Karyotype of *Pareas iwasakii*: The First Chromosomal Description of a Pareatine Snake (Colubridae)

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Abstract: The standard female karyotype of *Pareas iwasakii*, a species endemic to the southern Ryukyus, Japan, is described. This is the first report of the chromosomal arrangement for the subfamily Pareatinae. The present material had $2n=36$ chromosomes, consisting of eight pairs of banded macrochromosomes and ten pairs of apparently unbanded microchromosomes. Of these, pair 4 was heteromorphic, and thus is supposed to be the ZW-sex chromosome pair. The remaining pairs appeared homologous. The karyotype of this morphologically highly specialized snake largely fits the broadly prevailing henophidian/caenophidian format, which most likely represents a primitive state of the extant colubrid karyotypes.

Key words: *Pareas iwasakii*; Pareatinae; Karyotype; Chromosomal evolution; Ryukyu Archipelago

The colubrid subfamily Pareatinae consists of some 18 species belonging to three genera (*Pareas* Wagler, 1830; *Aplopeltura* Duméril, 1853; and *Asthenodipsas* Peters, 1864 [as the senior synonym of *Internatus* Rao et Yang, 1992]) of snail-eating snakes distributed in tropical and subtropical Asia (McDowell, 1987; Rao and Yang, 1992; David and Vogel, 1996; Ota et al., 1997). While being a well-defined monophyletic group characterized by several specialized features, this subfamily has very few morphological characters that allow inference of its relationships with other colubrid taxa (e.g., Underwood, 1967; McDowell, 1987). A recent molecular study also failed to reveal the phylogenetic status of the subfamily (Kraus and Brown, 1998).

Despite their possible taxonomic utility, karyotypes have been rather poorly studied for snakes in general (e.g., Bickham, 1984; Olmo, 1986). This is especially true with some morphologically highly divergent forms in the Old World (e.g., Ota et al., 1991; Das and Ota, 1998), and not a single pareatine species has ever been studied karyologically. Such a situation has made it difficult to discuss the status of Pareatinae from a cytotaxonomic view point.

I recently obtained one female of *Pareas iwasakii*, a species endemic to Ishigakijima and Iriomotejima Islands of the Yaeyama Group, southern Ryukyus (Ota et al., 1997), and prepared its meiotic karyotype successfully. In

this paper, I describe the metaphase chromosomes of *P. iwasakii* on the basis of this sample.

MATERIALS AND METHODS

The female *P. iwasakii* used for this study was collected from Hoshidate on Iriomotejima Island. In the laboratory, this individual, weighing 23.7 g at the time of the experiment, was intraperitoneally injected with 2.3 ml of colchicine solution (2 mg/ml), 18 hours before being narcotized with barbital. Bone marrow cells were extracted from ribs, and were treated with 0.06 M hypotonic KCl for 1 hour, followed by fixation in Carnoy solution (1:3 glacial acetic acid: absolute methyl alcohol). Metaphase cells, spread on a slide glass by the air-dry method, were photographed after being stained in 3% Giemsa solution for 40 min and rinsed in running water for 1–2 min.

The karyotype was determined on the basis of photographs of ten well-spread metaphase cells, and is described using the terminology of Levan et al. (1964), as modified by Green and Sessions (1991). The voucher specimen is deposited in the herpetological collection of Department of Zoology, Kyoto University (KUZ), under catalogue No. 47731.

RESULTS

The karyotype of the female *Pareas iwasakii* consisted of $2n=36$ chromosomes, of which pairs 1–8 were macrochromosomes; the remainder appeared to be microchromosomes. There were relatively distinct size gaps between pairs 2

FIG. 1. Standard karyotype of a female *Pareas iwasakii* from Iriomotejima Island of the Yaeyama Group, Ryukyu Archipelago (KUZ 47731). Thick bar equals 10 μ m. Note the apparent heteromorphism in pair 4.

and 3, and pairs 3 and 4, as well as between pairs 8 and 9. Heteromorphism was evident in pair 4: one of its components was submetacentric, whereas the other was metacentric. The remaining pairs appeared homologous, of which pairs 1, 3 and 8 were metacentric, pairs 2 and 7 submetacentric, and pairs 5 and 6 subtelocentric. All microchromosomes appeared telocentric (Fig. 1). Thus, the fundamental number equaled 52.

DISCUSSION

The presence of ZW-sex chromosomes (i.e., sex chromosomes heteromorphic in females and homologous in males) has been reported for a large proportion of colubrid species thus far examined karyologically (e.g., Bickham, 1984; Olmo, 1986; Ota and Iwanaga, 1997). Thus, it is likely that the components of pair 4 in the karyotype of the female *P. iwasakii* correspond to the Z and W sex chromosomes. This assumption needs verification especially from chromosomal data for males of this species.

It has been demonstrated that, in the family Colubridae, as well as in a few other snake families, a karyomorph consisting of 16 mostly biarmed macrochromosomes and 20 microchromosomes is by far the most broadly prevailing format both geographically and phylogenetically (Singh, 1972; Gorman, 1973; DeSmet, 1978; Bickham, 1984; Olmo, 1986). The karyotype of *P. iwasakii* fits this most general arrangement. Such a karyotypic condition of *P. iwasakii* is in sharp contrast to the unique, highly specialized morphology of this and other pareatine species among the colubrid

taxa (e.g., Underwood, 1967; McDowell, 1987).

The evolutionary significance of the broad prevalence of $2n=36$ format in snake karyotypes is still controversial. Most authors consider that such an arrangement represents a primitive state for karyotypes of this group of organisms (e.g., Beçak and Beçak, 1969; Gorman, 1973; Olmo, 1986). However, a few others, on the basis of the absence of homology between seemingly corresponding elements of the $2n=36$ karyotypes of the Boidae and Colubridae, argue that it may actually represent a terminal state of largely unidirectional derivations by a series of centric fissions from the ancestral karyomorph, which had actually consisted of a larger number of telocentric chromosomes (Mengden and Stock, 1980; Bickham, 1984).

The latter may be true with respect to the initial Henophidian and Caenophidian divergence. However, the presence of a number of karyotypes most reasonably interpreted as deriving from the $2n=36$ type within the Colubridae (e.g., Beçak and Beçak, 1969; Singh, 1972; Baker et al., 1972; Ota et al., 1991) suggests that this format is primitive at least for the extant members of this family, and that the morphologically highly specialized Pareatinae (as represented by *P. iwasakii* here) is chromosomally very conservative. Further studies on the basis of more colubrid materials and chromosome banding techniques are needed to verify this view.

ACKNOWLEDGMENTS.—I wish to express my sincere thanks to Iriomote Wildlife Conservation Center for providing this invaluable snake. I am also much in-

debted to Masafumi Matsui and Tsutomu Hikida for literature, to Saiko Yamashiro for laboratory assistance, and to Michihisa Toriba for calling my attention to the synonymy of the currently widely used generic name *Internatus*. This research was partially supported by a Grant-in-Aid from the Japan Ministry of Education, Science, Sports and Culture (B-08458165 to Masako Izawa).

LITERATURE CITED

- BAKER, R. J., G. A. MENGDEN, AND J. J. BULL. 1972. Karyotypic studies of thirty-eight species of North American snakes. *Copeia* 1972(2): 257-265.
- BEÇAK, W. AND M. L. BEÇAK. 1969. Cytotaxonomy and chromosomal evolution in Serpentes. *Cytogenetics* 8: 247-262.
- BICKHAM, J. W. 1984. Patterns and modes of chromosomal evolution in reptiles. In: A. K. Sharma and A. Sharma (eds.), *Chromosomes in Evolution of Eukaryotic Groups*, Vol. 2. p. 13-40. CRC Press, Florida.
- DAVID, P. AND G. VOGEL. 1996. *The Snakes of Sumatra*. Edition Chimaira, Frankfurt am Main. 260 p.
- DAS, I. AND H. OTA. 1998. A checklist of chromosome numbers of South Asian reptiles. *Hamadryad* 23(2): 179-193.
- DESMET, W. H. O. 1978. The chromosomes of 23 species of snakes. *Acta Zool. Pathol. Antverpiensia* 70: 85-118.
- GORMAN, G. C. 1973. The chromosomes of the Reptilia, a cytotaxonomic interpretation. In: A. B. Chiarelli and E. Capanna (eds.), *Cytotaxonomy and Vertebrate Evolution*. p. 349-424. Academic Press, New York.
- GREEN, D. M. AND S. K. SESSIONS. 1991. Nomenclature for chromosomes. In: D. M. Green and S. K. Sessions (eds.), *Amphibian Cytogenetics and Evolution*. p. 431-432. Academic Press, San Diego.
- KRAUS, F. AND W. M. BROWN. 1998. Phylogenetic relationships of colubroid snakes based on mitochondrial DNA sequences. *Zool. J. Linnean Soc.* 122: 455-487.
- LEVAN, A. K., K. FREDGA, AND A. A. SANDBERG. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.
- MCDOWELL, S. B. 1987. Systematics. In: R. A. Seigel, J. T. Collins, and S. S. Novak (eds.), *Snakes: Ecology and Evolutionary Biology*. p. 3-50. Macmillan Publ., New York.
- MENGDEN, G. A. AND D. STOCK. 1980. Chromosomal evolution in serpentes: A comparison of G and C chromosome banding patterns of some colubrid and boid genera. *Chromosoma* 79(1): 52-61.
- OLMO, E. 1986. Reptilia. In: B. John, H. Bauer, H. Kayano, and A. Levan (eds.), *Animal Cytogenetics*, Vol. 4: Chordata 3. p. 1-100. Gebruder Borntraeger, Berlin.
- OTA, H. AND S. IWANAGA. 1997. A systematic review of the snakes allied to *Amphiesma pryleri* (Boulenger) (Squamata: Colubridae) in the Ryukyu Archipelago, Japan. *Zool. J. Linn. Soc.* 121(3): 339-360.
- OTA, H., N. KAMEZAKI, T. HIKIDA, M. MATSUI, A. MORI, T. HAYASHI, AND S. TANABE. 1991. Karyotype of *Achalinus spinalis* from Japan: The first karyological description of a xenodermine snake (Colubridae). *Jpn. J. Herpetol.* 14(1): 12-14.
- OTA, H., J. LIN, T. HIRATA, AND S. CHEN. 1997. Systematic review of colubrid snakes of the genus *Pareas* in the East Asian islands. *J. Herpetol.* 31(1): 79-87.
- RAO, D. AND D.-T. YANG. 1992. Phylogenetic systematics of Pareinae (Serpentes) of southeastern Asia and adjacent islands with relationship between it and the geology changes. *Acta Zool. Sinica* 38(2): 139-149. (in Chinese, with English abstract)
- SINGH, L. 1972. Evolution of karyotypes of snakes. *Chromosoma* 38(3): 185-236.
- UNDERWOOD, G. 1967. *A Contribution to the Classification of Snakes*. The British Museum (Natural History), London. 179 p.

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