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THE KARYOTYPE AND CHROMOSOMAL BANDING PATTERNS OF THE GREEN TURTLE (*CHELONIA MYDAS*).—The karyotype of *Chelonia mydas* was first reported by Makino (1952) and was said to possess a diploid number of 56 in males and 55 in females. A subsequent study (Waddell and Sigel, 1956) confirmed the diploid number of 56 in an unreported number of individuals of unreported sex. Karyological data, both nondifferentially stained and banded preparations, from 5 specimens representing at least 3 and probably 4 distinct breeding aggregations are presented. The banding patterns are compared between sexes and breeding aggregations of *C. mydas*, as well as to the banded karyotype of another cryptodiran turtle, to determine any possible differences.

Materials and methods.—Tissue cultures were initiated from heart muscle excised from animals either in the field or in the lab. Cell cultures were grown in Medium 199 fortified with 20% fetal calf serum and chromosome preparations were made as described previously (Sites et al., 1979b). The G-band and C-band methods of Seabright (1971) and Sumner (1972) were used as described by Sites et al. (1979b).

The following specimens were studied: *Chelonia mydas*, Aves Island, Venezuela (15°40′N, 63°36′W), 2♂♂, Florida State Museum (UF) 42372, 42373; Philippine Islands, 1♀, UF 43674; Miskito Cays, Nicaragua, 1♂; Baboen Santi, Surinam (5°48′N, 53°57′W), 1 hatchling (sex unknown); *Chinemys reevesi*, 1♀, Texas Cooperative Wildlife Colllection No. 56736. The Aves Island, Surinam and Philippine Islands specimens are hatchlings from breeding aggregations at those sites. The exact locality for the Philippine specimen is unknown. The Miskito Cays specimen is from an immature animal captured by fishermen; no voucher specimen exists. Tag returns from breeding females indicate that this animal is a member of the Tortuguero, Costa Rica breeding aggregation; however, the possibility remains that it is derived from another site, such as Aves Island. No voucher specimen is available for the Surinam specimen but a series of hatchlings collected at the same time have been preserved and will be deposited in the Museum of Vertebrate Zoology, University of California, Berkeley.

Results.—*Chelonia mydas* has a diploid number of 56. The karyotypes of a ♂ and ♀ are presented in Fig. 1 and the chromosomes are arranged according to Bickham (1975). There are 7 pairs of group A (metacentric or submetacentric) macrochromosomes; 5 pairs of group B (telocentric or subtelocentric) macrochromosomes; and 16 pairs of group C microchromosomes. There are no heteromorphic sex chromosomes and all animals examined are karyotypically identical.

Fig. 2 is a comparison of the G- and C-banded macrochromosomes of *C. mydas* and a bata-

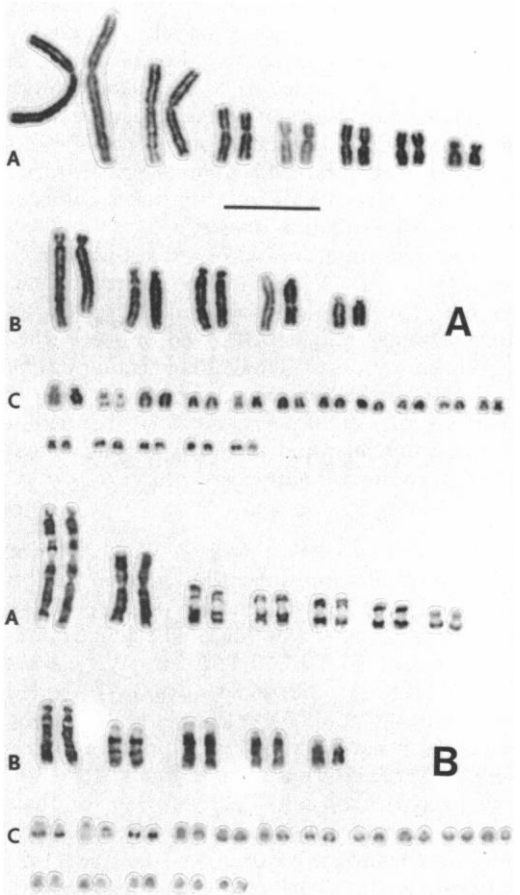

Fig. 1. Karyotypes of 2 individuals of *Chelonia mydas*. A) Standard karyotype of a ♂ from Aves Island (UF 42373). B) G-band karyotype of a ♀ from the Philippine Islands (UF 43674).

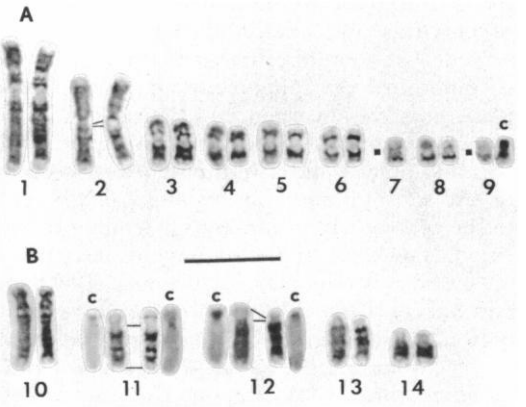

Fig. 2. Comparison of the G- and C-bands of the macrochromosomes (groups A&B) of *Chelonia mydas* and *Chinemys reevesi*. C is placed above each chromosome that has been C-banded and only those pairs are shown in which other than centromeric heterochromatin is present (pairs 9, 11 and 12). Chromosomes from *C. mydas* are placed as the left member of each pair. Pairs 7 and 9 of *C. reevesi* are absent from *C. mydas*. Bar is 10 microns.

gurine emydid (*Chinemys reevesi*). *Chinemys reevesi* has a diploid number of 52 and a 9:5:12 complement of pairs in groups A:B:C respectively. This karyotype appears identical to those of *Sacalia bealei* and 2 species of *Mauremys* and has been postulated to be the primitive karyotype of the Emydidae (Bickham, 1975, 1976; Bickham and Baker, 1976a). There are 6 pairs of group A chromosomes (nos. 1, 3, 4, 5, 6 and 8) and 3 pairs of group B chromosomes (nos. 10, 13 and 14) that are identical between *Chelonia* and *Chinemys*. Chromosome 2 differs by the presence of a large G negative band proximal to the centromere on the long arm in *Chinemys* that is not present in *Chelonia*. There are no apparent homologues in *Chelonia* to pairs 7 and 9 of *Chinemys*. The long arms of pair 11 are identical in the 2 species studied, but the short arms differ. *Chelonia* has a small, heterochromatic short arm and *Chinemys* has a larger euchromatic short arm on this chromosome. Chromosome 12 of *Chelonia* has a heterochromatic, G negative short arm that is not present in *Chinemys*. There are 16 and 12 pairs of microchromosomes in *Chelonia* and *Chinemys*, respectively; the second pair in *Chelonia* has a distinct secondary constriction not seen in *Chinemys*.

Discussion.—Data reported herein support earlier reports of *Chelonia mydas* having 56 chromosomes (Makino, 1952; Waddell and Sigel, 1965) but do not confirm the reported female heterogamety (Makino, 1952). The only instance of heterogamety that has been verified in turtles by modern cytological procedures is the report of male heterogamety in 2 species of *Staurotypus* (Bull et al., 1974; Sites et al., 1979a). We have not examined specimens from the population studied by Makino (*C. mydas japonica*); however, we feel it is highly unlikely that his report of heterogamety will be verified by workers using the methods now available.

The karyotype of *C. mydas* resembles in many respects the karyotypes of other cryptodiran turtles. Fig. 2 demonstrates the degree to which

the G-band patterns of the macrochromosomes of emydids and cheloniids have been conserved. A minimum estimate of the number of chromosomal rearrangements that have taken place since the divergence of *Chelonia* and *Chinemys reevesi* from a common ancestor is 6. Chromosome 2 underwent either an insertion or a small duplication on the long arm adjacent to the centromere in the lineage leading to the emydids. C-band studies of emydids have demonstrated this region as euchromatic (Bickham and Baker, 1976a). Two acrocentric microchromosomes were found to give rise to the biarmed macrochromosome number 7 of the emydid. Chromosome 9 of the emydid may have evolved through the addition of a heterochromatic short arm to a microchromosome that was already partially heterochromatic. The nucleolar organizer region in the emydid is located on this chromosome but is expressed as a secondary constriction on the second pair of microchromosomes in *Chelonia* (Fig. 1A). The secondary constriction of emydines is also found on a microchromosome (Bickham 1975; Bickham and Baker, 1976a, b). The events involved in the differentiation of chromosome 11 may have included the addition of a heterochromatic arm in *Chelonia* and the fusion of a microchromosome to the presumed ancestral acrocentric 11 in the emydid lineage. However, the primitive condition of 11 may be identical to that of *Chelonia*. Thus, the ancestral condition may have possessed a small heterochromatic short arm that was lost in the emydid lineage. Chromosome 12 has either undergone the addition of a heterochromatic short arm in the *Chelonia* lineage, or its deletion in the *Chinemys* lineage. It is unclear what condition is primitive because some batagurine emydids (*Rhinoclemmys, Siebenrockiella*) possess a G negative heterochromatic short arm on number 12 (Bickham and Baker, 1976a) but some other batagurines, all emydines and kinosternines do not (Bickham and Baker, 1976a; Sites et al., 1979b). In *Staurotypus* and the chelydrids this chromosome has undergone other rearrangements involving heterochromatin and the nucleolar organizer region.

The results of this study corroborate earlier findings that the rates of karyotypic change in cryptodiran turtles are conservative (Bickham and Baker, 1976a, 1979; Sites et al., 1979b; Stock, 1972). The two species compared in this report are members of different superfamilies (Gaffney, 1975), yet the banding patterns of the macrochromosomes can be easily related between the two. The results also indicate that karyological techniques are not sensitive enough to differentiate between breeding aggregations of green turtles. However, preliminary results from an electrophoretic survey of green turtle breeding aggregations indicate that significant genetic differentiation does exist.

Some data suggested a skewed sex ratio in *C. mydas* (Hirth, 1971), and led to unsuccessful attempts to develop a cytological sexing procedure (Owens et al., 1978) based on the earlier inaccurate report of female heterogamety. The radioimmunoassay procedure developed by Owens et al. (1978) permits sexing of subadult animals, but no rapid means of sexing hatchlings or young animals exists.

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ICTHYOLOGICAL NOTES

Copeia, 1980(3), pp. 543–545

A MODIFIED TECHNIQUE FOR FISH KARYOTYPE ANALYSIS USING SCALE EPITHELIUM.—Chromosomes have been studied in a variety of ways and from a variety of tissues for many years. The primary method for chromosome preparations was the squash technique (Ohno et al., 1965; Sharma and Sharma, 1972), but this did not always produce analyzable chromosomal spreads. In the 1950's, Ford and Hamerton (1956) introduced a technique for human chromosome preparation that revolutionized the field of cytogenetics. As a result of this, newer and better techniques were developed. Pretreatment with mitotic inhibitors was introduced to increase the number of metaphase chromosome spreads. Hypotonic solution pretreatment to aid in the separation and spreading of chromosomes was also introduced in addition to the air-drying method which improved the spread of chromosomes. These and other modifications have been introduced and are being employed in the study of chromosomes.

In this study, a modification of these techniques has been applied to the karyotypic analysis of fishes using scale epithelium without the need to sacrifice the animal. The colchicine treatment is done in vitro and is of value when looking at possible sequential alterations of the