4. Chromosome Evolution and Speciation of Reptiles. I

*Karyotype of the Japanese Mamushi, Agkistrodon blomhoffii blomhoffii (Viperidae, Crotalinae) with Special Regard to the Sex Chromosomes**

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The mamushi, *Agkistrodon halys-blomhoffii* complex, feared as a venomous snake, is distributed widely in Japan, Korea, China, USSR, Mongolia and Iran. They are classified into several species and subspecies from the morphological characteristics (Gloyd and Conant 1982). Among them *A. blomhoffii blomhoffii* (Boie) is endemic to Japan and distributes in Hokkaido, Honshu, Shikoku, Kyushu and some satellite islands. The chromosomes of the male germ cells in this species have first been reported by Nakamura (1927, 1935) by the classical cytogenetic techniques. According to him, the diploid chromosome number was $2n=36$ and $n=18$. Among them 5 pairs were V-shaped, 3 pairs were short and rod shaped and the remaining 10 pairs were dot like. The sex chromosome was not identified by him. The chromosome study of this species by the modern technique has been carried out by Itoh *et al.* (1970). According to them, the male specimen had $2n=36$ similar to the above study. Among them 16 were the macrochromosomes and the remaining 20 were microchromosomes. The sex chromosome was not identified either by them, because the observation of the female specimens was not successful. The present paper deals with the karyotypes of the male and the female of the Japanese mamushi, *A. blomhoffii blomhoffii*, by the modern cytogenetic technique, with special regard to the identification of the sex chromosomes in this species.

Material and methods. The Japanese mamushi, *Agkistrodon blomhoffii blomhoffii*, collected in Gifu-ken, Japan, were used in the present study. The chromosomes were observed from the direct preparations of the bone marrow cells by the following techniques. (1) About 0.3 ml of 0.1% (w/v) colchicine were injected in the peritoneal cavity. (2) Seven hours later the snake was anesthetized and 8 to 10 ribs were cut off. (3) The bone marrow cells were removed to centrifuge tube from the shaft of the bone in the physiological buffer solution (PBS) by use of the syringe. (4) The suspension was gently centrifuged at 1200 rpm for 5 min, after that the PBS was discarded. (5) About 3 ml of the hypotonic solution, 1% (w/v) sodium citrate, were added to the tube and suspended by pipette. (6) The suspension was left for 10 min in the room temperature. (7) Five ml of Carnoy 3:1 (glacial acetic acid 1 : absolute alcohol 3) were added to the tube and mixed with the hypotonic solution, and left for 10 min. (8) The suspension was centrifuged and the solution was discarded. Three ml of the Carnoy solution was added and left 10 min. (9) After another

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centrifuge, about 0.2 ml of Carnoy solution were added. (10) A drop of the fixed material was placed on a wet slide kept in 50% alcohol, dried quickly over a gas flame, (11) and stained with 2% Giemsa solution.

Results and discussion. 1) Male specimens. The males of the Japanese mamushi had 36 chromosomes (18 pairs) in the diploid cells which were the same as the previous investigations (Nakamura 1927, 1935; Itoh *et al.* 1970). Among them 8 pairs (nos. 1 to 8) were the macrochromosomes and the remaining 10 pairs (nos. 9 to 18) were the microchromosomes. Among the 8 macrochromosomes, the first two pairs (nos. 1 and 2) were characterized by the large metacentric and the submetacentric chromosomes. The next 3 pairs (nos. 3 to 5) were the second sized metacentric or submetacentric chromosome. The metacentric no. 3 was larger than the metacentric no. 4. In the no. 4 one arm was slightly shorter than the other one. The pair no. 5 was easily identified due to the submetacentric or subtelocentric characteristic. The remaining three pairs (nos. 6 to 8) showed the small subtelo- (nos. 6 and 8) and submetacentric (no. 7). The identification of 10 microchromosomes was difficult, because their size was so small, but only the largest one pair (no. 9) was characterized by the metacentric nature. The other 9 microchromosome pairs should be acrocentric (Fig. 2A).

2) Female specimens. The females had the same chromosome numbers as the male, $2n=36$. The karyotype was also the same as the male except the pair no. 4. This pair of the female was characterized by the heteromorphic; one member of the pair was metacentric similar to that found in the male, but the other one was subtelocentric and its length was about two third to the other partner (Fig. 1). This heteromorphic pair was strongly suggested to be the sex chromosome. The larger one metacentric chromosome would be the Z(X) and the smaller subtelocentric one was the W(Y). Therefore, the metacentric no. 4 found in the male specimens was the ZZ(XX) chromosomes. The pair no. 9 among the microchromosomes was also metacentric as seen in the male specimens (Fig. 2B).

The female heterogamety in the reptiles was first reported by Oguma (1934) in *Lacerta vivipara*. He described that the male specimens had $2n=36$, but the females has $2n=35$, and he assumed to be XO digamety in this species. On the other hand, Matthey (1943) did not observe any chromosome differences in both sex of the same *Lacerta* species and some other reptiles. In 1962, Kobel reported on the dimorphous pair of the sex chromosomes in the female snake, *Vipera berus*. After that the dimorphism of the sex chromosomes in the female snakes has been reported by several investigators (Becak *et al.* 1962; Becak 1968; Kobel 1967; Itoh *et al.* 1970; Baker *et al.* 1971, 1972; Singh 1972, 1978; Mengden and Stock 1980; Gutiérrez and Balaños 1980; Mengden 1985). The karyotype of the Japanese mamushi has been reported by Nakamura (1927, 1935) and Itoh *et al.* (1970). The latter authors have studied by the modern techniques, but they observed only the male specimens and then the sex chromosomes could not be identified in this species. By the present investigations the Z(X) chromosome was determined to be mediately sized and metacentric, and the W(Y) chromosome to be subtelocentric. The karyotype of the Chinese mamushi *A. blomhoffii brevicaudus* (*A. halys*) has been reported by Qu *et al.* (1981). Their study coincided with the present result, except the size and the shape of the sex chromosomes. Itoh *et al.* (1970) and Qu *et al.* (1981) have pointed out that 10 pairs of chromosomes of the mamushi in Japan and China were the dot-shape classified as the microchromosomes. All these chromosomes could not be identified from each other

Fig. 1. Metaphase chromosomes of the female Japanese mamushi, *Agkistrodon blomhoffii blomhoffii*. The sex chromosomes are denoted by Z and W. Arrows indicated the metacentric microchromosome pair no. 9.

Fig. 2. Karyotypes of the Japanese mamushi, *A. blomhoffii blomhoffii*. A: Male specimen. B: Female specimen. The pair no. 4 is the sex chromosomes. The sex chromosomes are denoted by the Z and W letters.

by them. De Smet (1978), however, observed that the largest microchromosome no. 9 in *A. contortrix* was metacentric. In the present specimens, it was also confirmed to be a metacentric nature and this pair was clearly distinguished from the other microchromosomes. It is a further problem, whether or not, this metacentric microchromosome no. 9 is observed in the other species or subspecies of the mamushi.

Summary. The karyotype of the Japanese mamushi, *Agkistrodon blomhoffii blomhoffii* collected in Gifu-ken is investigated directly from the born marrow preparations. The chromosome number of males and females of this species is $2n=36$, consisting of 8 macrochromosome pairs and 10 microchromosome pairs. The pair no. 4 is the sex chromosomes. In the male it is a metacentric homomorphic pair (ZZ), while in the female a metacentric and subtelocentric heteromorphic pair (ZW). The length of the W-chromosome is about two third of the Z-chromosome. Among 10 microchromosomes, the largest pair no. 9 is confirmed to be metacentric.

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