Karyotype of the Hawksbill Turtle, *Eretmochelys imbricata*, from Japan, with Notes on a Method for Preparation of Chromosomes from Liver Cells

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Abstract: The karyotype of the hawksbill turtle, *Eretmochelys imbricata*, from Japan was obtained from liver cells. This species has $2n=56$ homologous chromosomes forming a graded series. Comparison of the present karyotype with those of other cheloniids confirms the strong chromosomal conservativeness within the family. Methodological novelties of the present study are also discussed.

Key words: Cheloniidae; *Eretmochelys imbricata*; Karyotype; Liver cell

Karyotypes of the suborder Cryptodira show little variation, and all or most of the species of a single family appear identical with each other karyotypically (Bickham and Carr, 1983; Bickham, 1983). In the family Cheloniidae, karyotypes of three species, *Chelonia mydas*, *Lepidochelys olivacea* and *Caretta caretta* have been described (Bickham et al., 1980; Bhunya and Mohanty-Hejimadi, 1986; Kamezaki, 1989). These studies revealed that there are no recognizable differences among the standard karyotypes of the three species. On the other hand, Bickham (1981) and Bickham and Carr (1983) noted that the hawksbill turtle, *Eretmochelys imbricata*, without locality data, had 56 chromosomes, but without providing detailed description of the karyotype.

In this study, I investigated the karyotype of the hawksbill turtle from the Yaeyama Islands, Ryukyu Archipelago, using metaphase liver cells. The results further confirmed the assumption of low chromosomal variability within the family Cheloniidae.

MATERIALS AND METHODS

Two female hatchlings were used. These hatched on a beach of Kuroshima Island (124°E, 24°N) of the Yaeyama Islands, Ryukyu Archipelago, in 1988.

The hatchlings were injected with 0.02 ml of colchicine solution (1 mg/ml) and 0.01 ml of Phytohemagglutinin M (PHA; Difco) solution per 10 g of body weight. The injection of PHA followed Lin (1982). Twenty hours after the injection, the liver tissue was taken out and minced with scissors. The cell suspension was made by stirring the minced tissue in 0.1% trypsin (+0.02% EDTA) in a balanced salt solution for

ca. 25 min. The cells were treated with 0.07 M hypotonic KCl solution for 40 min, followed by fixation in Carnoy's solution. The fixative was changed three times at ten minute intervals. Metaphase chromosome spreads were obtained by an air-dry method and stained with 2% Giemsa solution in Sørensen phosphate buffer at pH 5.8. The karyotype was determined on the basis of 14 well-spread cells. Each specimen was sexed by microscopic investigation of gonadal sections.

RESULTS AND DISCUSSION

The karyotype consists of $2n=56$ chromosomes. Of these, pairs 2, 6, 8, 9, and 10 were regarded as metacentric, pair 1 submetacentric, pair 3 subterocentric, and pairs 4, 5, 7, and 11 acrocentric elements. The rest were classified as micro-chromosomes (Fig. 1).

The diploid number of the present sample agrees with that reported by Bickham (1981) and Bickham and Carr (1983) for this species. Chromosome morphology of *E. imbricata* agrees with those of *C. mydas*, *L. olivacea* and *C. caretta* at least in a standard Giemsa stained karyotype (Bickham et al., 1980; Bhunya and Mohanty-Hejimadi, 1986; Kamezaki, 1989).

The close resemblance among karyotypes of these species is in favor of the assumption that the karyotype is very conservative within the family Cheloniidae (Bickham, 1981). Karyological investigations of the remaining two cheloniid species, *L. kempfi* and *Natator depressa*, and further detailed analyses of chelonian karyotypes with banding techniques are required to draw a conclusion upon the variability of the karyotype in this family.

The method of obtaining isolated cells from the liver tissue was designed for the investigation on chromosomes of mice (e.g., Tanaka and

FIG. 1. Karyotype of a female *Eretmochelys imbricata* from the Ryukyu Archipelago, Japan. The scale indicates 10 μ m.

Ichihara, 1978), and this is the first application of this technique to the karyological study of Testudines. It has been pointed out that the karyotype of mouse liver cells varies in ploidy level (Epstein, 1967; Tulp et al., 1976). However, no such variation was recognized among the cells from *E. imbricata* examined in the present study. This seems to indicate the probable utility of this method for the karyological survey of turtles.

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要 旨 肝細胞をもちいたタイマイの核型分析

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八重山諸島で孵化したタイマイ *Eretmochelys imbricata* の幼体の肝細胞をもちいて、本種の核型を観察した。その結果、染色体数は $2n=56$ で、これまで報告されたウミガメ科3

種の核型と類似しており、この科内における核型の分化の低さを改めて確認した。また、肝細胞から分裂像を得る方法について述べた。

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