THE CHROMOSOME NUMBER IN SOME SALAMANDERS FROM NORTHERN JAPAN[1)]

BY

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(With 5 figures in text and 1 plate)

In parallel to the taxonomical study by INUKAI ('32) the present author undertook a study of spermatogonial chromosomes of some urodelan species belonging to *Hynobius* and *Salamandrella*, proposing to discover any chromosome differences which might exist between different species on the one hand, and, on the other hand to extend our knowledge on the chromosomes of urodeles in general.

As the results obtained from this study involve many important facts different from those hitherto reported on the urodelan chromosomes, it seems to be necessary to publish a short account at present.

This work was carried out under the direction of Prof. Dr. OGUMA, to whom the writer wishes to express his sincere obligation and appreciation. He is also greatly indebted to Prof. Dr. INUKAI who kindly placed numerous living specimens at his disposal and identified them. To Prof. Dr. UCHIDA the writer wishes also to acknowledge his indebtedness for valuable advice and encouragement.

Material and Method

Five different forms are included in this study, representing four species of *Hynobius* from Tohoku (Northeastern Honshu) and Hokkaido and one species of *Salamandrella* from Sakhalin. In all cases, the testes were removed from the animals in living condition, and the bodies were delivered to Prof. INUKAI for identification of the name.

1) Contribution No. 40 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

In every species, the testis consists of a simple club-shaped body showing only slight variation.[1] The testis was cut into three or four pieces and put into the fixative. In the earlier part of this work, BENDA's and MEVES' modifications of FLEMMING's solution were used for preservation of chromosomes, but thereafter, it was proved that FLEMMING's strong solution modified by MINOUCHI ('28) for mammalian chromosomes yields more favourable results than the former two. Sections were cut 15–25 micra in thickness. After bleaching the sections were pickled in CHURA's picro-acetic mixture. HEIDENHAIN's iron haematoxylin was applied for staining of chromosomes and light green as the counterstain in the usual way.

All drawings were made on the level of the desk on which the microscope was set, with the aid of ABBE's camera lucida using a ZEISS apochromatic objective 1.5 mm and a compensating ocular K. 20, t. l. 160 mm. For reproduction all the figures in text were reduced to approximately 3/5 the original size.

Observations

The observations recorded here are confined to the chromosomes of spermatogonial metaphase where of course the diploid number is present. Attention was paid primarily to the form, number, size and arrangement of the chromosomes in the following data.

Owing to their great number, the chromosomes in the later generation of spermatogonial cells tend to agglutinate with each other in the metaphase plate. Still more, there are generally found a lot of extremely small chromosomes occupying the central part of the spindle in metaphase, and it is these chromosomes that make precise observation difficult. The number of chromosomes, therefore, was only to be determined accurately from the metaphase plates of the primordial spermatogonia in excellently preserved condition. More than twenty, sometimes thirty equatorial plates were selected in each species for this purpose, and the number of chromosomes of

1) Concerning the testicular structure, see the writer's previous paper ('31).

each plate was counted after making drawings by the aid of a camera lucida. In every case, the chromosomes of a spermatogonium have been copied and arranged in pairs in a descending order with respect to size and form, for the sake of convenience of comparison.

1. *Hynobius retardatus* DUNN[1] (Fig. 1 & Pl. V, I)

The specimens used in this study were collected in the vicinity of Sapporo in early April, 1932. The material obtained from other localities of Hokkaido during the past three years was also used for comparison. The testes were fixed in FLEMMING'S strong solution without glacial acetic acid in which the salt was added in the proportion of about 0.7% for the total volume of the mixture. As compared with the other four species, it was rather difficult to obtain from the present material good figures of division in preservation, though the number of chromosomes is not very great.

The primary spermatogonia are usually found at the proximal portions of the seminiferous tubules (MAKINO, '31). They are solitary and surrounded by three or four follicle cells.

A spermatogonium possesses forty chromosomes (Fig. 1, a & b) which are usually arranged in a

Fig. 1. a & b. *Hynobius retardatus*. Spermatogonial metaphase, 40 chromosomes. ×2500.

1) *Hynobius lichenatus* which was used for the material in the writer's former work on the residual spermatogonia ('31) must be *H. retardatus*, as shown in the paper of INUKAI ('32).

rosette form, the larger ones always taking their position in the peripheral part of the spindle, while those of smaller size occupy the central space, being surrounded by the former. As is readily recognizable in the serial alignment (Pl. V, I), a chromosome garniture, as a whole, is composed of twenty-two V-shaped chromosomes with median or submedian fibre attachment, arranged at the periphery, and eighteen rod-shaped ones gradually decreasing in size, always occupying the central part of the spindle.

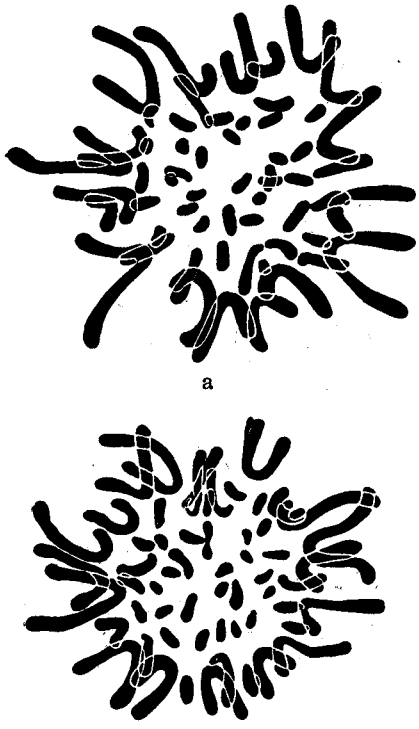

Fig. 2. a & b. *Hynobius lichenatus*. Spermatogonial metaphase, 58 chromosomes. ×2500.

Transverse sutures or constrictions appear very often in chromosomes, not only in the larger ones but also in those of smaller size. The tendency of constrictions to appear seems to be rather conspicuous in the present species as compared with the four other species studied.

There are no particular chromosomes to be considered as heterochromosomes, as has already been confirmed in the oocyte chromosomes of this species by the present author ('31). In this respect, all species observed in this study were the same.

2. *Hynobius lichenatus* BOULENGER (Fig. 2 & Pl. V, II)

The material employed in the study consisted of the testes from three males which were obtained in early April, 1930 from Aomori Prefecture. The animals were killed immediately after their arrival at the laboratory. For the fixative BENDA's fluid was applied.

As shown in Fig. 2, a & b, the chromosomes of the spermatogonia show the usual arrangement of a rosette form in metaphase.

At a glance, one can easily recognize that the chromosomes in this case are greater in number than in the preceding species. Counting shows that the spermatogonial number of chromosomes is unexceptionally fifty-eight.

The chromosomes may be divided into two main groups; one of which consists of nine pairs of atelomitic chromosomes, while the other is represented by twenty pairs of telomitic ones, as is clearly seen in the serial alignment of chromosomes (Pl. V, II). The atelomitic chromosomes varying in size and form are arranged radially in an outer circle of the equatorial plate with the apices of the V's directed toward the center of the spindle. The telomitic chromosomes range in shape from the long rods to dot-like ones, showing a gradual decrease in length. They always occupy the central space of the equatorial plane.

Hynobius retardatus from Hokkaido, had been considered to be the same species as the present species by many authors for a long time until the recent study of INUKAI ('32) established an indisputable difference between them from the anatomical point of view. The great difference in the chromosome number between these two species, such as forty and fifty-eight, also supports this view[1].

3. *Hynobius tokyoensis* TAGO[2] (Fig. 3 & Pl. V, III)

Two males were employed in the study, which were collected in Tochigi Prefecture in April, 1930 by the author. The animals were brought to the laboratory alive. For preservation of chromosomes BENDA's method was applied.

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1) A brief account concerning the chromosomes of these two species has been given by INUKAI ('32) in his paper.

2) The identification of species was based on the description of TAGO in "The salamanders of Japan", Tokyo ('31).

Fortunately, excellently clear figures of chromosomes in division were found in a great number in sections. Fig. 3, a & b, shows two representatives. There are fifty-six chromosomes in the spermatogonium, classified into ten pairs of the atelomitic and eighteen pairs of the telomitic type. As regards the arrangement and form of chromosomes the present species exhibits a similar appearance to the previous species, *H. lichenatus*. But a close comparison brings forth an important difference existing between the chromosomes of these two allied species (Pl. V, II & III). *H. lichenatus* possesses two more pairs of the telomitic chromosomes than *H. tokyoensis*, but has one pair less of the atelomitic ones. It is clear, therefore, that the former species has two more chromosomes than the latter. This difference in chromosomes between these two species suggests that in *H. lichenatus* a fusion might have taken place between certain four rod-shaped chromosomes by pairs so as to make two V's, thus decreasing the number and resulting in the chromosome count of *H. tokyoensis*. But there is no direct observation to prove such an explanation.

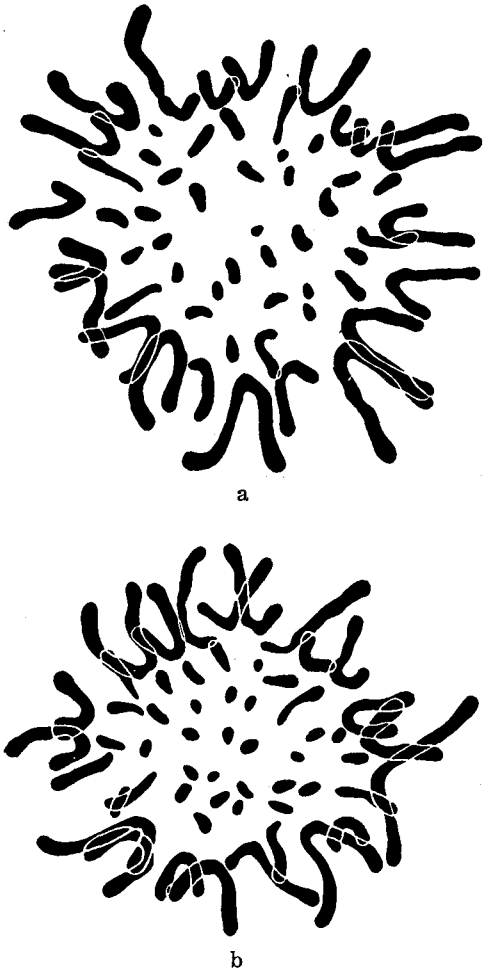

Fig. 3. a & b. *Hynobius tokyoensis*. Spermatogonial metaphase, 56 chromosomes. ×2500.

4. *Hynobius nigrescens* STEJNEGER (Fig. 4 & Pl. V, IV)

The salamanders used for material in the study were secured in April, 1932 in Niigata and Toyama Prefectures.[1] The testes from six males were fixed in FLEMMING's strong solution without glacial acetic acid.

Fig. 4, a was drawn from the material obtained from Niigata and b from Toyama. In both equatorial plates one can distinctly count fifty-six chromosomes, of which twenty are atelomitic, representing V-shape, while the remaining thirty-six are telomitic and rod-shaped. The V-shaped chromosomes are varied in form and size and take their position in the peripheral part of the equatorial plate as usual. The rod-shaped ones show the gradual diminution in size from long rods to small dot-like ones and are scattered well apart in the central space of the spindle.

The chromosomes of the present species are, as a whole, almost similar to those of *H. tokyoensis*, not only in number but also in morphological constitution. As is easily seen in comparison of chromosomes when

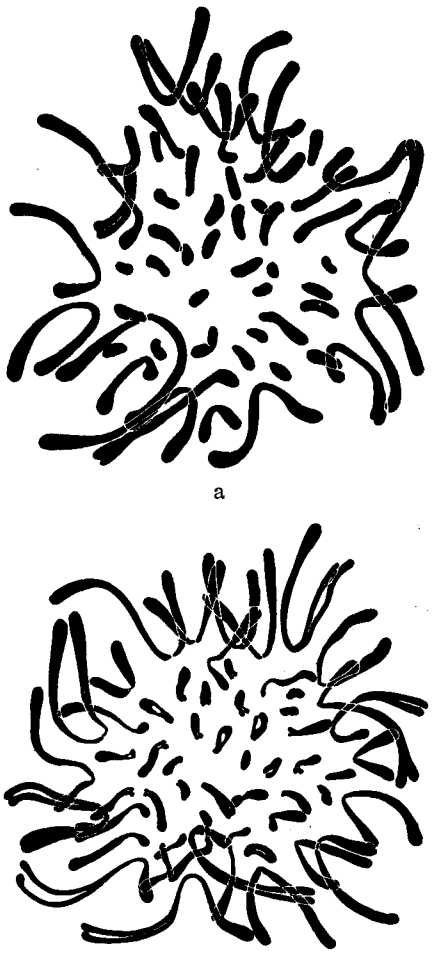

Fig. 4. a & b. *Hynobius nigrescens*. Spermatogonial metaphase, 56 chromosomes. ×2500.

1) For collection of the specimens the author is greatly indebted to Messrs. H. ISHIZUKA and R. HAYASHI, students of our institute.

serially arranged (Pl. V, III and IV), one can scarcely find any more marked differences between these two species than can be found in different germ cells of the same species.

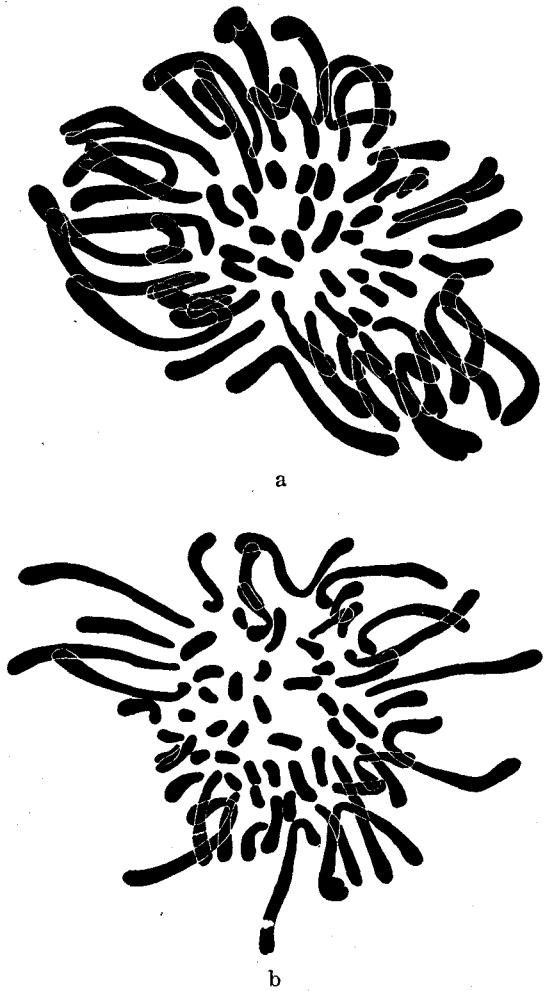

Fig. 5. a & b. *Salamandrella keyserlingii*. Spermatogonial metaphase, 62 chromosomes. ×2500.

5. *Salamandrella keyserlingii* DYBOWSKI (Fig. 5 & Pl. V, V)

The material on which the work was carried out consisted of six males collected in the vicinity of Toyohara, Sakhalin in early May, 1932[1].

As the fixative FLEMMING's strong solution without glacial acetic acid was employed as in the preceding case.

Fig. 5, a & b, shows the best examples obtained in the study of the spermatogonial chromosome. One can immediately find a remarkable difference existing between the present species and all hitherto observed species in regard to the chromosome constitution. In this species, there are found sever-

1) At this opportunity, the author wishes to express his hearty thanks to Messrs. M. UEDA and G. OKADA of the Toyohara Middle School, who kindly sent several living specimens at his request.

al J-shaped chromosomes and extremely long rod-shaped ones which are striking in contrast to the other species.

The equatorial plate of the spermatogonial metaphase contains sixty-two sharply defined chromosomes of which twelve are atelomitic and the remaining fifty are telomitic. Of twelve atelomitic chromosomes, some pairs have median or submedian fibre attachment being consequently V-shaped, while others are J-shaped resulting from subterminal attachment of fibres. Thus the atelomitic chromosomes show marked differences in their number and form from those observed already in *Hynobius*. Of twenty-five pairs of telomitic chromosomes, at least two are extremely long, as long as the largest V-chromosome. Such long straight chromosomes are not to be found in any species of *Hynobius*. The remaining pairs show gradual changes in their length as usual. These characters are clearly recognized in the serial alignment of chromosomes as shown in Pl. V, V.

So far as the present study goes, *Salamandrella* shows a distinct difference from *Hynobius* in its chromosome garniture, corresponding to the taxonomical difference between them. There has not been discovered any phylogenital correlation concerning the morphology of chromosomes at present.

Some Remarks on the Chromosome of the Urodele

In spite of many excellent works, our knowledge on the urodelan chromosome has been chiefly confined to *Salamandra* and *Triton*.[1] In these two the diploid number of the chromosome is twenty-four, which has been considered as the typical number in the urodele in general. The same number was known also from other urodelan amphibians, e. g., *Amblystoma*, *Amphiuma*, *Batrachoseps*, *Desmognathus*, *Diemyctylus*, *Geotriton*, *Necturus*, *Plethodon*, etc. In contrast to this a quite different number was reported recently by SMITH ('29) in the segmenting egg of *Cryptobranchus*

1) See OGUMA and MAKINO: Check-list of chromosome numbers in vertebrata ('31).

allegheniensis. He wrote as follows; "the number of chromosomes in the cleavage nuclei has not been determined beyond question, but is probably fifty-six." More recently IRIKI ('31) announced sixty-four chromosomes in the spermatogonium of *Megalobatrachus japonicus*.

So far as the present contribution is concerned, the chromosome number of hynobiid salamanders is not constant through different species, but varies from forty to fifty-eight. *Hynobius retardatus* possesses the lowest number and *H. lichenatus* the highest. Whether such a numerical difference is to be accounted for simply by linkage or by fragmentation of some chromosomes, as so explained in lizards by MATTHEY ('31), the present data are not sufficient to warrant the drawing of any decided conclusion.

There are found a number of small dot-like chromosomes in the diploid complex in any species of *Hynobius*. They vary both in number and size, by species. The difference of the chromosome number among species is mainly due to the number of these small sized chromosomes. On the other hand, the atelomitic V-shaped chromosomes do not show so remarkable difference in number between species; they are twenty-two in *H. retardatus* while eighteen in *H. lichenatus*. It is clear, therefore, that the numerical difference of the atelomitic chromosomes between species of *Hynobius* is by no means conspicuous as compared with that of the dot-like ones.

In the genus *Salamandrella* the atelomitic chromosomes seem to be much reduced in number, being represented by only six pairs. In this respect, this genus can be easily distinguished from *Hynobius*, in which there are more than nine pairs as mentioned above. The difference between these two genera is also emphasized by the shape of the chromosome; first, three pairs of large J-shaped chromosomes with subterminal fibre attachment, and second, the presence of the extremely long and straight rod-like chromosomes with telomitic attachment in *Salamandrella*. Generally speaking the appearance of the equatorial plate in *Salamandrella* exhibits rather a more close resemblance to that of *Megalobatrachus* (IRIKI, '31) than to *Hynobius*.

In every species observed in this study, there are no particular chromosomes to be considered as the sex chromosomes. In their number, form and arrangement the chromosomes of *Hynobius* and of *Salamandrella* as well, bear an apparent resemblance to those found in some sauropsida, e. g., pigeon (OGUMA, '27), snakes (NAKAMURA, '27, '28, MATTHEY, '31) rather than to those of any other groups of vertebrates.

Summary

1. The diploid chromosome numbers in five species observed are as below:

Hynobius retardatus	40 = 22 atelomitic+18 telomitic
Hynobius lichenatus	58 = 18 atelomitic+40 telomitic
Hynobius tokyoensis	56 = 20 atelomitic+36 telomitic
Hynobius nigrescens	56 = 20 atelomitic+36 telomitic
Salamandrella keyserlingii	62 = 12 atelomitic+50 telomitic

2. The chromosomes of *Salamandrella* differ remarkably from those of *Hynobius* in their constitution.

June, 1932

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Explanation of Plate V

Paired alignments of supposed homologous mates from spermatogonial chromosomes in a descending order.

I. *Hynobius retardatus.*
II. *Hynobius lichenatus.*
III. *Hynobius tokyoensis.*
IV. *Hynobius nigrescens.*
V. *Salamandrella keyserlingii.*

I. *Hynobius retardatus*

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