A STUDY OF THE CHROMOSOMES OF PSEUDACRIS NIGRITA TRISERIATA (WIED)

by

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B. S., Kansas State College
of Agriculture and Applied Science, 1948

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1949

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INTRODUCTION

The Amphibia have provided material for numerous cytological investigations. Of these investigations only a few have dealt with the family Hylidae. The purpose of the present study is to extend the knowledge of the chromosomes of members of the family Hylidae and to present additional evidence concerning the nature of the sex-chromosomes in this family.

There have been no reports in the literature concerning the chromosomes of the species investigated, Pseudacris nigrita triseriata (Wied). Iriki (1930, 1932) studied the chromosomes of Hyla arborea japonica Guenther; Galgano (1933a, 1933b) and Wickbom (1945) also studied the chromosomes of Hyla arborea. Bushnell et al. (1939) investigated Acris gryllus, Acris crepitans, Hyla avivoca, Hyla cinerea cinerea, and Hyla versicolor versicolor. These workers reported the haploid number as 12 in all species of Hyla investigated and 11 in the species of Acris. Witschi (1933) reported the probable haploid number of Hyla crucifer as 13.

Bushnell et al. (1939) reported a peculiar chromosome characterized by large size, odd shape, lighter staining, and precocious splitting in each of the five species investigated. Its morphology and behavior suggested to them that it is a sex-chromosome. In Hyla arborea japonica Guenther, Iriki (1930, 1932) reported an XX sex-chromosome mechanism in the male. His identification of the sex-chromosome pair was based primarily on its appearance and behavior at the equatorial plate in the

meiotic metaphase. Galgano (1933a, 1933b) did not think the chromosome pair described by Iriki was the sex-chromosomes, as he observed similar behavior in several chromosome pairs in some nuclei; in other nuclei only one pair demonstrated this behavior, but it was not always the same pair. Wickbom (1945) reported an "odd chromosome" in several Anurans, but shared Galgano's opinion that its form and behavior have nothing to do with its supposed sex-chromosome nature. In a discussion introducing his 1949 list of chromosome numbers in the Anura, Wickbom restated the opinion reviewed by Darlington (1937) and White (1948) that in the Anura no cytologically discernible sex-chromosomes are present.

The present study presents morphological evidence which emphasizes the need for further comparative cytological studies of the Hylidae in particular and of the Amphibia in general.

MATERIALS AND METHODS

The frogs and tadpoles used in this study were captured in the vicinity of Stockdale, Riley County, Kansas. They were identified with the aid of the keys and descriptions of Wright and Wright (1933). They were taken from a spring-fed roadside pool with argillaceous bottom and with abundant vegetation, principally Typha sp. and algae. Tissues from six adult males, five adult females, and more than twenty-four male and female tadpoles were examined.

Material was prepared for study by sectioning, by the tissue imprint method, and by the squash technique. Testes

and gravid ovaries were fixed for two hours in a mixture consisting of equal parts of Bouin's fixative and tap water. They were embedded in paraffin, sectioned at 15μ, and stained with Heidenhain's iron hematoxylin. The thickness at which the sections were cut necessarily limited the number of cells suitable for study, because of over-lap, but those cells which were not covered by others were observable in their entirety in a single section.

The tissue imprint method was used with testes only. A dissected testis was cut in half; the cut surface was touched lightly to the surface of a clean glass slide so as to leave a single layer of cells in a film on the slide. The slide so prepared was immediately immersed in a fixative consisting of absolute methyl alcohol, 6 parts; chloroform, 3 parts; and 5 per cent acetic acid, 1 part. Fixation was allowed to proceed for 10 minutes, after which the slide was rinsed in two changes of 90 per cent ethyl alcohol, carried through descending grades of ethyl alcohol to water, and stained by the usual method with Heidenhain's iron hematoxylin.

The squash technique was used principally with tissues of the tadpoles. The method was essentially that commonly used for temporary aceto-carmine preparations. The stain, however, was a 1 per cent solution of toluidine blue in 45 per cent acetic acid. Materials were examined with and without previous fixation; the latter condition produced more satisfactory results. A very small fragment of a dissected gonad was placed on a clean glass slide. A few drops of the toluidine blue solution were

immediately added. Staining was allowed to proceed for a period of 1-1/2 to 2 minutes with the slide placed on a hot-plate at 58° C. Following the staining period a few drops of water were added to dilute the excess stain. The fluid on the slide was then removed by blotting until the remaining quantity was just adequate to surround the tissue under a 22 mm square cover glass. The cover glass was applied and subjected to a firm, steady pressure without lateral movement. The resultant preparation displayed well-stained, adequately separated cells in which the chromosomes of dividing cells were clearly visible.

Small pieces of epidermis were prepared for examination, using the toluidine blue method described above, except that no pressure was applied to the cover glass.

OBSERVATIONS

Chromosomes of the Male

Mitotic. The number and morphology of the mitotic chromosomes were determined by the examination of numerous spermatogonial and somatic pro-metaphases and metaphases. The diploid complement consists of 12 pairs of chromosomes, all of which are metacentric (Plate I, Figs. 1, 2, and 3). Eleven of these pairs are equal and form a graded series ranging in size from about 15μ to about 4μ (Plate I, Fig. 4); a curve established from the lengths shows a sharp initial drop (about 15μ to about 10μ) followed by an even decline (about 10μ to about 4μ). The twelfth pair is unequal,

consisting of one large chromosome (about 20μ) and one small chromosome (about 3.5μ). The lengths given above are averages based on measurements of chromosomes in numerous spermatogonial metaphases.

No precocious splitting was observed; anaphasic movement was synchronous. The long chromosome of the unequal pair was usually quite conspicuous in mid- and late-anaphase figures.

Meiotic. In the sectioned material available, meiotic cells were almost exclusively at the diakinesis and pro-metaphase I stages. The number of chromosomes and their size relationships were confirmed; such cells showed 12 bivalents, three of which were usually much thicker than the rest. The bivalent formed from the unequal twelfth pair was conspicuous because of its greater length (Plate I, Figs. 10, 12, 13, 14, and 15). No anaphase I stages were observed.

No secondary spermatocyte divisions were present in the material available, hence the manner of separation of the unequal pair cannot be stated. That there is an unequal distribution of chromatin in the end-products of meiosis in the male is evident from the variation noted in the length of sperm heads of comparable stages of development. This variation was particularly conspicuous in the tissue imprint preparations. Since the sperm heads are crescent-shaped, no direct measurements were attempted; outline sketches made with the aid of a camera lucida were the basis for comparison of lengths. Sperm heads so measured constituted two distinct

categories based on length, with an average difference of about 3.5μ.

Chromosomes of the Female

Mitotic. Somatic mitoses were observed in the theca cells of ovarian tissue and in epidermal cells. Oogonial divisions were observed in the toluidine blue preparations from tadpole ovaries. The diploid complement of the female consists of 12 pairs of chromosomes, all of which are metacentric (Plate I, Figs. 6, 7, and 8). Eleven of these pairs correspond in relative lengths, morphology, and equality to the first eleven pairs of the male. The twelfth pair is also equal, consisting of two chromosomes homologous in morphology to the long chromosome of the unequal twelfth pair of the male (Plate I, Fig. 5).

As in the male, no precocious splitting was observed; anaphasic movement was synchronous.

Meiotic. In the sections of gravid ovaries a few prometaphases of the first meiotic division were observed. These cells confirmed the number and morphology; twelve bivalents were observed. The largest three or four of these were considerably thicker than the others, and the bivalent composed of the twelfth pair was conspicuous because of its greater length (Plate I, Figs. 9 and 11).

DISCUSSION

The chromosome formula established for Pseudacris nigrita triseriata (Wied) by the present study adds to the evidence that 12 is the most probable haploid number in the family Hylidae. The report of "probably 13" as the haploid number in Hyla crucifer (Witschi, 1933) and that of 11 as the haploid number in Acris crepitans and Acris gryllus (Bushnell et al., 1939) may indicate that an evolutionary divergence is in progress in the family, or, in view of the stable numbers of other families of the Anura, that a re-investigation of these species is in order.

While the number of chromosomes established by the present study is in accord with that reported for most other Hylids, the chromosome morphology presents a distinct point of variance. This point is the existence of what appears to be a well differentiated sex-chromosome mechanism of the XX-XY type, the male being the heterogametic sex. Although the material available did not allow a detailed study of the behavior of the sex-chromosomes in meiosis, the morphological evidence seems adequate to establish the existence of the XX-XY mechanism. In all cells examined in which chromosomes were observable, the X and Y chromosomes in the male and the two X chromosomes in the female were distinguishable. The homology of the X chromosome of the male with the X chromosomes of the female was consistently observed in both gonial and somatic pro-metaphases and metaphases. The appearance

of the XY bivalent at diakinesis and pro-metaphase of the first meiotic division in the male was distinctive in both the tissue imprint preparations and the sectioned material. The division of sperm cells into two groups on the basis of difference in length of the sperm heads has been described above.

If further investigations confirm the sex-chromosome mechanism described here, Pseudacris nigrita triseriata (Wied) will present a departure from the condition generally recognized in the class Amphibia; viz., that of a group of organisms exhibiting the simplest case of genotypic sex differentiation, in which no sex-chromosomes are distinguishable (Darlington, 1937; White, 1948; and Wickbom, 1945, 1949). More specifically, there will remain the problem of clarifying the nature of the sex-chromosomes in the family Hylidae.

The sex-chromosome in the male of Hyla arborea japonica was described by Iriki (1930, 1932) as a large V-shaped chromosome located in the periphery of the spindle. He concluded that each arm of this chromosome represented one of the homologous gonial chromosomes. He noted no splitting of this chromosome during the course of development, inferring a reductional separation in the first meiotic division. He explained the variation in the size of the arms on the basis of coiling of the chromosome and degree of separation of the turns. In view of the observations of the present study, Iriki's conclusion that "...the sex-chromosome of Hyla arborea japonica is of XX-type and this type is common in all anurans"

is in need of re-investigation.

As stated above, Galgano (1933a, 1933b) did not confirm Iriki's findings, and endorsed the opinion that no sex-chromosomes are morphologically detectable in the Anura.

The descriptions of Bushnell et al. (1939) were based on behavior and morphology of the chromosomes at diakinesis and metaphase I. They did not figure or describe spermatogonial or somatic mitoses, nor did they report on the chromosomes of the female. The shape of the chromosome which they suggested might be a sex-chromosome was described as dumbbell-shaped at first, and V-shaped later. They thought this chromosome was composed of equal elements, and noted a median constriction in each element; it was located at the periphery of the spindle in the first meiotic metaphase. These points of the morphology and behavior are suggestive of the V-chromosome described by Iriki in Hyla arborea japonica. Since meiotic cells were poorly represented in the materials used in the present study, these points cannot be adequately compared with the findings of Bushnell et al. and Iriki. It might be noted that the diakinesis and pro-metaphase chromosomes observed in the present study were frequently variable in outline and clumped together, so that definitive determination of the complement, particularly of the unequal pair in the male, was necessarily made from gonial and somatic pro-metaphase and metaphase chromosomes.

An obvious conclusion emerges from the survey of previous work on the family Hylidae and from the experience gained in

the present study: the clarification of the evolution of chromosome number and form and of the nature of the sex-chromosome mechanism in the family Hylidae awaits further comparative cytological study.

SUMMARY

1. The diploid complement of Pseudacris nigrita triseriata (Wied) consists of 24 metacentric chromosomes. In cells of the first meiotic division 12 bivalents are present.

2. One pair of chromosomes is unequal in the male; the corresponding pair in the female is equal and its members are morphologically homologous to the longer chromosome of the unequal pair in the male. On the basis of morphological evidence it is suggested that the sex-chromosome type in this species is XX♀:XY♂.

3. The relationships of these findings to the previous work on the family Hylidae are briefly discussed.

ACKNOWLEDGMENT

Unlimited gratitude is expressed to Dr. Mary T. Harman, whose inspiring instruction, excellent example, and capable guidance provided the source and sustenance of this study. Deepest appreciation is extended to Mary Anne Gugler; without her encouragement and patient understanding the project would not have been possible.

EXPLANATION OF PLATE I

Figures 1, 3, 6, 7, 9, 11, 12, 13, 14, and 15 were drawn from sectioned material stained with Heidenhain's iron hematoxylin. Figures 2 and 8 were drawn from temporary toluidine blue squash preparations. Figure 10 was drawn from a tissue imprint preparation stained with Heidenhain's iron hematoxylin. All figures were drawn with the aid of a camera lucida at table level. The plate was reduced one-third for reproduction. Optics: Spencer 1.8 mm objective, N.A. 1.25; Spencer 16X ocular.

Figs. 1 and 3. Chromosomes of spermatogonial metaphase.

Fig. 2. Chromosomes of somatic metaphase from the epidermis of a tadpole.

Fig. 4. Diploid complement of the male, traced from Fig. 1.

Fig. 5. Diploid complement of the female, traced from Fig. 7.

Figs. 6 and 7. Chromosomes of somatic metaphase from the theca of a female frog.

Fig. 8. Chromosomes of oogonial metaphase.

Figs. 9 and 11. Primary oocytes at pro-metaphase.

Fig. 10. Chromosomes of a primary spermatocyte at pro-metaphase.

Fig. 12. Primary spermatocyte at diakinesis.

Figs. 13, 14, and 15. Chromosomes of primary spermatocyte pro-metaphases.

PLATE I

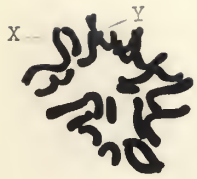

Fig. 1.

Fig. 2.

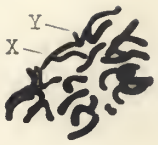

Fig. 3.

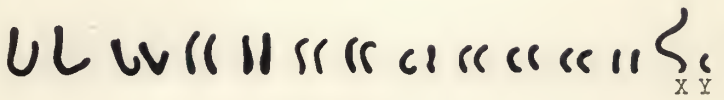

Fig. 4.

10μ

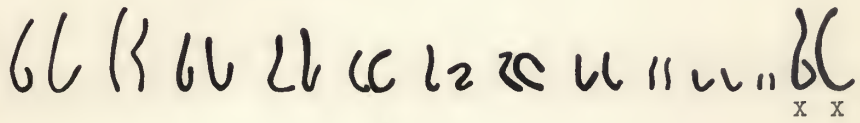

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

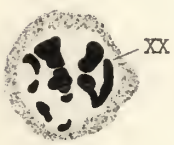

Fig. 9.

Fig. 10.

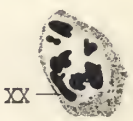

Fig. 11.

Fig. 12.

Fig. 13.

Fig. 14.

Fig. 15.

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