CHROMOSOME STUDIES IN THE MYRIAPODA V. A CHROMOSOMAL SURVEY IN SOME CHILOPODS WITH A CYTO-TAXONOMIC CONSIDERATION

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The Myriapoda is a group of arthropods in which the chromosome survey has progressed much less than in other groups as Insecta, Crustacea and Arachnida, and despite their world wide distribution, our knowledge of their chromosomes has remained incomplete. The literature pertaining to the chromosomes of Myriapoda is filled with discrepancies. Earlier accounts published by Blackmann (1903, 1905, 1907, 1910), Medes (1905), Oettinger (1908) and Bouin (1920, 1922, 1925) gave no sufficient evidence appreciable at present (see the chromosome-list by Makino, 1950). No accurate and clear-cut evidence has been presented for the morphology of chromosomes and the sex chromosome mechanism in any of these earlier investigations. Only the paper lately published by Makino and Niiyama (1942) concerning *Scolopendra damnosa* is remarkable and highly appreciable in giving an accurate account on the morphology of chromosomes. In view of this fact, further study of chromosomes in this field is highly desirable. During recent years, the present author has engaged in a comparative study of the chromosomes of Myriapoda to contribute something in this unexplored field of cytology, especially with reference to the taxonomical significance of cytological characters (Ogawa 1950a, 1950b, 1951a, 1951b, 1951c). The present paper deals with the chromosomes of eight forms of the Chilopoda, with a general discussion of some cytological characters and their bearing on taxonomical relationships in this group.

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Material and method: The present investigation has been undertaken with eight species of chilopods which cover three families, as shown in Table 1. The chromosomes were studied exclusively with the testicular material. The animals coming under study were collected in both Shizuoka and Aichi Prefectures; the greater part was obtained in the city of Hamamatsu and its suburbs. Immediately after their collection in the fields they were dissected alive and the gonads together with digestive canals were put into the fixative. For the fixatives, modified Navashin's fluid, Carother's fluid and Bensley's

solution were employed with satisfactory results. The slides were prepared through the usual paraffin technique and stained with Heidenhain's iron-haematoxylin.

Observations

Scolopendromorpha—Scolopendridae

1). *Scolopendra subspinipes mutilans* L. Koch: The chromosomes were observed in both spermatogonia and spermatocytes. Chromosome counting revealed that the number of chromosomes was 28 in diploid (Fig. 1) and 14 in haploid. Considered from the external feature, the chromosomes seem to be of simple rod-type, and show a little variation in size. They arrange in a radial manner in the metaphase equatorial plate. The observations carefully carried out through both mitotic and meiotic divisions failed to demonstrate any special chromosomes which can be regarded as the sex chromosome, either in morphological characters or in behavior. Thus the chromosomes of the present species are nearly similar to those of *Scolopendra damnosa* reported by Makino and Niiyama (1942), not only in the number but also in other morphological features.

2). *Scolopendra subspinipes japonica* L. Koch: Careful examination of several metaphase plates of both spermatogonia and spermatocytes showed that there are 18 chromosomes in diploid (Fig. 2) and 9 in haploid. It is surprising that the present species shows a much lower number of chromosomes than the former species, in spite of the taxonomical kinship between them. It is a noticeable fact that the diploid complement of this species is characterized by containing 10 dumb-bell-shaped elements together with 8 simple rod-shaped ones (Fig. 2). This evidence is favorable for the interpretation of the numerical relationship of chromosomes in these species. Some

Figs. 1-16. Chromosomes of 8 forms of Chilopoda. All are metaphase polar views of diploid groups. $\times 3000$. 1, *Scolopendra subspinipes mutilans*. Spermatogonium. 2, *Scolopendra subspinipes japonica*. Spermatogonium. 3-4, *Otocryptops rubiginosus*. Spermatogonia. 5-6, *Otocryptops* sp. Spermatogonia. 7-8, *Bothriopolys asperatus*. Spermatogonia. 9-10, *Esastigmatobius longitarsis*. Somatic metaphases male digestive canal. 11-12, *Thereuonema hilgendorfi*. Spermatogonia. 13, *Thereuonema hilgendorfi*. Somatic metaphases in female digestive canal. 14-15, *Thereuopoda clunifera*. Spermatogonia. 16, *Thereuopoda clunifera*. Oogonium.

mention will be made later on this respect.

Cryptopidae

3). *Otocryptops rubiginosus* (L. Koch): The chromosomes of spermatogonia alone came under study in this species, due to the unfavorable season of collection. The number of chromosomes was determined as 27 (Figs. 3-4) without any slight doubt. Most of the chromosomes appear as condensed rod-type, showing a slight variation in length, and a few larger ones assume dumb-bell-shape.

The odd number of chromosomes naturally suggests the presence of an unpaired heteromorphic chromosome. At present, it is difficult to demonstrate the heteromorphic element at this stage with certainty. For its final determination, the thorough study of both mitotic and meiotic divisions in future is required.

4). *Otocryptops* sp: Through the observations of both spermatogonial and meiotic divisions, the present species was found to possess the diploid number of 22 (Figs. 5-6) and the haploid number of 11. By reference to the spermatogonial metaphases (Figs. 5-6), it is evident that the chromosomes of this species are more slender and longer than those of the former species (*Otocryptops rubiginosus*). From their superficial configuration, they seem to be of telomitic nature. There is no evidence for the presence of particular chromosome which is peculiar in nature.

Lithobiomorpha—Lithobiidae

5). *Bothropolys asperatus* (L. Koch): Based on the observations of both spermatogonial and spermatocyte divisions, it became clear that the present species has 33 chromosomes in diploid (Figs. 7-8) and 19 in haploid. The diploid complement is characterized by containing one pair of V-shaped elements of medium-size, one pair of large J-shaped ones and the remaining rod-shaped ones of varying sizes which range in shape from long rod-shaped ones to minute spheroidal bodies. They are arranged in radial manner in the equatorial plate. No particular element can be detected either in the morphological structure or in meiotic behavior.

Henicopidae

6). *Esastigmatobius longitarsis* Verhoeff: The male diploid number of chromosomes was determined as 20 (Figs. 9-10) in some somatic metaphases observed in the digestive canal, and the haploid number of 10 was obtained in both the primary and secondary spermatocytes. The present species is characterized by having unusual karyotype, in striking contrast to those of the former species. As seen in Figs. 9-10, the diploid complex comprises two remarkably huge elements showing undulation; they lie always at the central position of the equatorial plate and are nearly equal in size. The remaining 18 chromosomes are all of minute spheroidal shape having very slight size-difference, and scatter surrounding the huge ones. Thus, on account of their prominent huge size, the former two elongated elements show a marked distinction from the latter minute elements. In the course of separation at anaphase, the huge elongated elements longitudinally split into identical halves and pass to opposite poles, holding their long axis

parallel to the equatorial plate. This evidence suggests the occurrence of diffused kinetochores in these chromosomes. Thus, the present species is very remarkable among others by its unique karyotype containing unusual chromosomal elements, and by the occurrence of the diffused kinetochore.

Scutigeromorpha—Scutigeridae

7) *Thereuonema hilgendorfi* Verhoeff: The male diploid chromosomes, 36 in number, were observed in the spermatogonial division, and 18 haploid chromosomes were found in both the primary and secondary divisions. Further the female diploid number of chromosomes was proved to be 36 through the observations of both oogonial and somatic division in the digestive canal (Fig. 13). The karyotype of this species closely resemble that of the former species; namely, the diploid complement consists of two elongated huge elements and the remaining 34 minute spheroidal ones showing slight size variation. The demarcation between the former and latter elements is very striking. The huge elements always occupy the central part of the equatorial plate, surrounded by the minute ones.

It is of interest that this species represents the sexual difference regarding the huge chromosomes. The morphological comparison of the chromosomes between the two sexes revealed that the male cell shows the XY mechanism of the sex chromosomes. The sex chromosomes are represented by two elongated huge elements. In the male cell they are unequal in size, thus indicating the XY complex, whereas they are identical in size in the female cell showing the XX complex. In the first meiotic division the X and Y conjugate end-to-end forming the XY complex. Then a longitudinal splitting appears in the XY complex along the long axis of both X and Y. The separation of the XY complex is taken place at anaphase along the longitudinal splitting, thus giving rise to two identical bodies, each of which consists of the X and Y. Hence, the first division is unusually equational for the XY complex. The XY complex disjoins in the second meiotic division, the X and Y passing to the opposite poles. Finally the post-reductional separation of the sex chromosomes is characteristic of this species. The preliminary notes of the sex chromosomes were published in one of the former papers (Ogawa 1950b), and further detailed accounts will be given elsewhere.

8). *Thereuopoda clunifera* (Wood): The chromosomes of the male and female were observed in germ-cells, and it was made clear that the diploid number is 36 in both sexes (Figs. 14, 15, 16). The haploid number was found to be 18 in the male meiotic divisions. The karyotype of this species is nearly resemblant to those of the foregoing two species. That is to say, the diploid set of this species comprises also two elongated huge elements together with the remaining 34 minute spheroidal ones with a slight variation of size.

The present species is also characterized by having the X and Y chromosomes in the male. The X and Y elements are represented by the elongated huge chromosomes; they differ in size in the male cell, while in the female cell they are identical in size. Thus the XY-XX sex-mechanism is clearly established in this form. The size-difference between the X and Y elements is smaller in this species than in *Thereuonema hilgendorfi*. Detailed accounts will be described elsewhere.

Remarks

The taxonomical studies of the Chilopoda of Japan are referable to some papers published by Takakuwa (1940, 1941, 1943) and Takashima (1949). According to them the chilopods are covered by 4 orders including 9 families and 25 genera. The species studied by the present author are taxonomically arranged in Table 1. On the basis of the present investigation, some remarks will be given in the following, with special regard to the bearing of cytological characters upon taxonomy.

Scolopendra subspinipes mutilans ($2n$, 28) and *Scolopendra subspinipes japonica* ($2n$, 18): It is remarkable that these two related species show a highly different chromosome number. The former species possesses 10 chromosomes more than the latter. The chromosomes of the former species are all characterized by the simple rod-shape, while the diploid complement of the latter species contains 10 chromosomes (in 5 pairs) assuming a distinct dumb-bell-structure. The question of the numerical difference of chromosomes between these two related species will be well answered if the dumb-bell-shaped elements found in *Scolopendra subspinipes japonica* be taken as multiples, each being made up of two elements due to an end-to-end connection resulting in dumb-bell-structure.

Otocryptops rubiginosus ($2n$, 37) and *Otocryptops* sp. ($2n$, 22): The two species here considered are closely related forms belonging to the same genus of Cryptopidae. Despite their taxonomical kinship, the chromosomes of these two species are surprisingly dissimilar, not only in number but also in other morphological respects. The chromosomes of *Otocryptops rubiginosus* are condensed rod-shaped, while those of *Otocryptops* sp. assume slender elongated form. From the cytological point of view, these data seem to suggest a wrong taxonomic association of these two species.

Bothropolys asperatus ($2n$, 38) and *Esastigmatobius longitarsis* ($2n$, 20): These two forms belong to the same order (Lithobiomorpha), but to the different family, the former species belonging to the Lithobiidae, while the latter is of the Henicopidae. These two species are chromosomally different in a considerable degree. The former species has larger number of chromosomes than the latter. Further difference follows in that the chromosomes of *Bothropolys asperatus* show an usual radial arrangement at metaphase, whereas those of *Esastigmatobius longitarsis* show a circular metaphase arrangement. Considered from the behavior of the chromosomes in the course of division, the chromosomes of *Esastigmatobius longitarsis* seem to carry diffused kinetochores.

Thereuonema hilgendorfi ($2n$, 36) and *Thereuopoda clunifera* ($2n$, 36): These two species are regarded to be closely related in the family Scutigerae of Scutigeraomorpha, since they have the same number of chromosomes which show a circular metaphase arrangement. Thus, the chromosomes seem to carry diffused kinetochores in both species. Furthermore, they are characterized by having an X-Y mechanism of the sex-chromosomes which undergo the post-reductional separation. Only difference by which the two species are distinguishable lies in the size of the sex-chromosomes; the size-difference between the X and Y is larger in *Thereuonema hilgendorfi* than in *Thereuopoda clunifera*.

So far as the data at hand are concerned, it can be said that all the species belonging to the Scolopendromorpha here under study uniformly show an usual arrangement, whereas the species belonging to both Lithobiomorpha and Scutigermorpha can be classified into two groups as regard to the metaphase arrangement of chromosomes; the one is represented by *Bothropolys asperatus* of the Lithobiidae (Lithobiomorpha) showing a radial arrangement, and to the other group belong *Esastigmatobius longitarsis* (Henicopidae; Lithobiomorpha), *Thereuonema hilgendorfi* and *Thereuopoda clunifera* (Scutigeridae; Scutigermorpha) which are characterized by a circular arrangement of chromosomes. For further statement a wider cytological sampling of the included species should be necessary.

Summary

The chromosomes of eight forms of the Chilopoda were comparatively investigated in this study, especially with reference to the relationship of the cytological characters upon taxonomy. The taxonomic arrangement of the studied species and their chromosome numbers here established are shown in Table 1.

Table 1.

Species	Chrom.-number		Composition of karyotype	Metaphase arrangement of chroms.
	2n	n		
SCOLOPENDROMORPHA				
Scolopendridae				
1. <i>Scolopendra subspinipes mutilans</i>	28s	14♂ (I, II)	Isomorphic composition. Sex-chrom. unknown.	Radial arrangement.
2. <i>S. s. japonica</i>	18s	9♂ (I, II)	Isomorphic or dimorphic composition. Sex-chrom. unknown.	"
Cryptopidae				
3. <i>Otocyrtops rubiginosus</i>	27s		Prob. isomorphic composition. Unpaired X-chrom. is supposed.	"
4. <i>O. sp.</i>	22s	11♂ (I, II)	Isomorphic composition. Sex-chrom unknown.	"
LITHOBIOMORPHA				
Lithobiidae				
5. <i>Bothropolys asperatus</i>	38s	19♂ (I, II)	Polymorphic composition. Sex chrom. unknown.	"
Henicopidae				
6. <i>Esastigmatobius longitarsis</i>	20m	10♂ (I, II)	Dimorphic composition. Sex-chrom. unknown.	Circular arrangement.
SCUTIGEROMORPHA				
Scutigeridae				
7. <i>Thereuonema hilgendorfi</i>	36s, o, m	18 (I, II)	Dimorphic composition. X-Y sex-chrom. occur.	"
8. <i>Thereuopoda clunifera</i>	36s, o	18♂ (I, II)	"	"

The chromosomal relationship between *Scolopendra subspinipes mutilans* and *S. s.*

japonica was interpreted on the basis of both the morphology and the number of the chromosomes.

Though *Otocryptopus rubiginosus* and *O. sp.* are closely related belonging to the same genus, the chromosomes of these two species failed to show any morphological kinship.

The chromosomes of *Bothropolys asperatus* and *Esastigmatobius longitarsis* belonging to the Lithobiomorpha showed a remarkable difference not only in the number of chromosomes but also in other morphological features.

Thereuonema hilgendorfi and *Thereuopoda clunifera* were proved to show similar chromosomal feature, being characterized by having the XY-XX sex-chromosome mechanism.

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