The chromosomes of the order Crocodilia[1]

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Abstract. The somatic karyotypes of the 21 surviving species of the order Crocodilia are presented. The population surveyed totals 71 animals, with representatives of both sexes in 13 species. No karyotypic differences were observed among the multiple specimens within a given species or subspecies. There was no evidence for consistent heteromorphism that might be indicative of a sex-chromosome pair. All chromosomes possessed distinct morphology and were classified as macrochromosomes in the absence of an absolute distinction between macro- and microchromosomes.

The diploid number in this order ranges from 30 to 42; however, the fundamental number (N.F.) shows only 10% variation (56 to 62). Two obvious marker chromosomes were evident – a satellited submetacentric characteristic of alligators and a telocentric with a marked secondary constriction observed in nine species of crocodiles. Evolutionary pathways of karyotype development are discussed from the consideration of both centromeric 'fusion' and 'fission'.

Introduction

The 21 species comprising the order Crocodilia today represent the remnants of a formerly highly successful group (WERMUTH and MERTENS, 1961). The crocodilians enjoy world-wide distribution in the tropical and subtropical zones of all continents, and several species are represented by geographical races. The taxonomic classification

of this group is derived primarily from osteological characters, with heavy emphasis on those of the skull (WERMUTH, 1953). It would therefore be interesting to compare the pattern thus established with one based on cytogenetic criteria that represent an entirely different character system, which has been subject to distinct patterns of evolution and selective pressures.

The problem seemed particularly appropriate since it was demonstrated earlier (COHEN and CLARK, 1967) that crocodilian cells for chromosome study could easily be obtained without risk to the animal. It was thus possible to sample the rich collections in various zoos in the United States. The present communication reports karyotypes of 71 animals representing all Recent species of crocodilians. Wherever possible, analysis was based on samples from multiple specimens and representatives of both sexes.

Materials and methods

Table I lists the number of animals, sex and karyotype description for each species. Detailed data on the individual specimens and comments on their identification and origin are given in Appendix I. The animals were sampled at the Buffalo Zoological Gardens, Buffalo, New York (B.Z.), the New York Zoological Park, Bronx, New York (N.Y.Z.), the Philadelphia Zoological Gardens, Philadelphia, Pennsylvania (P.Z.), the Ross Allen Reptile Institute, Silver Springs, Florida (R.A.R.I.), and the Crandon Park Zoological Gardens, Key Biscayne, Florida (C.P.Z.). Care was expended in checking the taxonomic identification and sex of specimens. We are grateful to Mr. PETER J. BRAZAITIS for advice in the sexing of crocodilians (BRAZAITIS, 1968). The common names of species in this order are listed in Appendix II.

Blood samples were obtained in a heparinized syringe from a large vessel lying close to the ventral surface at mid-abdomen (most probably the portal vein and not the heart). Approximately six to eight drops of whole blood from a 20-gauge needle were used as the cell inoculum.

Chromosomal preparations were obtained from phytohemagglutinin (PHA) stimulated peripheral leukocytes using a commercially available microculture method (Grand Island Biological Company, Chromosome Medium 1A, Grand Island, New York). The cultures were incubated at 29° to 30°C for varying lengths of time. Although there was some interspecies fluctuation in the optimum time for obtaining mitoses, multiple cultures harvested after three, four and five days of incubation yielded sufficient metaphase cells to derive the karyotype of each species. The cultures were exposed to Colcemide (0.05 μg/ml) for the final six hours of the incubation period.

Table I. Number of specimens, sex and morphologic distribution of chromosomes among the crocodilians.

Species	No.	Sex ♀	Sex ♂	No. of cells	2N	NF	Metacentric L	Metacentric S	Sm	T	Marker[2]
Paleosuchus trigonatus	2	1	1	59	42	58	0	10	6	26	None
P. palpebrosus	3	–	3	81	42	58	0	10	6	26	None
Melanosuchus niger	3	1	1[1]	129	42	60	0	12	6	24	None
Caiman latirostris	3	2	1	82	42	60	0	12	6	24	Sat. Sm.
C. crocodilus	15	3	8[1]	443	42	62	0	16	4	22	None
Alligator mississippiensis	6	1	5	308	32	60	10	14	4	4	Sat. Sm.
A. sinensis	1	–	1	28	32	60	10	14	4	4	Sat. Sm.
Gavialis gangeticus	1	1	–	38	32	60	10	10	8	4	Sat. Sm.
Crocodylus siamensis	2	2	–	64	34	58	8	10	6	10	2° Constr. T
C. porosus	5	2	3	200	34	58	8	12	4	10	2° Constr. T
C. moreletii	2	–	2	68	32	56	8	10	6	8	2° Constr. T
C. johnsoni	2	1	1	70	32	58	8	10	8	6	2° Constr. T
C. acutus	4	3	1	106	32	58	8	10	8	6	2° Constr. T
C. intermedius	1	–	1	44	32	58	8	10	8	6	2° Constr. T
C. niloticus	4	2	2	162	32	58	8	12	6	6	2° Constr. T
C. novaeguineae	4	2	2	94	32	58	8	12	6	6	None
C. cataphractus	3	2	1	88	30	58	10	10	8	2	Sat. Sm.
C. rhombifer	1	–	1	50	30	58	10	10	8	2	2° Constr. T
C. palustris	2	–	2	85	30	58	10	12	6	2	2° Constr. T
Osteolaemus tetraspis	3	1	2	122	38	58	4	10	6	18	None
Tomistoma schlegelii	4	1	3	154	32	58	10	14	2	6	None

[1] Remaining animals too young to be sexed.

[2] Sat. Sm. = satellited submetacentric; 2° Constr. T = secondary constriction telocentric.

After centrifugation, the supernatant was discarded and the cells resuspended in a hypotonic sodium-citrate solution (1.0%) for 30 min at 37°C. Fixation was achieved by at least three changes of 3:1 methanol:acetic acid solution. In some cultures, additional fixative changes were required to lyse superfluous red cells and to yield sharper chromosome morphology. Slides were prepared by a combination of air-drying and ignition techniques and stained with 2% acetic orcein.

The diploid number of each species was determined by counting a minimum of 25 cells per animal; however, in most cases many more than the minimum number were counted. At least 10 well-spread metaphase cells per animal were photographed and analyzed by 'cutting and matching' to establish the representative karyotypes for the species. The chromosomes were classified on morphological grounds as either large or small metacentrics, submetacentrics or telocentrics.

Results

No karyotypic differences were observed among the multiple specimens within a given species. There was no evidence for a consistent heteromorphism of any given pair of chromosomes which might have indicated the existence of sex chromosomes. The chromosomes of each animal possessed definite morphology, and since there is no uniformly accepted definition of microchromosomes, all elements were considered macrochromosomes.

Paleosuchus trigonatus (SCHNEIDER) and *P. palpebrosus* (CUVIER) (2N = 42; NF = 58). Both species in this genus have identical karyotypes (Fig. 1a) consisting of 10 small metacentrics, 6 submetacentrics and a graded series of 26 telocentrics. Of this last group, four large telocentric chromosomes are distinguishable on the basis of size.

Melanosuchus niger (SPIX) (2N = 42; NF = 60). The karyotype (Fig. 1b) consists of 12 small metacentric elements, 6 submetacentric chromosomes and 24 telocentrics. The four large telocentrics and six submetacentrics of *M. niger* and the species of *Paleosuchus* appear identical. However, *M. niger* has two additional small metacentrics instead of the smallest telocentrics.

Caiman latirostris (DAUDIN) (2N = 42; NF = 60). The karyotype of this species (Fig. 1c) is similar to that of *M. niger* in the distribution of the various morphological types of chromosomes, *i.e.*, 12 small metacentrics, 6 submetacentrics and a series of 24 telocentric elements, including the four large telocentrics seen in all the above species. The distinguishing characteristic of *C. latirostris* is the obvious marker in
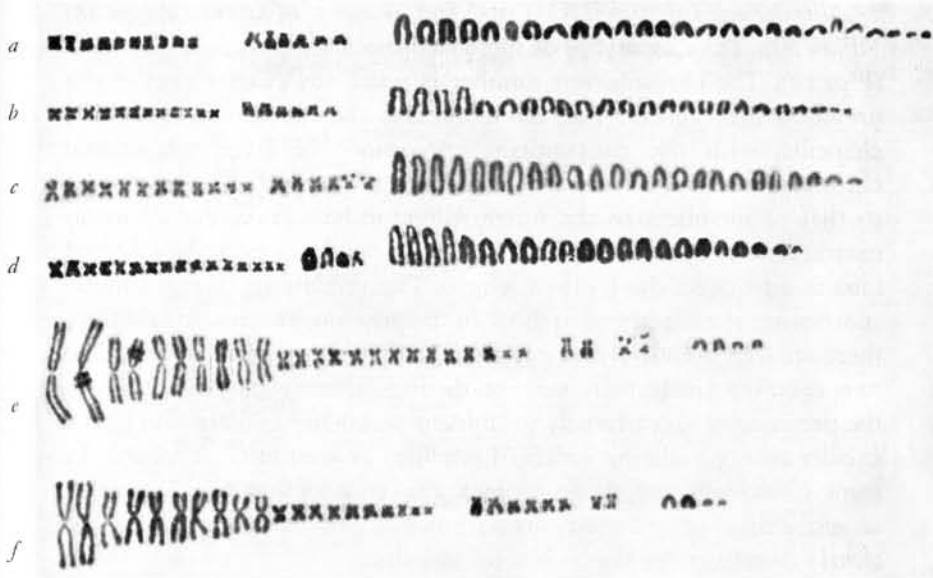
Fig. 1. The karyotypes of the crocodilians: *(a) Paleosuchus palpebrosus* (2N = 42); *(b) Melanosuchus niger* (2N = 42); *(c) Caiman latirostris* (2N = 42); *(d) Caiman crocodilus* (2N = 42); *(e) Alligator mississippiensis* (2N = 32); *(f) Gavialis gangeticus* (2N = 32). Note satellited submetacentric chromosome in *C. latirostris, A. mississippiensis* and *G. gangeticus*.

the submetacentric group. This chromosome bears a deep secondary constriction in the short arm subtending very large satellites. A similar marker also occurs in several other species described below.

Caiman crocodilus (LINNAEUS) (2N = 42; NF = 62). The 15 specimens available to us included four animals that were definitely identified as belonging to the subspecies *C. crocodilus yacare* (DAUDIN) and one to the subspecies *C. crocodilus fuscus* (COPE). The karyotypes among these subspecies are indistinguishable (Fig. 1d). There are 16 small metacentrics, only 4 submetacentrics and a graded series of 22 telocentrics, which include the four large telocentric chromosomes. When compared to *Paleosuchus* and *Melanosuchus*, *Caiman crocodilus* lacks one pair of submetacentric and several pairs of telocentric elements but has several additional small metacentric chromosomes. The karyotypes of the genera *Paleosuchus* and *Melanosuchus* as well as those of *Caiman crocodilus* lack obvious morphological landmarks; neither satellites nor obvious secondary constrictions were observed.

Alligator mississippiensis (DAUDIN) and *A. sinensis* FAUVEL ($2N = 32$; $NF = 60$). The karyotypes of these two species appear to be identical (Fig. 1e). The chromosome number is much lower than that of the previous three genera. This reduction is at the expense of telocentric elements, with the concomitant appearance of large metacentric chromosomes. However, the fundamental number is very similar to that of members of the other Alligatoridae. There are 24 metacentric chromosomes, of which 10 are large and can easily be grouped into five pairs on the basis of length. The remaining 14 small metacentrics are similar in size to those of the previous species. In addition, there are four submetacentric and four telocentric chromosomes. The two submetacentric pairs may be distinguished from each other by the presence of an extremely prominent secondary constriction in the smaller pair, simulating enlarged satellites as seen in *C. latirostris*. In some overcontracted chromosomes, this constriction may appear as an elongation of the short arms; however, the karyotype (Fig. 1e) clearly demonstrates that it is a constriction.

Gavialis gangeticus (GMELIN) ($2N = 32$; $NF = 60$). The karyotype of this species consists of 20 metacentric chromosomes easily arranged into five large and five small pairs (Fig. 1f). There are eight submetacentric and four telocentric elements. Certain karyotypic similarities exist between *Gavialis* and *Alligator* in the presence of the 10 large metacentric chromosomes and the 'satellited' marker submetacentric element. However, *Gavialis* does have four fewer small metacentrics and four more submetacentrics than does *Alligator*.

The 11 species of the genus *Crocodylus* show general agreement in having a number of large metacentric chromosomes, an almost characteristic marker and a very constant fundamental number. They also exhibit marked interspecific differences and are hence discussed separately, grouped according to their chromosome arrangements.

Crocodylus siamensis SCHNEIDER ($2N = 34$; $NF = 58$). This species has 18 metacentric chromosomes, 8 large elements that can be distinguished as four pairs by length plus a series of 10 small metacentrics (Fig. 2a). There are three identifiable pairs of submetacentrics. Ten telocentric elements complete the karyotype, comprising one large and one small pair and six remaining telocentrics of approximately the same length. However, one of these pairs (the smallest) can be recognized by a deep secondary constriction in the proximal third of the arm. Although in many cells the constriction was not apparent

Fig. 2. The karyotypes of the crocodilians: *(a) Crocodylus siamensis* (2N = 34); *(b) Crocodylus porosus* (2N = 34); *(c) Crocodylus moreletii* (2N = 32); *(d) Crocodylus johnsoni* (2N = 32); *(e) Crocodylus intermedius* (2N = 32); *(f) Crocodylus novaeguineae* (2N = 32). All species except *C. novaeguineae* possess a deep secondary constriction in a medium-sized telocentric.

in both homologues, this, in all probability, reflects contraction variability among the chromosomes.

Crocodylus porosus Schneider (2N = 34; NF = 58). There are 20 metacentric chromosomes including 4 large pairs, 2 obvious pairs of submetacentrics and a graded series of 10 telocentric elements (Fig. 2b). The species has one more pair of metacentrics, one fewer pair of submetacentrics and a series of telocentrics apparently identical to those of *C. siamensis*. However, the marker telocentric chromosome almost invariably shows the secondary constriction in both members of the pair.

Crocodylus moreletii Dumeril, Bibron and Dumeril (2N = 32; NF = 58). This species has 18 metacentrics, of which 8 are large, and a series of 10 small elements (Fig. 2c). There are six submeta-

centrics that form three distinct pairs. A series of eight telocentric chromosomes is comprised of one large and one small pair and two of intermediate size. Two of the four intermediate-sized telocentrics show the secondary constriction.

Crocodylus acutus CUVIER and *Crocodylus johnsoni* KREFFT (2N = 32; NF = 58). These species show identical karyotypes of 18 metacentric chromosomes, 8 large and 10 small (Fig. 2d). They differ from that of *C. moreletii* in having eight rather than six submetacentric chromosomes (the additional pair being smaller) and in possessing only six telocentrics, lacking the smallest pair. The constricted telocentric marker pair of chromosomes is present.

Crocodylus niloticus LAURENTI and *Crocodylus intermedius* GRAVES (2N = 32; NF = 58). Both of these species have identical karyotypes (Fig. 2e) with 20 metacentric chromosomes, 8 of which form large pairs, the remaining 12, small pairs. They also have six submetacentric chromosomes, apparently very similar to those of *C. siamensis*, and six telocentric elements, the smallest pair bearing the deep constriction.

Crocodylus novaeguineae SCHMIDT (2N = 32; NF = 58). The four specimens examined belonged to the subspecies *C. novaeguineae mindorensis*. The karyotype is identical to those of *C. niloticus* and *C. intermedius* except for the lack of the marker telocentric (Fig. 2f).

The karyotypes of the last four species are thus quite similar, and all of them share a diploid number of 2N = 32 and NF = 58.

Crocodylus cataphractus CUVIER (2N = 30; NF = 58). This species has 20 metacentric chromosomes arranged in five pairs of large elements and a series of 10 smaller ones, a condition quite distinct from that in other members of the genus (Fig. 3a). With these, it shares the occurrence of four large metacentric pairs; it differs in possessing a slightly smaller fifth pair. There are eight submetacentrics, of which one pair has enlarged satellites similar to the submetacentric marker observed in *Alligator* and *Caiman latirostris*. The two telocentric chromosomes do not bear the marker constriction common to other species of Crocodylidae.

Crocodylus rhombifer CUVIER (2N = 30; NF = 58). This form (Fig. 3b) has five large and five small pairs among its 20 metacentric chromosomes, as does *C. cataphractus*. However, it also has eight submetacentric chromosomes, with one large pair similar to that of *C. acutus* and *C. johnsoni*. Both of the telocentric chromosomes of this species bear deep secondary constrictions.
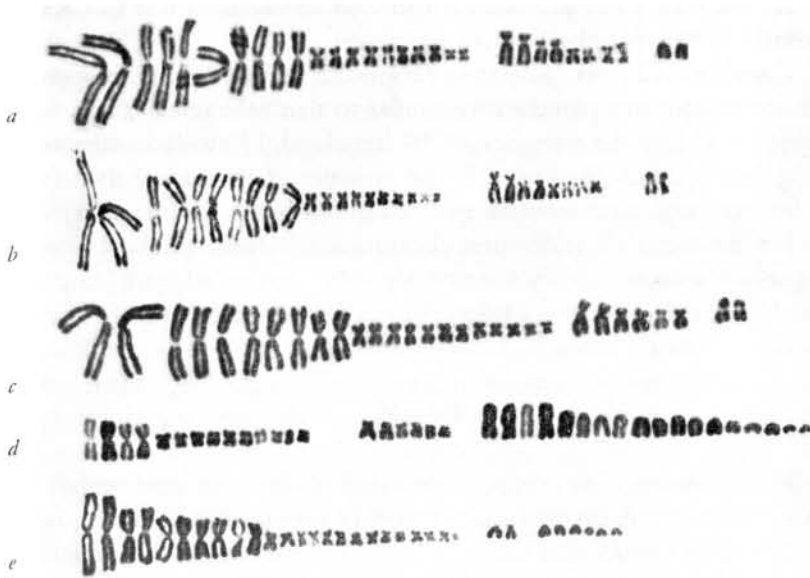
Fig. 3. The karyotypes of the crocodilians: *(a) Crocodylus cataphractus* (2N = 30); *(b) Crocodylus rhombifer* (2N = 30); *(c) Crocodylus palustris* (2N = 30); *(d) Osteolaemus tetraspis* (2N = 38); *(e) Tomistoma schlegelii* (2N = 32). Note the secondary constriction in the telocentric of *C. rhombifer* and *C. palustris. C. cataphractus* possesses a satellited submetacentric similar to that seen in the Alligatoridae.

Crocodylus palustris LESSON (2N = 30; NF = 58). This species has 10 large metacentrics identical to those of *C. rhombifer* (Fig. 3c). However, it has one more pair of small metacentrics and two fewer pairs of submetacentrics than does *C. rhombifer*. The two telocentric chromosomes again bear the deep secondary constriction.

Osteolaemus tetraspis COPE (2N = 38; NF = 58). One of the three specimens examined belonged to the subspecies *O. t. osborni* (SCHMIDT), the others to *O. t. tetraspis*. No chromosomal differences could be discerned between them. The karyotype (Fig. 3d) consists of 14 metacentrics, of which 4 are large and 10 are small, as well as 6 submetacentric and 18 telocentric chromosomes. This species has four large telocentric elements similar to those seen in the genera *Paleosuchus*, *Melanosuchus* and *Caiman*, but only two pairs of large metacentrics,

fewer than found in any other form. Neither satellites nor secondary constrictions were observed.

Tomistoma schlegelii (Muller) (2N = 32; NF = 58). This species has a chromosome pattern very similar to that seen in *Alligator*, with which it shares the presence of 10 large and 14 small metacentrics (Fig. 3e). It differs, however, in the presence of two rather than four submetacentric elements (the least observed in the entire order) and in having six small telocentric chromosomes. None of the chromosomes shows a secondary constriction.

Discussion

The relatively few cytogenetic studies thus far performed on crocodilians have, except for the report of Fishman *et al.* (1968), been reviewed by Cohen and Clark (1967). The present findings confirm the previously reported diploid numbers of *Alligator mississippiensis* (Risley, 1942; Matthey, 1947; Makino, 1951), *A. sinensis* (Fishman *et al.*, 1968) and *Caiman crocodilus* (Beçak *et al.*, 1964). We are now able to reconfirm the suggestion of Beçak *et al.* (1964) that the Crocodilia lack microchromosomes.

The question of reptilian sex-chromosome mechanisms is currently under active investigation. Several workers have described obvious female heterogamety among snakes and amphibians (Beçak *et al.*, 1962; Kobel, 1962; Weiler and Ohno, 1962; Hauschka and Brunst, 1964), while others have observed a heteromorphic sex chromosome pair in certain male lizards (Pennock *et al.*, 1969). Additional reports document reptilian species in which obvious sex chromosomes do not occur (Risley, 1942; Matthey and Van Brink, 1956; Van Brink, 1959; Seto and Pomerat, 1965). The present studies suggest that the crocodilians belong with the latter group. We have observed specimens of both sexes in 13 species without noting a constant heteromorphism that might be indicative of a sex-chromosome pair. The karyotype of a female *A. sinensis* (Fishman *et al.*, 1968) appears to be identical to the male karyotype reported in the present study. However, in the karyotypes of four of the species presented in this paper (*Crocodylus porosus*, *C. novaeguineae*, *C. moreletii* and *Tomistoma schlegelii*) the smallest metacentric element appeared unpaired. This discrepancy was not present in all cells examined in any individual

nor in all specimens and probably does not reflect true heteromorphism. Therefore, sex determination via a chromosomal mechanism is certainly not obvious among crocodilians. The differentiation of the sex chromosomes may be yet too subtle to be morphologically noticeable, or sex may be determined on a genic rather than chromosomal level.

Considerable variation in both chromosome number and morphology is encountered among the Crocodilia, yet certain constant features may be noted. The total number of chromosome arms (MATTHEY's 'nombre fondamentale', or NF) is very stable throughout the order and, with a range from 56 to 62, shows a variation of only 10%. Although obvious karyotypic shifts seem to have occurred, the conservation of the genome is striking. The occurrence of identical marker chromosomes in *Caiman latirostris*, *Alligator*, *Gavialis* and *Crocodylus cataphractus* is of interest. Similarly, nine species of *Crocodylus* exhibit a telocentric element with a marked secondary constriction.

There are numerous reports of karyotypic differences between primate species presumed to be closely related on other grounds (CHU and BENDER, 1961, 1962; HAMERTON, 1963). Such differences have been noted also in rodents (WAHRMAN and ZAHAVI, 1958; MATTHEY, 1963; RAO and SHARDA, 1964) and reptiles (KOBEL, 1963; GORMAN, 1965). The most probable mechanisms for the observed chromosomal rearrangements include centric fusion of the Robertsonian type, pericentric inversions, reciprocal translocations and isochromosome formation. Each of these phenomena may act individually or in concert with others to yield a progression from one karyotype to another. The most important of these mechanisms are Robertsonian translocation (both centromeric 'fusion' and possibly 'fission') and pericentric inversions, with numerous suggested examples of each.

It is generally accepted that reduction of the total chromosome number seems most frequently to occur by Robertsonian 'centric fusion', *i.e.*, through translocations between acrocentric or telocentric elements. Such a phenomenon implies the loss of telocentric chromosomes and the simultaneous emergence of biarmed elements. ROBERTSON (1916) also suggested the possibility of 'centromeric fission', but though this mechanism is theoretically possible, there has been little evidence to substantiate its occurrence. In discussing principles of karyotype evolution, WHITE (1963) cites 'fission' as a definite

mechanism, although much less common than 'fusion'. A theoretical consideration of the 'fission' *vs.* 'fusion' controversy for both sex chromosomes and autosomes has been rendered by TODD (1967, 1969). WAHRMAN *et al.* (1969) cited the importance of 'fission' in the chromosomal evolution of several forms of the rodent *Spalax*. Additional examples of this phenomenon have also been described recently in insects (SOUTHERN, 1969) and in lizards (GORMAN *et al.*, 1967, 1969). One difficulty of the 'fission concept' is the need to generate an additional centromere in order to accommodate the new chromosome. As yet, we lack sufficient information about centromeric ultrastructure and function to predicate a mechanism for this. Although COLE and CORREY (1969) have suggested a quadripartite structure of meta- and submetacentric Chinese hamster chromosomes, yielding four chromatids which may function independently, the bulk of empiric and experimental evidence supports the view that chromosome numbers tend to be reduced by translocation and 'centric fusion'.

It has been suggested (CHU and BENDER, 1961) that those species having a larger number of biarmed chromosomes are more 'specialized'. The implication is that a large number of telocentric elements will, in contrast, be associated with more 'primitive' or less 'specialized' forms. Such arguments appear both polemic and academic since it must be considered that the karyotypes presently being investigated are generally those of closely related species. It is also highly likely that these species have been separated for a period that would be relatively short in terms of evolutionary history and that such species would generally have been derived from a common progenitor. Furthermore, it seems hardly likely that all organisms or even all animals have been derived via the same mechanism. If the direction of change is indeed from 'telocentricity' toward 'metacentricity', we might expect all Recent forms to have become entirely biarmed by now. The rigid restrictions for the regulation of mitosis and, particularly, meiosis most likely maintain karyotypic modification within narrow constraints and restrict viable variants to certain limited types of chromosomes. This may explain the observed stability that permits major structural changes of the organism itself with relatively minor variation of the chromosomal complement.

Yet, the assumption that 'centromeric fusion' and inversion are the basic (*i.e.*, most probable) mechanisms inducing changes of karyotype permits certain theoretical formulations concerning the chromosomal
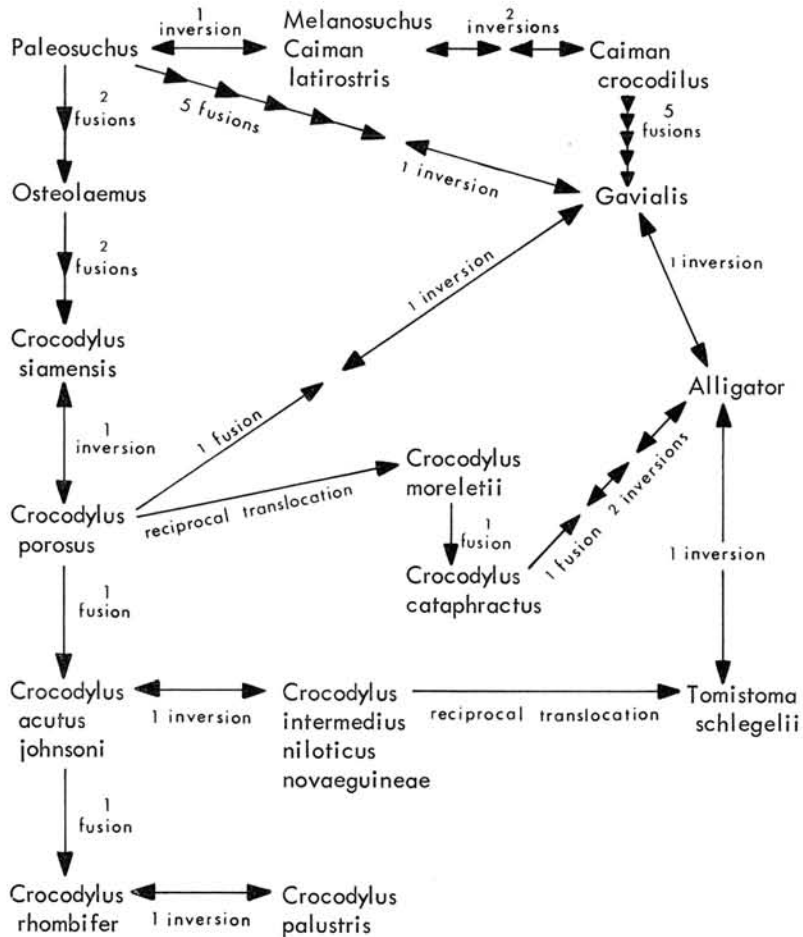
Fig. 4. Arrangement of crocodilian karyotypes based upon the assumptions that (1) telocentricity is primitive, (2) 'fusions', inversions and translocations are the operative mechanisms of chromosomal alteration and (3) the least number of steps between two karyotypes represents the probable path of change. The connection between *Caiman crocodilus* and *G. gavialis* indicates the possible confusion of such schemes if the last restriction is eliminated.

interrelationships among the Crocodilia. Such schemes may be, at best, tenuous but allow a consideration of the minimum number of intermediate steps required to obtain the various karyotypes observed. The sequence by which the existing crocodilian karyotypes could have evolved (Fig. 4) may reflect the affinities of the species they characterize. Past phylogeny might be reconstructed from the comparison of numerous such statements (or diagrams), each for a different characteristic of the organism.

The first grouping that immediately becomes apparent among the crocodilian karyotypes comprises the three genera *Paleosuchus*, *Melanosuchus* and *Caiman*, all of which are characterized by the presence of large numbers of telocentric chromosomes and absence of large metacentrics. The karyotype of *Paleosuchus* could give rise to that of *Melanosuchus* and *C. latirostris* by a pericentric inversion of a small telocentric into a metacentric pair. The complement of *Caiman crocodylus* could be achieved by assuming two further inversions in the karyotype of *M. niger* or *C. latirostris* of a telocentric and a submetacentric element, respectively, in order to yield two additional metacentric pairs.

Only *Osteolaemus* and eight species of the genus *Crocodylus* (*acutus*, *intermedius*, *johnsoni*, *moreletii*, *niloticus*, *novaeguineae*, *porosus* and *siamensis*) resemble these forms in the possession of a number of large telocentric chromosomes. These are lacking in *Alligator*, *Gavialis* and *Tomistoma* and in the remaining species of the genus *Crocodylus* (*cataphractus*, *palustris* and *rhombifer*). These forms do have an increased number of large metacentric chromosomes. The large metacentric elements found in all crocodilians but *Caiman*, *Melanosuchus* and *Paleosuchus* might well have resulted from a variable number of Robertsonian translocations in the several species since their presence is accompanied by a concomitant reduction of telocentrics. It is, for instance, possible to create a sequence in which the karyotype of *Paleosuchus* is changed into that of *Gavialis* by five centric 'fusions' and one pericentric inversion of a telocentric pair.

Since these are shifts involving a complex sequence of changes for karyotypic modification, it seems simpler to proceed via a series of smaller steps. The karyotype of *Osteolaemus* may be derived from that of *Paleosuchus* by inducing two telocentric 'fusions', leading to two large metacentric pairs and the loss of four telocentrics. The step from *O. tetraspis* to *Crocodylus siamensis* may again be bridged by assuming two additional 'fusions' that yield two more pairs of large

metacentrics. The remainder of the karyotype of *C. siamensis* is then identical with that of *Osteolaemus* except for the appearance of the marker telocentric in *C. siamensis*.

The chromosomal complement of *Crocodylus siamensis* can again be modified to that of any of the species of the genus. All species except *C. acutus*, *C. johnsoni*, *C. porosus*, *C. rhombifer* and *C. palustris* have six submetacentric chromosomes. These shifts, therefore, could be explained by telocentric to metacentric rearrangements via Robertsonian translocations. A pericentric inversion within the karyotype of *C. siamensis* from submetacentric to metacentric yields the karyotype of *C. porosus*. A translocation between the smallest and of one intermediate-sized telocentric pairs to create an additional large pair of submetacentrics would change the karyotype of *C. rhombifer* into that of *C. acutus* or *C. johnsoni*. Some possible support for the latter mechanism would be provided by the observation that the second pair of large metacentrics of *C. rhombifer* has arms of unequal lengths, which would be expected if this pair had been produced by fusion of the two unequal-sized telocentrics of *C. acutus* or *C. johnsoni*.

It is possible to derive the karyotypes of *C. niloticus*, *C. intermedius* and *C. noveaguineae* from those of *C. acutus* or *C. johnsoni* by an inversion of one of the smaller submetacentrics leading to an additional metacentric element. The karyotype of *C. porosus* could have been transformed into that of *C. moreletii* by a reciprocal translocation very close to the centromere of a medium-sized telocentric with a small metacentric. This would yield an additional submetacentric and a small fragment which would have to be lost in order to reduce the number of centromeres to that observed. The karyotype of *C. cataphractus* may be obtained directly from that of *C. moreletii* by a Robertsonian 'fusion' between two larger telocentrics to yield a fifth pair of large metacentrics. In both forms, the third largest pair is slightly submetacentric.

Tomistoma again differs from *Crocodylus niloticus* and *C. novaeguineae* in having two additional metacentric pairs (one large and one small) and in lacking two submetacentric pairs. A reciprocal translocation near the centromere of the two submetacentric elements resulting in long arm-long arm and short arm-short arm segregants would produce the pattern seen in *Tomistoma*. The karyotype of this species could also be transformed into that of *Alligator* and *Gavialis* through a series of inversions.

Table II. Distribution of chromosomal arms among the crocodilians based on assumption of 'centromeric fission'.

		Group A Robertsonian chromosomes			Group B[1] Inversion chromosomes	Corrected No. of arms
	2N	M	T	Arms	Arms	
Paleosuchus (2 species)	42	—	24	24	18	42
Melanosuchus niger	42	—	24	24	18	42
Caiman latirostris	42	—	24	24	18	42
Caiman crocodilus	42	—	24	24	18	42
Osteolaemus tetraspis	38	4	16	24	18	42
Crocodylus siamensis	34	8	8	24	18	42
Crocodylus porosus	34	8	8	24	18	42
Crocodylus intermedius	32	10	4	24	18	42
Crocodylus niloticus	32	10	4	24	18	42
Crocodylus johnsoni	32	10	4	24	18	42
Crocodylus acutus	32	10	4	24	18	42
Crocodylus novaeguineae	32	10	4	24	18	42
Crocodylus moreletii	32	10	4	24	18	42
Alligator (2 species)	32	10	4	24	18	42
Tomistoma schlegelii	32	10	4	24	18	42
Gavialis gangeticus	32	10	4	24	18	42
Crocodylus cataphractus	30	12	—	24	18	42
Crocodylus rhombifer	30	12	—	24	18	42
Crocodylus palustris	30	12	—	24	18	42

[1] All chromosomes of group B are counted as one-armed elements (as if derived from previously split metacentrics).

Fig. 5. Crocodilian karyotypes rearranged according to the concept of centromeric 'fission' of metacentric chromosomes. Note the constancy of 'corrected arms' suggesting conservation of the genome in this order: *(a) Paleosuchus palpebrosus; (b) Melanosuchus niger; (c) Caiman latirostris; (d) Caiman crocodilus; (e) Osteolaemus tetraspis; (f) Crocodylus siamensis; (g) Crocodylus porosus; (h) Crocodylus intermedius; (i) Crocodylus johnsoni; (j) Crocodylus novaeguineae; (k) Crocodylus moreletii; (l) Alligator mississippiensis; (m) Tomistoma schlegelii; (m) Gavialis gangeticus; (o) Crocodylus cataphractus; (p) Crocodylus palustris; (q) Crocodylus rhombifer.*

Alternatively, the karyotype of *Gavialis* could be produced from that of *Crocodylus siamensis* by a single 'fusion' plus an inversion, or from *C. porosus* by a single 'fusion' plus two inversions. The karyotype of *Alligator* could again be produced from that of *Gavialis* by two inversions.

The foregoing scheme describes the possible karyotypic evolutionary history based upon the tacitly accepted primary assumption of centromeric 'fusion'. As indicated previously, however, centromeric 'fission' may also occur, and when a model similar to that proposed by WAHRMAN *et al.* (1969) is applied to crocodilian chromosomes, an interesting result obtains.

The chromosomes of crocodilians can be classified into two groups: Group A, those meta- or submetacentric elements capable of undergoing centromeric 'fission' and yielding two independently functioning elements (Robertsonian chromosomes); Group B, those elements resulting from the fission of Group A members into two original telocentric chromosomes which may have undergone further morphological modification through inversion (Inversion chromosomes). The chromosomes of Group B are considered equal in genetic content to the corresponding arms of the original metacentric chromosomes (Group A). The small meta- and submetacentrics of this group are similarly considered as one-armed elements.

With these considerations, a basic 'crocodilian' karyotype can be constructed. It consists of six pairs of Group A (Robertsonian) chromosomes and nine pairs of Group B (Inversion) chromosomes. The karyotypes of all species of the order can now be easily derived. Karyotypes constructed so as to restore the possible metacentricity of the original Group A chromosomes are shown in Figure 5. They emphasize the remarkable constancy in the amount of genetic material. The numerical relationships between the crocodilian karyotypes are presented in Table II. Although this approach is undoubtedly arbitrary, it is readily apparent from such an analysis that conservation of the genome is indeed striking since each species contains 42 'corrected' chromosome arms of approximately identical length.

It is primarily of academic interest to demonstrate the possibility of the above sequences since such manipulations do not prove that the change actually occurred, let alone in the directions described. Only a few of the chromosomes can be individually recognized, and it is rarely possible to determine whether one is dealing with remnants
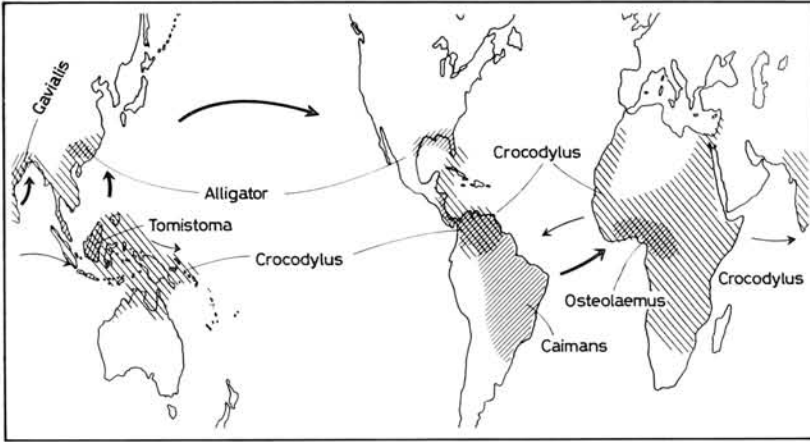
Fig. 6. A map in schematic form showing the approximate ranges of the species discussed in this paper. The sequence of arrows indicates one possible view of affinities within the Recent members of the order, independent of the fossil record. It is *not* presented as an estimate of zoogeographical pattern.

of steps in a gradual sequence or with independent forms that have achieved similar karyotypes via historical accident. Only the simultaneous evaluation of multiple systems of morphological and biological characteristics may permit the distinction between evolutionary parallelism or convergence.

The potential for spurious interpretation is documented by the four cases in which the karyotypes were indistinguishable between two species. These instances *(Paleosuchus trigonatus* and *P. palpebrosus; Alligator mississippiensis* and *A. sinensis; Crocodylus acutus* and *C. johnsoni;* and *C. niloticus* and *C. intermedius)* involve pairs of species easily considered distinct on the basis of morphological and, of course, biological criteria. We are not aware of any report suggesting more than generic affinity between the members of any of these pairs of species; indeed, in three of the pairs the members occur on different continents.

The evidence deduced from these chromosomal studies does cast an interesting light on the taxonomic classification proposed for the crocodilians on the basis of other characteristics. The three genera *Paleosuchus, Melanosuchus* and *Caiman* seem to form a distinct group. There is no strong evidence here to support linking them with

Alligator in a family or subfamily, nor is the karyotype of *Gavialis* sufficiently distinct to suggest that it belongs in a grouping different from either *Alligator* or *Crocodylus*. The minimal postulated shifts required to transform one chromosome pattern to another are mapped in Figure 4. If centric 'fusion' is indeed the major evolutionary mechanism, this would suggest the origin of all crocodilian karyotypes from a *Paleosuchus*-like pattern with change proceeding in one direction toward the pattern shown by *Melanosuchus* and *Caiman* and in the other direction toward those karyotypes characterizing *Osteolaemus* and *Crocodylus*, the latter again giving rise to the karyotypes of *Alligator*, *Gavialis* and *Tomistoma*.

Such a pattern may contain a modicum of zoogeographical and ecological merit (SILL, 1968). *Paleosuchus*, *Melanosuchus* and *Caiman* would then be considered old South American forms representing primarily freshwater species that seem to have been long isolated on that continent. *Osteolaemus* is a freshwater form of the equatorial forests of West Africa, whereas *Crocodylus*, a genus that ranges in the coastal regions of South America, north to the Antilles and Florida, across Africa and through southern Asia and the island chain to Australia, has many members that tolerate or prefer estuarine or marine situations. *Gavialis* and *Tomistoma* are species of limited distribution in eastern Asia, though the latter is known from the fossil record of eastern North America. *Alligator* occurs in China and the southeastern United States. If additional character systems support the pattern shown by the karyotypes observed in the caimans and *Osteolaemus*, this assemblage presents another example of trans-South Atlantic affinities which may exist. Similarly, the distribution patterns of *Alligator* and *Tomistoma* suggest affinity between the faunas of eastern Asia and eastern North America (Fig. 6).

Appendix I

Available identification parameters of the 71 crocodilians investigated including (where possible): sex, location, date of blood sample, identification number, weight, length and point of origin.

Paleosuchus palpebrosus: ♂, (NYZ), 7/25/67, No. 650615, 4.5 kg, 96.5 cm; ♂, (NYZ), 2/16/68, No. 67694, 0.8 kg, 60.4 cm; ♂, (NYZ), 2/16/68, No. 67695, 0.2 kg, 40.6 cm.

Paleosuchus trigonatus: ♂, (NYZ), 7/25/67, No. 620000, 3.1 kg, 83.8 cm; ♀, (NYZ), 9/21/67, No. 67415, 1.6 kg, 63.5 cm.

Melanosuchus niger: ♀, (NYZ), 2/1/67, No. 650432, 2.3 kg, 85.0 cm; ♀, (NYZ), 9/21/67, No. 640039, 5.1 kg, 152.4 cm; ?, (NYZ), 1/30/69, No. 68429, 0.4 kg, 52.7 cm.

Caiman latirostris: ♀, (CPZ), 4/17/69, 58.9 cm; ♀, (CPZ), 4/17/69, 55.8 cm; ♂, (CPZ), 4/17/69, 123.2 cm.

Caiman crocodilus: ♂, (BZ), 3/15/66; ♂, (BZ), 3/15/66; ♀, (NYZ), 2/1/67, No. 65089; ♂, (NYZ), 2/1/67, No. 650305, 10.9 kg, 137.2 cm; ♀, (NYZ), 2/1/67, No. 640462, 7.4 kg, 121.9 cm; ♂, (NYZ), 2/1/67, No. 66119, 7.2 kg, 121.9 cm, Colombia; ?, (NYZ), 2/1/67, No. 66618; ?, (NYZ), 10/19/67, No. 67582, 0.5 kg, 27.0 cm; ?, (NYZ), 10/19/67, No. 67578, 0.4 kg, 23.6 cm, Colombia; ♀, (RARI), 1/6/69, 54.6 cm, Peru.

Caiman crocodilus yacare: ♂, (NYZ), 2/1/67, No. 66175, 0.7 kg, 59.0 cm; ♂, (NYZ), 2/1/67, No. 66512, 8.5 kg, 129.5 cm; ♂, (NYZ), 10/19/67, No. 67579, Paraguay; ♂, (RARI), 1/6/69, 40.6 cm, Paraguay.

Caiman crocodilus fuscus: ?, (NYZ), 2/1/67, No. 66008, 9.5 kg, 138.0 cm, Colombia.

Alligator mississippiensis: ♂, (BZ), 3/15/66; ♂, (BZ), 3/15/66; ♂, (NYZ), 2/1/67, No. 67052; ♂, (NYZ), 2/1/67, No. 66689, 93.3 kg, 228.6 cm, Florida; ♀, (BZ), 8/13/67; ♂, (BZ), 3/20/69, 199 cm.

Alligator sinensis: ♂, (NYZ), 9/21/67, No. 560502, 25.2 kg, 172.7 cm.

Gavialis gangeticus: ♀, (NYZ), 9/21/67, No. 650668, 5.1 kg, 129.5 cm.

Crocodylus siamensis: ♀, (NYZ), 7/25/67, No. 650092, 26.8 kg, 182.9 cm; ♀, (NYZ), No. 650093, 14.6 kg, 160.0 cm.

Crocodylus porosus: ♂, (BZ), 3/15/66; ♂, (BZ), 3/15/66; ♀, (NYZ), 10/19/67, No. 67572, 0.1 kg, 37 cm; ♂, (NYZ), 10/19/67, No. 67573, 0.1 kg, 40 cm; ♀, (NYZ), 10/19/67, No. 67574, 0.1 kg, 38.0 cm.

Crocodylus moreletii: ♂, (NYZ), 9/21/67, No. 67495, 0.9 kg, 60 cm; ♂, (NYZ), 9/21/67, No. 66173, 8.1 kg, 128.3 cm, Honduras.

Crocodylus johnsoni: ♂, (NYZ), 1/30/69, No. 69000, 0.3 kg, 48.5 cm; ♀, (NYZ), 1/30/69, No. 68494, 0.4 kg, 51 cm.

Crocodylus acutus: ♀, (NYZ), 7/25/67, No. 620025, 50.8 kg, 224.8 cm, Ecuador; ♀, (NYZ), 7/25/67, No. 620001, 37.4 kg, 210.8 cm; ♀, (NYZ), 7/25/67, No. 640035, 31.0 kg, 182.9 cm; ♂, (RARI), 1/6/69, 69.9 cm.

Crocodylus intermedius: ♂, (CPZ), 4/17/69, 190.5 cm.

Crocodylus niloticus: ♂, (BZ), 3/15/66; ♂, (NYZ), 7/25/67, No. 610009, 22.4 kg, 175.3 cm; ♀, (NYZ), 7/25/67, No. 610008, 33.7 kg, 193.0 cm; ♀, (NYZ), 10/19/67, No. 67594, 0.5 kg, 62 cm.

Crocodylus novaeguineae mindorensis: ♀, (NYZ), 9/21/67, No. 67317, 0.3 kg, 51.0 cm; ♀, (NYZ), 9/21/67, No. 67315, 0.5 kg, 58.0 cm; ♂, (NYZ), 9/21/67, No. 67314, 0.4 kg, 55.0 cm; ♂, No. 67316, 0.6 kg, 62.4 cm.

Crocodylus cataphractus: ♀, (NYZ), 2/1/67, No. 650719, 2.2 kg, 91.0 cm; ♂, (NYZ), 7/25/67, No. 610503, 33.3 kg, 202.0 cm, Liberia; ♀, (CPZ), 4/17/69, 54.6 cm.

Crocodylus rhombifer: ♂, (NYZ), 7/19/68, No. 580001, 138.9 kg, 259.1 cm.

Crocodylus palustris: ♂, (PZ), 4/15/69, 204.3 kg, 259.0 cm; ♂, (CPZ), 4/17/69, 104.1 cm.

Osteolaemus tetraspis: ♂, (NYZ), 2/1/67, No. 650163, 6.8 kg, 109.2 cm; ♀, (RARI), 1/6/69, 72.4 cm.

Osteolaemus tetraspis osborni: ♂, (NYZ), 7/25/67, No. 67111, 2.0 kg, 83.8 cm.

Tomistoma schlegelii: ♂, (BZ), 3/15/66; ♂, (NYZ), 7/25/67, No. 66151, 4.1 kg, 111.8 cm; ♂, (NYZ), 10/19/67, No. 66152, 2.2 kg, 96 cm; ♀, (RARI), 1/6/69, 102.9 cm.

BZ = Buffalo Zoological Gardens, Buffalo, New York.
CPZ = Crandon Park Zoological Gardens, Key Biscayne, Florida.
NYZ = New York Zoological Park, Bronx, New York.
PZ = Philadelphia Zoological Gardens, Philadelphia, Pennsylvania.
RARI = Ross Allen Reptile Institute, Silver Springs, Florida.

Appendix II

Common names of the crocodilians

Species	Common name
Paleosuchus palpebrosu.	Dwarf caiman
P. trigonatus	Smooth front caiman
Melanosuchus niger	Black caiman
Caiman latirostris	Broad nose caiman
C. crocodilus	Spectacled caiman
Alligator mississippiensis	American alligator
A. sinensis	Chinese alligator

Appendix II (continued)

Gavialis gangeticus	Gavial
Crocodylus siamensis	Siamese crocodile
C. porosus	Salt water crocodile
C. moreletii	Morelett's crocodile
C. johnsoni	Australian crocodile
C. acutus	American crocodile
C. intermedius	Orinoco crocodile
C. niloticus	Nile crocodile
C. novaeguineae	Phillipine crocodile
C. cataphractus	Slender snouted crocodile
C. rhombifer	Cuban crocodile
C. palustris	Mugger crocodile
Osteolaemus tetraspis	Broad nose crocodile
Tomistoma schlegelii	False gavial

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