Chromosome Studies in the Myriapoda
VII. A chain-association of the multiple sex-chromosomes found in Otocryptops sexspinosa (Say)

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Many examples of the multiple sex-chromosomes are known in plants (Ono 1950). In spite of the extensive literature that has appeared on the chromosomes of various kinds of animals (cf. Makino 1951), such multiple sex chromosomes are very rare in animals, having been infrequently reported in Insecta, Arachnida and Nematoda. Most of the reported cases are of a multiple X-chromosome type with or without a Y-element, such as the 8X-Y type occurring in Ascaris incurva. Recently, Piza (1946) reported an interesting X-2Y type of the sex-chromosome in Enoptera surinamensis, a species of Gryllidae.

In the present paper is to be reported the sex-chromosomes of a multiple X-Y mechanism found in Otocryptops sexspinosa which is remarkable as a new example in animals.

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Material and method: According to Takakuwa (1940) and Takashima (1949), Otocryptops sexspinosa is said to have a wide distribution through Japan, Korea and Formosa. The material for this study was obtained in the vicinity of Hamamatsu, Shizuoka Prefecture. The animals were dissected alive, and the gonads, together with other viscera, were put into the fixative. For the fixatives, modified Navashin's solution and Carothers' mixture were employed with satisfactory results. The slides were prepared according to the ordinary paraffin technique and stained with Heidenhain's iron-haematoxylin.

Observations
1. Chromosomes of the spermatagonium and the male somatic cell. The testicular and male somatic material from the digestive canal supplied

1 The species described as Otocryptops sexspinosa sexspinosa in the previous paper by the present author (1952) was erroneously identified; it differs essentially from the present species here concerned.
many excellent dividing cells which allow a critical study of the male diploid chromosomes (Figs. 1-4, 32). Observations revealed that the diploid number of chromosomes was 15, which consist of three large V-shaped elements, one large J-shaped one, six large rod-shaped ones and five small rod-shaped ones.

Figs. 1-16. All camera-lucida drawings. ×2800. 1. Somatic metaphase, from the male digestive canal, 15 chromosomes. 2-4. Spermatogonial metaphases, 15 chromosomes in each. 5-8. Somatic metaphases, from the female digestive canal, 14 chromosomes in each. 9. Alignmental arrangement of the spermatogonial chromosomes, based on Fig. 2. 10. Alignmental arrangement of the female somatic chromosomes, based on Fig. 5. 11. Nucleus at diakinesis, showing a chain-combination of the sex-chromosomes in deep black. 12-13. Primary spermatocyte metaphases, polar view, showing three autosome bivalents and a chain of the sex-chromosomes. 14. A chain of the sex-chromosomes, taken from the diakineti nucleus. 15. The same, from the first spermatocyte pro-metaphase. 16. The same, from the first spermatocyte metaphase.
The male diploid complement is represented by the formula of \(3V's + 1J + 6R's + 5r's\) (Fig. 9). The J-shaped chromosome has its centromere near its proximal end, so that it looks occasionally like a rod-chromosome. Four of the six rod-shaped chromosomes (6R's) are of nearly similar size, while the remaining two are slightly smaller. Five small rod-shaped chromosomes vary in size: two of them are relatively smaller than the others of which one is considerably longer. From the results of the above observations it becomes evident that the chromosomal elements, 2V's and 4R'S, constitute three homologous pairs, while 1V, 1J, 2R's (R1 and R2) and 5r's (r1, r2, r3, r4 and r5) are particular ones.

2. Chromosomes of the female somatic cell. Fortunately many metaphase plates of the female somatic cells were found in the digestive organs, as shown in figures 5 to 8 and 33. Careful observations made it clear that the diploid number of chromosomes in the female was 14 having the complex of \(4V's + 6R's + 4R's\) (Fig. 10). Noticeable is the fact that the V-shaped and rod-shaped elements are in pair, each having a homologous mate. The diploid complement of the female is therefore represented by \(2 \times (2V's + 3R's + 2r's)\).

3. Chromosomes of the first spermatocyte. The nucleus of the early growth period showed a large chromatin-nucleolus deeply stained with haematoxylin. This chromatin-nucleolus was observable in the nucleus through various stages of the growth period until at the end of diakinesis. Then the chromatin-nucleolus developed into a chain of chromosomes (Fig. 11). There were discernible in the nucleus at this stage three bivalent chromosomes and a chain of chromosomes. It is remarkable that the chromosomes forming the chain were always constant in order of combination (Figs. 11, 14). At metaphase three bivalents and a chain of chromosomes arrange themselves in the equatorial plate (Figs. 12, 13, 17). From their morphological configurations there is no doubt that the three bivalents are derived from two V-shaped chromosomes and four rod-shaped chromosomes, which were all paired (Figs. 12, 13, 17, 20). The chain of chromosomes lies in the equatorial plate in a zigzag manner, with the same order of combination as that observed in the diakinetic stage (Figs. 14–16). Interesting and noticeable is the structure of the chain of chromosomes, as clearly observed through the metaphase to anaphase stage (Figs. 17–22). Careful observations made it clear that the chain is composed of the following 9 elements, 1V, 1J, 2R's and 5r's. They arrange in the following order and manner: \(V_{r_1} r_2 r_3 r_4 r_5 R_1 R_2\). Thus, the three bivalents derived from 2V's and 4R's, and nine elements forming a chain (1V, 1J, 2R's and 5r's) constitute the haploid set in the primary spermatocyte of this species. In the first division, two sister groups of chromosomes result due to a peculiar separation of the chain chromosomes. One of the groups receives 1V, 2R's and \(V + r_1 + r_2 + R_1\), being seven in total number, while the other contains eight elements consisting of 1V, 2R's and \(J + r_1 + r_2 + r_3 + R_1\). This feature is shown in figure 23, a and b.
4. **Sex-chromosomes.** Here, a comparison of chromosomes between the male and female cells is needed. As already mentioned, the serial alignment of the female somatic chromosomes shows seven paired chromosomes (Fig. 10). The half set of these seven pairs fairly corresponds in their
morphological feature to the seven chromosomes occurring in the male cell (1V, 2R’s and V + r2+ + R1). Further, these seven elements apparently correspond to the seven members of chromosomes which migrate to one of the poles in the first spermatocyte division (Figs. 19, 22). Thus, a set of chromosomes consisting of V− r2− r1− R1 found in the male cell is present in the female cell, while the other set, J− r1− r3− r5− R2, is completely lacking in the female cell. In the first spermatocyte metaphase, these two sets of chromosomes associate into a chain, J− V− r1− r2− r3− r1− R1− R2. In the first division, these elements separate into two groups forming a combination in each and moving to the opposite poles: one group consisting of V− r2− r1− − R1 migrates to one pole and the other group comprising J− r1− r3− r5− R2 runs to the opposite pole. Hence, there is good reason to assume that the nine chromosomes forming a chain in the first spermatocyte are no other than the sex chromosomes. That is to say, according to the general rule of the sex chromosomes, V− r2− r1− R1 should be X1− X2− X3− X4, while J− r1− r3− r5− R2 should be Y1− Y2− Y3− Y4− Y5. Hence it follows that the male chromosome formula is to be represented by 2n, 15 = 6A + X1− X2− X3− X4− X5+ Y1− Y2− Y3− Y4− Y5, while the female formula has a constitution of 2n, 14 = 6A + 2X1− 2X2− 2X3− 2X4 (A designates the autosomal elements).

5. Chromosomes of the secondary spermatocyte. Resulting from the first division, there are two different groups of chromosomes in the secondary spermatocyte: one contains seven chromosomes, while the other eight (Figs. 24–25). Morphological comparison of the chromosomes in the two groups revealed that the constitution of the seven-chromosome group is 2V’s + 3R’s + 2r’s, while that of the eight-chromosome group is 1V + 1J + 3R’s + 3r’s. From the results of the foregoing observations it is evident that the seven-chromosome group consists of 3 autosomes plus 4X’s, whereas the eight-chromosome group comprises the same autosomal set plus 5Y’s. In the second division, all the elements separate into equal halves. Thus there are produced two different kinds of spermatids, one containing 8A + 4X’s (Fig. 30, a−b), and the other having 3A + 5Y’s (Fig. 31, a−b).
Remarks

In his preceding papers (Ogawa 1950, 1952 a, b, 1953) the present author has reported on a new type of the sex-chromosomes in two species of the Scutigeridae (Chilopoda) which are characterized by the X-Y chromosomes undergoing post-reductional division. He reported their interesting features and behavior. In this paper a clear-cut example of the multiple sex-chromosomes was demonstrated in Otocryptops sexspinus. The sex-chromosome mechanism found in this species is of a 4X-5Y complex. So far as the author is aware, such a complex as presented here has never been reported in any kind of animals so far (cf. Makino 1950). Previously, a 2X-Y complex has been known to occur in several species of mantis (Oguma 1921, 1946, Hughes-Schrader 1950). Guénin (1948, 1952) has found a 3X-Y complex in Cicindela (Coloptera), and Blaps (Col.). Further the same author has described a 4X-Y type in Blaps gigas (1949). Piza (1946) reported an X-2Y mechanism in Eneoptera surinamensis (Gryllidae). Recently, Suzuki (1952) demonstrated two or three X-chromosomes without Y in the male of some spiders. Such multiple X’s have been known to occur in many species of Heteroptera (Schrader 1928), and of Plecoptera (Matthey and Aubert 1947). Corresponding examples of the multiple sex-chromosomes have been found in plants, such as Humulus (Kihara 1928, 1929, Sinotó 1929 a, b, c, Kihara & Hirayoshi 1932, Ono 1937), and Rumex (Kihara & Ono 1923, Ono 1935).

White (1940) stated that the multiple or compound sex-chromosomes originated as the result of translocation. The chain-association of chromosomes has been explained by the mechanism of translocation in plants by many authors (cf. Yamashita 1951). Similarly the association of the X chromosome with autosomes occurring in Paratylothropidia brunneri (Acrididae) has been explained as the result of translocation by King and Beams (1938). For the present case, there is no positive evidence to account for the chain-like association of the 4X- and 5Y-chromosomes. But an application of the mechanism of translocation may also be possible in this case for the explanation of their origin, because of the well-accepted view in this field of plant cytology.

Summary

The present paper describes the multiple sex-chromosomes found in Otocryptops sexspinus, a species of Chilopoda. It was found that the present species possesses a 4X-5Y mechanism of the sex-chromosomes in the male. They show the chain-like association at the first metaphase. The formula of the chromosomes is $6A+X_1-X_2-X_3-X_4+Y_1-Y_2-Y_3-Y_4-Y_5$ in the male, and $6A+2X_1-2X_2-2X_3-2X_4$ in the female.
Literature


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Addendum: After this paper was completed and ready to send to press, the author found the paper by Guénin (1953) published in Rev. Suisse Zool. 60: 462-466. Guénin reported a 12X-6Y mechanism of the sex-chromosomes in the male of Blaps polychresta (Coleoptera). At the first metaphase these sex-chromosomes appear in the form of a chromosomal mass lying in the central area of the equatorial plate, without showing the chain-association. In the first division, the sex-chromosome complex divides into two groups, 12X and 6Y which migrate to opposite poles. In both structural feature and behavior, therefore, the multiple sex-chromosomes of Blaps essentially differ from those of the present species.