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Sex Chromosomes of the Tree Frog, *Hyla arborea japonica*¹⁾

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(With 16 Text-figures)

Beginning with the pioneer work of Carnoy et Lebrun (1899), a considerable number of papers have been published pertaining to the chromosomes of Amphibia (cf. list of Makino, 1956). The major interest has been centered in the discovery of the sex-determining mechanism, though the problem is not solved as yet. For instance, Witschi (1922, 1924, 1929, 1933) reported an X-Y sex chromosome mechanism to occur in male anurans, while Iriki (1930, 1932) emphasized the existence of an XX-mechanism in them. In contrast to the above views, Makino (1932), Galgano (1933), Wickborn (1945), and some others have failed to demonstrate the sex chromosome elements in either males or females of anurans under microscope.

The chromosomes of the tree frog, *Hyla*, have been studied by Iriki (1930) for a Japanese species, by Bushnell *et al.* (1939) for American forms, by Galgano (1933) for the European variety, and by Wickborn (1945) for German species. These investigations are in agreement in reporting the number of chromosomes of *Hyla* as 24 in diploid, and 12 in haploid, though the sex-chromosome mechanism has remained in controversy. The present author has had an opportunity to examine the chromosomes of *Hyla arborea japonica*, using an improved squash technique; results dissimilar to those of the previous workers have been gained.

Before going further, the author wishes to acknowledge here his indebtedness to Professor Sajiro Makino of Hokkaido University for his keen interest in the problem and his kindness in improving this manuscript. Gratitude is also extended to Dr. Taku Komai for valuable advice.

Material and method

The tree frogs, *Hyla arborea japonica*, used as material, were collected in the suburbs of Misima. The preparations of the material were subjected to the authors' new squash technique (Yosida & Ishihara 1956) as follows: To small pieces of the testis placed on a slide glass, were added two or three drops of hypotonic Ringer's solution (1 Ringer: 10 water) for metaphase chromosomes, and N/100 solution of FeCl₂ for prophase chromosomes. The testis pieces were left in the solution for 10 to 15 minutes; the solution was removed

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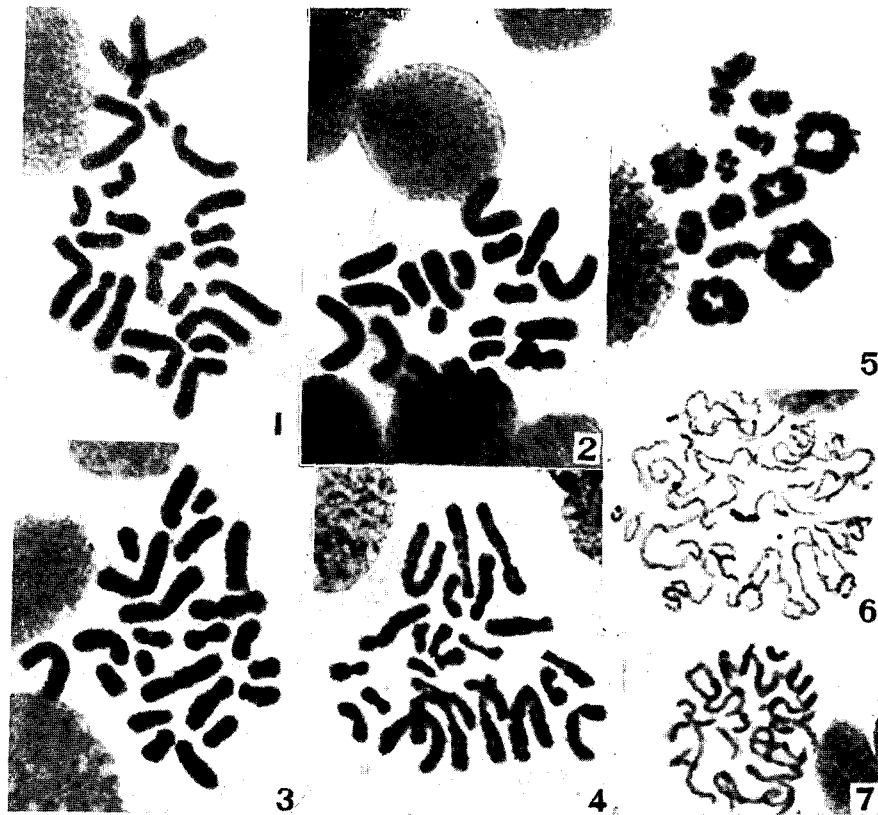
Dedicated to Professor Tohru Uchida in honor of his 60th birthday.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 13, 1957 (Prof. T. Uchida Jubilee Volume).

by the use of blotting paper. Finally the material was fixed and stained with acetic orcein. After about 20 minutes a cover slip was placed over the material with a light pressure, and the slip was sealed with paraffin.

Observations

1. *Spermatogonial chromosomes at metaphase*: The diploid number of 24 was proved in many spermatogonia, in agreement with the report by Iriki (1930) (Figs 1,2,4, 8, and 9). Aneuploid or polyploid cells are rarely observed. An example of the aneuploid cells having 23 chromosomes is shown in Figures 3 and 10. Serial alignment shows that the member of pair No. 6 are single (Fig. 15). The normal diploid complement is provided with 11 paired elements and 2 ele-



Figs. 1-7. Photomicrographs of chromosomes in the frog, *Hyla arborea japonica*. 1-4, spermatogonial metaphase. 5, metaphase of the first spermatocyte. 6, early stage of the spermatogonial prophase. 7, late stage of the spermatogonial prophase. Figs. 1-5, $\times 1640$. Figs. 6 and 7, $\times 1200$.

ments which are slightly unequal in size (Fig 12-15). The first three pairs (Nos. 1, 2 and 3) are characterized by having a median or submedian centomere. The next three pairs (Nos. 4, 5 and 6) are of subterminal attachment. The remaining five pairs (Nos. 7 to 11) are small in size and median or submedian in attachment. The larger one of the two unequal chromosomes falls in size between Nos. 6 and 7, while the smaller one ranks between Nos. 7 and 8. The former is characterized by a subterminal centromere, and the smaller one by a median centromere. In reference to the general rule as to the sex-determining mechanism, it is most probable that the heteromorphic pair here under consideration may be the sex-element, and that the larger one is the X while the smaller one is the Y. The Y chromosome often shows a remarkable bending at the centromere region (Figs. 1-4, 8-10 and 12-15).

The length of chromosomes was measured on a micron basis in three excellent metaphasl plates. The data are shown in Table 1. A diagram of metaphase chromosomes is given in Figure 16. The whole length of the largest chromosome is $9.11 \pm 1.18 \mu$, that of the shortest one is $2.5 \pm 0.29 \mu$. The length of longer arms is $5.18 \pm 0.69 \mu$ in the former, and $1.35 \pm 0.17 \mu$ in the latter. The whole length of the X and Y chromosomes is $4.03 \pm 0.41 \mu$ and $3.26 \pm 0.4 \mu$, respectively. The length of the longer arms was obtained as $2.56 \pm 0.41 \mu$ in the X, while it was $1.7 \pm 0.24 \mu$ in the Y. In size the X chromosome lies between chromosomes No. 6 ($5.14 \pm 0.53 \mu$) and No. 7 ($3.67 \pm 0.29 \mu$). The size of the Y chromosome is almost the same as No. 8 chromosomes ($3.28 \pm 0.33 \mu$), though the two are dissimilar in respect to position of the centomere.

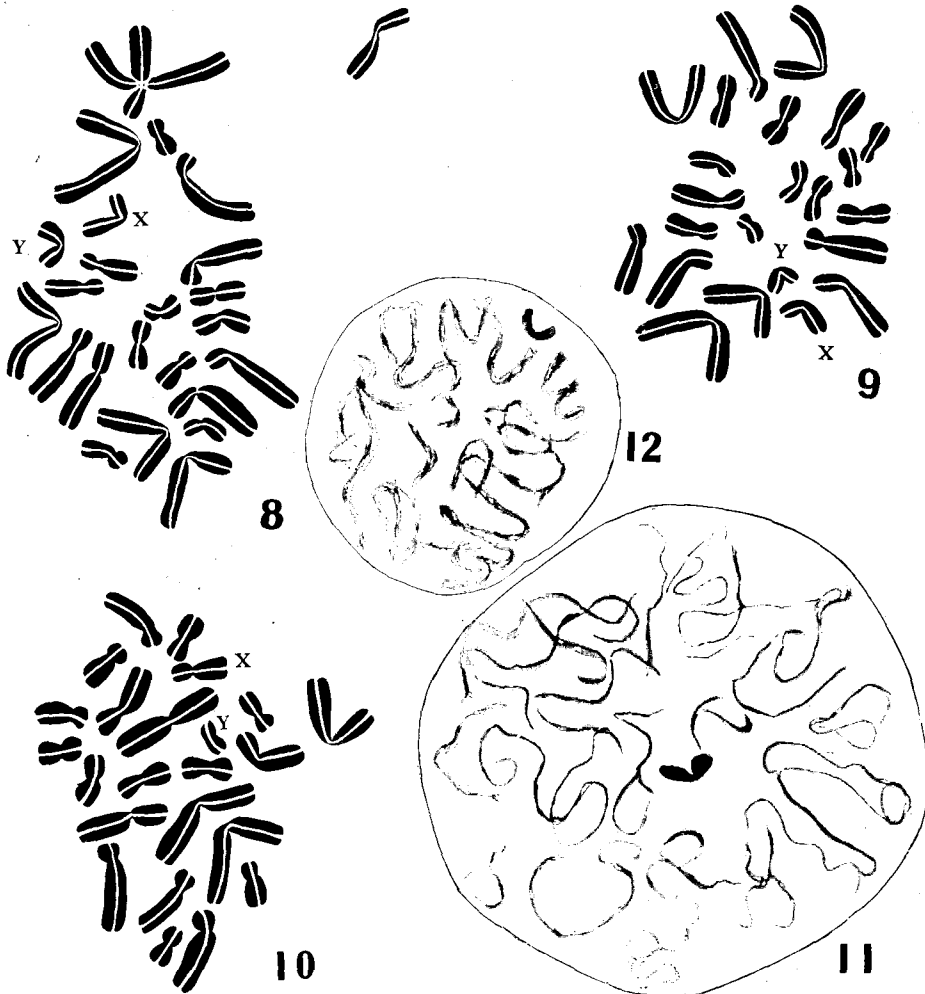
The length of the long arm in percentage of the whole length of each chromosome was calculated for the eleven pairs of autosomes and sex chromosomes

Table 1. Whole length of each chromosome and length of long arm of the chromosome with its percentage. Average of three metaphase plates.

Pair No.	1	2	3	4	5	6	X	7	Y	8	9	10	11
Whole length of chromosome	9.11 ± 1.18	7.73 ± 0.63	6.50 ± 0.51	6.16 ± 0.58	5.36 ± 0.56	5.14 ± 0.53	4.03 ± 0.41	3.67 ± 0.29	3.26 ± 0.4	3.28 ± 0.33	3.08 ± 0.15	2.83 ± 0.14	2.50 ± 0.29
Length of long arm	5.18 ± 0.69	4.23 ± 0.34	3.93 ± 0.64	4.83 ± 0.41	3.75 ± 0.22	3.68 ± 0.5	2.56 ± 0.31	1.85 ± 0.14	1.7 ± 0.24	2.33 ± 0.25	1.7 ± 0.18	1.71 ± 0.19	1.35 ± 0.17
Length of long arms in percent of whole length of the chromosome	57 ± 1.35	55 ± 3.16	65 ± 3.28	79 ± 2.12	72 ± 4.28	71 ± 2.49	64 ± 1.29	51 ± 1.41	52 ± 0.24	70 ± 4.07	55 ± 3.74	61 ± 6.13	54 ± 4.03
Classification of chromosomes by position of centromere	S.M.	M.	S.M.	S.T.	S.T.	S.T.	S.M.	M.	M.	S.M.	M.	S.M.	M.

Remarks. M. = Median centromere; S.M. = Submedian centromere; S.T. = Subterminal centromere.

(Table 1). For the sake of convenience all chromosomes have been classified into median, submedian and subterminal groups, according to the proportions of the long arms such as 50-55%, 56-70% and over 71%. It was then found that four out of eleven autosome-pairs were median, four were submedian and

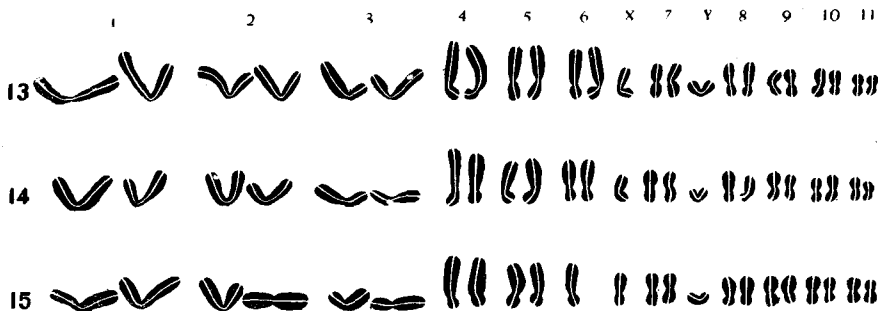


Figs. 8-10. Chromosomes in spermatogonial metaphase. 8, the same as Fig. 1. 9, the same as Fig. 2. 10, the same as Fig. 3. Figs. 11 and 12. Chromosomes in spermatogonial prophase. 11, the same as Fig. 6. 12, the same as Fig. 7. All figures are drawn by camera lucida ($\times 2000$). X = X-chromosome, Y = Y-chromosome.

remaining three were subterminal, whereas the X and Y chromosomes were submedian and median, respectively (Table 1).

2. *Spermatogonial chromosomes at prophase*: In the early prophasic nucleus most chromosomes appear as fine chromonema, while one element sometimes remains heteropicnotic (Figs. 6, 11). At late prophase when the ordinary chromosomes become thicker, this particular element still maintains a different configuration (Figs. 7, 12). It is similar in shape and size to the Y chromosome as observed at metaphase. Its length is 4.5μ at early prophase being slightly longer than the Y at metaphase. It is remarkable that the peculiar element is smaller in size in the late prophase nucleus than in the early prophase nucleus (Figs. 7, 12). The length of this element in the late prophase is 3.4μ , being approximately equal to the length of the Y at metaphase. The length of its long arm is 1.9μ , being also similar to that of the Y at metaphase. Further, the peculiar element exhibits a prominent bending at the centromere at prophase as occurred likewise at metaphase. On the basis of the above findings, it is highly probable that the peculiar body, heteropycnotic at prophase, may be the Y chromosome.

3. *Chromosomes of the first meiotic metaphase*: The first meiotic metaphase invariably shows 12 bivalent chromosomes (Fig. 5). In the light of the findings on the diploid complement it is evident that there exists a heteromorphic element consisting of a large X and a small Y. The present observations failed to detect the X-Y complex, most probably due to the fact that the difference in size between the two is not very remarkable.



Figs. 13-15. Serial alignments of chromosomes in spermatogonial metaphase. 13, from Fig. 8. 14, from Fig. 9. 15, from Fig. 10.

Discussion

Reference to the list of Makino (1956) indicates that the chromosomes of anuran amphibians have so far been studied in about 53 species since Carnoy et Lebrun (1899). There are considerable controversies as to the sex-determining mechanism in this group

of animals: some ones claim male homogamety while some others emphasize male heterogamety of an XY mechanism. On the other hand, there are a few authors who have failed to identify the sex chromosomes.

Iriki (1930), who emphasizes male homogamety in frogs, has reported in *Hyla arborea japonica* that the largest two V-shaped elements represent the sex-chromosomes of an XX type, since they form a bivalent of peculiar behavior at meiosis. In contrast to the above view, Galgano (1933) has informed in *Hyla arborea* that there is no element which may be referred to as the sex-chromosome. A similar view has been expressed by Wickbom (1945) for *H. arborea* from Germany, and by Bushnell *et al.* (1932) for several species of American tree-frogs. The present investigation has revealed that the largest pair in the material under discussion does not correspond to the sex-chromosome.

Here, one's attention is attracted to Witschi's studies (1922, 1924, 1933), which dealt with sex-chromosomes in several anurans. He has reported that the male diploid number of chromosomes in *Rana temporaria* is 26, containing an XY pair which is distinguishable from others by its somewhat peculiar behavior

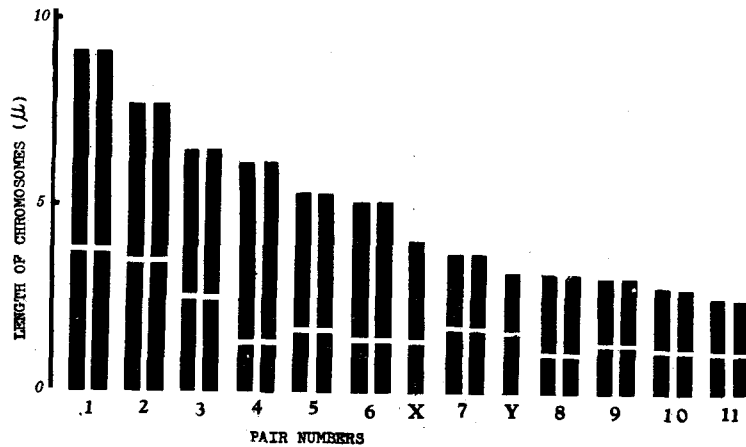


Fig. 16. Diagram of spermatogonial chromosomes of *Hyla*.

during meiosis. The sex-chromosomes identified by him rank fourth in order of decreasing size. He found a similar feature in several species of toads (Witschi 1933), in which the sex chromosomes are represented by the fifth element. It is then evident that the present author's findings support the view of Witschi.

The length of chromosomes of *Hyla arborea* L. was measured by Wickbom (1945). There is a considerable similarity between the results of Wickbom and those of the present author.

Tjio and Levan (1956) have demonstrated in the rat that the Y chromosome is heteropycnotic through the whole cycle of mitosis in the spermatogonial tissue, while the X element is not so. This evidence is comparable to that presented for *Hala* by the present author.

Summary

1. The regular germ line of the tree-frog, *Hyla arborea japonica*, shows the diploid number of 24 and the haploid number of 12 in males. The male diploid complement consists of 11 pairs of autosomes and two unequal, X and Y, chromosomes.

2. Karyological analysis of the metaphase chromosomes shows that there are present three large pairs provided with a median or submedian centromere, three pairs carrying a subterminal centromere, and the remaining twelve smaller elements which are median or submedian in attachment.

3. The X chromosome ranks in size order between chromosomes 6 and 7, and the size of the Y chromosome is almost the same as No. 8. The former is characterized by a submedian centromere, the latter by a median centromere. The whole length of the X and Y chromosomes was measured as $4.03 \pm 0.41 \mu$ and $3.26 \pm 0.4 \mu$, respectively.

4. The Y element is often positive heteropycnotic in the spermatogonial prophase.

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