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Cytogenetic studies of Hynobiidae (Urodela). IX. Karyological characteristics of *Hynobius abei* Sato by means of R- and C-banding

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Summary. Chromosomal characteristics of the salamander species *Hynobius abei*, from Ohimya (Kyoto) were revealed by the techniques of R- and C-banding. The karyotype of *H. abei* was characterized by the shortness of an R-negative (C-positive) band in the terminal region of the long arm of chromosome 2 and a band encompassing the whole short arm of chromosome 10. These two bands in *H. abei* were the shortest among those of the various *Hynobius* species that have been examined. Otherwise no differences could be detected between *H. abei* and seven other pond-type species of *Hynobius* (2n = 56) in terms of the banding patterns of 18 specifically identifiable pairs of their chromosomes.
Key words. *Hynobius abei*; banding karyotype; R-banding; C-banding.

In order to examine the process of differentiation in the genus *Hynobius* from a cytogenetic perspective, banding analyses of the karyotypes are required for all members of the genus. Since banding analysis was first applied to the chromosomes of salamanders by Kohno et al.[1] (1983, C-banding) and by Kuro-o et al.[2] (1986, R-banding), the banding characteristics of the karyotypes of eight species of the genus *Hynobius* have been described[3-7]. However, for the remaining eight of the 16 *Hynobius* species found in Japan and Korea, including *H. abei*, no information on banded karyotypes is available in the literature.

Hynobius abei is distributed endemically on the Tango peninsula of Kyoto prefecture in Japan where it has been protected since 1983, because the size of the population has decreased significantly. Since the material is hard to obtain, only one report has been published on the chromosome number (2n = 56) and karyological characteristics, excluding banding of the species[8].

Here we report the results of a detailed karyological analysis of *H. abei* performed by R- and C-banding.

Materials and methods

Two egg-sacs of *H. abei* were collected from Ohmiya (Kyoto) with the permission of the Board of Education of Kyoto prefecture. Ten embryos were used for chromosome analysis.

The R-banding (RBA) technique established by Dutrillaux et al. (1973)[9] was used with slight modifications (RBG). Details of the technique have been described elsewhere[2].

For C-banding (CBG), chromosome preparations were made by the method previously used for embryonic cells of *Hynobius*[4], and C-banding was carried out following the method of Sumner (1972)[10].

Results

Karyotype analyses were performed on a total of 48 excellent metaphase spreads from 10 embryos. It was clearly shown that the chromosome number was 56 (2n), which is identical to that given in the report of Seto and Matsui (1984) and also to all the other pond-type species of *Hynobius* studied, with the exception of *H. retardatus*. Banding karyotypes of *H. abei* are shown in figures 1 (R-banding) and 2 (C-banding). Banding analysis allowed 18 out of 28 chromosome pairs to be specifically identified. There are 9 large-sized chromosome pairs (nos. 1–9; meta-, subtelo- and submetacentric), 4 medium-sized chromosome pairs (nos. 10–13; subtelo-, submeta- and metacentric) and 5 pairs of small-sized chro-

Figure 1. Karyotype of *Hynobius abei* with R-banding.

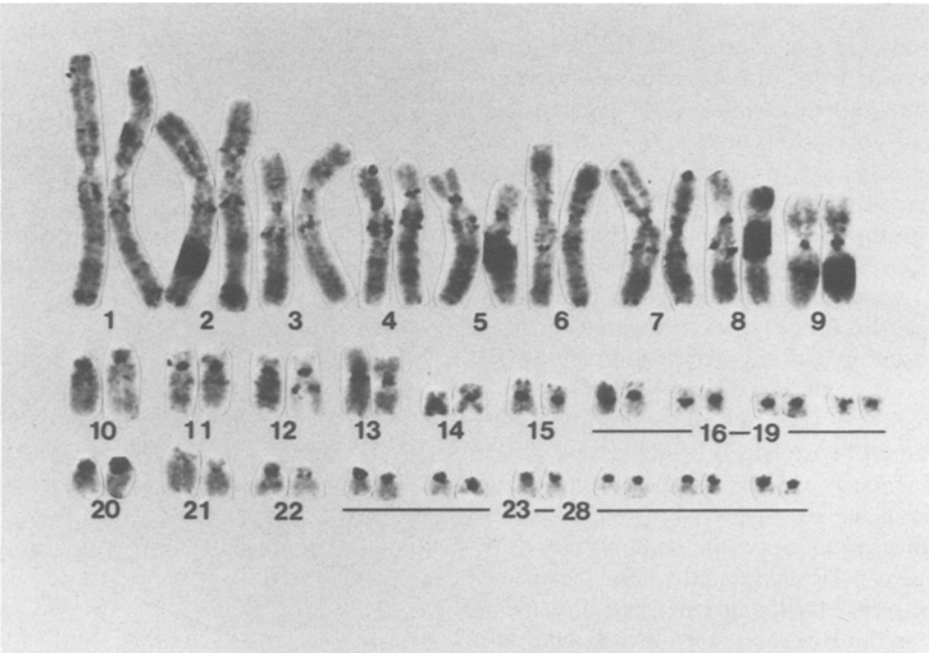

Figure 2. Karyotype of *Hynobius abei* with C-banding.

mosomes (nos. 14, 15, 20, 21 and 22; submeta- and acrocentric). The 15 small chromosome pairs consist of 6 meta- or submeta- and 9 acrocentric chromosomes.

A comparison of the R-banding patterns of five pond-type species of *Hynobius* revealed that the terminal band on the long arm of chromosome 2 was reduced in size, in the following order: *H. leechii*, *H. nebulosus*, *H. tokyoensis*, *H. nigrescens*, and *H. abei*[5]. The terminal band of *H. abei* was smaller than that of *H. tokyoensis* (table). In contrast, the short arm of chromosome 10 was decreased in size in the following order: *H. leechii* (from the northern part of Korea, Sogumgang, Yangju and Kangnung), *H. retardatus*, *H. lichenatus*, *H. tokyoensis*, *H. nigrescens*, *H. abei*, and *H. nebulosus*[5, 11, 12]. The short arm of chromosome 10 of *H. abei* was found to be almost the same size as that of the homologous chromosome of *H. nebulosus* from Nagasaki. In the latter species, the short arm of chromosome 10 is the shortest found in all

Variations in the terminal bands on the long arm of chromosome 2 and the short of chromosome 10, in 4 pond-type *Hynobius* species*

Species	Collection site	Chromosome 2 Length of terminal band as a percentage of the length**	Chromosome 10 Length of the short arm as a percentage of the length of the long arm***	Reference
H. leechii	Northern Korea	12.29 ± 1.28 (14)	51.28 ± 5.59 (14)	4
H. retardatus	Tohbetsu	-	49.40 ± 4.40 (20)#	11
H. lichenatus	Inawashiro	-	43.29 ± 4.30 (20)#	11
H. tokyoensis	Kantoh district	8.37 ± 0.71 (19)	42.19 ± 4.50 (19)	12
H. nigrescens	Nakakubiki	7.50 ± 1.70 (12)	29.75 ± 3.19 (20)#	5 & 11
H. nebulosus	Nagasaki	11.38 ± 1.35 (12)	21.60 ± 2.70 (5)	12
H. abei	Ohmiya	5.24 ± 0.13 (4)	22.37 ± 3.00 (4)	present study

( ): The numbers in the parentheses show the numbers of measured chromosomes.
*: The lengths were measured on R-banded chromosomes except in 3 cases (marked #) in which C-banded chromosomes were used.
**: The value is calculated as the length of the terminal (R-negative) band divided by the length of the chromosome (from which the length of the terminal band has been subtracted) × 100.
***: The value is calculated as the length of the short arm of chromosome 10 divided by the length of the long arm × 100.
-: not measured

the *Hynobius* species analyzed (table). Thus, *H. abei* was revealed to have the smallest terminal band on chromosome 2 and one of the smallest short arms of chromosome 10 found in all of the *Hynobius* species examined by the C- and R-banding techniques[1–7].

Discussion

In a previous study[8] of two adult specimens of *H. abei* from Takeno (Kyoto), using squash technique and staining by conventional Giemsa, it was demonstrated that the small-sized pairs of chromosomes in *H. abei* consisted of 5 submeta- or meta- and 10 acrocentric pairs. However, all the specimens from Ohmiya (Kyoto) in the present study showed 6 submeta- or meta- and 9 acrocentric chromosome pairs. The banding karyotypes (especially C-banding) clearly indicated that none of the 6 submeta- or metacentric pairs in the group of small-sized chromosomes examined by us had any heterochromatin in its short arm. Thus, the addition of one pair of chromosomes to the small-sized submeta- or metacentric group in our results cannot be explained by elongation of the short arm of an acrocentric chromosome pair by means of multiplication of the heterochromatin.

In Japan and Korea, 16 species of the genus *Hynobius* are found, and these can be divided into pond types and mountain-brook types. Excluding one species, namely, *H. retardatus* found in Hokkaido, the other 9 pond-type species of *Hynobius* in Japan and Korea have the same chromosome number, 2n = 56. They also exhibit similar karyotypes, except for the shape of chromosome 10 and the length of the terminal band on chromosome 2[2]. These two variations are interspecific, although the short arm of chromosome 10 also shows intraspecific differentiation between localities[4,11]. The variations are dependent on the length of one C-positive and one R-negative region in these two chromosome pairs. Each of these two regions in *H. abei* was one of the smallest among all corresponding regions of the several *Hynobius* species for which banding has been described. In contrast, the same regions in *H. leechii*, from the northern part of Korea, were the largest observed. These two species, *H. abei* and *H. leechii*, represent the extremes in variability for chromosomes 2 and 10 among the species of *Hynobius* examined.

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