Chromosome morphology of the Madagascar tree boa *Sanzinia madagascariensis*

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The karyotype of *Sanzinia madagascariensis* (2n = 34, NF = 50) was obtained from peripheral lymphocytes. A survey of chromosome morphology in the Boidae reveals that the karyotype of *S. madagascariensis* is unique, and not easily derivable from that of other pythons and boas. The significance of this in relation to the zoogeographic anomaly presented by the endemic Madagascar boas *Sanzinia* and *Acrantophis* is discussed.

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Die kariotipe van *Sanzinia madagascariensis* (2n = 34, NF = 50) is verkry van periferiese limfosiete. 'n Opname van chromosoom-morfologie in die Boidae toon dat die kariotipe van *S. madagascariensis* uniek is en nie maklik afgelei kan word van dié van luislange en boas nie. Die betekenis hiervan, in verhouding tot die dieregeografie/zoogeografie soos vertoon deur die endemiese Madagaskar boas *Sanzinia* en *Acrantophis*, word bespreek.

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The taxonomic affinities of pythons, boas and related snakes have recently been the subject of active debate (Underwood 1967, 1976; Dowling 1975; McDowell 1975). Concomitant with this the application of biochemical and chromosomal data as adjuncts to morphological analysis has increased. Madagascar boas of the genera *Acrantophis* and *Sanzinia* represent a zoogeographic anomaly, being endemic to the island, but apparently being closely related to the Neotropical boids *Boa* and *Corallus* respectively, with which they were long classified. Chromosomal data exist for a number of Neotropical and Eurasian boids, but none of the Madagascar genera have previously been karyotyped. The observations reported here provide some data on this subject.

Materials and Methods

Chromosomes were collected from an adult male *Sanzinia madagascariensis* (Dumeril & Bibron) born in captivity to a gravid female collected in 1974 in the vicinity of Perinet in the eastern rain forests of central Madagascar (Branch & Erasmus 1976).

Peripheral lymphocytes were stimulated to divide *in vivo* by injections of phytohemagglutinin P (Difco Ltd), arrested in metaphase with colchicine, and collected in haematocrit tubes as outlined elsewhere (Branch 1978). Cells were treated with hypotonic sodium citrate, fixed with acetic methanol (1:3), flame-dried, and stained in a May–Grünwald Giemsa solution.

Well-spread mitotic figures were located using a Leitz microscope. The modal diploid number was determined and five suitable spreads having the modal karyotype were photographed on high contrast Copex film, ASA6 (Agfa Ltd). The karyotypes were constructed and measurements taken of the relative length of each macrochromosome to determine its percentage contribution to the total haploid macrochromosome length; centromere index, expressed as the length of the short arm relative to the total length of the chromosome; and the arm ratios. Chromosomes were classified according to the terminology of Cole (1970). The 'Nombre Fondamental' (NF = the total number of arms in the karyotype) was determined by scoring 2 for metacentric and submetacentric chromosomes, and 1 for telocentric, subtelocentric and microchromosomes.

The snake is deposited in the herpetological collection of the British Museum (Natural History) as accession BM 1977. 1209.

Table 1 Chromosomal data for the Boidae

Species	2n	V	I	m	NF*	Author
Erycinae						
Charina b. bottae	36	8	8	20	44	Gorman & Gress 1970
Lichanura r. roseofusca	36	8	8	20	44	Gorman & Gress 1970
Eryx j. johnii	34	8	8	18	42	Singh *et al.* 1968
Eryx jaculus	34	8	8	18	42	Werner 1959
Gonglyophis conicus	34	8	8	18	42	Singh *et al.* 1970
Boinae						
Boa c. constrictor	36	8	8	20	44	Becak *et al.* 1966
Boa c. amarali	36	8	8	20	44	Becak *et al.* 1966
Eunectes murinus	36	8	8	20	44	Becak *et al.* 1966
Epicrates cenchria crassus	36	8	8	20	44	Becak *et al.* 1966
Epicrates s. striatus	36	8	8	20	44	Gorman & Gress 1970
Sanzinia madagascariensis	34	16	4	14	50	Present study
Corallus caninus	44	—	24	20	44	Becak *et al.* 1966
Corallus enydris cookii	40	4	16	20	44	Gorman & Gress 1970
Pythoninae						
Python curtus	36	8	8	20	44	Fischman *et al.* 1972
Python molurus	36	8	8	20	44	Singh *et al.* 1968
Xenopeltinae						
Xenopeltis unicolor	36	8	8	20	44	Cole & Dowling 1970
Loxocemus bicolor	36	8	8	20	44	Fischman *et al.* 1972

*The NF shown may differ from that of the original source, due to different methods of calculating the NF. V = Metacentric and submetacentric chromosomes; I = Subtelocentric and telocentric chromosomes; m = Microchromosomes.

Results and Discussion

Mitotic figures were not exceptionally numerous, although adequate for the karyotype to be determined with reasonable accuracy. Counts of 39 suitable spreads gave a modal diploid number (2n) of 34 chromosomes, the first 20 of which may be considered as macro-chromosomes, with 14 micro-chromosomes remaining (Fig. 1). However, the distinction between macro- and micro-chromosomes is not as prominent as the distinction found in the 'typical ophidian karyotype' (Werner 1959; Gorman & Gress 1970), and the chromosomes show a gradual reduction in size. Of the macro-chromosomes, pairs 1, 3, 4, 5 and 10 are metacentric, or essentially so; pairs 2, 6 and 9 are submetacentric; pair 7 is subtelocentric with very short small arms; and pair 8 is telocentric. The morphology of the micro-chromosomes is not usually resolvable, although the first 4—6 appear to be telocentric, or almost so. No secondary constrictions or obvious heteromorphic pairs were observed, and this accords with previous findings for the Boidae.

For the most part chromosome morphology within the Boidae is relatively conservative (Table 1), with most of the species having 16 macro-chromosomes and 20 micro-chromosomes (2n = 36). This typical ophidian pattern is also widely distributed among other groups, notably the Viperidae and Colubrinae (Gorman 1973).

The presence of such a karyotype in the genera *Lichanura, Charina, Eunectes, Epicrates, Boa, Python, Xenopeltis* and *Loxocemus* indicates that it is the primitive boid condition, and that three other lines of chromosomal evolution within the Boidae are evident.

— Old World sand boas of the genera *Eryx* and *Gonglyophis* have lost a pair of microchromosomes, reducing the diploid number and NF of their karyotypes to 34 and 42 respectively. A similar loss has occurred in some Old World colubrines including members of the genera *Dasypeltis, Chamaetortus* (=*Dipsadoboa*), and *Crotaphopeltis* (Branch unpubl. obser.). The New World erycines *Charina* and *Lichanura* have retained the typical condition, and this is in agreement with the views of Hoffstetter and Rage (1972) and Underwood (1976) who consider the New World erycines to be more primitive. Erycines first appear during the early Tertiary in North America and subsequently radiated from west to east into Europe before the separation of the two conti-

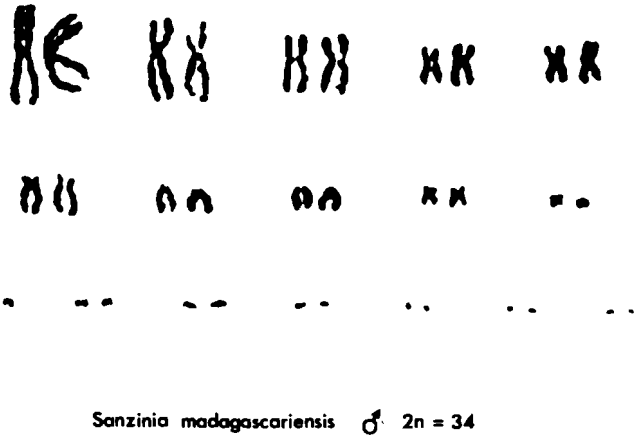

Fig. 1 The karyotype of *Sanzinia madagascariensis*.

Table 2 Morphometric data on the macro-chromosomes of *Sanzinia madagascariensis*

	Macro-chromosome pairs									
	1	2	3	4	5	6	7	8	9	10
Relative length										
Percentage	22,1	18,6	13,5	9,3	8,7	8,7	6,5	5,0	5,0	2,6
±S.E.	1,24	2,50	2,21	0,84	0,82	0,79	0,65	0,52	0,99	0,20
Centromere index										
Percentage	47,8	40,4	44,0	44,5	46,3	24,3	13,3	—	5,4	44,2
±S.E.	6,75	5,31	5,20	5,8	4,9	2,10	0,99	—	5,71	6,75
Arm ratio	1,09	1,48	1,28	1,25	1,16	3,11	6,48	—	1,82	1,26
±S.E.	0,31	0,38	0,29	0,29	0,21	0,46	0,51	—	0,09	0,06

nents and the formation of the North Atlantic. *Eryx* is not considered to have entered Africa before the Miocene contact between the African and Eurasian plates (Underwood 1976), but no chromosomal data are available for any of the three African species.

— Neotropical arboreal boas of the genus *Corallus* have increased diploid numbers (*C. caninus*, 2n = 44; *C. enydris cookii*, 2n = 40), but retain a NF similar to other boids. As Gorman and Gress (1970) suggested, this probably resulted from centric fission of some or all of the metacentric and submetacentric macro-chromosomes. Although it is generally assumed that chromosomal evolution proceeds by centric fusion, leading Becak, Becak and Nazareth (1966) to claim that *Corallus caninus* was the most primitive boid, it is now known that occasionally diploid numbers may be increased by centric fission, as for example in the iguanid lizard *Plica plica* (Gorman & Holzinger 1967) and the African amphisbaenid *Monopeltis sphenorhynchus* (Branch unpubl. obser.).

— The unusual karyotype of *Sanzinia madagascariensis* is not easily derivable from the typical boid condition. Although having a similar diploid number (34) to *Eryx* and *Gonglyophis* the NF of *Sanzinia* is much higher than that of any other boid. As noted earlier there is not an obvious break in the size of macro-chromosomes and micro-chromosomes in *Sanzinia*, and although pair 10 chromosomes have been classified as macro-chromosomes they could also be considered as large micro-chromosomes. If this was the case then the typical boid karyotype could be obtained from that of *Sanzinia* by the addition of two pairs of micro-chromosomes and centric fusion of pairs 7 and 8, with associated changes in the centrometric index of the individual macro-chromosomes resulting from pericentric inversions. However, such a mechanism does not fully agree with morphometric data for the macro-chromosomes of *Sanzinia* (Table 2). The combined relative lengths of chromosome pairs 7 and 8 do not fall within the ranges of any other boid macro-chromosomes summarized in Gorman and Gress (1970). Various other mechanisms can be postulated for the derivation of the *Sanzinia* karyotype, but in the absence of banding data all have little to support them. Comparative chromosomal banding may give some indication of the evolutionary steps involved. However, chromosomal studies on snakes are still in their infancy, and such data for boids, or for any other snakes, is almost totally lacking.

Underwood (1976) has shown that the relations of the postorbital bones and the vidian canals associate *Acrantophis* and *Sanzinia*, and he shares the view of Mertens (1972) that both are evolved from a single trans-oceanic colonist. Chromosomal data for *Acrantophis* are urgently required for comparison with the unique karyotype of *Sanzinia madagascariensis*.

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