

Karyotype of a Japanese Salamander *Hynobius kato* and Its Implication on Breeding Ecology (Amphibia: Caudata)

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ABSTRACT—Karyotype of a Japanese small salamander, *Hynobius kato*, was first described. All individuals examined had $2n=58$ chromosomes, consisting of nine pairs of biarmed macrochromosomes, four pairs of biarmed medium-sized chromosomes, six pairs of biarmed microchromosomes, and 10 pairs of unarmed microchromosomes, although distinction of the second and the third groups of chromosome pairs was not clear. All pairs appeared homologous and no sexual dimorphism was found. Possession of $2n=58$ chromosomes in *H. kato* strongly suggests its lotic-breeding habits as was expected from the number and size of eggs and adult morphology. When compared morphology of chromosomes among lotic-breeders with $2n=58$ chromosomes, metacentric nature of No. 10 seems to characterize the karyotype of *H. kato*.

Key words: *Hynobius kato*, chromosome, karyotype, breeding habit, taxonomy

INTRODUCTION

Hynobius kato is a small salamander recently described from central Japan whose breeding habits are unknown (Matsui *et al.*, 2004).

Generally, species of the genus *Hynobius* are split into the lowland, still-water breeding type (lentic-breeder) and stream breeding type (lotic-breeder) (Sato, 1943). Matsui *et al.* (2004) inferred *H. kato* to be a member of the lotic-breeders, from its morphological and some reproductive characteristics (*e.g.* small clutch size, large, non-pigmented egg, and thick tail of metamorphs), and supposed unique breeding habit of underground breeding. However, the result of electrophoretic analysis indicated this species to be grouped unequivocally neither with lotic-breeding *H. naevius* and *H. kimurae* nor lentic-breeding *H. tokyoensis* compared (Matsui *et al.*, 2004).

Karyotypes of all Japanese *Hynobius* salamanders except for *H. kato* have already been examined, and the numbers of chromosomes are divided into the following three types, $2n=58$ [*H. boulengeri*, *H. kimurae*, *H. naevius*, *H. stejnegeri* (lotic-breeders)], $2n=56$ [*H. abei*, *H. dunni*, *H. hidamontanus*, *H. lichenatus*, *H. nebulosus*, *H. nigrescens*,

H. takedai, *H. tokyoensis* (lentic-breeders), and *H. okiensis*, *H. tsuensis* (lotic-breeders)], and $2n=40$ [*H. retardatus* (lentic-breeder)] [Seto and Matsui (1984), Seto *et al.* (1986), Ikebe *et al.* (1986), Iizuka and Kakegawa (1989), Kuro-o *et al.* (2002) and references therein].

In contrast, karyotypes of congeners from outside of Japan are not studied well, but, from the data currently available, two groups are recognized: $2n=56$ (*H. amjiensis*, *H. chinensis*, *H. leechi*, *H. quelpartensis* and *H. yangi* from China and South Korea) and $2n=58$ (*H. arisanensis*, *H. formosanus*, and *H. sonani* from Taiwan) [Seto and Utsunomiya (1987), Iizuka and Kakegawa (1989), Cha and Lee (1995), Ikebe *et al.* (1998), Kuro-o *et al.* (2002) and references therein]. Of these, species with $2n=56$ chromosomes are all known to be lentic-breeders (Kim *et al.*, 2003; Fei *et al.*, 2005), while those with $2n=58$ chromosomes are proved or strongly supposed to be lotic-breeders (Kakegawa *et al.*, 1989).

Thus, the chromosome number roughly corresponds to the type of breeding, with most of lotic-breeders having $2n=58$ chromosomes and all lentic-breeders having $2n=56$ or 40 chromosomes.

From these lines of available information, karyological approach is expected to be an effective tool to estimate breeding habit of *H. kato*. We thus examined the standard karyotype of this species.

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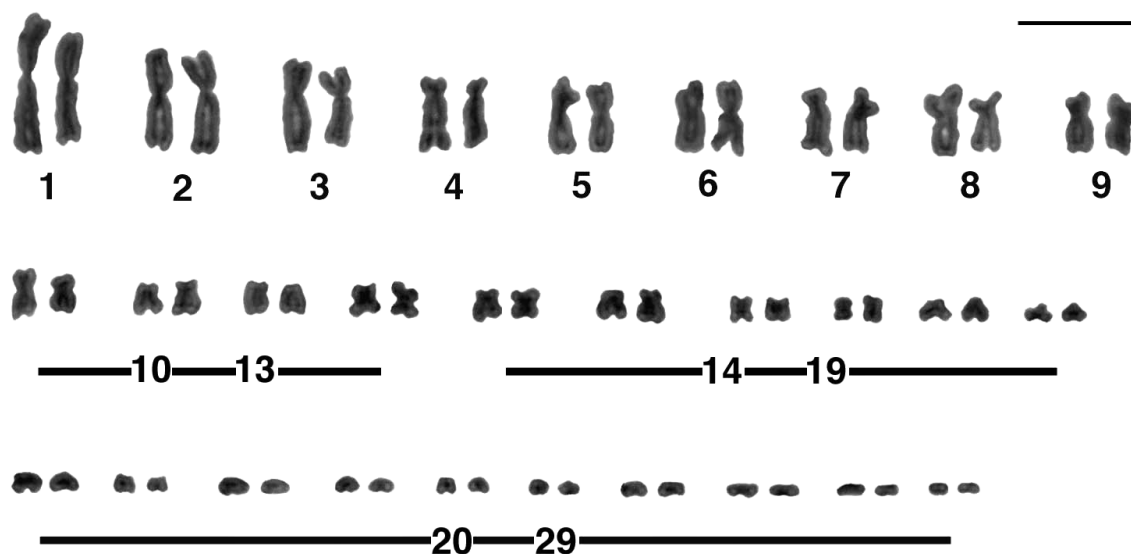


Fig. 1. The Giemsa stained karyotype of male *Hynobius katoi* from Misakubo-cho, Shizuoka Prefecture (KUHE 34789). A bar at the right top equals 10 μ m.

MATERIALS AND METHODS

Chromosomal analyses were conducted on two males, one female, and two juveniles of *H. katoi* collected from Fujieda-shi (type locality) and Misakubo-cho of Shizuoka Prefecture from late April to early May. Sex was determined for each specimen by direct observation of gonads. Chromosomes were studied on the basis of squashed preparations of intestinal epithelium as described by Kezer and Sessions (1979) with a minor modification, using a 24 hour incubation period at 15°C after an intraperitoneal injection of colchicine solution (0.2 mg/ml). Colchicine-arrested metaphase chromosomes were stained with conventional 4% Giemsa solution. In the following description, the nomenclature follows Green *et al.* (1980). Voucher specimens are deposited in the Graduate School of Human and Environmental Studies, Kyoto University (KUHE 34753, 34789-90, 34792, 34883).

RESULTS

We obtained a total of 16 well-spread metaphase plates for all of the five individuals of *H. katoi* (Fig. 1). The diploid chromosome number was $2n=58$. Sexual dimorphism was not observed at the Giemsa staining level. The 29 homologous pairs could be divided into four groups by size and shape (classification according to Seto *et al.*, 1983 and Seto and Matsui, 1984) (Fig. 1). The first group contained large biarmed chromosomes (Nos. 1–9), of which Nos. 4 and 7 were subtelocentric, Nos. 8 and 9 were submetacentric, and the remaining five pairs were metacentric. Nos. 10–13 composed the second, medium-sized group of biarmed chromosomes in nearly equal sizes. This group contained one subtelocentric (No. 12), one submetacentric (No. 11), and two metacentric chromosomes (Nos. 10 and 13). Six pairs of the third group (Nos. 14–19) were biarmed (meta-, submeta- or subtelocentric) microchromosomes, of which two pairs (Nos. 14 and 15) were larger than the remaining pairs. The fourth

group included 10 (Nos. 20–29) unarmed (*i.e.*, telocentric) microchromosomes.

DISCUSSION

The diploid number, $2n=58$, found in the present study of *H. katoi* indicates that this species shows karyological affinity with most of the lotic-breeding *Hynobius* species from Japan and Taiwan. This result agrees with the fact that *H. katoi* has other characteristics common to lotic-breeders (Matsui *et al.*, 2004: see above) and strongly suggests that it is a member of lotic-breeders.

The karyotype of *H. katoi* is unique in having metacentric No. 10, because in other species with $2n=58$ chromosomes, No. 10 is submetacentric (*H. naevius*, *H. arisanensis*, and *H. sonani*), subtelocentric (*H. Boulengeri* and *H. formosanus*), or telocentric (*H. kimurae*) (Iizuka and Kakegawa, 1989; Ikebe *et al.*, 1986). No comparable information is available for *H. stejnegeri* (Seto and Matsui, 1984).

Of the six pairs of chromosomes composing the third size group in the *H. katoi* karyotype (*i.e.*, Nos. 14–19), the two largest pairs, though referred to as microchromosomes here like the remainder, were actually intermediate in size between chromosomes composing the second size group and the other microchromosomes. Thus, although we followed conventional classification of $2n=58$ chromosomes into four size groups (Seto *et al.*, 1983), it is equivocal to separate the second and third groups in *H. katoi* based on the relative size of chromosomes contained.

Similar tendency is reported for Nos. 14 and 15 (Iizuka and Kakegawa, 1989) or No. 14 alone (Ikebe *et al.*, 1986) in *H. kimurae*, and No. 14 in *H. naevius* (Iizuka and Kakegawa, 1989). By contrast, *H. Boulengeri* (Ikebe *et al.*, 1986) and Taiwanese species with $2n=58$ chromosomes (Iizuka

and Kakegawa, 1989), have Nos. 14 and 15 that are similar in size with the remaining microchromosomes of the third group.

Electrophoretically, *H. katoi* was not always grouped with *H. kimurae* and *H. naevius* which have $2n=58$ chromosomes (Matsui *et al.*, 2004), probably these species have already achieved extremely great genetic differentiation from each other. Variations found among karyotypes of these species may support such an idea.

As mentioned above, the number of chromosomes, as well as some external characters in metamorphs, does not completely coincide with the breeding habit in the *Hynobius* salamanders. Indeed, *H. okiensis* from Dogo, Oki Islands and *H. tsuensis* from Tsushima Island are lotic-breeders, but they have $2n=56$ chromosomes (Seto *et al.*, 1986, 1987) like lentic-breeding congeners. In postmetamorphic morphology (*e.g.* flattened tail and thin limbs), the two species also resemble lentic-breeders with $2n=56$ chromosomes rather than other lotic-breeding species.

Because phylogenetic relationships within *Hynobius* are not yet established, we cannot at present estimate the order of changes among breeding habit, morphology, and karyotype in each species of this genus. However, it is likely that lotic sites (*i.e.* small mountain streams) would have been more abundant than the lentic ones (*e.g.* paddies) in islands with steep geomorphology like Tsushima and Dogo (Oki), before the land was inhabited and exploited by human. If the breeding habits are less conservative than the adult morphology and/or chromosome number, these two insular species would have changed breeding sites from lentic to lotic ones. This hypothesis would be tested by future phylogenetic studies of the genus.

We could not detect sexually dimorphic pairs in our standard karyotype of *H. katoi*, but a series of recent studies using various banding techniques (*e.g.* Kuro-o *et al.*, 2002) revealed the microchromosome pair of No. 21 to be sexually dimorphic in some *Hynobius* species with $2n=56$ chromosomes. Future applications of such techniques may reveal their presence in *H. katoi*, as well as in other species with $2n=58$ karyotypes.

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