its synchronous behaviour in the second division. All the chromosomes synchronise in their poleward movement (fig. 18). Out of nearly 150 second anaphase figures counted, in none of them a variation or deviation from the normal behaviour is noticed.

Discussion

The above description furnishes a very interesting behaviour of the sex chromosome during the heterokinetic division. It *always lags*, irrespective of its position at metaphase I. It may lie anywhere in the cell—may be nearer one pole (precession) or on the equator or any where intermediate between the two extreme conditions. In the second division, however, I have never noticed a lagging chromosome—all synchronise their movements.

gen) all are from hematoxylin preparations. The sex chromosome is marked in the figures by the letter-s; the nucleolus by the letter-n. 1-3, pachytene stage showing the autosome bivalents which are paired and are parallel. See the sex chromosome showing the split (1, 2) and unsplit (3) condition. 4-6, metaphase I. Note the distribution of the sex chromosome. In fig. 4 (polar view) and fig. 6 (side view) it is on the equator and eccentrically situated. Fig. 5 (side view) shows the same near one pole (precession). A clear space is visible. 7-9, early Anaphase I. The sex chromosome lying in the same position which it had at metaphase. Fig. 9 (side view) shows the dumbbell nature (split) of the sex chromosome—its long axis turned poleward. 10-12, late Anaphase I. See the sex chromosome lagging (succession), while the rest of the autosomes have reached the poles (figs. 10, 11—side view). Fig. 12, polar view of the same. The sex chromosome is incorporated in one of the two cells. 13, telophase I (side view). The sex chromosome is still lagging. Note the dumbbell (split) and the long axis of the dumbbell turned poleward. 14, anaphase I in a tetraploid cell (Feulgen preparation). See the two sex chromosomes lagging just like in the diploid cell. Also note that they are segregating to the same pole. 15, spermatogonial metaphase showing 25 chromosomes. 16, metaphase II (polar view, a) showing 13 chromosomes the sex chromosome cannot be distinguished. b) showing only 12 chromosomes. Note all the chromosomes appear round. 17, metaphase II (side view). All the chromosomes appear dumbbell, showing the chromatid split in the chromosomes (not all chromosomes drawn). 18, anaphase II (side view). All the chromosomes synchronise in their movement (compare figs. 10 and 13).

There are many interesting views to account for the behaviour of the sex chromosome during heterokinetic division-precession, succession or synchronism (for details see, Schrader 1928).

It may be assumed that due to heteropycnosis (characteristic feature of the sex chromosome, in male) it lags. But there are instances where it condenses sooner as in all Orthoptera and in the membracid, *Atymna castana* (Boring 1907) but still precession is a more common feature and not succession (lagging).

To attribute succession to the fact that the chromosomes involved have no equivalent partner and therefore are hindered in some way in the following normal division, is not much helpful to the problem. Such a hypothesis might find some support in the hybrid meiosis where the chromosomes are irregularly distributed and as such a lack of homology is accountable. But it should not be forgotten that lagging has been observed in the case of chromosomes which are certainly partners—for instance the two sex chromosomes (tetraploid cell) of the male *Poophilus* (fig. 14).

It is also not the pairing nor any other meiotic phenomenon that may be entirely responsible for Oguma (1921) has described in *Tenodera* a lagging in the spermatogonial divisions.

The other alternative, that due to some as yet unknown reason the equational halves of the sex chromosome are parted from each other only with difficulty and thus succession is the outcome of it. For again in Orthoptera where no equational division of X occurs in the first division and which therefore might be regarded as an extreme retardation in separation of the equational halves of the X, precession and not succession is the common phenomenon.

To me the better explanation seems to be that proposed by Piza (1945 a, b), as follows:

The variability in the behaviour of the sex chromosome is due to the fact that they can have any position with respect to the rest of the autosomes, in the cell. And this is due to the fact that the sex chromosome being a stationary body, remains even in anaphase I, in the place which it had in the metaphase I. If it is on the equator, it is in line with the rest of the autosome bivalents. On the other hand if it is away from the equator, it is nearer one pole (precession at first metaphase).

What makes the sex chromosome show precession or succession? It is the weak kinetochore it has, that is responsible to show a weak response to the polar influences, where ever it stays and as such cannot orient itself. It is only later when the cell begins to divide that the sex chromosome is incorporated in one of the cells, escaping the influence of the other, and later joining the rest of the autosomes as was assumed by Piza. On the other hand in the second division contrary to first division, no lagging has been observed but synchronous behaviour.

In *Poophilus*, in certain cells the sex chromosome is already nearer one pole at first metaphase (fig. 5). There is no reason why it should not reach the pole earlier as in *Atymna castana* in the first anaphase than the autosomes. There are instances in *Vanduzes* (Boring 1907) where both precession and succession is noticed. However, in *Poophilus* such a thing never happens—it *always lags*. Though it is nearer one pole, precession is not seen in anaphase I, only succession. It reaches the poles only after the rest of the chromosomes have reached the pole i.e., in late anaphase I or telophase I.

The probable reason is—the kinetochore of the sex chromosome is weak and remains as such as late as first anaphase or telophase. Only later when the anaphasic or telophasic forces begin to operate, the sex chromosome begins to respond to the polar influences by which time the rest of the autosomes have already reached the poles. When once the kinetochore becomes active (which seems to take place in late anaphase) the activity is continued until the completion of the second division and hence no lagging is noticed in the second meiotic division.

And much depends on the position of the chromosome. It is very clear in *Poophilus*, that it remains always near the periphery of the nucleus. A similar case is shown by Piza (1945 b) in some Brazilian Orthoptera where he has shown that if this position happens to coincide with that of the one of the pole in a dividing cell, the sex chromosome will naturally appear nearer the pole in the first metaphase. On the other hand if the angle formed by the spindle axis with that of the sex chromosome increases, the latter will appear far away from the pole and ultimately rests in the equatorial plane. Intermediary positions between the two extremes are possible. In *Poophilus* all these conditions—nearer one pole; lying on the equator, or intermediate condition, are seen.

What matters most is the kinetic activity of the sex chromosome itself. If the kinetochore is active earlier than the act of disjunction of autosomes, precession is the result. If it becomes active after the autosomes have reached the poles, as in *Poophilus*, succession is the only alternative.

It is not without interest to note that the so-called exceptions (lagging of the X chromosome) observed by Boring (1907), Rao (1955, 1956) and by others (Kurakawa, 1953a, b) with the fact observed in *Poophilus* (described here) probably represent a normal behaviour in Homoptera Auchenorrhyncha where in the majority of XO species studied so far the X chromosome shows the phenomenon of lagging (succession).

Summary

1. The diploid number in *Poophilus* is 25, and the haploid number is 12AA and X.

2. The sex chromosome segregates in the first division typical of Homoptera Auchenorrhyncha.

3. The sex chromosome lags (succession) in the first division and *not* in the second meiotic division. A detailed behaviour of the same is discussed.

Acknowledgements

I am grateful to Professor M. A. Moghe for his interest in the present work. It is a pleasure to record my indebtedness to Professor Franz Schrader and Professor S. de Toledo Piza Jor, for their criticisms. I thank Dr. M. Puttarudraiah, Govt. Entomologist, Department of Agriculture, Bangalore, for helping me with the identification of the insect. I also wish to acknowledge the Ministry of Education, Government of India, for the Senior award of Scholarship.

Author's abstract

The sex chromosome in the first heterokinetic division always lags (succession) but not in the second. This behaviour is the out-come of the kinetic activity of the kinetochore. Because of the weak kinetochore it lags in the first division, but when the normal activity is restored (which seems to happen in late anaphase I or telophase I) it shows its synchronous behaviour in the second division, along with the rest of the autosomes.

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Addendum

After this paper was sent to prees, I have received “A note on the chromosome numbers in the Homoptera Auchenorrhyncha” published in Hereditas 43: 465-466 (1957). The author in question (Halkka) has described the chromosome number and the sex chromosome mechanism in 90 species of Finnish Homoptera Auchenorrhyncha.