Cytogenetic studies of Hynobiidae (Urodela) XIX. Morphological variation of sex chromosomes pairing behavior of sex lampbrush chromosomes in *Hynobius quelpaertensis* (Mori) from Cheju Island, South Korea

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Abstract

Using Giemsa staining, C-banding and Ag-NOR staining techniques, we analyzed chromosomes in adult male and female *Hynobius quelpaertensis* and in embryos of this species in egg sacs collected from eight localities of Cheju Island, South Korea. Chromosome pair 21 was consistently homomorphic in male specimens, while it was heteromorphic in female specimens, suggesting the occurrence of ZZ/ZW sex chromosome constitution in this species. The W chromosome, being much larger than the Z chromosome, was of three morphologically distinct types: W^A^, W^B^ and W^C^. Lampbrush chromosomes examined in the oocytes of one female specimen having the W^A^ chromosome showed that the short arm of the W^A^ chromosome and the long arm of the Z chromosome paired closely and hence are genetically homologous. We also tried to analyze the structural relationship among the three types of W chromosomes based on their C-banding and Ag-NOR patterns.

Introduction

Chromosome studies have not been very successful in salamander species of the genus *Hynobius* (family Hynobiidae) distributed throughout East Asia (Sato 1943, Zhao & Adler 1994). It is not yet clear, for example, whether the sex chromosomes are morphologically differentiated in most species. The situation has been further complicated by difficulties in the taxonomy of individual species.

Our earlier cytogenetic studies using embryos of five *Hynobius* species have detected several specimens having a heteromorphic chromosome pair (*H. dunni*: Ikebe et al. 1987; *H. lichenatus* and *H. tokyoensis*: Kuro-o et al. 1987; *H. leechii*: Kohno et al. 1987; *H. kimurae*: Ikebe & Kohno 1991). We could not conclude, however, whether the heteromorphic pair represents morphologically differentiated sex chromosomes or autosomal polymorphism, since the sex of the embryos were
not determined. Recent cytogenetic studies using adult male and female specimens proved the existence of morphologically distinguishable Z and W sex chromosomes in *H. tenuis* (now identified as *H. hidamontanus* according to Matsui et al. 2002) (Ikebe et al. 2000) and *H. tokyoensis* (Kuro-o et al. 2002). It is likely, therefore, that morphologically differentiated sex chromosomes are also present in *H. dunni, H. lichenatus, H. leechii* and *H. kimurae*, although there is no hint of such sex chromosomes in remaining *Hynobius* species so far examined (Kuro-o et al. 2002).

*Hynobius leechii* has been found in northern China, and the Korean peninsula (Sato 1943), with Cheju Island being the southern limit. Recent biochemical studies (Lee & Jung 1993, Lee et al. 1998, Yang et al. 1997) have shown, however, that the populations of *H. leechii* from Cheju Island and the small islands off the south coast of the Korean peninsula are genetically different from other *H. leechii* populations. Thus, Yang et al. (2000) proposed to designate animals from Cheju Island and other Korean islands as an independent species, *Hynobius quelpaertensis*. In addition, the population from Busan, South Korea, has also been identified as a new species, *Hynobius yangi*, on the basis of morphological characteristics (Kim et al. 2003).

In the present study, we analyzed the mitotic chromosomes of adult male and female specimens and embryos by Giemsa staining, C-banding and Ag-NOR staining to identify the sex chromosomes of *H. quelpaertensis* collected from eight localities in Cheju Island. We also examined the paring behavior of the sex lampbrush chromosomes in the meiotic division of oocytes to help delineate homologous and nonhomologous regions in the sex chromosome bivalents.

**Materials and methods**

**Materials**

Cheju Island has an area of 1826 km$^2$ and lies 90 km off the Korean Peninsula (Figure 1a). Adult male and female *H. quelpaertensis*, and/or embryos in egg sacs, were collected from the following eight localities: Wolpyongdong (altitude: 400 m), Kyoraeri (500 m), Songpanak (700 m), Ipsokdong (300 m), Sanghyo (200 m), Chongwangsa (600 m), Okusu (900 m), and Yongshil (1100 m) (Figure 1b). These collection sites extend from the lowlands to halfway up Mt. Halla (1950 m).

**Chromosome preparation**

For mitotic chromosome observation, we used the testis and intestinal epithelium from males, the intestinal epithelium from females, and whole bodies from tail-bud-stage embryos. Chromosome preparation from the adult testis was made as reported by Kuro-o et al. (1998). We used the method of Kezer and Sessions (1979) for chromosome preparation of the intestinal epithelium, and the method reported by Kohno et al. (1987) for the embryonic materials. C-banding was carried out according to the CBG technique (Sumner 1972), and NORs were stained by the technique reported by Howell and Black (1980).

A female specimen collected from Songpanak was used for meiotic chromosome observation. Lampbrush chromosomes were prepared by the method of Gall (1966) with some modifications (Ohtani 1990).

**Results**

**Mitotic chromosomes**

Mitotic chromosomes from 37 males, 25 females, and 68 embryos in 11 egg sacs were analyzed by a conventional Giemsa staining (Table 1). Mitotic cells from 26 specimens (5 males, 8 females and 13 embryos in 5 egg sacs) and 30 specimens (10 males and 20 females) were analyzed by C-banding and Ag-NOR staining technique, respectively (Table 2). The numbers of metaphase plates examined are shown in Tables 1 and 2.

The chromosome number 2n = 56 was confirmed in all the specimens examined. Male cells contained 28 homomorphic chromosome pairs, which correspond to those reported previously by Seto and Iizuka (1993). On the other hand, the second largest acrocentric pair (chromosome 21) was consistently heteromorphic in female cells (Figures 2 and 3). The heteromorphic pair was found in 36 out of 68 (52.9%) embryos examined: 17 out of 31 embryos collected in Kyoraeri, 6 out of 8 in Song-panak,