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The Chromosomes of the Sea Turtle, *Chelonia japonica*, with Evidence of Female Heterogamety[1]

With 12 Text-figures

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Over a period of many years, chromosome research in relation to sex determination has been a subject of intense interest in the field of animal cytology. In the higher vertebrates the sex-chromosome mechanism has been fully established on a sufficient cyto-genetic basis in these years (see Makino's list, 1951). In mammals it is quite beyond discussion that the male is heterogametic. The existence of female heterogamety in birds has been built up as a well-established fact on both cytological and genetic evidence. Thanks to the extensive studies of Oguma (1934, 1937), it was made clear that in reptiles, the heterogametic condition occurs in the female sex as in birds, from the discovery of differences of the chromosome constitutions by sexes in both the lizard (*Lacerta vivipara*) and the soft-shelled turtle (*Amyda japonica*). The results of Oguma's studies are, moreover, of importance in proving the close relationship in phylogeny between birds and reptiles from the cyto-genetic stand-point. However, less progress has so far been made in this field of study in the reptiles than in the birds. Recently Nakamura (1937, 1949) confirmed female heterogamety in the sea turtle, *Caretta caretta olivacea*, by finding an unpaired heteromorphic chromosome in the female cell. Encouraged by the results of these studies, the present author and Asana (1948) made a comparative

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study of the chromosomes in both sexes in the agamid lizards, *Calotes versicolor* and *Sitana ponticeriana*, and obtained a similar result. In striking contrast to these reported evidences, Matthey (1943) and Margot (1946), one of his followers, reported a surprising fact showing that in *Chamaeleon vulgaris*, *Anguis fragilis* and *Lacerta vivipara* the chromosomes exhibit numerical difference by sexes. The present investigation concerning *Chelonia japonica*,[1] the data from which constitute this paper, yielded further evidence of a heterogametic condition in the female sex by the finding of an unpaired sex chromosome.

The author sincerely expresses deep gratitude to Emeritus Professor Kan Oguma, now the Director of the National Institute of Genetics at Mishima, for his valuable criticism.

MATERIAL AND METHOD

Chelonia japonica on which the present study was carried out, is a kind of sea turtle belonging to the Chelonidae, being very common in tropical waters. These sea turtles are abundantly captured in the coastal waters around the Bonin Islands during the summer time, when they gather for spawning. The female lays eggs, over one hundred in number, in the sand of the shore from July to August, and the embryonic development is said to be completed in 60–70 days. The material for the present study was obtained by the author at Chichi-jima, one of the Bonin Islands, when he visited there in August 1936 for the collection of some cytological material.

The material consists of both adult and embryonic gonads. The chromosomes of the male were studied in both adult and embryonic testes, those of the female in the embryonic material alone. The embryonic gonads were obtained by dissecting the embryos out of developing eggs of various ages, at 30, 40, 50 and 60 days after spawning. As the fixative, Hermann's mixture with reduced acetic acid was exclusively employed with satisfactory results. The microscopical observations were carried out on the usual paraffin sections stained by the iron-haematoxylin method according to Heidenhain.

In histological structure, the embryonic gonads exhibit a clear differentiation into the ovary and the testis. The embryonic ovary is easily distinguishable from the testis by having a thick and elongated cortical layer of primordial germ cells which show active proliferation. The testis is a little shorter in length but much thicker than the ovary

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[1] Concerning the chromosomes of *Chelonia japonica*, there is a brief reference by Oguma (1942) in an address given before the 49th Annual Meeting of the Anatomical Society of Japan at Tokyo, April 1942.

of the same age. The testis is further characterized by showing the primordial germ cells which develop as solid cords of irregular shape towards the medullary region of the gonads. Thus the structural features of the embryonic gonads are quite identical to those described by Oguma (1937) for *Amyda japonica*. The sexes of the embryos can therefore be clearly distinguished from an examination of the histological characters of the respective gonads.

THE CHROMOSOMES OF THE MALE

The male diploid chromosomes were investigated in germ cells in both adult and embryonic testes. Study of the spermatogonial division made it clear that the diploid complement of the male sex contains 56 chromosomes (Figs. 1–3). The same number of chromosomes was observed as an additional evidence, to occur in the dividing somatic cells. The components of the diploid complement were found to fall into two different groups according to their size and shape. One group comprises the chromosomes assuming a distinct V-shape of varying sizes, being 14 in number. They appear to show submedian spindle fiber

Figs. 1–6. Chromosomes of the male germ cells. 1, 2, spermatogonial metaphases from embryonic testes. 3, the same from adult testis. 56 chromosomes in each. 4, 5, primary spermatocyte metaphases, 28 bivalents in each. 6, secondary spermatocyte metaphase, showing 28 elements.

attachment, the lengths of the arms forming the V's being dissimilar. Among them two pairs are very prominent on account of their size, which is markedly larger than the others. The other group consists of simple rod-shaped chromosomes; they are 42 in number and vary in shape from elongated rods to minute spheroidal bodies. Four pairs among them are clearly distinguished from the others by their remarkably elongated form, sometimes bent or undulated. All the rod-shaped elements taper toward their inner ends, except for the longest ones, each of which carries a globular part at the proximal end. In view of their external form it appears that the point of the spindle fiber attachment is probably terminal in them.

Morphological analysis based on the mating up cf the supposedly homologous chromosomes by means of a comparison of their shape and size, reveals that the male diploid complement consist of 28 distinct pairs of homologous elements, comprising 7 homologous pairs of V-shaped chromosomes and 21 homologous pairs of rod-shaped ones. There is no element left unpaired. An example of the alignmental arrangement thus prepared is shown in Figure 11.

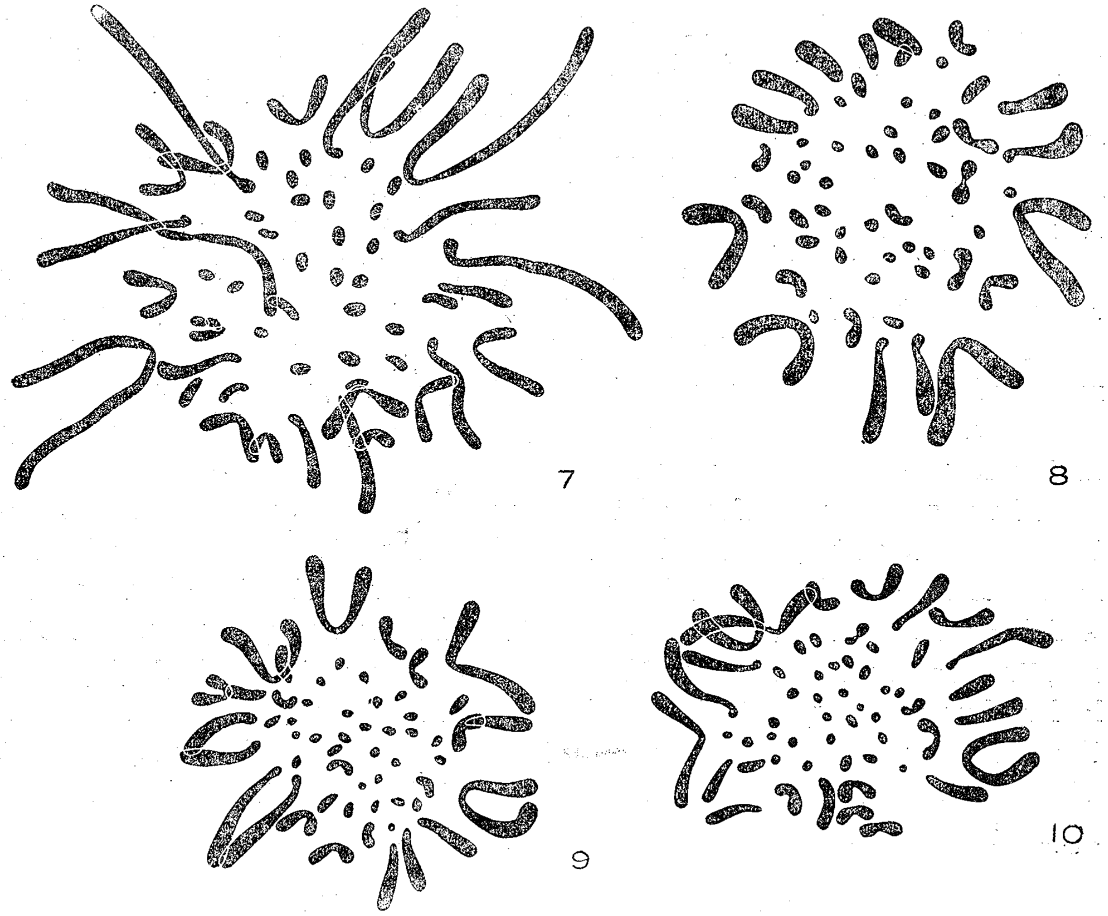
The chromosome counts made in the haploid condition including both the primary and secondary spermatocytes consistently gave the reduced number as 28 (Figs. 4–6). By this observation, the diploid number of 56 formerly established is finally confirmed. The chromosomes of the primary spermatocyte are all ordinary bivalents in structure, and there is no element which assumes a heteromorphic configuration (Figs. 4–5).

The evidence here obtained is thus sufficient to indicate that the male is homogametic as regards the sex chromosomes, although the conclusive identification of the sex chromosomes is difficult from a study of the male chromosomes alone.

Here, some comparison of the chromosomes may be pertinent between the present species and its related species, *Caretta caretta olivacea*. Nakamura (1949) in the male cell of *Caretta* reported 58 chromosomes which consist of 10 V-shaped elements and 48 rod-shaped ones. *Caretta caretta olivacea* bears a considerable resemblance to *Chelonia japonica* in its chromosomes, but the numerical and morphological differences of the chromosome complements here involved are such that they can not 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 CHROMOSOMES OF THE FEMALE

The observations on the female chromosomes were made exclusively in the ovaries from embryonic specimens. The oogonial metaphases studied in many dividing cells consistently showed the diploid number as 55 in the female cell, as seen in Figures 7–9. The same number of chromosomes was confirmed again in some somatic cells (Fig. 10). The female cells thus contain one chromosome less in total number than do the male cells in which 56 chromosomes were found. The odd number of chromosomes in the female cell naturally suggests the existence of an unpaired element. To identify the unpaired chromosome, a morphological analysis of the chromosomes was made by comparing their shape and size. The female diploid complement is here also grouped into two sorts, of V-shaped and rod-shaped chromosomes, as was the case in the male cell. The V-shaped chromosomes are again 14 in number, and are quite identical in form with the corresponding ones of the male cell. There are here again 7 homologous pairs of submedian chromosomes, two of which are very outstanding for their large size. Evidently, there are neither an unpaired element nor a hetero-



Figs. 7–10. Chromosomes of the female cells. 7–9, oogonial metaphases from embryonic ovaries. 55 chromosomes in each. 10, somatic metaphase from the epithelial cell of the nephric tubule of the embryo, showing 55 elements.

morphic pair in the group consisting of the V-shaped chromosomes. Thus the V-shaped chromosomes exhibit no difference in the two sexes.

In contrast with the V-shaped, the number of rod-shaped chromosomes is odd, being 41. This odd number obviously indicates the presence among them of an unpaired element which remains without a homologous mate. The paired alignment of chromosomes, arranged according to their size and shape, made it clear that the chromosome marked with x is destitute of its appropriate partner (Fig. 12). It is therefore this chromosome which is responsible for the numerical difference between the chromosome numbers of the two sexes. Thus the sexual difference of chromosomes is attributable to the fact that the chromosome marked with x is unpaired in the female, whereas it is in a paired state in the male. The chromosome x is nothing other than the sex chromosome of this species.

The sex chromosome thus determined is represented by one of the shorter rod-shaped chromosomes in order of size, as recognized in a serial arrangement of chromosomes (Figs. 11, 12). Corroborative evidence has previously been furnished in the cases of other turtles such as *Amyda japonica* (Oguma 1937) and *Caretta caretta olivacea* (Nakamura 1949).

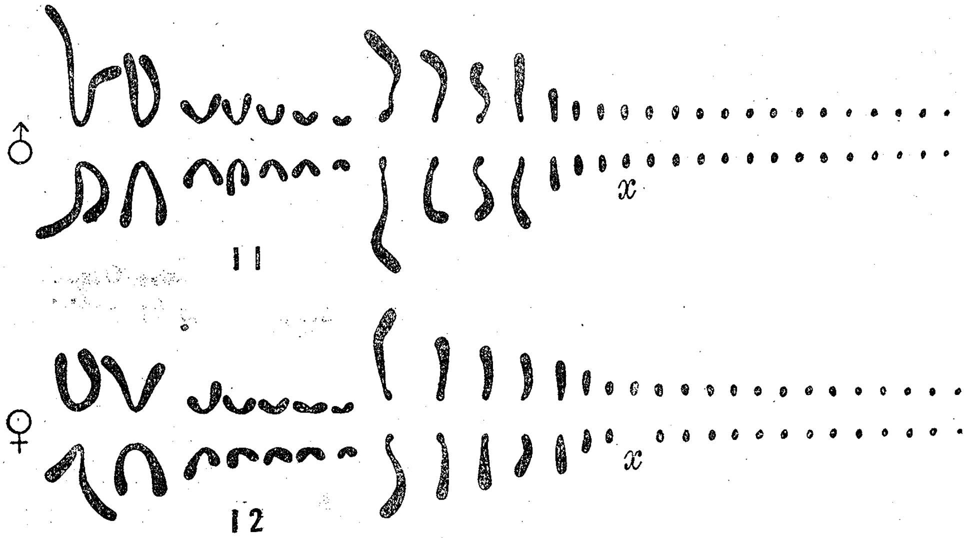
THE SEX CHROMOSOME

From the foregoing account of the chromosomes of *Chelonia joponica* in both sexes, it is apparent that the chromosomes of the female are 55 in number and those of the male 56. The numerical difference in two sexes is attributable to the condition of the sex chromosome; in the male it is present as a homologous pair, while in the female it stands solitarily, having no mate. It is this particular chromosome which causes the sexual difference between the chromosomes of the present species, and therefore this fact is well understood if the particular element be taken as the sex chromosome.

The sex chromosome here identified was found to be one of the short rod-shaped chromosomes, as detected in the alignmental arrangement of supposed homologous pairs (see x in Figs. 11, 12). It is thus clear that the female is heterogametic as regards the unpaired sex chromosome.

With reference to the literature there are six species of reptiles including the species of this study, in which the sex chromosome has been discovered (see Makino's list, 1951); they are *Lacerta vivipara* (Lacertidae; Oguma 1934), *Calotes versicolor* (Agamidae; Makino and Asana 1948), *Sitana ponticerina* (the same as above), *Amyda japonica*

(Trionycnidae ; Oguma 1937), *Caretta caretta olivacea* (Chelonidae ; Nakamura 1949), and *Chelonia japonica* (Chelonidae ; this paper). It has been demonstrated that in every of them the sex chromosome is uniformly represented by one of the short rod-shaped chromosomes. This uniformity of the sex chromosome is very remarkable in its nature and remains unaltered although the karyotypes of the studied species vary considerably. This finding seems sufficiently to indicate that the sex chromosome of reptiles is generally not of a large size but rather minute, being represented by one of the short rod-shaped chromosomes. This feature is also regarded as one of the distinguishing cytological characteristics of the reptiles, standing in contrast to the birds, a related group of animals, in which the sex chromosome is generally of medium size. Also, the evidence appears to indicate that the sex chromosome of the reptiles has developed through its own particular evolutional course which is independent from that of the autosomal group.



Figs. 11, 12. Alignmental arrangements of paired chromosomes. 11, from a spermatogonial cell. 12, from an oogonial cell. *x* denotes the sex chromosome.

In view of the above findings here cited, it is very surprising to find the papers of Matthey (1943) and Margot (1946) informing that they failed to find a numerical difference of chromosomes in the two sexes of *Chamaeleon vulgaris, Anguis fragilis* and *Lacerta vivipara.* Matthey (1943) found 24 chromosomes in both male and female cells of *Chamaeleon.* Margot (1946) reported 44 and 36 chromosomes in *Anguis* and *Lacerta,* respectively, which show no visible difference by sexes. It is not clear why these authors could not find the unpaired chromosome in the female cell. If it be permissible to make some criticism of their studies, one thing which is most probable is that their results involve something questionable in the research of the micro-chromosomes in which the sex chromosome is included. The author would like to mention here the results of a reinvestigation on *Lacerta vivipara* which became possible through the courtesy of Dr. Oguma; it resulted in finding again an unpaired sex chromosome in the female cell, in complete agreement with the results formerly obtained by Dr. Oguma (1934).

SUMMARY

The chromosomes of the sea turtle, *Chelonia japonica*, were investigated in the two sexes with special reference to the sexual difference of the chromosomes. The diploid number of the male is 56 and that of the female is 55. The cause of the numerical difference lies in the fact that one particular chromosome is always unpaired, having no mate, in the female cell, whereas it is in a paired condition with a homologous partner in the male. It is this particular chromosome which is responsible for the sexual difference of chromosomes between the two sexes, and therefore is regarded as the sex chromosome of this species. Based on the alignmental arrangement of the homologous chromosomes by means of the comparison of their shape and size, the sex chromosome was identified as one of the short rod-shaped chromosomes. It was established therefore that the female is heterogametic as regards the sex chromosome.

LITERATURE CITED

Makino, S. 1951. An atlas of the chromosome number in animals. Iowa State College Press, Ames.

Makino, S. and J.J. Asana 1948. Chromosoma **3** : 208-219.

Margot, A. 1946. Rev. Suisse Zool. **53** : 555-595.

Matthey, R. 1943. Arch. Jul. Klaus-Stift. Vererb. **13** : 1-16.

Nakamura, K. 1937. Jap. Jour. Genet. **13** : 240.

——— 1949. La Kromosomo **5-6** : 205-213.

Oguma, K. 1934. Arch. Biol. **45** : 27-46.

——— 1937. Jour. Genet. **34** : 247-264.

——— 1942. Kaibōgaku-Zasshi **20** : 157-172.