

**A Study on the Chromosomes of a Spider,  
*Heteropoda venatoria*, with Special Reference  
to  $X_1$ -,  $X_2$ - and  $X_3$ -chromosomes**

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**A Study on the Chromosomes of a Spider,  
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to  $X_1$ -,  $X_2$ - and  $X_3$ -chromosomes<sup>1)</sup>**

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(With Plates I-III)

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## INTRODUCTION AND HISTORY

Some cytological investigations in spiders were done by CARNOY (1885), WAGNER (1896a, b) and BÖSENBERG ('04, '05), but most of these are of only classical values and as far as the chromosomes are concerned, we can hardly find any available informations among them. After that time WALLACE ('00, '05, '09), MONTGOMERY ('05) and BERRY ('06) studied on the chromosomes in the spermatogenesis of some spiders. They reported some considerations upon the occurrence of sex-chromosomes in these arachnids. Among them, especially WALLACE ('09) described for the first time an  $X_1X_2-0$  type of sex-determining mechanism in the male of *Agelena naevia*. This multiple sex-chromosome mechanism was confirmed by PAINTER ('14). PAINTER's work was based on the thirteen representatives of different families, his technique, however, was crude and the observations made were not so satisfactory. He could not succeed in making accurate counts of the chromosome numbers in the diploid chromosome sets in any species of his materials. SOKOLSKA ('25) discovered another interesting multiple sex-chromosome mechanism of an  $X_1X_2X_3-0$  type in *Tegenaria domestica* males. The spermatogenesis of American Lycosid-spiders was studied by CHICKERING and HARD ('35), especially HARD ('39) succeeded first in confirming an  $X_1X_2-0$  mechanism in *Lycosa crassipes* based on the observation of both diploid and haploid chromosome sets.

Recently many important cytological studies of spiders have been published by REVELL ('47), PÄTAU ('48), HACKMAN ('48) and SUZUKI ('49, '50). The latter two workers made researches on sixty nine and twenty one species of spiders, respectively. From these authors a great deal of important knowledges have been added and a notable progress has been made in this field.

REVELL (l. c.) employing modern technique (squashes in Lacmoid stain, DARLINGTON and LA COUR, '42) as well as normal method, confirmed an  $X_1X_2X_3-0$  type in two species of *Tegenaria domestica* and *T. derhamii* males. HACKMAN (l. c.) reported in his painstaking works that he could have found an  $X_1X_2-0$  type in 62 species, an  $X-0$  type in 5 species and an  $X_1X_2X_3-0$  type in one definite and one doubtful species. SUZUKI (l. c.), one of the present authors, also found occurrence of an  $X_1X_2-0$  type in 18 species and an  $X-0$  type in 3 species.

To summarize the entire cases reported in the literature up till now, excluding doubtful one, the frequency of the above mentioned types of sex-determining mechanism is as follows:  $X-0$  type 8,  $X_1X_2-0$  type 93 and  $X_1X_2X_3-0$  type 2. Consequently it would seem that in

spiders an  $X_1X_2-0$  type is the most usual (90 per cent), an  $X-0$  type is the next (8 per cent) and an  $X_1X_2X_3-0$  type is the least in cases (2 per cent).

Two cases of true occurrence of the 3 X's mechanism were found in *Tegenaria domestica* (SOKOLSKA '25 and REVELL '47) and *Tegenaria derhamii* (REVELL '47 and HACKMAN '48); another doubtful case of this type was reported in *Micrommata viridissima* by HACKMAN (l. c.). HACKMAN thinks that *T. domestica* may be synonymous with *T. derhamii*. If it is so, certain instances of this interesting 3 X's mechanism will only be a single case.

A new instance of the multiple sex-chromosome mechanism of an  $X_1X_2X_3-0$  type has been found recently by the present authors in *Heteropoda venatoria* (Family Sparassidae). We have succeeded fortunately in demonstrating these three multiple sex-chromosomes not only in both male and female germ cells but also in the somatic cells of the female. As far as the literature is concerned, the sex-chromosomes of spiders, except an  $X-0$  type, have never been distinctly identified in the spermatogonial chromosomes. In many cases they were distinguished exclusively during meiosis, and the various authors considered that the sex-chromosomes in the spermatocytes may probably be derived from respective number of spermatogonial chromosomes. In the present study we have recognized the  $X_3$ -chromosome, one of the 3 multiple sex-chromosomes, in the diploid chromosome sets of both sexes. The precise morphological characters of chromosomes, particularly those of sex-chromosomes, and their behaviors throughout the mitotic and meiotic cycles will be described in this paper.

Before going further, the authors wish to express their grateful thanks to Prof. S. MAKINO for his cordial advices and criticisms throughout this work.

## MATERIALS AND METHODS

*Heteropoda venatoria* used in this study is a common house spider in central and southwest Japan. All the materials have been collected in the school house of the Hamamatsu Branch, Department of Education of the Shizuoka University throughout every spring to autumn since 1947 to 1949. The testis before July contains the spermatogonial cells alone, and during July to September that is filled with the cells in successive stages of meiosis, and in the testis taken after October are observed mainly mature sperms, spermatids and some of spermatocytes.

As soon as the testis is taken out, it is put into the fixative. For

the preservation of chromosomes various kinds of fixatives are employed. In order to preserve nucleus NAKAMURA's modified NAVASHIN's solution (with glacial acetic acid  $\frac{1}{2}$  as normal) is used. It offers the excellent result throughout the entire stages of mitotic and meiotic divisions. But this fluid has one defect; it tends to stain the cytoplasm more or less and gives some unsatisfactory conditions. CAROTHER's solution is particularly good for the fixation of the spermatogonial and spermatocytic chromosomes. And modified BENSLEY's solution for histology shows the most favorable result in preserving the chromosomes of meiotic division and female germ cells.

For embedding the materials into paraffin buthyl alcohol instead of ethyl alcohol is used. Following the usual treatment of the paraffin method, they are cut at various thickness from 6 to 8 micra and stained according to the iron hematoxylin method of HEIDENHAIN. In the staining of preparations also buthyl alcohol is employed.

All drawings are outlined at table level with the camera lucida using a compensating ocular K. 20 and a LEITZ  $\frac{1}{2}$  oil immersion objective, at a magnification of approximately 5,000 diameters. All plates are reduced to approximately  $\frac{1}{4}$  diameters for publication.

## OBSERVATIONS

### I. Spermatogonia

A large number of metaphase plates were obtained from the materials which had been fixed before July. In most of the metaphase plates innumerable number of chromosomes are present and some of them overlap one another, so that it makes us impossible of penetrating observations of them. Among them twenty excellent figures are selected and employed for the precise observations. In Pl. I, Figs. 1-4, the representatives of those spermatogonial metaphase plates are shown. The alinement arrangements of supposed homologous chromosomes in Pl. I, Figs. 1 and 4 are shown in Pl. I, Figs. 9-10.

As is shown in Pl. I, Figs. 1-4, the number of chromosomes is invariably 41. All the chromosomes are essentially of the simple rod-type. Some chromosomes are almost straight, but many of them are slightly curved and tapering towards their inner ends. The chromosomes are mostly of smooth outlines, some of them, however, especially larger ones are of more or less irregular thickening and sometimes such chromosomes as having knob-like thickening exhibit bizarre

contours. The chromosomes are arranged generally on the equatorial plate in a radial manner, their tapering ends pointing towards the centre of that plate. It makes us believe that all the chromosomes are acrocentric. The serial alinements of Pl. I, Figs. 9-10, include 20 pairs of homologous chromosomes and an unpaired one. Excepting an unpaired chromosome, all the homologous pairs can be arranged in length from 1st to 20th in the approximate order. The unpaired chromosome, designated as  $X_3$ , placed at the extreme left in Pl. I, Figs. 9-10, is considered as representing one of the sex-chromosomes. In order to identify the sex chromosome distinctly, peculiar care has been paid.  $X_3$  is the largest one of all the metaphasic chromosomes; it is larger in length than the largest pair of homologous chromosomes, and in the finest case, it is provided with one transverse constriction in the locus of its about  $\frac{1}{3}$  from the apical end, by which the chromosome is divided into two successive segments, so that it assumes a distinct bipartite structure, which is the most marked characteristic for the  $X_3$ -chromosome (Pl. I, Fig. 6 a-f). It is most clearly observed in the  $X_3$ -chromosome in the primary spermatogonia, and its constricted position in the  $X_3$ -chromosome is variable a little in the different individuals. In some cases, when  $X_3$  is slightly condensed such as in the secondary spermatogonia, the constriction is not so much distinct, but even in such cases, the  $X_3$  is constricted more or less in the position which is supposed to be the locus of constriction.  $X_3$  is almost straight or slightly curved, but in extreme cases as shown in Pl. I, Fig. 6a, it is curved in the point of constriction and assumes approximately a V-shaped form. Prior to the metaphase, namely in the prometaphase also,  $X_3$  is evidently detectable by its peculiar morphological feature. Two of  $X_3$ 's taken in the prometaphasic figures are pictured in Pl. I, Fig. 5a-b. As shown in the figure,  $X_3$  in that stage is already provided with one transverse constriction and showing a fine bipartite appearance, in this case however, some differences exist. Namely in the prometaphase the basal segment of  $X_3$  is almost similar in structure with that in the metaphase, while the apical segment in this stage is somewhat thin and assumes a spiral feature, so that it forms a marked contrast to the basal segment. Thus  $X_3$  has the largest size among all the metaphasic chromosomes and always is provided with one constriction and as a result, it assumes a distinct bipartite structure, therefore, in the excellent metaphase plate it is quite clearly distinguished from other chromosomes. In the equatorial plate,  $X_3$  usually lies at the peripheral circle of the metaphasic chromosomes.

As it will be verified later, 3 sex-chromosomes, designated as  $X_1$ ,  $X_2$  and  $X_3$  in this paper, are contained in the present species. Conse-

quently these 3 sex-chromosomes must be included in the spermatogonial chromosomes also. Of these sex-chromosomes  $X_3$  is above identified, so that the rest two, i. e.  $X_1$  and  $X_2$  ought to be detected. It has been mentioned above that except for  $X_3$ , all the remaining 40 chromosomes have no particular morphological feature, beyond that of length. So we think, though  $X_1$  and  $X_2$  are contained in the spermatogonial chromosomes, they can not be clearly identified in that stage, probably due to the failure of the distinct form and size.

## II. Meiosis

### (1) Growth period

It is known by some authors that the sex-chromosomes during growth period are recognized as strongly heteropycnotic bodies. The similar feature can be seen in the present species. In the leptotene a small clear cavity is formed in the so-called proximal position of the nucleus. In this cavity small heteropycnotic, condensed mass of undefinitive form can be seen (Pl. II, Fig. 12). It is not similar in structure and contains more or less deeply stained chromatins, but in this stage its affinity for the stain is weak; it is approximately equal to the leptotene threads. As the previous workers stated, that compact mass is considered to be the condensed sex-chromosomes. In the zygotene some differentiations take place in the structure of that body and three elements appear from it (Pl. II, Fig. 13). Of these three elements, one is a ring-shaped body; it consists of small number of segments, and bears somewhat indistinct contour. The rest two are short rods; they are segmented more or less and have indefinite outlines. Those three bodies lie in the cavity closely in contact, usually the ring-shaped body stays more distally than the rest, but occasionally the reverse is the case. It seems to be probable that the two rod-shaped bodies represent  $X_1$  and  $X_2$ , and the ring-shaped one  $X_3$ . As the stage goes forward, the staining capacity of 3 X's increases gradually. In the pachytene (Pl. II, Fig. 14) 3 X's become more evident in structure and they stand apart completely one another. By the time of this stage  $X_1$  and  $X_2$  elongate and are of slightly ambiguous outline. They stay side by side and lie in parallel to the axis of autosome threads. On the other hand,  $X_3$  remains a segmented ring form. After that time the ring is broken at one point and becomes a curved elongate chromosome of irregular outline. Those three sex-chromosomes during this period are considerably deeply stained.  $X_1$ ,  $X_2$  and especially  $X_3$  are variable in morphological feature and arrange-

ment. Various features of them are illustrated in P1. II, Fig. 15 a-e. The arrangement of 3 X's in this stage is almost similar with that of the previous stage, but sometimes 3 X's stand in parallel to each other or one of 3 X's lies apart distantly from the rest two (P1. II, Fig. 15 a-e). By the time of later pachytene, those 3 X's become more condense and increase staining capacity. During first half of diplotene, 3 X's are fused into one markedly condensed, deeply stained heteropycnotic mass, in which 3 X's are recognizable as 3 small swellings (P1. II, Figs. 16-17). In the second half of diplotene, the structure of the compact mass loosens gradually and from that body appear three bodies once more. In that time various kinds of complex processes can be seen (P1. II, Fig. 18 a-d). At first appears a small spherical, transparent space in the compact mass, and from it two rings of different size are formed. After that time two small, rod-shaped chromosomes are produced from one ring. Another ring remains as it was and afterwards these bodies separate completely. This ring-shaped body consists of some segments, but later the ring is broken at one point. It seems that two rod-like chromosomes correspond to  $X_1$  and  $X_2$ , and the ring body represents  $X_3$ . In the late diplotene, those 3 X's are condensed respectively, and exist as independent three compact bodies (P1. II, Fig. 19). They are arranged on the proximal position of the nuclear cavity, of which the largest one, probably representing  $X_3$ , is of dumb bell form. In the diakinesis 3 X's increase their length; usually  $X_3$  lies quite apart from the  $X_1X_2$  group, while the latter two take a parallel arrangement (P1. II, Fig. 20).  $X_1$  and  $X_2$  are of almost equal size and with slightly irregular contour, on the other hand  $X_3$  is always larger in length than the former. The ends of  $X_1$  are curved, and it assumes somewhat a ring shaped form. In this stage 3 X's are less stained than autosomes. To our interests, such figures are occasionally observed, as a nucleolus-like body comes in contact with the  $X_3$ -chromosome. The association of  $X_3$  and nucleolus-like body can be found also at early diakinesis. In respect to this phenomenon, however, more precise future study may be necessary.

## (2) Primary spermatocytes

Some metaphasic figures are shown in P1. III, Figs. 23-27. In the figures 22 distinct chromosomes are clearly observed. In the slides which were fixed with modified NAVASHIN's solution, all the chromosomes are heavily stained and as a result, they assume swollen appearance (P1. III, Figs. 26-27). In such figures the chromosomes are arranged closely each other and some of them are overlapped; therefore, the accurate count of chromosome number or



the precise observation of chromosomes is very difficult. On the other hand, the preparations which had been treated by modified BENSLEY's solution afforded the most excellent results. On the equatorial plate of such slides, all the chromosomes, excluding the peculiar three chromosomes, are distributed well apart at nearly equal distance each other; consequently it allows us to bring the precise observation of individual chromosome (Pl. III, Figs. 23-25). Of 22 chromosomes in the primary spermatocytic figure, 19 are bivalent and seem to be autosomes, while the rest three are univalent, which probably represent sex-chromosomes. Some tetrads, especially smaller ones are constricted in the centre and assume a typical dumb bell form. The rest are of V or horse-shoe shape. In the excellent figures such as shown in Pl. III, Figs. 23-25, all the tetrads are seen to be consisted of two knob-like swellings, which contact together at apex. These facts are very likely to indicate that the tetrads of the present species are all composed of the transverse rod-ones with bi-terminal spindle attachment. In the polar view of metaphasic plate, three chromosomes lie in the most peripheral zone of the general circle, sometimes in the extreme cases they situate on the outside of the autosome group. They are always univalent and distinctly recognizable from autosome tetrads. It is really evident that these three chromosomes represent sex-chromosomes. They are not equal in size, among them two are always similar in size, but the remaining one is invariably larger than the former. It seems that the former indicate  $X_1$  and  $X_2$  and the latter  $X_3$ . These three X's stand in the metaphase nearly vertical to the equatorial plate, approximately parallel to the axis of the spindle (Pl. III, Fig. 28). In many cases all the 3 X's lie closely, particularly  $X_1$  and  $X_2$  remain always side by side, but  $X_3$  often lies apart distantly from the  $X_1$   $X_2$  group. In the polar views, 3 X's assume short rods, and particularly in the excellent figures they are represented as three dots; in the lateral aspects, however, they are long rods of different size (Pl. III, Fig. 28). Among them  $X_3$  is invariably larger than the rest two, so that  $X_3$  is distinguished from the latter without difficulty. As pointed out,  $X_1$  and  $X_2$  bear almost an equal size and show parallel arrangement during the primary meiotic division; consequently it makes us impossible to do more penetrating analysis of two chromosomes. They are rather likely to be homologous, but regarding this point we have some questions.

Excepting three sex-chromosomes, all the autosome tetrads in the anaphase separate into two equal daughter halves throughout their whole length. The separation of autosomes is quite synchronous. The three sex-chromosomes do not divide in this division and go

ahead in the early stage of anaphase into one pole (Pl. III, Figs. 29-31). In many cases, therefore we can see that in early stage of anaphase when the autosome tetrads are still staying on the equatorial plate, 3 X's already are staying in the intermediate position between the equatorial plate and one pole. Usually 3 X's remove simultaneously with close connection, but sometimes  $X_3$  passes over to the same pole separately from the  $X_1X_2$  group.

SOKOLSKA (1. c.) reported the abnormal separation of sex-chromosomes in *Tegenaria domestica* males of an  $X_1X_2X_3-0$  type. She described that in some cases 3 X's remove to one pole, but the remaining one stays in the vicinity of the opposite pole. Furthermore, she observed a different kind of abnormality, in which both poles of one dividing cell contain 2 X's respectively. In the present materials such abnormalities of sex-chromosomes have never been found.

As above mentioned, in the primary spermatocytic division 3 X's are distinctly identified by the particular arrangement and behavior as well as the morphological character. In respect to size, remarkable differences can be found between 3 X's. In comparing the spermatogonial with the primary spermatocytic chromosomes, it may well be concluded that the three sex-chromosomes,  $X_1$ ,  $X_2$  and  $X_3$  in the primary spermatocytes arise from three univalent chromosomes existed in the spermatogonia.

### (3) Secondary spermatocytes and spermatids

As all the three sex-chromosomes pass over undivided into one pole in the first division, two sorts of the secondary spermatocytes are produced regarding the sex-chromosomes. One half contains 19 autosomes and three sex-chromosomes ( $19A + X_1X_2X_3$ ); one half includes 19 autosomes and none of sex-chromosomes ( $19A$ ) (Pl. III, Figs. 33-36). In this stage the cell is distinctly small in size, compared with that of the first division. All the chromosomes, including 3 X's, assume straight or slightly curved short rods. They do not show such regular arrangements as seen in the first division, but are arranged rather irregularly more or less and some of them are overlapped one another. Though particular attentions were paid to distinguish the sex-chromosomes, unluckily in many cases it was rather difficult to identify them with certainty. Nevertheless, in rare cases, as shown in Pl. III, Fig. 33, we could recognize them. In the excellent figure, 3 X's stay on the slightly different level from the equatorial plate, but among them the larger one, probably representing  $X_1$ , lies in the peripheral circle of autosomes, and the

rest two, representing  $X_1$  and  $X_2$ , are smaller than the former, and stand side by side each other on the outside of the general circle of other chromosomes. All the chromosomes, including 3  $X$ 's, divide mitotically in the second division.

As is expected, two kinds of spermatids are formed from secondary spermatocytes. One contains 3  $X$ 's and 19 autosomes, and the other bears 19 autosomes alone. The spermatids bearing 3  $X$ 's contain one deeply stained, heteropycnotic mass, which is considered as indicating sex-chromosomes, in the resting nuclei (Pl. II, Figs. 21-22). The spermatids of this kind are found in about one half of the figures.

### III. Chromosomes in the oogonia as well as in the female somatic cells

In order to demonstrate the multiple sex-chromosome mechanism decidedly, the chromosomes in the female germ cells or somatic cells must be undertaken. But as a matter of fact, such works are so difficult, that not a single accurate description of diploid chromosomes in the female has never been published by the previous authors up to the present (recently SUZUKI obtained an excellent oogonial figure in *Cyclosa octotuberculata*, unpublished). For that purpose numerous materials of females were cut. Several slides which had been preserved in early summer offered some excellent diploid chromosome figures. Two figures shown in Pl. I, Figs. 7-8, are obtained in the oogonial plate and the somatic mitosis in the epithelial cell of ovary.

The chromosome number is invariably 44 in both figures. The morphological feature and arrangement of metaphasic chromosomes are approximately similar with those in the spermatogonial plate. The alinemental arrangement of the supposed homologous chromosomes in Pl. I, Fig. 7 is given in Pl. I, Fig. 11. All the 44 chromosomes consist of 22 pairs of homologous chromosomes. The first pair of chromosomes, designated as  $X_1$  in Pl. I, Fig. 11, is the largest of all. Those two chromosomes are curved in the position of about terminal one third, and at that point both chromosomes are slightly constricted. The same feature is also seen in 2  $X_2$ 's pictured in Pl. I, Fig. 8; in this case a more distinct constriction can be seen in the two chromosomes. Thus the first two chromosomes in Pl. I, Fig. 11 are larger in size and of more distinct morphological features than any other chromosomes, therefore, it makes us believe that they indicate two  $X_1$ -chromosomes. These two  $X_1$ 's are situated on the opposite position in the peripheral circle of the chromosomes, but in Pl. I, Fig. 7, they lie in symmetric position. No other than

2  $X_3$ 's, any pair of homologous chromosomes has never such distinct morphological character. Namely they are essentially rods and arranged in length from the longest 1 to the shortest 21 in the approximate order.

As stated in the spermatogonial plate, here also  $X_1$  and  $X_2$  can not be identified in the diploid chromosomes, due to the failure of the distinct morphological feature.

In comparing the chromosome number of the spermatogonia with that of the oogonia or somatic cells, it is easily known that the oogonial and female somatic chromosomes contain 3 supernumerary chromosomes. These three chromosomes are considered as representing one set of three multiple sex-chromosomes,  $X_1$ ,  $X_2$  and  $X_3$ . Therefore, the oogonial and female somatic chromosomes will seem to contain 38 autosomes and two sets of three multiple sex-chromosomes, i. e.  $38A + 2X_1 + 2X_2 + 2X_3$ .

## DISCUSSION

### I. On the multiple sex-chromosome mechanism of an $X_1X_2X_3-0$ type in spiders

As already mentioned, three different types of sex-chromosome mechanism,  $X-0$ ,  $X_1X_2-0$  and  $X_1X_2X_3-0$ , are known in spiders, and among them the second type is the most usual, the first is the next and the last is the least in cases. The multiple sex-chromosome mechanism of an  $X_1X_2X_3-0$  type in spiders was first reported by SOKOLSKA (1. c.) in *Tegenaria domestica* males, and this was confirmed by REVELL (1. c.) in two species of *Tegenaria derhamii* and *Tegenaria domestica*. Quite recently HACKMAN (1. c.) announced the occurrence of this type in *T. derhamii*. By HACKMAN *T. domestica* is likely to be synonymous with *T. derhamii*. If HACKMAN's view is correct, *T. derhamii* becomes only a single case of a true  $X_1X_2X_3-0$  type.

By above three authors *T. domestica* and *T. derhamii* have three sex-chromosomes, but strange to say, the chromosome number of these species is variable by the different authors. Namely the haploid chromosome number of *T. domestica* is 21? ( $18?A + X_1X_2X_3$ ) by SOKOLSKA and 23 ( $20A + X_1X_2X_3$ ) by REVELL, that of *T. derhamii* is 23 ( $20A + X_1X_2X_3$ ) by REVELL and 19? ( $16?A + X_1X_2X_3$ ) by HACKMAN. If these two species are synonymous as HACKMAN stated, why is the chromosome number variable by the different authors?

At any rate, the demonstration of an  $X_1X_2X_3-0$  type in spiders has not been much satisfactory and more careful study should be

undertaken. We have found another case of three sex-chromosome mechanism in *Heteropoda venatoria*. In the present material the 3 sex-chromosomes have been clearly traced throughout the entire stages of spermatogenesis; moreover, the validity of this interesting multiple sex-chromosome mechanism has been verified by the direct observation of the oogonial and female somatic chromosomes.

HACKMAN (1. c.) added one doubtful case of this type in *Micrommata viridissima* belonging to the same family of the present species. Consequently the occurrence of the 3 X's mechanism is known only in one species of family Agelenidae and one definite and one doubtful case of family Sparassidae at present. All the species of both families of Agelenidae and Sparassidae studied cytologically have larger chromosome number than any species of other families of the same suborder Arachnomorphae; as to the chromosome number, above two families bear rather closer relationship to the suborder Mygalomorphae than to the suborder Arachnomorphae. (Recently SUZUKI (1. c.) reported that *Heptathela kimurai*, one species of the suborder Liphistiomorphae, has a very high number of chromosomes ( $2n=80$ )). It seems to be much interesting from the viewpoint of phylogeny, that some species of these two families are characteristic for the chromosome number and the sex-determining mechanism.

## I. On the $X_1$ -, $X_2$ - and $X_3$ -chromosomes

The sex-chromosome of spiders was researched by many workers such as MONTGOMERY ('05), BERRY ('06), WALLACE ('09), PAINTER ('14), SOKOLSKA ('25), HARD ('39), REVELL ('47), PATAU ('48), HACKMAN ('48), SUZUKI ('49, '50) and so forth. But the precise morphological analysis of sex-chromosome throughout the entire stages of mitotic and meiotic divisions was very few. The previous studies on the sex-chromosome have been based mostly on the observations during the various stages of meiosis and not on the mitotic cycles. BERRY (1. c.) found the "odd chromosome" (sex-chromosome) in *Epeira scolopetaria* (synonym of *Aranea sericata*) males. She reported that odd chromosome is a small round chromosome and exist as a single body in the spermatogonial plate. But as is well known from her figures of maturation division, her conclusion on the sex-determining mechanism was incorrect, so that we cannot always approve her descriptions on the odd chromosome in the spermatogonial plate completely. After that time PAINTER (1. c.) concluded that two sex-chromosomes arose from the two univalent spermatogonial chromosomes. He found two chromosomes of the peculiar position, size and behavior, in the spermatogonial plate and considered them as sex-chromosomes. In

this case, however, as he said himself, the identification of sex-chromosomes was not satisfactory and the exact features were remained unsolved. Recently REVELL (l. c.) stated in his paper as follows, "In spermatogonial metaphases the pairs of autosomes and two or three X's show strong somatic pairing,.....The X's can be recognized by their greater length." His description is thus too short and not a single figure was given, so that it is difficult to draw the detailed informations about them.

As fully described before, the sex-chromosomes of spiders are distinctly ascertained during meiosis, but it is difficult to identify them morphologically in the mitotic chromosomes. As pointed out, HACKMAN and SUZUKI studied numerous species of spiders and found many important results on the sex-chromosomes; in many cases, nevertheless, they failed in identifying the sex-chromosomes morphologically in the spermatogonial plates, though a single exceptional case of *Oxyopes ramosus* was described by HACKMAN. But in this case *Oxyopes ramosus* was a species of X-0 type.

*Heteropoda venatoria* has three multiple sex-chromosomes in the male and of which  $X_3$  is the largest one in the spermatogonial chromosomes, and in the excellent figures it is provided with one transverse constriction, and as a result, it assumes a distinct bipartite feature. Thus  $X_3$  is always recognized in that plate. The same is seen in the oogonial and female somatic chromosomes. This is the first case which one of the multiple sex-chromosomes was distinctly identified in the diploid chromosomes of spiders. Contrary to  $X_3$ , the identification of the rest  $X_1$  and  $X_2$  in the diploid chromosome sets is difficult. But the existence of  $X_1$  and  $X_2$  is exactly proved through the observation of chromosomes in meiosis and the comparison of diploid and haploid chromosome number. As the previous workers stated, here also both  $X_1$  and  $X_2$  are considered to be sex-chromosomes, because of their particular form, position and behavior, throughout the entire stages of the growth period of meiosis and the two maturation divisions.

In comparing  $X_1$ ,  $X_2$  and  $X_3$ , the first two bear marked similarity, but they differ evidently from the last one in many respects. The differences between 3 X's were thoroughly described before. The similarity between the  $X_1$  and  $X_2$  in the present species makes us remember the similar condition of them in an  $X_1X_2-0$  type. Concerning the relation between two X's in an  $X_1X_2-0$  type, some interpretations are found. WHITE ('40, '48), based on the results of PAINTER (l. c.) and HARD (l. c.), stated with a little question that the multiple sex-chromosomes of spiders of an  $X_1X_2-0$  type studied cytologically are homologous, because two sex-chromosomes are always equal

in length. We think, however, WHITE's view is no more than a presumption and we can not always approve his conclusion. Recently some questionable cases have been reported by some authors, for example, REVELL (1. c.) in *Tegenaria atrica*, HACKMAN (1. c.) in four species of *Philodromus* and one species of *Tibellus oblongus*, and SUZUKI (1. c.) in *Agelena opulenta*, found that in those species of an  $X_1 X_2-0$  type, two X's, i. e.  $X_1$  and  $X_2$  have unequal sizes. Here we can not state more consideration on this point; it will be fully reported by SUZUKI based on the vast materials in the near future.

### SUMMARY

1. Chromosomes in the male and female germ cells as well as in the female somatic cells of *Heteropoda venatoria* (Fam. Sparassidae) were studied.

2. In the male the chromosome number is 41 in diploid and 22 in haploid. That of the female is 44 in diploid.

3. The diploid chromosomes contain three sex-chromosomes,  $X_1$ ,  $X_2$  and  $X_3$  in the male, and two sets of them,  $2X_1$ ,  $2X_2$  and  $2X_3$  in the female, and consequently the male is heterogametic.

4. The spermatogonial, oogonial and female somatic chromosomes are essentially rod-shaped and acrocentric.

5.  $X_1$  is the largest one in the diploid chromosome sets and is provided with one transverse constriction, so that it assumes a distinct bipartite feature, and as a result, it is always distinctly identified in the spermatogonial plate. The same is seen in the oogonial and female somatic chromosomes also.

6.  $X_1$  and  $X_2$  can not be detected in both gonial plates and somatic mitosis, due to the failure of the distinct morphological feature, but they are clearly distinguished by the peculiar behavior, arrangement and morphological character, during all the stages of meiosis.

7. All the three sex-chromosomes show marked heteropycnosis in the course of growth period of meiosis, during that stage they are distinctly traced.

8. At the primary spermatocytic metaphase, 3 X's lie close to one another, side by side, outside the autosome circle, and can be identified beyond dispute. In the lateral aspect, they stand parallel to the axis of spindle, but as to the arrangement some irregularities are observed; among them, particularly  $X_3$  lies apart distantly from the  $X_1 X_2$  group.

9. In the first division 3 X's pass over undivided into one pole, and as a result, two kinds of secondary spermatocytes are formed,

in respect to the sex-chromosomes.

10. All the autosome tetrads in the primary spermatocytes are of bi-terminal spindle attachment and are transverse rod-tetrads.

11. Throughout the entire stages of spermatogenesis  $X_1$  and  $X_2$  show marked similarity in many characters and are considered more or less to be homologous, but  $X_n$  bears marked difference from them.

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## EXPLANATION OF PLATES

## PLATE I

- Figs. 1-4. Spermatogonial chromosomes, polar view, showing the diploid number (41) of chromosomes. (CAROTHER's solution)
- Fig. 5 a-b.  $X_3$ -chromosomes taken from the spermatogonial chromosomes in the prometaphase. (CAROTHER's solution)
- Fig. 6 a-f. Various shapes of  $X_3$ -chromosomes taken from the spermatogonial chromosomes in the metaphase. d, e, f correspond to  $X_3$ 's in Figs. 3, 2, 1, respectively. (CAROTHER's solution)
- Fig. 7. Oogonial metaphase, polar view, showing the diploid number (44) of chromosomes. (Modified BENSLEY's solution)
- Fig. 8. The polar view of the equatorial plate of the epithelial cell of the female. 44 chromosomes are seen. (Modified BENSLEY's solution)
- Figs. 9-10. Two sets of the spermatogonial chromosomes arranged into pairs according to size. 9, 10 corresponding to Figs. 1, 4, respectively.
- Fig. 11. Paired arrangement of homologous mates from oogonial chromosomes in a descending order. The figure corresponds to Fig. 7.

## PLATE II

- Figs. 12-20. Primary spermatocyte stages of growth period. (12, 13, 14, 16, 17, 19: Modified NAVASHIN's solution; 15 a-e, 18 a-d: CAROTHER's solution)
- Fig. 12. Leptotene stage showing heteropycnotic mass of sex-chromosomes.
- Fig. 13. Zygotene stage showing the early appearance of three sex-chromosomes.  $X_1$ ,  $X_2$ ,  $X_3$ ; three multiple sex-chromosomes.
- Fig. 14. Pachytene stage.  $X_3$  is clearly distinguished from  $X_1$ ,  $X_2$ -chromosomes.
- Fig. 15 a-e. Various shapes and arrangements of 3 X's at pachytene.
- Fig. 16. Early diplotene stage showing the condensation of 3 X's.
- Fig. 17. Still later stage.
- Fig. 18. a-d. Successive process of the development of 3 X's during diplotene.
- Fig. 19. 3 X's in the later diplotene stage.
- Fig. 20. Diakinesis stage showing the elongation of 3 X's.
- Figs. 21-22. Spermiogenic stages. (Modified NAVASHIN's solution). Fig. 21 with, and Fig. 22 without a heteropycnotic mass of 3 X's.

## PLATE III

- Figs. 23-27. Primary spermatocytes, polar view of metaphase plates, with three sex-chromosomes. Note the difference in size between  $X_1$ ,  $X_2$  and  $X_3$ .  $X_1$ ,  $X_2$ ,  $X_3$ : three multiple sex-chromosomes. (Figs. 23-25: Modified BENSLEY's solution; Figs. 26-27: Modified NAVASHIN's solution)
- Fig. 28. Chromosomes of the primary spermatocyte in side view, showing three sex-chromosomes. (Modified NAVASHIN's solution)
- Figs. 29-31. Chromosomes in the anaphase of the primary spermatocyte division, showing the precocious segregation of 3 X's. (Fig. 29: Modified BENSLEY's solution; Figs. 30-31: Modified NAVASHIN's solution)
- Fig. 32. Chromosomes in the telophase of the primary spermatocyte division. (Modified NAVASHIN's solution)
- Figs. 33-34. Polar view of chromosomes of the secondary spermatocyte metaphase showing 22 dyads. (Modified NAVASHIN's solution)
- Figs. 35-36. The same showing 19 dyads. (Modified NAVASHIN's solution)





