Karyotype of *Hynobius leechii* from Cheju Island, Korea

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Abstract: The karyotype of *Hynobius leechii* from Cheju Island, Korea is described. Previous studies indicated that the Cheju population is phenotypically dissimilar to the populations from mainland Korea. However, the karyological results obtained from squash preparations of intestinal epithelia indicate that the Giemsa-stained karyotype in this population is quite similar to those of mainland populations.

Key words: *Hynobius leechii*; Korean salamander; Cheju Island; Karyotype; Squash technique

Cheju Island (Cheju-do) is the largest island in Korea and is located near the southern end of the Korean Peninsula. The karyotype of a Korean hynobiid salamander, *Hynobius leechii*, was analyzed by Seto et al. (1986) and Kohno et al. (1987). These studies, however, dealt with mainland populations and did not include specimens from Cheju Island.

The Cheju population of this species has been thought to be not identical with the mainland populations. In 1928, Mori considered the Cheju population as a distinct subspecies and proposed the name *H. l. quelpaertensis*. Okada (1935) included this subspecies in his list of Japanese salamanders, whereas Sato (1937, 1943) did not admit its distinct subspecific position and treated it as a mere local variety of *H. leechii*. Since then no comprehensive studies have been made on the Cheju population of this species until recent years.

Lately, Uh et al. (1992) reported the geographic variation in the external morphology of *H. leechii* within Korea. According to this report, the Cheju population differs significantly from populations from mainland Korea, which was shown by morphological comparisons with 16 skeletal characters among five populations. This report prompted us to conduct the present karyological studies of the Cheju population. It seems of significance to provide karyological evidence that has never been included in previous karyological studies of *Hynobius leechii*.

**Materials and Methods**

Male salamanders were collected from the following seven localities on Cheju Island during 26–31 March 1993: Wha Bukdong, Cheju City; Yongdong, Cheju City; Chonwangsa, Cheju City; Yaksu, Pukcheju-gun; Kaewol Bridge, Pukcheju-gun; Suakkyegok, Namcheju-gun; and Sanghyo, Sogwipo City. Each of these localities differed in altitude, and varied in the water temperature (from 5.0°C at Chonungsa to 12.0°C at Wha Bukdong) and in the degree of water flow in the stream.

In order to analyze karyotypes, somatic chromosomes were prepared by the squash technique applied to the adult intestinal epithelium, as described by Kezer and Sessions (1979). Intraperitoneal injection of colchicine solution (0.2 mg/g of body weight; SIGMA) was made 48 hr before fixation. Conventional Giemsa stain (5% in buffer solution at pH 6.8) was used.

**Results**

Fifty-four metaphase cells from 15 adult males were analyzed. The somatic chromosomes counted 56 for the Cheju population of *H. leechii* (Fig. 1). This number is the same as those previously reported for populations from mainland Korea (Seto et al., 1986; Kohno et al., 1987).

Twenty-eight homologous pairs were divided into four groups according to size and shape. The first group contained large chromosomes; besides a pair of subtelocentrics (No. 4) and two pairs of submetacentric elements (Nos. 7 and 8), large metacentric chromosomes (Nos. 1, 2, 3, 5, 6 and 9) characterized this group. The second group consisted of a pair of submetacentrics (No. 10), metacentrics (No. 13), and two subtelocentric pairs (No. 11 and 12). The third and fourth groups in the karyotype comprised six small biarmed chromosomes (Nos. 14–19) and nine uniarmed pairs (Nos. 20–28), respectively.

The second group was especially noticeable because the size of No. 10 chromosome as compared to those of other three members of the group (Nos. 11–13), and the size of the short arm of this pair (10 p) showed slight geographic variations, although statistical significance has not been detected.

**Discussion**

Although species of the genus *Hynobius* are usually split into two types, i.e., mountain stream types and lowland still-water types (Sato, 1943), this distinction is not always clear, as Matsui (1987) pointed out. *Hynobius leechii* is widely distributed in Korea and has been recognized as a lowland still-water type (a lentic breeder;
FIG. 1. Karyotype of Hynobius leechii from Cheju Island, Korea. The chromosome preparation was made by a squash technique with intestinal epithelia from adult male. Conventional Giemsa stain. Scale denotes one section = 10 μm.

Sato, 1943). As far as we observed, the Cheju population inhabits various habitats; some specimens were found in mountain streams at a water temperature of around 5°C at altitudes of 600 m (Chonwangsa) and 900 m (Yaksu) above sea level, and others were in lowland lentic habitat with water temperatures around 11–12°C (Wha Bukdong) at less than 50 m above sea level.

From morphological comparisons with 16 skeletal characters among five populations of H. leechii, Uh et al. (1992) noted that the Cheju population differed significantly from populations from mainland Korea. Yang et al. (1982) and Kim et al. (1990) also reached the same conclusion from the isozyme analyses in Korean salamanders using starch gel electrophoresis. Thus, results of both phenotypic and genotypic examinations suggest that the Cheju population might represent a distinctive taxon.

Comparing the present karyological results with those of the mainland populations of H. leechii (Seto et al., 1986; Kohno et al., 1987), the Cheju population is judged to resemble the mainland populations, especially those from Yangju and Chindo as presented by Kohno et al. (1987) that have a karyotype nearly identical with the present one. From the Kyongju population (Seto et al., 1986) the Cheju population is differentiated by only a minor difference in the number of small biarmed pairs in the third group; six pairs are present in the Cheju population in contrast to five in the Kyongju population. A similar intraspecific variation has been reported in the Japanese H. nebulosus, which has a wide distribution range (Ikebe and Kohno, 1979; Seto et al., 1983; Ikebe et al., 1987).

A karyotype that is quite similar to the Cheju population of H. leechii was found in H. okiensis (Seto et al., 1987). The latter species is endemic to Dogo Island of the Oki Islands, which are geographically close to Cheju Island and the Korean Peninsula. From the larval morphology and lotic breeding habitats, this species has been regarded as a mountain-brook type (Sato, 1943). The karyological similarity of H. okiensis and H. leechii from Cheju Island, however, once again underscores the difficulty of classifying the Hynobius species into mountain stream and lowland still-water types.

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要旨　韓国済州島のチョウセンサンショウウオの核型

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チョウセンサンショウウオの核型は、すでに韓国本土産の個体について報告されている。しかし従来から別亜種の可能性を示唆されている済州島の個体はこれまでにその核型が分析されたことはなかった。そこで済州島内の7ヶ所で採集した雌雄体を用い、腸上皮から得られた分裂中期細胞を押しつぶし法により染色体観察をした。その結果、染色体数は2n＝56でギムザ染色された核型からみる限り、染色体の形態は韓国本土産の個体と類似性が高く、外部形態、骨格、酵素タンパクなどの表現型で知られるような済州島集団の独自性は核型には現れていないことが示された。

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