Karyotypes of Some Neotropical Turtles

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Analyses of meiotic and mitotic chromosomes are presented for eight species of Neotropical turtles. *Geochelone carbonaria* differs from *G. denticulata* by centromere position of one of the smaller macrochromosomes. *Geochelone carbonaria* is considered primitive in this respect because its karyotype is similar to that of the Old World batagurines. Three taxa of Greater Antillean *Chrysemys* (*C. terrapen, C. stejnegeri vicina, C. decorata*) possess a typical emydine karyotype and do not differ from other *Chrysemys* or other emydine genera that have been karyotyped. *Rhinoclemys punctularia* has two pairs of microchromosomes that are not found in *R. pulcherrima. Rhinoclemys punctularia* is considered to be karyotypically primitive to *R. pulcherrima* due to its closer similarity to the Old World batagurine karyotype. The karyotype of *Kinosternon scorpioides* from Trinidad is similar to previous reports of specimens from Brazil and of other species of *Kinosternon*.

NEOTROPICAL turtles have received little attention from cytogeneticists. This study presents karyological data from 27 specimens of eight taxa representing the families Kinosternidae, Testudinidae and Emydidae from the Carribbean region and Central America. The species include *Kinosternon scorpioides, Geochelone carbonaria, G. denticulata, Chrysemys* (= *Pseudemys*) *terrapen, C. stejnegeri vicina, C. decorata, Rhinoclemys pulcherrima,* and *R. punctularia*. Karyotypes of the first three species listed above were studied by other workers; (Barros et al., 1972; Sampaio et al., 1971; Stock, 1972) those of the last five taxa have not been reported previously. Analysis of both mitotic and meiotic chromosomes are presented here, and the phylogenetic implications are discussed.

MATERIALS AND METHODS

The family, species, number and sex of individuals and collecting localities are given in Table 1. Specimens were collected from natural populations (Table 1, localities 1–4, 6–8, 10) or purchased at food markets (Table 1, localities 5 and 9). The specimens from locality 10 (Table 1) are deposited in the U. S. National Museum; all others are deposited in The Museum, Texas Tech University.

The turtles were processed for chromosome preparations from spleen as described by Bickham (1975). Some individuals were also karyotyped from primary tissue cultures established from heart tissue and grown in Ham's F-10 medium fortified with 16% fetal calf serum.

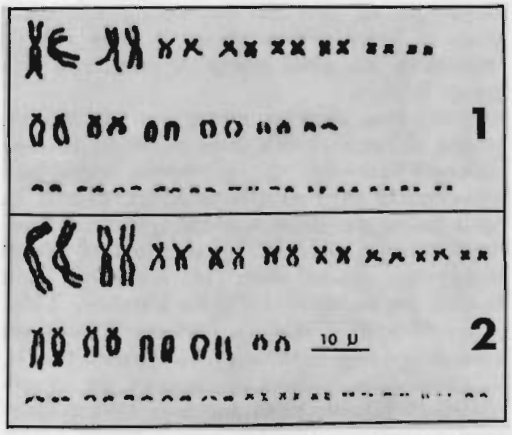

Fig. 1. Karyotype of *Geochelone denticulata*, ♂, TTM R-7385, 2n = 52, with an 8:6:12 complement of groups A:B:C.
Fig. 2. Karyotype of *Geochelone carbonaria*, ♂, TTM R-7399, 2n = 52, 9:5:12.

consists of six pairs of group A macrochromosomes, five pairs of group B macrochromosomes and 15 pairs of group C microchromosomes.

Rhinoclemys punctularia (Daudin), Fig. 7, 2n = 56. Variation between individual cells of this species was greater than that encountered in any other species examined with the diploid counts ranging from 52 to 60. Frequency of diploid numbers in 70 cells was 52 = 5.6%, 53 = 10%, 54 = 30%, 55 = 15.4%, 56 = 31%, 57 = 4.2%, 58 = 1.4%, 60 = 1.4%. Variation in chromosome number in both spleen and heart fibroblasts was evident in all seven individuals karyotyped. The modal number was 2n = 56. Karyotypic analysis revealed the variation usually was due to the loss of one or two microchromosomes. The karyotype possesses six group A pairs and five pairs of group B macrochromosomes and 7 pairs of group C microchromosomes (Table 1).

Kinosternidae.—Kinosternon scorpioides (Linnaeus), Fig. 8, 2n = 56. There are seven pairs of group A macrochromosomes, six pairs of group B macrochromosomes and 15 pairs of group C microchromosomes (Table 1).

Meiotic analyses.—The results of meiotic analyses are summarized in Table 2. Meiotic chromosomes in *Chrysemys* appear to be identical to the meiotic chromosomes of *Clemmys guttata* as described by Bickham (1976). Twenty-five bivalents are present at diakinesis and 25 chromosomes, representing a normal haploid set, are present at prophase II. No precociously

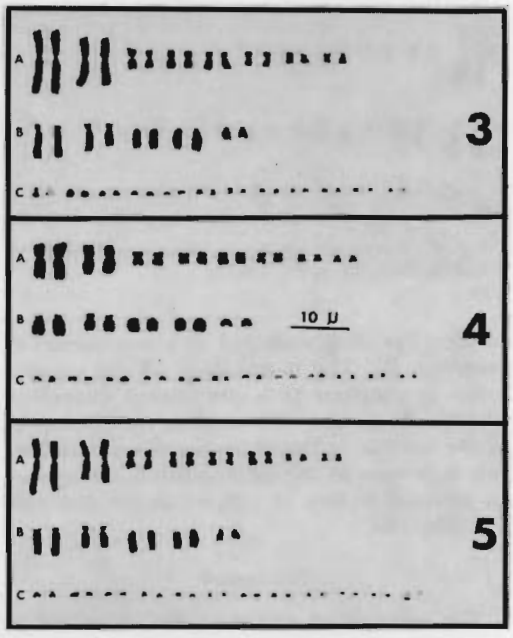

Fig. 3. Karyotype of *Chrysemys terrapen*, ♂, TTM R-7396, 2n = 50, 8:5:12.
Fig. 4. Karyotype of *Chrysemys decorata*, ♂, TTM R-7394, 2n = 50, 8:5:12.
Fig. 5. Karyotype of *Chrysemys stejnegeri vicina*, ♂, TTM R-7406, 2n = 50, 8:5:12.

condensed regions are evident. Meiosis from the specimen of *Geochelone denticulata* was described previously (Bickham, 1976).

An analysis of the meiotic chromosomes of *Rhinoclemys punctularia* (Table 2) reveals 28

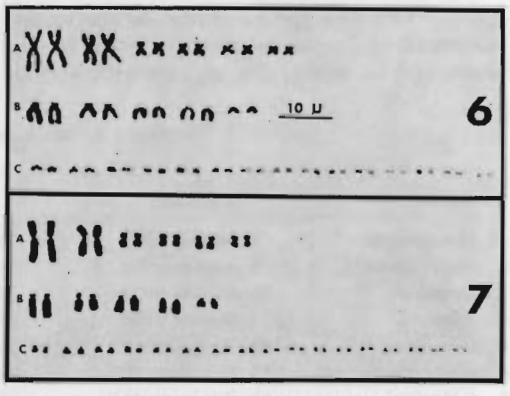

Fig. 6. Karyotype of *Rhinoclemys pulcherrima*, ♀, TTM R-7434, 2n = 52, 6:5:15.
Fig. 7. Karyotype of *Rhinoclemys punctularia*, ♀, TTM R-7384, 2n = 56, 6:5:17.

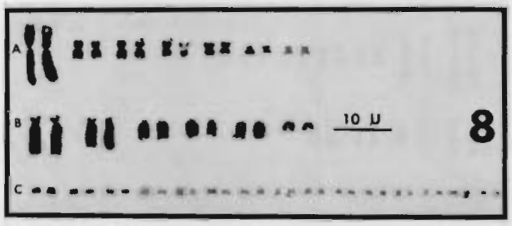

Fig. 8. Karyotype of *Kinosternon scorpioides*, ♀, TTM R-7380, 2n = 56, 7:6:15.

bivalents in diakinesis and 28 chromosomes in prophase II. The morphology of the chromosomes in prophase II is not distinct enough to arrange in groups. There does not appear to be the notable variation in chromosome number that is present in the somatic tissues. There are no obvious regions of precocious condensation in pachytene.

Discussion

The two species of testudinids examined in this study were examined by Sampaio et al. (1972). They reported karyotypes which differ by centromere placement of chromosome 12 (their terminology), which is acrocentric in *G. denticulata* and metacentric in *G. carbonaria*. Stock (1972) examined only *G. carbonaria* and concluded, after an inspection of the karyotypes figured by Sampaio et al. (1972), that there is no karyotypic difference between the two species. Stock (1972) suggests that Sampaio et al. (1972) may have inadvertantly changed positions of chromosomes 12 and 13 of *G. denticulata* and that pair 13 of *G. denticulata* should be compared to pair 12 of *G. carbonaria*. Our data (Figs. 1 and 2) agree with Sampaio et al. (1972). The two species differ in centromere placement of one of the smallest macrochromosomes, and because of this we have arranged the karyotypes so that *G. denticulata* has eight group A pairs and six group B pairs and *G. carbonaria* has nine group A pairs and five group B pairs.

Geochelone appears to be one of the few genera of turtles which show intrageneric chromosomal variation. *G. carbonaria*, which possesses 9:5:12 pairs of groups A:B:C (Table 1), has a karyotype which is indistinguishable from the karyotype possessed by several Old World batagurine genera such as *Mauremys* and *Sacalia*; see Bickham (1975) for a review. Thus, it would appear that *G. carbonaria* possesses a karyotype that is more primitive than that of *G. denticulata*. These two species are closely related (Williams, 1960) and may have evolved from a common ancestor with a 9:5:12 karyotype. The evolution of *G. denticulata* involved a pericentric inversion or deletion of a heterochromatic short arm of one of the three smallest ancestral group A chromosomes giving rise to an 8:6:12 karyotype.

There is no apparent chromosome difference between the three species of Greater Antillean turtles studied, *Chrysemys decorata*, *C. stejnegeri vicina* and *C. terrapen*. Their karyotypes, 8:5:12 (Table 1), are identical to all other emydine species which have been reported in the literature (Bickham, 1975). Stock (1972) and Forbes (1966) published karyotypes of other species of *Chrysemys* (= *Pseudemys*). The lack of variation within the genus and within the subfamily suggests that the Emydinae is a natural assemblage, having evolved from a single ancestor.

The emydid subfamily Batagurinae is primarily an Old World group. Most of the species reported in the literature possess 2n = 52, 9:5:12 karyotypes (Bickham, 1975) although Stock (1972) reported 2n = 50 for *Ocadia* and *Siebenrockiella*. Bickham (1975; 1976) considered the batagurine 2n = 52, 9:5:12 karyotype to be primitive for the family. This con-

Table 2. Summary of Meiotic Data.

Species	Date Collected	Date Killed	Locality (See Table 1)	Meiosis	Sperm Present
R. punctularia	6 August 1974	26 October 1974	5	+	+
R. punctularia	6 August 1974	15 November 1974	5	–	?
C. decorata	29 August 1974	26 October 1974	9	–	–
C. decorata	29 August 1974	24 January 1975	9	+	+
C. stejnegeri	29 August 1974	25 October 1974	9	–	?
C. stejnegeri	23 February 1975	17 March 1975	10	+	+
C. stejnegeri	23 February 1975	17 March 1975	10	+	+
C. terrapen	10 July 1974	26 October 1974	3	+	+
C. terrapen	10 July 1974	26 October 1974	3	+	+

TABLE 1. CHROMOSOMAL DATA FOR SOME NEOTROPICAL TURTLES.

Family and Species	2n	Group	Specimens ♂♂	Specimens ♀♀	Locality
Testudinidae					
Geochelone carbonaria	52	9:5:12	1		7
G. denticulata	52	8:6:12	1		5
Emydidae, Emydinae					
Chrysemys terrapen	50	8:5:12		2	2
C. terrapen	50	8:5:12	2	2	3
C. decorata	50	8:5:12	2	1	9
C. stejnegeri vicina	50	8:5:12	3	2	9
C. stejnegeri vicina	50	8:5:12	2		10
Emydidae, Batagurinae					
Rhinoclemys pulcherrima	52	6:5:15		1	1
R. punctularia	56	6:5:17	2	3	5
R. punctularia	56	6:5:17		1	6
Kinosternidae					
Kinosternon scorpioides	56	7:6:15		1	4
Kinosternon scorpioides	56	7:6:15		1	8

Localities
1. Nicaragua: Dept. Chinandega; 18 km S. Honduras Border on Hwy 24.
2. Jamaica: St. Ann Parish; Orange Valley.
3. Jamaica: St. Ann Parish; Green Castle.
4. Trinidad: St. George Co.; San Rafael.
5. Trinidad: St. Patrick Co.; Market at Oropuche.
6. Trinidad: St. George Co.; Maracas Valley.
7. Trinidad: Mayaro Co.; Guayaguayare.
8. Trinidad: St. George Co.; Village of Four Roads.
9. Haiti: Dept. de l'Ouest; Market at Port-au-Prince.
10. Republica Dominica: Prov. Samana; the cienaga near Sanchez.

Meiotic preparations were made from testicular tissue as described by Bickham (1976).

Chromosomes are arranged in the figures in groups as described by Bickham (1975). Group A consists of metacentric or submetacentric macrochromosomes, Group B consists of telocentric or subtelocentric macrochromosomes and Group C consists of microchromosomes. The terms for centromere placement are defined by Levan et al. (1964). Five metaphases were counted for each individual of all species whenever possible, and in *Rhinoclemys punctularia* usually 10 to 25 cells were counted.

Specimens from Trinidad were identified using the keys of Underwood (1962). Greater Antillean specimens were identified from Barbour and Carr (1940) and Cochran (1941). Meyer and Wilson (1973) was used for the specimens from Nicaragua.

RESULTS

Karyotypic data from somatic cells are summarized in Table 1. A brief description of the karyotypes of each species follows.

Testudinidae.—Geochelone denticulata (Linnaeus), Fig. 1, 2n = 52. This species possesses eight pairs of group A macrochromosomes, six pairs of group B macrochromosomes and 12 pairs of group C microchromosomes.

Geochelone carbonaria (Spix), Fig. 2, 2n = 52. There are nine pairs of group A macrochromosomes, five pairs of group B macrochromosomes and 12 pairs of group C microchromosomes.

Emydidae (Emydinae).—Chrysemys terrapen (Lacépède), Fig. 3, 2n = 50. There are eight pairs of group A macrochromosomes, five pairs of group B macrochromosomes and 12 pairs of group C microchromosomes. The first pair of microchromosomes possess a distinct secondary constriction near the centromere.

The karyotype of *Chrysemys decorata* (Barbour and Carr), Fig. 4, 2n = 50, and *Chrysemys stejnegeri vicina* (Barbour and Carr), Fig. 5, 2n = 50 appear to be identical to that of *C. terrapen*.

Emydidae Batagurinae.—Rhinoclemys pulcherrima (Gray), Fig. 6, 2n = 52. The karyotype

clusion is based on the widespread occurrence of this karyotype in the presumably primitive Batagurinae, along with the presence of a large heterochromatic region observed in pachytene of the batagurine karyotype (*Mauremys*) but not in the emydine karyotype. Bickham (1976) has suggested that this region may have been deleted during the evolution of the emydine karyotype. An examination of pachytene of the three emydine species studied here reveals an absence of such a heterochromatic region.

Rhinoclemys is the only batagurine genus present in the New World. We examined two species, *R. pulcherrima* and *R. punctularia*, and found that their karotypes differ from each other and from other emydids examined thus far. *Rhinoclemys pulcherrima* possesses 2n = 52, but the karyotype shows a 6:5:15 complement (Table 1, Fig. 6) which differs from the proposed primitive batagurine 9:5:12 karyotype. *Rhinoclemys punctularia* possesses a modal number of 2n = 56 which is the first emydid shown to possess a diploid number other than 50 or 52. The karyotype shows a 6:5:17 complement, which differs from *R. pulcherrima* by the presence of two more pairs of group C microchromosomes. There is always some variation in diploid number resulting from the techniques required to prepare the cells on microscope slides for examination. This technically induced variation usually results in a lower diploid number and chromosomes seem to be randomly lost. In this case the reduction in diploid number is greater than generally is characteristic of our techniques and seems to be restricted to a reduction in microchromosomes. This leads us to conclude that the variation is a natural phenomenon and not an artifact.

These two species differ from the primitive batagurine karyotype in a way that no simple mechanism can explain. Probably the three smallest group A macrochromosomes of the primitive batagurine karyotype underwent the deletion of heterochromatin (repetitive DNA) and/or centric fission. The heterochromatic region seen in pachytene in the primitive batagurines may represent about half of one of the three smallest group A macrochromosomes (Bickham, 1976). Deletion of this region would leave a small acrocentric microchromosome. The centric fission of the remaining two smallest group A chromosomes would result in a 2n = 56, 6:5:17 karyotype with no heterochromatic region in pachytene. This is exactly the situation in *R. punctularia*, which has a 2n = 56, 6:5:17 karyotype. Analysis of pachytene in a single male *R. punctularia* (Table 2) shows no heterochromatic region to be present.

The karyotype of *R. pulcherrima* can be derived from *R. punctularia* by the deletion or translocation elsewhere in the karyotype of two pairs of group C microchromsomes. Deletion could be possible if the microchromosomes in question consist mostly of repetitive DNA. The application of C-band procedures could perhaps shed some light on this situation.

We interpret the data for *Rhinoclemys* to indicate that, karyotypically, *R. punctularia* is primitive to *R. pulcherrima*. The fact that the somatic cells of *R. punctularia* possess a great deal of variation in the number of group C microchromosomes suggests that up to four microchromosomes can be lost without being lethal to the cell. An analysis of meiosis reveals a modal number of n = 28 for *R. punctularia*, this gives strength to the conclusion that the correct diploid number is 2n = 56 for this species. The genus *Rhinoclemys* presents the cytogeneticist with an interesting possibility to study the process of speciation and associated karyotypic evolution which appears to involve the deletion of heterochromatin.

Bickham (1975) suggested that the emydine 8:5:12 karyotype was derived from the primitive batagurine 9:5:12 karyotype by the deletion of most or all of one of the smallest group A chromosomes from the batagurine karyotype. It is most likely that the Emydinae evolved from a batagurine ancestor and because *Rhinoclemys* is the only New World batagurine genus it is an attractive possibility as an ancestor for the Emydinae. Based on karyological considerations this seems unlikely because the emydine karyotype is more similar to the primitive batagurine karyotype than it is to either of the *Rhinoclemys* karyotypes presented here. Further studies of other *Rhinoclemys* species could perhaps clarify the uncertainty as to the origin of the Emydinae.

The primitive batagurine karyotype seems to be restricted to the Old World. The extreme similarity of the karyotype of *Geochelone carbonaria* to the primitive batagurine karyotype suggests *G. carbonaria* possesses a "primitive" testudinid karyotype. If the batagurine and testudinid karyotypes are identical it seems likely that the testudinid and batagurine lineages diverged in the Old World with a testudinid possessing a primitive karyotype subsequently reaching the New World. *Geochelone carbonaria* has inherited this primitive karyotype. Other New World testudinids which have been karyotyped, such as *Gopherus* (Stock,