Mem. Fac. Educ., Shimane Univ. (Nat. Sci.) Vol. 16. pp. 15-20, December 1982.

Karyotype Analysis of Two Species of Hynobiid Salamanders, *Hynobius nebulosus nebulosus* (Schlegel) and *Hynobius naevius* (Schlegel)

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Abstract: Somatic chromosomes of two representative species of genus *Hynobius* were observed with the intestinal epithelium by the method of Kezer and Sessions (1979). Diploid number of *H. n. nebulosus* and *H. naevius* was confirmed to have 56, which is an uniform number with other majority of species in genus *Hynobius*. Twenty-eight pairs of homologues were classified into four groups to facilitate the comparison of each karyoytpe. The chromosome morphology constituting both karyotypes was similar in general but not identical with each other. An unambiguous difference occurred in the medium-sized chromosomes (Nos. 10-13). Onsequently it brought about the different fundamental number (FN) between two species, 90 for *H. n. nebulosus* and 92 for *H. naevius*.

The karyotype of *H. n. nebulosus*, which is a common species and is widely distributed in western Japan, was not exactly the same with that analyzed by the previous workers. The minor difference found in the small-sized chromosome groups (Nos. 14-18 & 19-28) may indicated a chromosomal polymorphism according to the locality.

Introduction

Habitats of salamanders of family Hynobiidae are restricted to the eastern Asia and they are thought to be the most primitive of the living Urodela as is indicated not only by many structural features but also by their mode of reproduction in which fertilization is external.

According to the Makino's list (1956), chromosome observations of hynobiids were undertaken in 15 species based on the testis-sectioning method during the 1930s and 1940s. However detailed karyotype analyses of these species have not been adequately done since the earlier works, mainly due to becoming difficulty of securing living materials in recent years and of obtaining mitotic cells for the cytological preparations.

Because of their unique ecological and life history patterns the hynobiids have much of karyological interest when we consider the amphibian cytotaxonomy and karyoevolution. This paper presented accurate karyotypes of two representative species of genus Hynobius which have not yet been studied fully.

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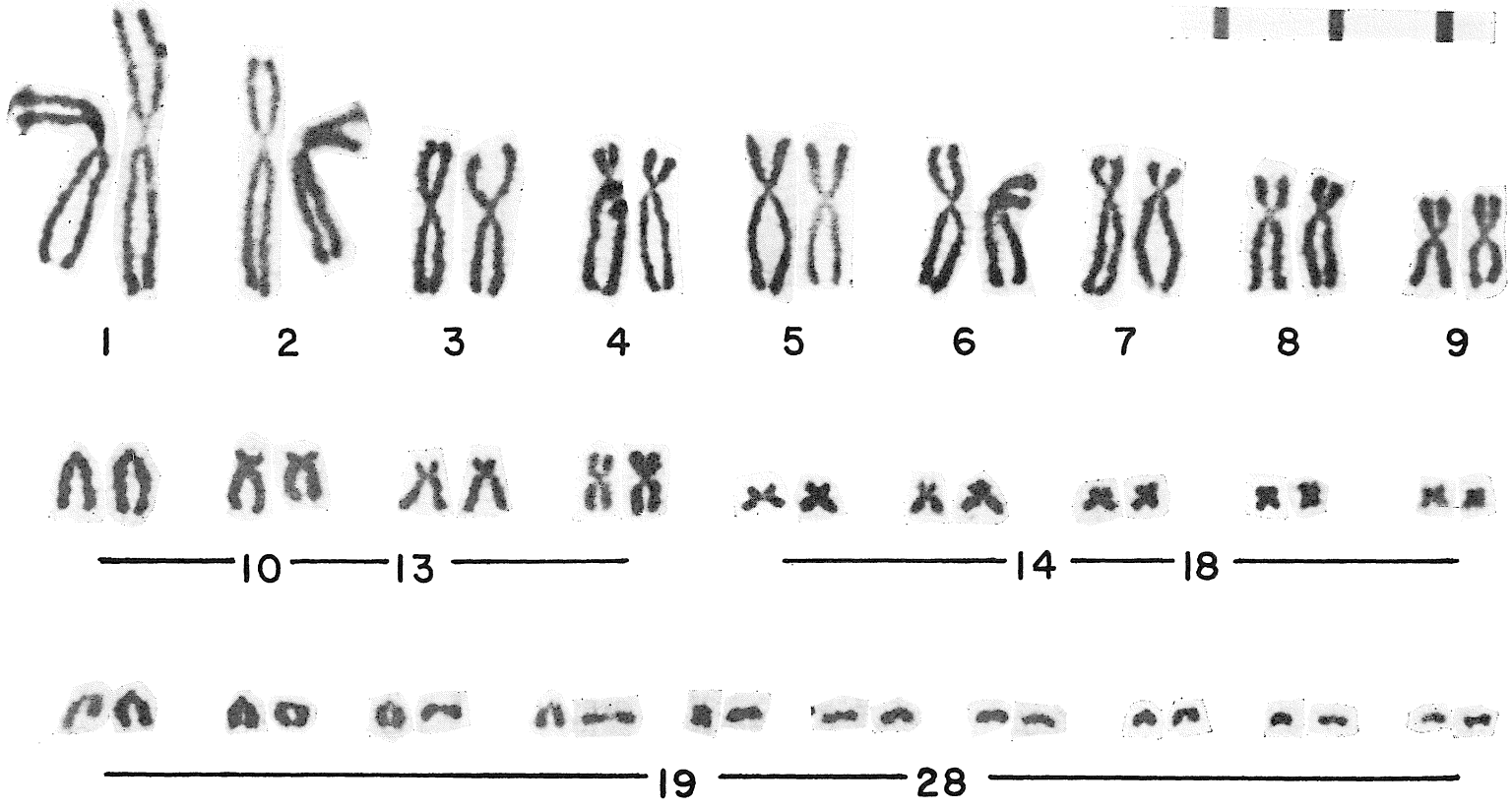

Figure 1. Karyotype of *Hynobius nebulosus nebulosus* (female). Scale : 1 division=10μm.

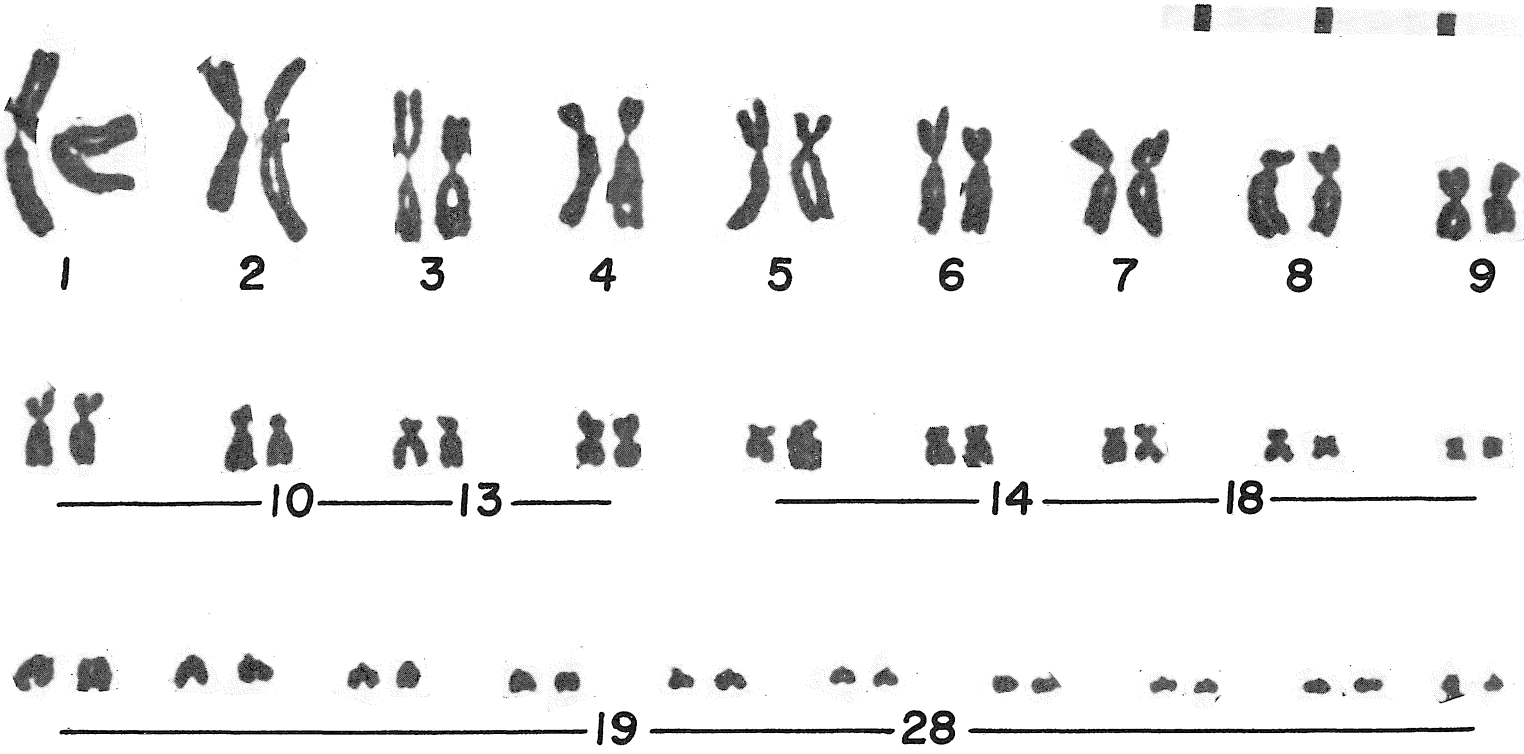

Figure 2. Karyotype of *Hynobius naevius* (male). Scale: 1 division=10μm.

Materials and Methods

The adult female of *Hynobius nebulosus nebulosus* was collected in Yoshii-cho, Okayama-ken, and male and female individuals of *H. naevius* were procured in Yuki-cho, Hiroshima-ken. Larval specimens of *H. naevius* were secured in Masuda-shi, Shimane-ken.

The animals were injected with a colchicine solution intraperitoneally at 0.1 m*l* of the 2 mg/m*l* solution per gram of body weight, and were kept in a cool water at 15°C for 24 hours. The entire gut truct was isolated from the dissected animals and fixed in an acetic-alcohol mixture after 30 minutes treatment of hypotonic solution (distilled water, pH adjusted 7.0).

Somatic chromosomes were prepared from the epithelial cells by the mehtod of Kezer ane Sessions (1979). Chromosomes were stained with conventional Giemsa stain. Nomenclature and abbreviations indicating the shape of chromosomes are those proposed by Levan *et al.* (1964).

Results

The chromosome number of our specimens of *H. n. nebulosus* and *H. naevius* was both 2n=56, confirming the earlier works of Makino (1934) for *H. nebulosus* and Makino (1939) for *H. naevius*. It is the same chromosome number with that of all other examined species of genus *Hynobius* endemic to Japan, with an exception of *H. retardatus* (2n=40; Makino, Azumi & Sasaki 1971). The chromosome morphology, however, was recognized to be unlike between two species.

The karyotype of *Hynobius n. nebulosus* (Fig. 1): Twenty-eight pairs of chromosomes in the complement were classified into four groups to facilitate the comparison with other karyotypes. A large-sized chromosomes in the first group included 5 metacentric pairs (Nos. 1, 2, 3, 5, & 9) and 4 submetacentric pairs (Nos. 4, 6, 7, & 8). Second group consisted of 4 medium-sized chromosomes (Nos. 10-13); 1 pair of metacentrics, 1 pair of acrocentrics and 2 pairs of submetacentric elements. Third and fourth groups were small-sized chromosomes; meta- and submetacentric shapes were in the third group (Nos, 14-18) and acro- or telocentric elements in the fourth group (Nos. 19-28).

The karyotype of *Hynobius naevius* (Fig. 2): General feature of the chromosome constitution is identical with the former species. Four chromosome groups constituting the karyotype were 9 large-sized pairs (Nos. 1-9), 4 medium-sized pairs (Nos. 10-13), 5 small-sized meta- and submetacentric pairs (Nos. 14-18) and 10 pairs of acro- or telocentric chromosomes (Nos. 19-28).

In comparing the two karyotypes we noticed an obvious difference in the second group as illustrated in Figure 3. *H. n. nebulosus* bears 1 acrocentric, 2 submetacentric and 1 metacentric pairs as compared with *H. naevius* that has 2 submetacentric and 2 metacentric pairs in the group. A minor dissimilarity was also found in the small-sized group of *H. n. nebulosus* between the present observation and the previous study by Ikebe and Kohno (1979b). They described 7 pairs of meta- and submetacentric chromosomes in the third group and 8 pairs of acrocentrics in the fourth group. In contrast we observed 5 pairs of meta- and submetacentrics and 10 pairs of acrocentric chromosomes in each group. Therefore the fundamental number (FN) counted as 90 in our

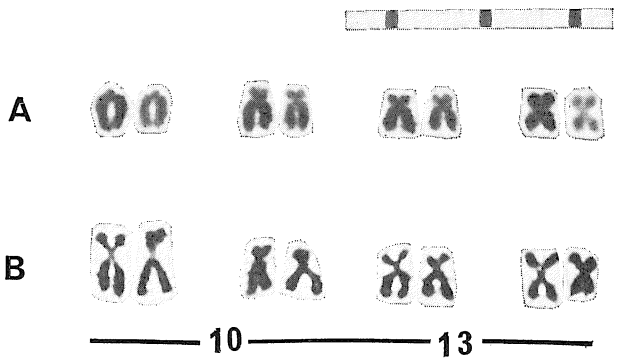

Figure 3. A comparison of the second chromosome group in two kinds of *Hynobius* karyotypes. A, from *H. n. nebulosus*. B, from *H. naevius*. Scale: 1 division=10μm.

karyogram in comparison with 94 in their karyogram.

So far as we examined adult specimens of *H. naevius,* morphology of male and female karyotypes were identical and no heteromorphic pair of chromosomes as the sex element was detected between both karyotypes.

Discussion

There have been four papers describing the karyotype of Hynobiids, which were observed with the squashed preparations (Azumi & Sasaki 1971, Ikebe & Kohno 1979a, 1979b, Morescalchi *et al.* 1979). Among them, studies by Ikebe and Kohno (1979a, 1979b) presented precise karyograms of four species; *H. n. nubulosus H. n. tokyoensis, H. lichenatus,* and *H. nigrescens.* Their unambignous illustrations supply sufficient information for comparing chromosome morphology in the respective species. In comparison of our karyograms of *H. n. nebulosus* and *H. naevius* with those of above four species we hold an interest in the four pairs of medium-sized chromosomes (Nos. 10-13).

As illustrated in Figure 3, four pairs of chromosomes in the second group represented the most characteristic difference between two karyotypes. It has long been described by amphibian workers that genus *Hynobius* was grouped into two, *nebulosus*-group and *naevius*-group with the feature of the life history and with morphological and ecological characteristics between these types (Dunn 1923). Present observation provided a karyological evidence representing the difference in accord with the above grouping.

A minor morphological difference in the small-sized chromosome group of the *H. n. nebulosus* karyotype by Ikebe and Kohno (1979b) in compared with the present observation is another interesting problem. The difference of the chromosome constitution in the third and fourth group results the difference of the fundamental number (FN) between two specimens of the same species. As Sato (1937) described, *H. n. nebulosus* is a common species and widely distributed in the western Japan with some local variations. This karyological variation in the species suggests an indication of chromosome polymorphism according to the locality.

Acknowledgments : I am indebted to Doctors Yasuaki Utsunomiya and Taeko Utsunomiya of Hiroshima University, and Mr. Atsunori Kageyama of Masuda Senior High School, Shimane-ken, for providing me the specimens used in this study. I am also indebted to Professor Hirochika Oue for his valuable comments on the manuscript.

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