Karyotypes of Japanese Species of the Genus *Trimeresurus*

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Abstract: The karyotypes of four species of Japanese *Trimeresurus* are described. They are similar to each other and the diploid number is $2n=56$ with 16 macro- and 20 microchromosomes. In all species, the sex chromosomes of ZW type are recognized. The three species of them have the second pair similar to that of the new world pit-vipers, while remaining *T. okinavensis* has a slightly larger arm ratio which approaches that of Asian *Agkistrodon*. C-banded karyotypes are described for *T. flavoviridis* and *T. okinavensis*. The latter has a portion heavily stained on the longer arms of 1st and 2nd pairs, which correspond to the secondary constrictions observed in some metaphase plates. These are not observed in the former.

Key Words: Crotalinae; *Trimeresurus*; Karyotype; C-banded pattern; Secondary constriction

There are four species of the genus *Trimeresurus* in the Ryukyu Archipelago, Japan. Among them, *T. flavoviridis* and *T. okinavensis* were reported to have the diploid number $2n=36$ by Makino and Momma (1949) using classical techniques. Similar figures were also reported on two species of the genus in Taiwan, namely *T. stejnegeri* and *T. mucrosquamatus*, by Nakamura (1935). The karyotypes of these two species were later examined using modern techniques by Qu et al. (1981) and the observation by Nakamura was confirmed by them.

In the present study, the karyotypes of all four species of the genus *Trimeresurus* in Japan are described and compared with those of other crotaline snakes. In *T. flavoviridis* and *T. okinavensis*, C-banded karyotypes were examined in addition to the Giemsa stained karyotypes.

MATERIALS AND METHODS

The origins and the number of specimens examined are as follows:

- T. flavoviridis*, two females and two males from Tokunoshima Is. and a female* from Okinawa Is.
- T. tokarensis*, two females from Takarajima Is.
- T. elegans*, three females from Ishigakijima Is.
- T. okinavensis*, two females* from Okinawa Is.

Among them, C-banded karyotypes were observed in the specimens with the asterisks.

The procedure for observing metaphase chromosomes was the same as that described earlier by me (Yosida and Toriba, 1986a;

Toriba, 1987) and the chromosomes were observed from bone marrow of the ribs. On the C-banded karyotypes, the BSG method of Sumner (1972) was applied with a slight modification. The preparations were dried without using a gas flame, then treated consecutively with 0.2N HCl for 1 hr; 5% Ba(OH)₂ at 50°C for 30 to 40 min; 2×SSC at 60°C for 1 hr; and stained with 4% Giemsa solution in phosphate buffer of pH 6.8. The measurements were made on 10 to 15 well-spread metaphase plates, and nomenclature follows Levan et al. (1964).

RESULTS

Numbers of well-spread metaphase plates were observed for all four species, in which the diploid number was $2n=36$, with 16 macro- and 20 microchromosomes. The karyotypes are shown in Fig. 1 and the measurements in Table 1. As seen in them, the karyotypes of these species are similar to each other. The fourth largest pair is sex chromosomes and composed of a metacentric Z and submeta- or subtelocentric W-chromosome. Among autosomes, the first, 3rd, 4th and 6th pairs are metacentric in all the forms.

On the other hand, three other pairs show larger variation. The second pair has larger value of arm ratio than the first pair with that of *T. okinavensis* largest. The 5th and 7th pairs are submeta- or subtelocentric with the exception of the 7th pair of *T. elegans* which is metacentric. However, the 7th pair was small and the preparation of the metaphase chromosomes of *T. elegans* was relatively poor among four species. Thus this difference should not be considered seriously. Among ten pairs of microchromosomes the largest one was recognized as metacentric and others seemed to be

TABLE 1. The relative length (RL, %) and arm ratio (AR) of the macrochromosomes of Japanese species of the genus *Trimeresurus* and some other crotaline species for the comparison.

		Number								
		1	2	3	4	5	6	7	Z	W
<i>T. flavoviridis</i>	RL	26.0	21.9	15.2	8.9	6.6	6.1	5.5	10.0	7.9
	AR	1.2	1.5	1.2	1.3	3.2	1.6	2.5	1.2	3.7
<i>T. tokarensis</i>	RL	26.8	20.1	13.9	9.0	7.3	6.8	6.0	10.3	7.8
	AR	1.1	1.7	1.2	1.4	2.6	1.3	2.3	1.2	3.7
<i>T. elegans</i>	RL	27.2	20.8	14.7	8.9	6.5	6.5	5.5	10.2	7.7
	AR	1.2	1.6	1.2	1.5	2.0	1.2	1.6	1.5	2.3
<i>T. okinavensis</i>	RL	26.9	22.1	15.2	8.5	6.9	6.3	5.2	9.0	8.1
	AR	1.3	2.2	1.1	1.5	3.2	1.2	3.0	1.5	1.9
<i>B. jararaca</i> ¹⁾	RL	26	21	15	8	7	7	6	10	7
	AR	1.1	1.5	1.2	1.6	2.7	1.5	2.3	1.1	3.5
<i>C. durissus</i> ¹⁾	RL	26	21	15	9	7	7	6	10	6
	AR	1.1	1.6	1.0	1.6	2.6	1.5	2.2	1.2	2.2
<i>A. contortrix</i> ²⁾	RL	25.7	20.4	13.6	9.5	7.3	6.6	6.1	10.9	
	AR	1.0	1.5	1.1	1.4	1.2	2.7	1.8	1.2	
<i>A. blomhoffii</i> ³⁾	RL	26.9	21.3	15.0	8.7	6.5	6.2	5.5	9.7	7.5
	AR	1.1	2.5	1.2	1.8	2.9	1.9	2.8	1.1	3.1
<i>T. stejnegeri</i> ⁴⁾	RL	24.1	20.0	15.2	9.0	8.0	7.2	6.1	10.2	
	AR	1.1	2.0	1.5	1.8	6.7	2.1	11.5	1.5	

¹⁾ Beçak (1965). ²⁾ recalculated from De Smet (1978). ³⁾ *A. b. blomhoffii*, present study. ⁴⁾ Qu et al. (1981).

subtelo- or telocentric.

In some of the metaphase plates of *T. okinavensis*, a structure like a secondary constriction was seen in the longer arm of the largest pair (Fig. 2). A similar one was more feebly seen in the second pair. In the C-banded karyotype of this species, the corresponding positions of these secondary constrictions were best stained, although centromeres of each pair and the W-chromosome were only weakly stained (Fig. 2). On the other hand, the C-banded karyotype of *T. flavoviridis* did not show such a structure, and only centromeres of each pair and W-chromosomes were well stained (Fig. 3). Although a special structure was not recognized in Giemsa stained karyotype, a slightly well stained portion was recognized in the distal end of the longer arm of the second pair.

DISCUSSION

The present results agree well with those of Makino and Momma (1949). The diploid

number of $2n=36$ with 16 macro- and 20 microchromosomes are similar to most other crotaline snakes so far examined by previous workers (Beçak, 1965; Baker et al., 1972; Zimmerman and Kilpatrick, 1973; Gutiérrez et al., 1979; Qu et al., 1981). Although De Smet (1978) considered that *Bothrops schlegelii* has 24 macro- and 12 microchromosomes, observation of his figure and a later study by Gutiérrez et al. (1979) on this species indicate similarity of this species to other species. This pattern is also found in many colubrid and boid snakes.

The submeta- or subtelocentric W-chromosome is also common in crotaline snakes with the exception of a telocentric W in *Agkistrodon contortrix*, *A. ussuriensis* and *Sistrurus catenatus* (Baker et al., 1972; Yosida and Toriba, 1986b).

Among the slight differences found in the centromeric positions of the pair nos. 2, 5 and 7, I am most interested in the second pair, because most other species of new world Crotalinae have

FIG. 1. The female karyotypes of four species of the genus *Trimeresurus* with those of the genus *Agkistrodon* for comparison. (A) *T. flavoviridis* from Tokunoshima Is. (B) *T. tokarensis* from Takarajima Is. (C) *T. elegans* from Ishigaki-jima Is. (D) *T. okinavensis* from Okinawa Is. (E) *Agkistrodon blomhoffii blomhoffii* from Gifu, Japan. (F) *A. halys caraganus* from USSR. Sex chromosomes are denoted by Z and W.

FIG. 2. Giemsa stained (upper) and C-banded (lower) karyotypes of female *Trimeresurus okinavensis*. Arrows indicate secondary constrictions and Z and W show sex chromosomes.

FIG. 3. Giemsa stained (upper) and C-banded (lower) karyotypes of female *Trimeresurus flavoviridis* from Okinawa Is. Z and W show sex chromosomes.

a 1.2–1.7 arm ratio, according to Beçak (1965) and De Smet (1975) and similar value in my calculation from the figures by other workers mentioned above (see also Table 1). On the other hand, Asian *Agkistrodon* have a far larger value as seen in Fig. 1 (E, F) and Table 1. Among the four species of the genus *Trimeresurus* examined in the present study, three species have a value similar to the new world crotaline snakes, and *T. okinavensis* has a slightly larger value which approaches Asian *Agkistrodon*. According to Qu et al. (1981), however, *T. mucrosquamatus* and *T. stejnegeri* have an intermediate value between them (Table 1). Therefore future study is needed to clarify the problem, though this pair seem to have had some role in the evolution of crotaline snakes.

The difference in the C-banded karyotypes of *T. flavoviridis* and *T. okinavensis* suggests their remote relationship as claimed by Burger (1971) and Hoge and Romano-Hoge (1981). However, there is no other species whose C-banded karyotype has been examined. The study on the C-banded karyotypes of other species of crotaline snakes will be interesting.

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要旨 日本産ハブ属の核型

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日本産ハブ属4種の核型を記述した。すべて $2n=36$ (大染色体18, 小染色体20) でよく似た核型を持つ。しかし第2染色体の腕比がヒメハブで他のものより大きかった。またC—バンド

染色をすると、ハブとヒメハブで違いがみられた。

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