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Intraspecific Karyotype Variation in *Hynobius kimurae* Dunn (Urodela, Hynobiidae) by Analysis of C-banding[1]

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ABSTRACT—C-banding analysis was performed on two previously reported karyotypes of *Hynobius kimurae*. A difference in the amount of centromeric heterochromatin was observed between these two karyotypes. The karyotype with the larger amount of centromeric heterochromatin was assumed to be a derived type, since the amount of heterochromatin was unusual in comparison with the C-banded karyotypes of other *Hynobius* species. We observed the same C-banding patterns in the arms of chromosomes Nos. 1–9 and 11–14 in the two karyotypes, except for the C-bands in the centromere reions. Intra-populational karyotype variation was also found.

INTRODUCTION

The salamander, *Hynobius kimurae,* is distributed from west to central Honshu, Japan. On the basis of its breeding habitats and larval characteristics, this species has been classified as a mountain-brook type of *Hynobius* [1].

The karyotypes of *H. kimurae* from five localities have been reported previously by Ikebe *et al.* [2]. The chromosome number of *H. kimurae* was found to be 2n=58. The existence of intraspecific karyotype variation in this species was also described. Populations with one karyotype were found in two separate districts (Tokyo Prefecture and Hyogo Pref.), whereas those having another karyotype were found in the area between the above two prefectures.

The C-banded karyotype of *H. kimurae* from Hakuba-mura (Nagano Pref.) has been reported by Iizuka and Kakegawa [3]. However, no analysis of intraspecific karyotype variation using banding techniques has yet been published.

In this paper, we present details of C-banding analysis of the two previously reported karyotypes of *H. kimurae*. Also, intra-populational karyotype variation in specimens from Miyama-cho, Tokyo is newly described.

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MATERIALS AND METHODS

Egg-sacs of *H. kimurae* were collected from Miyama-cho (Hachioji-shi, Tokyo Pref.) and Oshimizu-machi (Hakui-gun, Ishikawa Pref.), representing the eastern and central areas of its range, respectively. Tail-bud stage embryos were used for chromosome analysis. Numbers of analyzed egg-sacs, embryos and metaphases are shown in Table 1.

The techniques of chromosome preparation and C-banding used in this study have been described previously by Kohno *et al.* [4]. The C-banding technique employed is the method of Sumner (CBG technique) [5] with slight modifications.

TABLE 1. Years and sites of collection of *H. kimurae*, and numbers of egg-sacs, embryos and metaphases analyzed by C-banding

Collection site and year		Analyzed number of		
		egg-sacs	embryos	metaphases
Miyama-cho	1987	1	8	38
Miyama-cho	1988	2	8	34
Oshimizu-machi	1988	3	6	30

RESULTS

Karyotypes of *H. kimurae* from Miyama-cho and Oshimizu-machi are shown in Figure 1. The karyotypes of *H. kimurae* from Miyama-cho and Oshimizu-machi correspond to the previously designated types 1 and 2, respectively [2]. There are differences between the two karyotypes in the features of chromosomes Nos. 10 and 15–29. With regard to chromosomes Nos. 15–29, we observed 5 pairs of metacentric chromosomes and 10 pairs of acrocentric chromosomes in type 1, and 8 pairs of meta- and submetacentric chromosomes and 7 pairs of subtelo- and acrocentric chromosomes in type 2.

C-banded metaphases from specimens at the two localities at approximately the same level of condensation are shown in Figure 2. All of the centromere regions on chromosomes from Oshimizu-machi specimens have particularly large C-

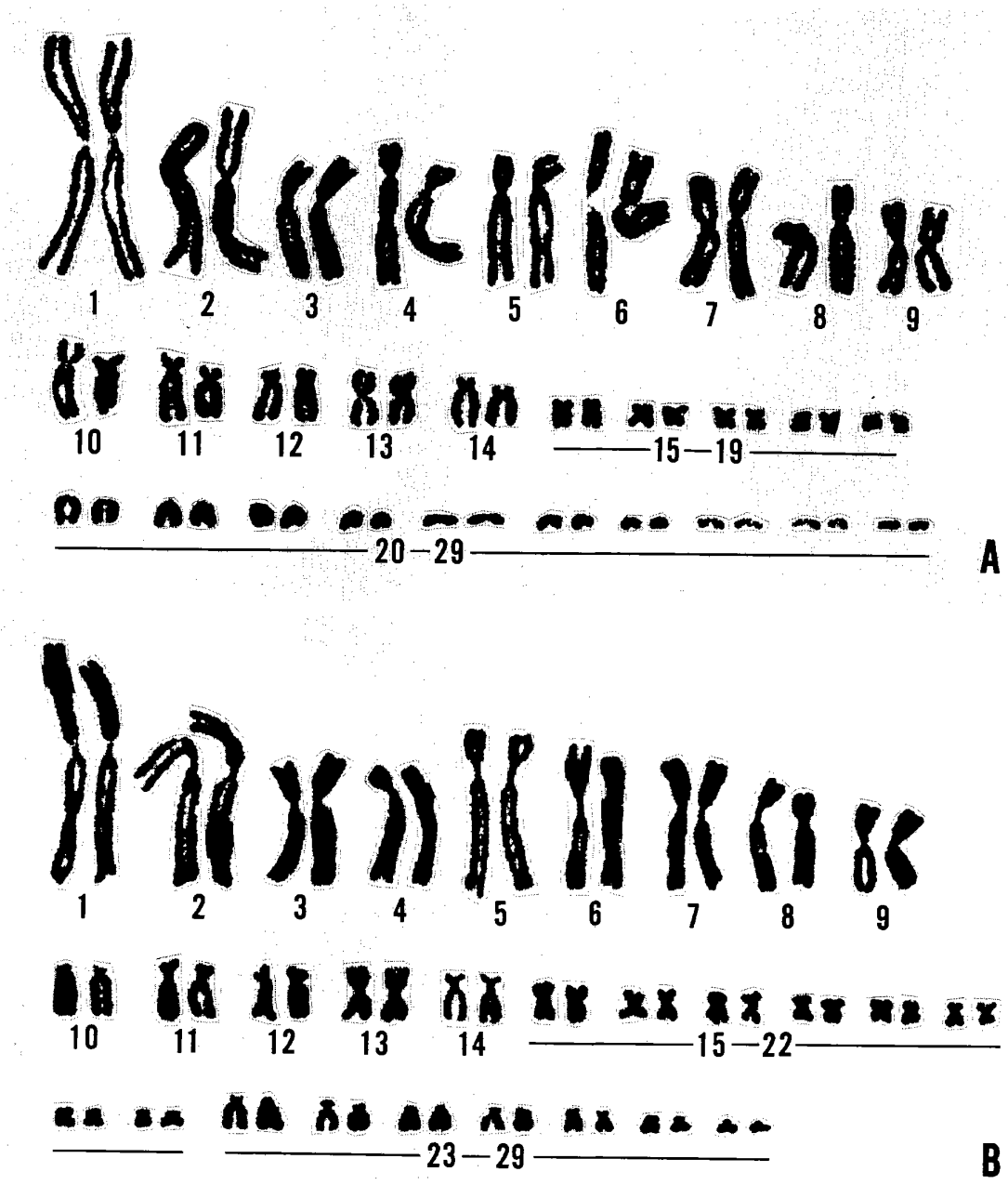


FIG. 1. Conventional Giemsa-stained karyotypes of *H. kimurae* from two localities. A: Miyama-cho (type 1) B: Oshimizu-machi (type 2).

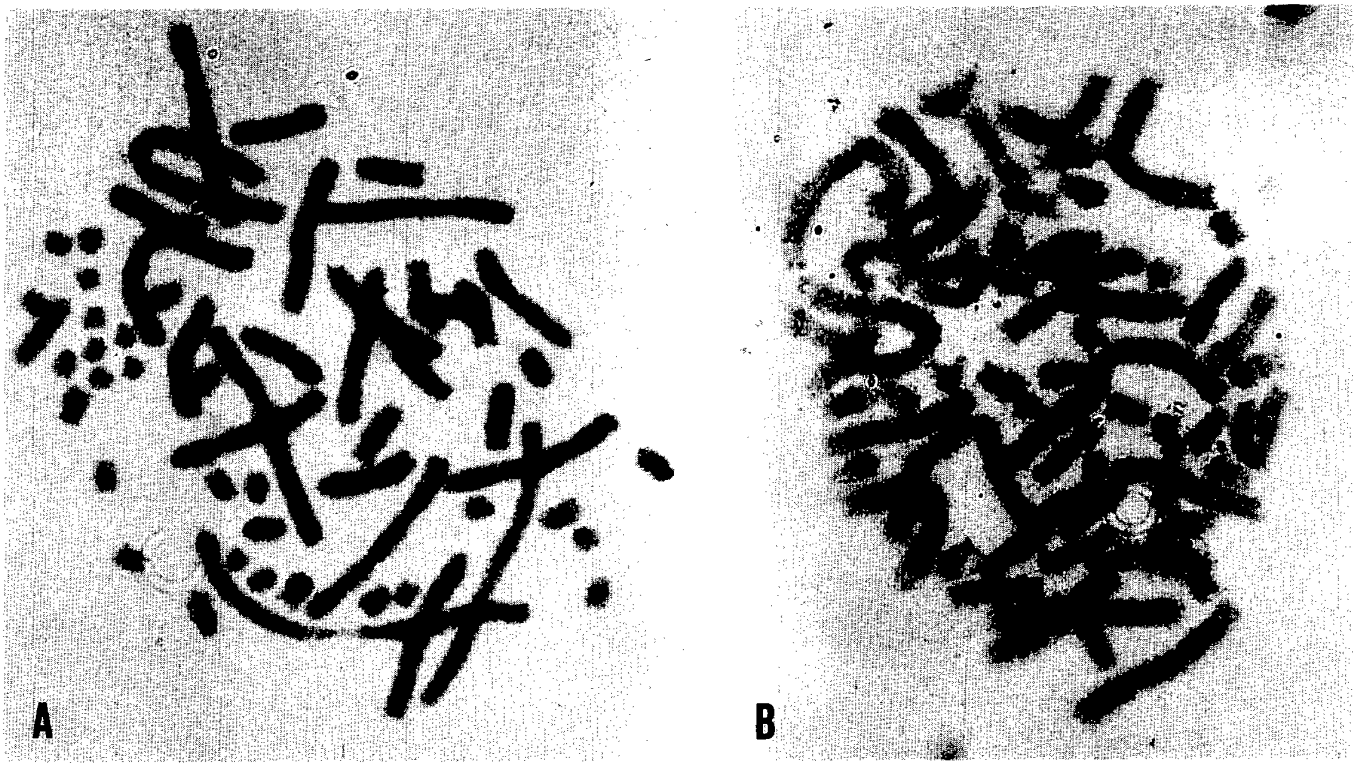


FIG. 2. C-banded metaphases of *H. kimurae* from two localities.
A: Miyama-cho B: Oshimizu-machi.

positive bands (Fig. 2-B). C-banded karyotypes from specimens at the two localities are shown in Figure 3. We observed the same C-banding patterns in the arms of chromosomes Nos. 1–9 and 11–14 in the two karyotypes, except for the C-bands in the centromere regions. In chromosomes Nos. 15–29, we identified only three pairs (Nos. 20–22) of type 1 (Fig. 3-A).

In the specimens from Miyama-cho, we observed intra-populational karyotype variation (Fig. 4). Four of eight embryos analyzed in 1987, and three of eight embryos analyzed in 1988 had a marker chromosome, indicated by an arrow in Figure 4. We were unable to observe the marker chromosome as a pair. This marker chromosome had particularly dark C-bands in the regions of the centromere and the telomere of the long arm (Fig. 5). Furthermore, one metacentric chromosome among chromosomes Nos. 15–19 was absent from all specimens which contained the marker chromosome.

DISCUSSION

According to our C-banding analysis, there was a difference in the amount of centromeric heterochromatin between the two karyotypes of *H. kimurae*. Green [6] reported such a phenomenon between subspecies of the frogs, *Rana aurora aurora* and *Rana aurora draytonii* distributed in North America. The karyotypes of these subspecies were very similar, except that all the chromosomes of *R. a. draytonii* had greatly enlarged centromere regions, which were darkly stained by the C-banding technique. *Rana a. draytonii* was found to have nearly twice as much centromeric heterochromatin per cell as *R. a. aurora*. Green [7] assumed that the karyotype of *R. a. aurora* was a primitive type because the unusually large amount of centromeric heterochromatin of *R. a. draytonii* in comparison to the C-banded karyotypes of other *Rana* species. In *H. kimurae*, we also assume that the karyotype in specimens from Miyama-cho (type 1) is a primitive type, whereas that in specimens from Oshimizu-machi (type 2) is a derived type. We arrived at this

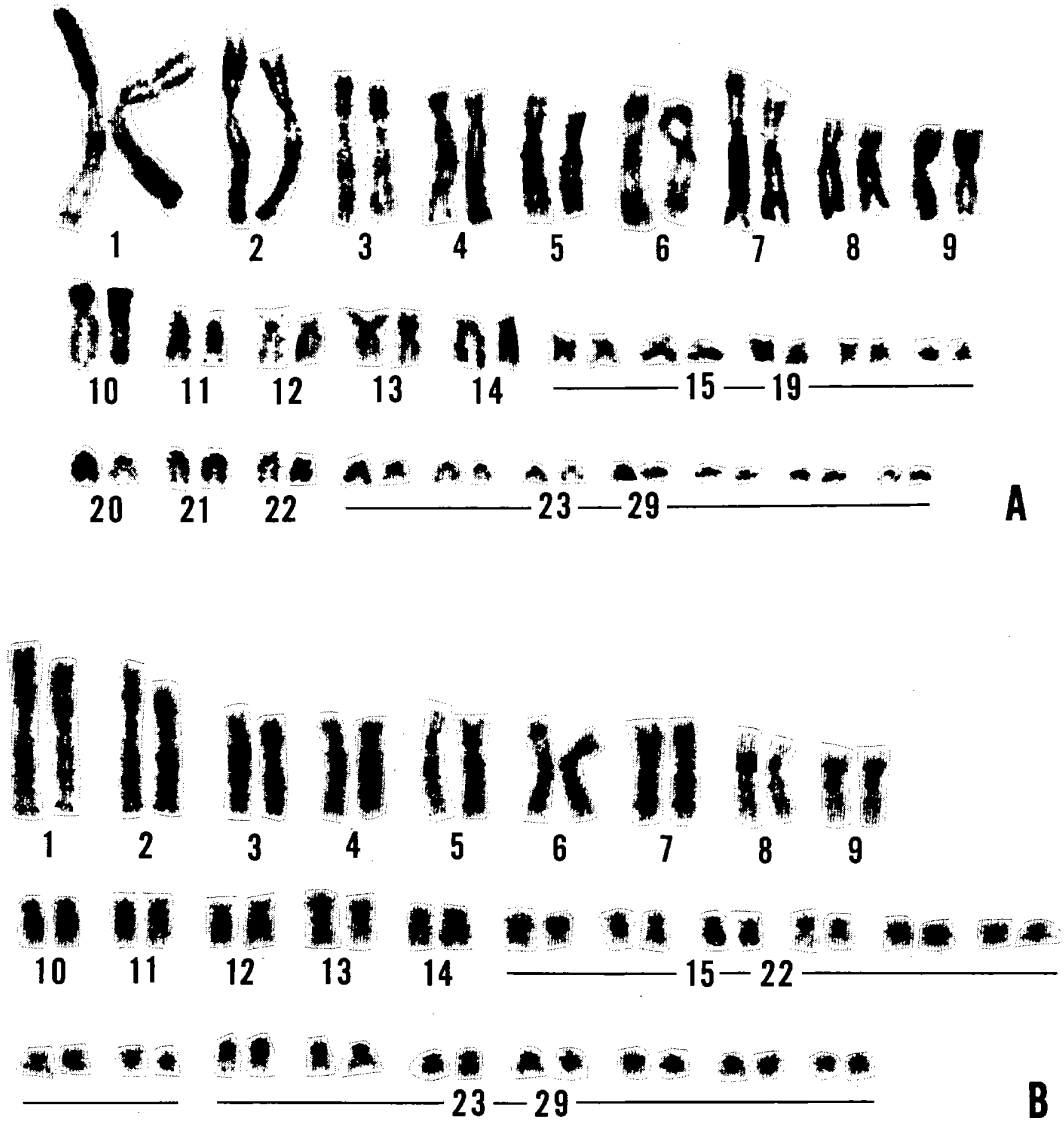
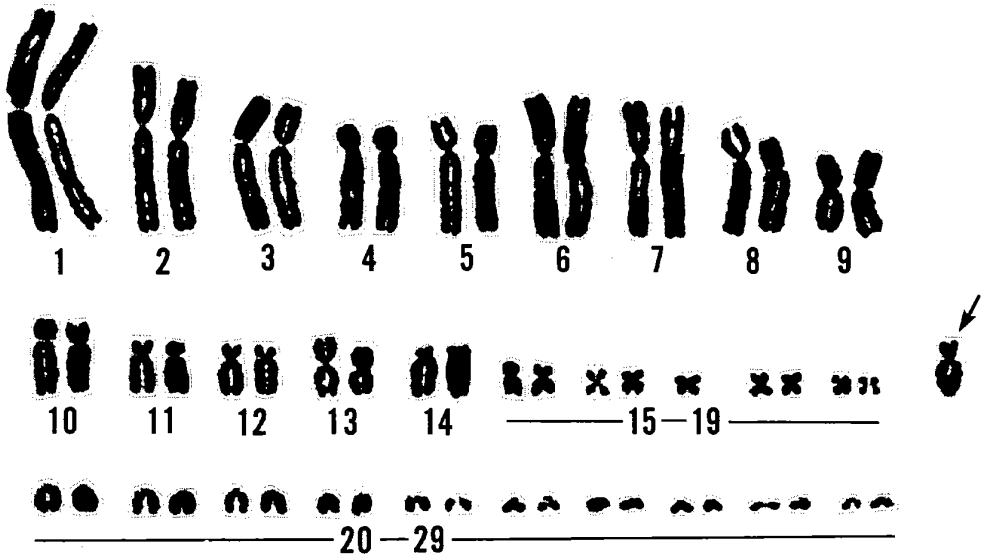


FIG. 3. C-banded karyotypes of *H. kimurae* from two localities. A: Miyama-cho B: Oshimizu-machi



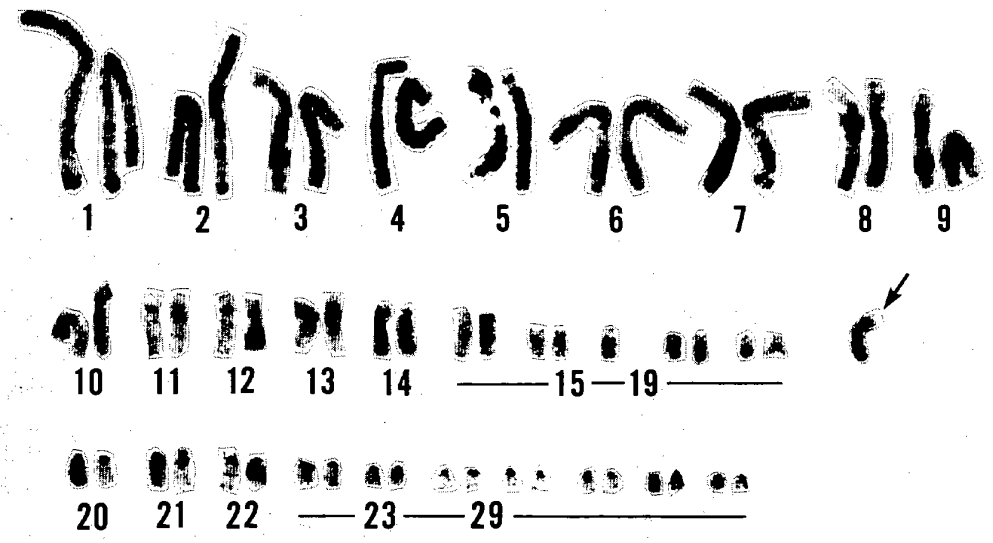


FIG. 5. C-banded karyotype of *H. kimurae* having the marker chromosome of specimens from Miyama-cho.

conclusion because we have not observed such a large amount of centromeric heterochromatin as that of type 2 in C-banding analyses of other *Hynobius* species [4, 8–10]. It is noteworthy that similar centromeric mutations occurred in both American Anura and Japanese Urodela. Green [6] considered that the amplification of centromeric heterochromatin in *R. a. draytonii* is best explained as a case of selective enhancement of non-coding, simple sequence DNA. The finding in *H. kimurae* could also be interpreted in a similar way.

Intraspecific morphological variations of chromosome No. 10 have been observed not only in *H. kimurae* but also in other *Hynobius* species [4, 9]. We have inferred that the evolutionary change of chromosome No. 10 in ten pond-type *Hynobius* species involves a diminution in the length of the short arm, which is stained C-band positive [11]. In *H. kimurae,* the length of the short arm of chromosome No. 10 would also decrease. As stated above, the type 2 karyotype, having a smaller short arm in chromosome No. 10, is the derived type.

There were differences in the features of chromosomes Nos. 15–29 between the two karyotypes of *H. kimurae.* These differences seem to have resulted from amplification of the centromeric heterochromatin in these 15 chromosome pairs, and the formation of a short arm in more than 3 chromosome pairs in type 2.

We observed the same marker chromosome as that shown in Figure 4 in specimens from Okutama-machi (Nishitama-gun, Tokyo Pref.) by conventional Giemsa-staining (Ikebe, unpublished data). Therefore, an presence of the marker chromosome is not a feature peculiar to the population at Miyama-cho. The specimens from both Miyama-cho and Okutama-machi had the type 1 karyotype. We were unable to observe the marker chromosome in specimens having the type 2 karyotype. According to the karyotype analysis, this marker chromosome seems to have resulted from transformation of one metacentric small-sized chromosome. We cannot conclude with certainty whether this marker chromosome indicates the presence of morphologically differentiated sex-chromosomes or autosomal polymorphism, since embryos were used in this study.

FIG. 4. Conventional Giemsa-stained karyotype of *H. kimurae* having the marker chromosome of specimens from Miyama-cho.

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REFERENCES

1 Sato, I. (1943) A Monograph on the Japanese Urodeles. Nippon Shuppan-sha, Osaka, pp. 218–234 (In Japanese)

2 Ikebe, C., Yamamoto, T. and Kohno, S. (1986) Karyotypes of two Japanese Hynobiid salamanders, *Hynobius Kimurae* Dunn and *Hynobius boulengeri* (Thompson). Zool. Sci., **3**: 109–113.

3 Iizuka, K. and Kakegawa, M. (1989) Comparative karyology in five rare species of Hynobiid salamanders from Taiwan and Japan. In "Current Herpetology in East Asia". Ed. by M. Matsui, T. Hikida and R. C. Goris, Herpetological Society of Japan, Kyoto, pp. 95–105.

4 Kohno, S., Kuro-o, M., Ikebe, C., Katakura, R., Izumisawa, Y., Yamamoto, T., Lee, H. Y. and Yang, S. Y. (1987) Banding karyotype of Korean salamander: *Hynobius leechii* Boulenger. Zool. Sci., **4**: 81–86.

5 Sumner, A. T. (1972) A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res., **75**: 304–306.

6 Green, D. M. (1985) Differentiation in amount of centromeric heterochromatin between subspecies of the red-legged frog, *Rana aurora*. Copeia, 1985: 1071–1074.

7 Green, D. M. (1986) Systematics and evolution of Western North American frogs allied to *Rana aurora* and *Rana boylii*: karyological evidence. Syst. Zool., **35**: 273–282.

8 Kohno, S., Ohhashi, T. and Ikebe, C. (1983) Cytogenetic studies of Hynobiidae (Urodela). II. Banding karyotype of *Hynobius tokyoensis* Tago. Proc. Jpn. Acad., **59B**: 271–275.

9 Ikebe, C., Aoki, K. and Kohno, S. (1987) Karyotype analysis of two Japanese salamanders, *Hynobius nebulosus* (Schlegel) and *Hynobius dunni* Tago, by means of C-banding. Zool. Sci., **4**: 621–626.

10 Izumisawa, Y., Ikebe, C., Kuro-o, M. and Kohno, S. (1990) Cytogenetic studies of Hynobiidae (Urodela). IX. Karyological characteristics of *Hynobius abei* Sato by means of R- and C-banding. Experientia, **46**: 104–106.

11 Ikebe, C., Kuro-o, M., Yamada, H. and Kohno, S. (1990) Cytogenetic studies of Hynobiidae (Urodela). X. Morphological variation of chromosome 10 in ten pond-type *Hynobius* from Korea and Japan, with comments on phylogenetic relationships. J. evol. Biol., **3**: 155–170.