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**Notes on the Chromosomes of the Pangolin,
Manis pentadactyla (Edentata)¹⁾**

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(With 7 Text-figures)

At the present time, one of the dominant interests underlying mammalian chromosome studies concerns the chromosome mechanism in connection with the evolutionary changes shown in the several orders of mammals. Few zoologists doubt at present that the eutherian mammals have arisen from a common stem. This being the case, the knowledge about the chromosomes in the primitive orders of the eutherian mammals, such as in the Edentata and Sirenia, is interesting and important for the theoretical consideration associated with evolution. But, our cytological knowledge of these primitive mammals is very meagre or has remained deficient. Because these mammals are generally rare in occurrence and at the same time very restricted in their distribution, their chromosome survey has progressed less than in other orders. The literature pertaining to the chromosomes of lower eutherian mammals refers only to two classical papers which dealt with the chromosomes of the armadillo, a remarkable species of the order Edentata (cf. the list published by Makino, 1951). Newman and Patterson (1910) and Newman (1912) recorded the approximate number as 31-32 in diploid and 10-14 in haploid in the nine-banded armadillo (*Tatusia novemvinctum*) reporting an XO chromosome mechanism. Working probably with the same species, Painter (1925) reported an approximate chromosome number of 60 for the diploid complex in the observation of amnion cells. Thus there is a considerable disagreement in the reported cases and therefore a thorough exploration of the chromosomes of these mammals is highly desirable.

The present paper deals with the chromosomes of the pangolin *Manis pentadactyla* (L.), belonging to the Manidae of the Edentata. This is a rare mammal found in the mountainous regions of Formosa. The testes which were removed

1) Contribution No. 261 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

from a single male obtained in Formosa and fixed in Flemming's solution with a trace of acetic acid provided the material for the present study. Because of the unfavourable season for collection, the material was not available for a thorough study of chromosomes throughout spermatogenesis. But in view of the unsatisfactory status of the chromosome survey in this order, the publication of even such a fragmentary account seems justified.

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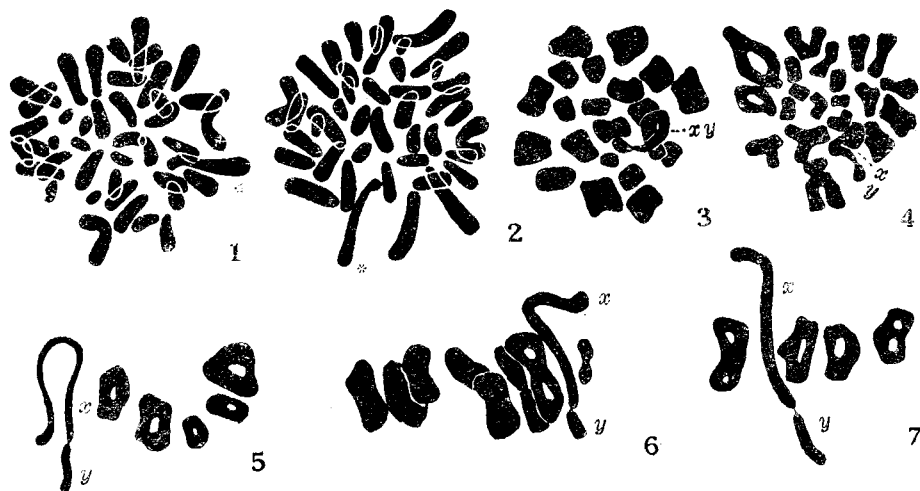
The material was fixed and embedded in paraffin by the senior author in Formosa (S.T.), and then was sent to the junior author (S.M.) for further work. The sections were prepared by means of the iron-haematoxylin method after Heidenhain. The cytological work on the chromosomes together with the arrangement of the data in the manuscript was done by the junior author (S.M.).

Observations

The material under observation provides a sufficient number of dividing spermatogonial cells to allow a close investigation of chromosomes. Careful counting of several metaphase plates of the spermatogonia gave 42 as the diploid number in every case. Examples are shown in Figures 1 and 2. The diploid complex is remarkable not only for its comparatively low number but also for the fact that the majority of the chromosomes are rod-shaped. The constituent members of the diploid complement are clearly shown in the alignment of the supposed homologous pairs in a serial order (Fig. 8). Close examination reveals that the complement contains few chromosomes of striking shape and size. Two chromosomes are very prominent because of the remarkable V-shape of the submedian structure. They evidently constitute a homologous pair because of their similar size. The remaining chromosomes seem to be rod-shaped from their external appearance, varying in size. Among them, however, there is a strikingly elongated element which is in fair contrast to the others due to its remarkable feature. It is the largest of all and carries a conspicuous globular part at the proximal end of the main body thus displaying a clear J-shape. It is thus evident from its external appearance that it is not simple rod-shaped but to be regarded as a subterminal chromosome. Furthermore, on account of its solitary and outstanding occurrence in the diploid complex, it is undoubtedly the one corresponding to the X-chromosome. The even number of chromosomes herein established naturally suggests the occurrence of an XY-pair of sex chromosomes, as is the general rule in the mammals. The identification of the Y chromosome, however, presents some difficulty, due to the abundance of the chromosomes of similar size. To judge from the morphology of the sex chromosome pair as disclosed in the first meiotic division, the Y element is probably represented by a little larger element than the members constituting

the smallest autosomal pair.

The chromosomes found in the first meiotic metaphase are observable with extreme clearness due to the reduced number of elements. The chromosomes were counted therefore with certainty and this gives final confirmation of the diploid number obtained in the spermatogonia. Every metaphase plate under study showed consistently 21 chromosomes (Figs. 3-4). The chromosomes are all bivalent in structure. The haploid complex consists of 20 autosomal bivalents, each being made up of two equivalent components showing an ordinary structure and an XY bivalent.



Figs. 1-7. Chromosomes of *Manis pentadactyla*. Figs. 1-2, spermatogonial metaphases, 42 chromosomes in each. The element marked with an asterisk indicates the X chromosome, the Y being not identified. Figs. 3-4, primary spermatocyte metaphases, 21 elements in each. Figs. 5-7, profile views of the first meiotic spindles, showing the XY-complex. 5, prometaphase. 6-7, metaphases. $\times 3800$.

The XY bivalent is readily distinguishable from the autosomal bivalents by having a strikingly heteromorphic conformation. It generally occupies the peripheral position together with the larger autosomal elements in the equatorial arrangement. It consists of the J-shaped X element of larger size with slender outline and the Y element of small size. The latter is attached to the extremity of the long arm of the X. At metaphase, the X is placed so that its longer arm connected with the Y at its extremity is vertical to the equatorial plate, while the shorter arm lies in most cases parallel to the equatorial plate (Figs. 5-7). To judge from the structural configuration, the spindle fibers of the X seem to attach to the point where the long arm and short arm join, while in the Y chromosome they apparently attach to its free end. The whole configuration displayed by the XY

bivalent, as outlined in the above description, is clearly shown in the side view of the metaphase spindle of the first meiotic division (Figs. 5-7). Thus, the X is distinctly characterized by a submedian structure and to its longer arm the Y element comes in contact. In all probability, during the division of the autosomal elements, the X also disjoins from the Y. As a result of the first meiotic division, there are to be produced two kinds of secondary spermatocytes, the one having the X element and the other the Y.



Fig. 8. Serially aligned arrangement of the paired chromosomes.

Remarks

The present authors were surprised at finding that the chromosome complex of the pangolin (*Manis pentadactyla*) here under study is highly different from that of the armadillo (*Tatusia novemcinctum*), whilst they had expected the the karyotypes of these two animals would show a close approach to each other, owing to the taxonomical kinship existing between them. According to Painter (1925), the number of chromosomes of the armadillo shows 60 in the diploid set. Referring to the figures presented in his paper (Figs. 16-17 of Painter 1925), it is apparent that the constituent elements of 60 chromosomes of the armadillo are all of the rod-type. If one compares the karyotype of the pangolin herein studied with that of the armadillo just mentioned, the dissimilarity is at once apparent. The numerical and morphological differences of the chromosome complements involved are too great to be accounted for on the basis of the formation of multiple chromosomes by fusion of rod-elements, two by two, into V-shaped ones.

The karyotype of the pangolin is rather simple in structure among those of the eutherian mammals so far studied, since, excepting one pair of V-shaped autosomes and the J-shaped X-element, the chromosomes are all simple rod-shaped. Rough comparison of the karyotypes carried out in the eutherian mammals shows some resemblance of karyotype between the pangolin and certain of the murid rodents and of the insectivores.

Painter (1925) counted the chromosome number in 10 species of eutherian mammals covering 7 different orders, and emphasized that the chromosome number of 48 seems to be the typical and is probably the basal number for the mammals. During the past years, the present junior author (S.M.) has engaged in a comparative study of mammalian chromosomes, extending his survey to 49 species which represent 1 species of the Edentata, 2 species of Chiroptera, 25 species of the Rodentia, 7 species of the Carnivora, 8 species of the Artiodactyla, 3 species of the Perissodactyla, 1 species of the Cetacea and 2 species of the Primates. The

results of these studies indicate that there is a striking diversity in the number of chromosomes of the species studied, ranging from 30 to 78, and further, the chromosome number of 48 was proved to exist in only four out of 42 species studied. Thus the occurrence of 48 chromosomes is very rare in mammals and can no longer be regarded as the basal number. The same conclusion was reached by Matthey (1948) in an extensive survey of the mammalian chromosomes.

No accurate and clear-cut evidence has ever been demonstrated for the sex chromosome mechanism of the Edentata. Painter (1925) working with the armadillo, was unable to observe the sex chromosomes of this species. The evidence presented in the present study clearly showed that the XY mechanism of sex determination occurs in the pangolin. The X is represented by the large J-shaped element and the Y by one of the small rod-shaped ones. At metaphase, the X is so placed that its longer arm connected with the Y at its extremity is vertical to the equatorial plate. Similar configuration of the XY-complex as seen in the pangolin has been found to occur in the Okinawa fruit bat (*Pteropus dasymallus inopinatus*) and the nutria (*Myocastor coypus*) (Makino 1947, 1948).

Summary

The chromosomes of the pangolin (*Manis pentadactyla*) were investigated in male germ cells. The diploid number was 42 in the spermatogonia and the haploid number was 21 in the spermatocytes. Sex chromosomes of the typical XY-type were found to occur in this species. The X element is represented by the largest element assuming the J-shape, while the Y is an element a little longer than the members forming the smallest autosomal pair.

The karyotype of this species is rather simple in structure among those of the eutherian mammals so far sampled, since, excepting one pair of V-shaped autosomes and the J-shaped X-element, the chromosomes are all simple rod-shaped.

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