Chromosoma (Berl.) 79, 53–64 (1980)

CHROMOSOMA
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Chromosomal Evolution in Serpentes; A Comparison of G and C Chromosome Banding Patterns of Some Colubrid and Boid Genera

Gregory A. Mengden [1] and A. Dean Stock [2]

[1] Houston Zoological Gardens, Houston, Texas, U.S.A. Current address: Department of Population Biology, Research School of Biological Sciences, Australian National University, Canberra, A.C.T., Australia;
[2] Biomedical Research, Exceptional Child Center, UMC 68, Utah State University, Logan, Utah, U.S.A.

Abstract. G and C-chromosome banding techniques have been used to compare the structure of the karyotype in a variety of colubrid and boid snakes. The comparison of G-band patterns indicates that while some band sequences have been conserved, either as whole chromosomes or entire arms, there is also evidence of considerable rearrangement especially in the smaller chromosomes. In the colubrid *Elaphe subocularis* there is also evidence that there has been a relocation of the centromere on chromosome 2 without any accompanying inversion in the sequence of G-bands. Finally, G-banding has facilitated the demonstration of a simple pericentric inversion distinguishing the Z and W chromosomes in *Acrantophis dumereli*. This represents the first report of differentiated sex chromosomes in a boid snake. The combined banding data thus indicates that snake chromosomes are certainly not lacking in variability. The use of C-banding to detect constitutive heterochromatin has confirmed that in some boids and colubrids macrochromosomes have been derived from microchromosomes by the additions of heterochromatin.

Introduction

Chromosome banding pattern analysis has greatly facilitated both the matching of homologous chromosomes and the tracing of the cytogenetic changes which may have occurred between related populations as they diverged phyletically. Such studies in reptiles have demonstrated that the conclusions arrived at by the comparison of gross karyotypes have often been unwarranted (Stock and Mengden, 1975).

The present study was initiated to compare the results of banding pattern analysis with those obtained from the study of conventional karyotypes (Baker et al., 1971) to test the assumed lack of chromosomal variation between snake genera and the assumption of a primitive 36-chromosome karyotype for snakes (Becak and Becak, 1969).

0009-5915/80/0079/0053/$02.40

Methods and Materials

Tail skin biopsies were taken from the Scrub Python, *Liasis (Python) amethystinus,* (2n=36); Boelen's Python, *Liasis (Python) boeleni,* (2n=36); Papuan Olive Python, *Liasis olivaceus papuanus,* (2n=36); Dumerel's Ground Boa, *Acrantophis dumereli* (2n=34); Madagascar Tree Boa, *Sanzinia madagascarensis,* (2n=34), (Boidae); Lindhiemer's Rat Snake, *Elaphe obsoleta lindhiemeri,* (2n=36); Transpecos Rat Snake, *Elaphe subocularis,* (2n=36); and Marcy's Garter Snake, *Thamnophis marcianus,* (2n=36), (Colubridae). Samples were minced and set up in McCoy's 5A medium with 20% fetal calf serum and incubated at room temperature. Cells from fibroblast lines were harvested with colcemid arrest and hypotonic pretreatment, and air dried slides were made. The G-banding patterns were obtained by a combination of the trypsin (Seabright, 1972) and the urea (Shiraishi and Yoshida, 1972) techniques as described by Stock et al. (1974). The C-band technique follows the alkaline SSC technique of Stefos and Arrighi (1971). Samples from the boids and *Elaphe subocularis* were obtained from specimens in zoological garden collections. The *Elaphe obsoleta* was collected in Harris County, Texas, while the *Thamnophis marcianus* was collected from three miles east of Elmendorph, Bexar County, Texas, U.S.A.

Results

Centromeric Heterochromatin. Heterochromatin is present in the centromeric regions of all autosomes of all the species of *Elaphe* and *Thamnophis* examined in this study. It is also present in the Z chromosomes of these species except in the case of *E. subocularis* where the Z apparently lacks centromeric constitutive heterochromatin (Fig. 1 a). *Elaphe subocularis* possesses a large block of heterochromatin next to the centromeric region of chromosome 2 which stains less intensely than the heterochromatin associated with the centromere. The species of the genus *Liasis* show centromeric heterochromatin on all macrochromosomes, but some of the microchromosomes either lack or else possess very

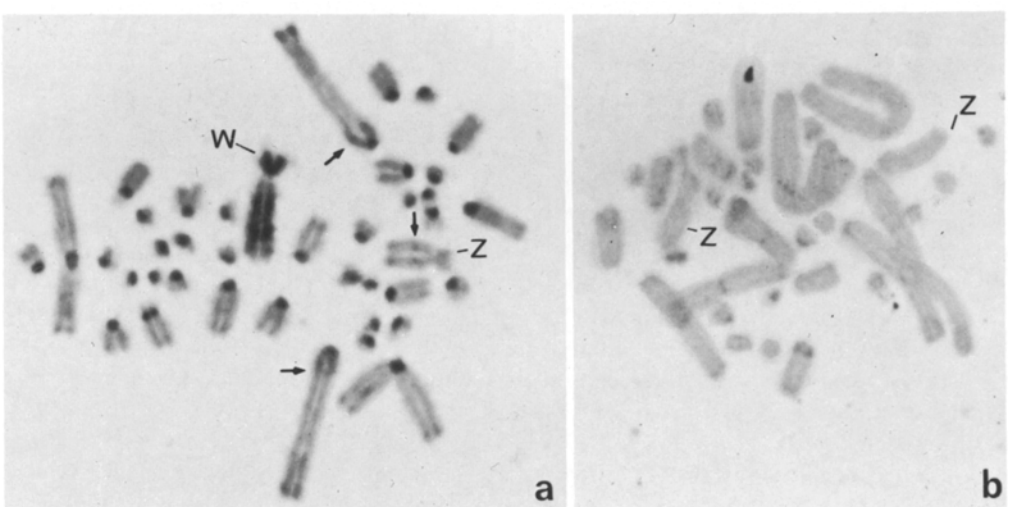

Fig. 1 a and b. C-banded metaphase chromosomes of *Elaphe* species: **a** *Elaphe subocularis,* note that the Z has a small interstitial C-band and that the no. 2 chromosome has a centromeric het block with distinctive staining properties; **b** *Elaphe obsoleta*

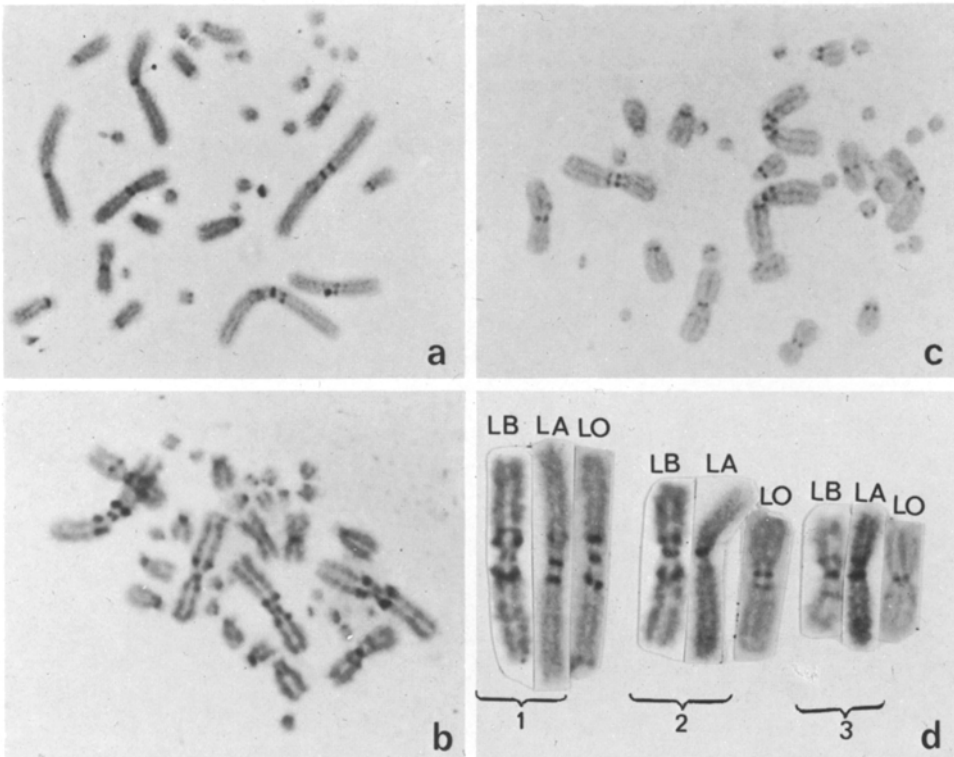

Fig. 2a–d. C-banded metaphase chromosomes of boid species: **a** *Liasis amethystinus;* **b** *Liasis boeleni;* **c** *Liasis olivaceus,* note that in addition to the centromeric C-bands each species has a specific pattern of interstitial C-banding; **d** A comparison of chromosomes 1, 2, and 3 of the three *Liasis* species

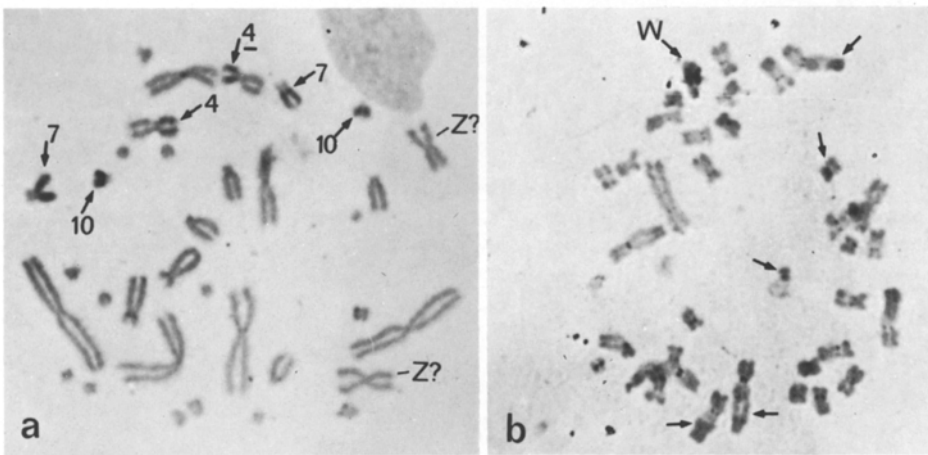

Fig. 3a and b. C-banded metaphase chromosomes of **a** *Sanzinia madagascarensis,* note whole arm blocks of heterochromatin in chromosomes 4, 7 and 10, and **b** *Thamnophis marcianus,* note large blocks of het (arrows) some of which are telomeric and some whole arm

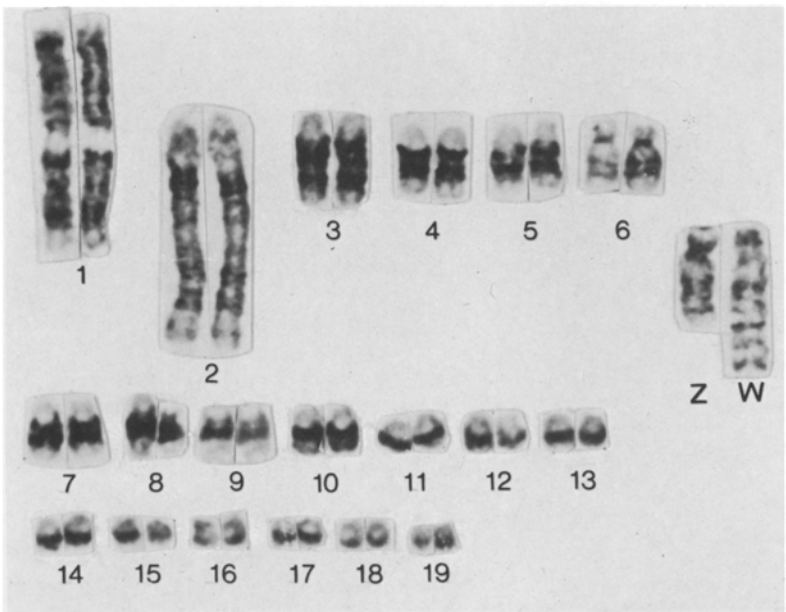

Fig. 4. G-banded karyotype of *Elaphe subocularis*, note that the W-chromosome also shows a G-band like pattern

small amounts of constitutive heterochromatin (Fig. 2a–c). *Sanzinia* lacks heterochromatin in the centromeric regions of most of the chromosomes (Fig. 3a).

Telomeric Heterochromatin. Heterochromatin is found in the telomeric regions of several chromosomes in both species of *Elaphe*. *Thamnophis* possesses considerable amounts of telomeric heterochromatin; consequently many of the smaller pairs of chromosomes in this genome are almost completely heterochromatic (Fig. 3b).

Interstitial Heterochromatin. Of the boids studied, all the species of the genus *Liasis* showed small interstitial regions of heterochromatin on the three largest chromosomes. In comparing these chromosomes between species within the genus, we find that, though they all possess heterochromatic regions associated with the centromere, each species nevertheless is distinguishable by a unique combination of interstitial C-bands (Fig. 3d). *Elaphe subocularis* also possesses small interstitial bands on the Z chromosome (Fig. 1a). This may be accompanied by an inversion in the Z as compared to *E. obsoleta*.

Whole-Arm Heterochromatin. Sanzinia is unique among the snakes studied in that it possesses three pairs of macrochromosomes (4, 7 and 10, Fig. 3a) with entirely heterochromatic arms (Fig. 3b). These areas are all G-band negative (Fig. 10). *Thamnophis* also possesses smaller chromosomes with completely heterochromatic short arms (Fig. 3b).

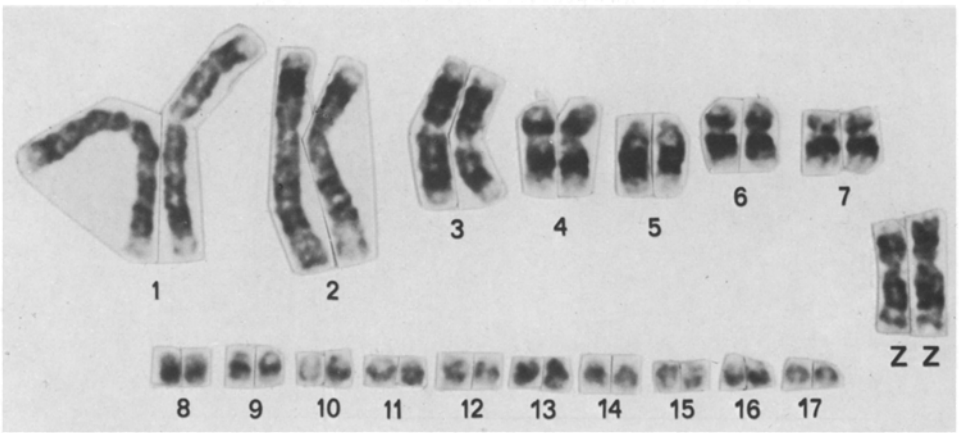

Fig. 5. G-banded karyotype of *Elaphe obsoleta*

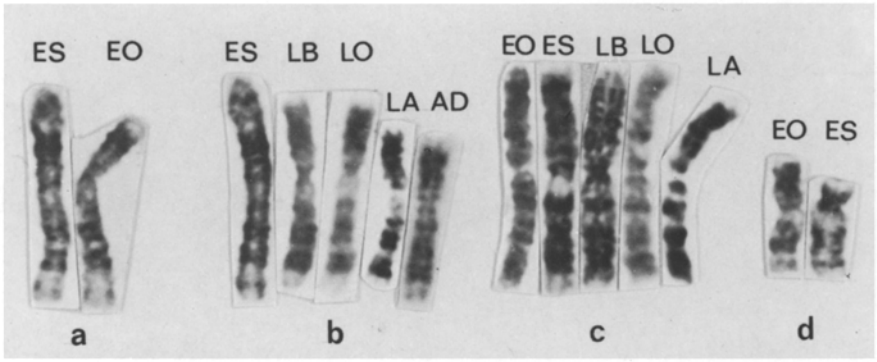

Fig. 6a–d. Comparison of G-banded chromosomes. **a** Chromosome 2 of the *Elaphe* species, note similarity of banding pattern but difference in centromeric position; **b** chromosome 2 of 5 species; **c** chromosome 1 of 5 species; **d** Z chromosomes of the *Elaphe* species, note the lack of homology in the short arms which cannot be accounted for by the presence of heterochromatin (see Fig. 1a). Abbreviations equal the following: *ES Elaphe subocularis; EO Elaphe obsoleta; LB Liasis boeleni; LA Liasis amethystinus; LO Liasis olivaceus; AD Acrantophis dumereli*

Sex Chromatin. The W-chromosome of *Elaphe subocularis* appears to be largely heterochromatic and C-band positive (Fig. 1a). It is the third largest chromosome in the genome and almost twice the size of the Z. Unlike the heterochromatic arms on autosomes in *Sanzinia,* which are G-band negative, the large W chromosome in *E. subocularis* possesses alternating Giemsa positive and negative regions which give it a G-band pattern resembling G-banded euchromatin (Fig. 4).

Colubrid G-bands. Differences in G-band patterns between *Elaphe subocularis* (2n = 40, Fig. 4) and *E. obsoleta* (2n = 36, Fig. 5) can be attributed to the differ-

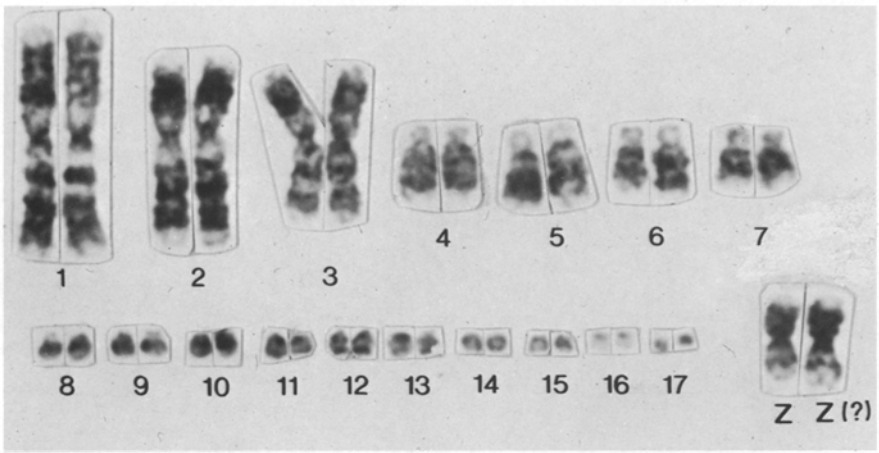

Fig. 7. G-banded karyotype of *Liasis boeleni*

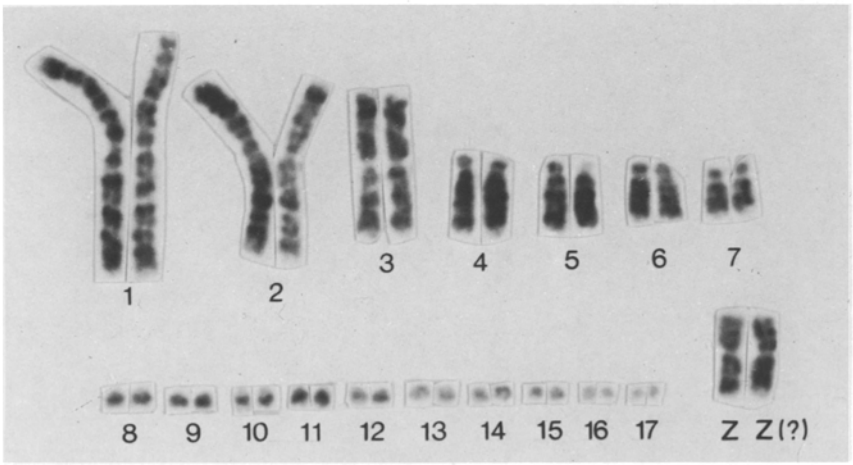

Fig. 8. G-banded karyotype of *Liasis amethystinus*

ences in the amount and position of heterochromatin and to inversions and Robertsonian-type changes. Chromosome 2 in *E. subocularis* is telocentric, whereas its homolog in *E. obsoleta* is submetacentric. This change in centromeric position has occurred without detectable rearrangement of the G-band pattern (Fig. 6a). Chromosomes 3 and 4 of *E. subocularis* appear to be homologous to the single chromosome 3 of *E. obsoleta*. Similarly, chromosomes 5 and 9 of *E. subocularis* appear homologous with chromosome 4 of *E. obsoleta*. The long arm of chromosome 6 of *E. subocularis* is homologous to chromosome 5 of *E. obsoleta*, while chromosomes 8 and 10 of *E. subocularis* provide combined homology with chromosome 6 in *E. obsoleta*. Chromosome 7 of *E. subocularis*

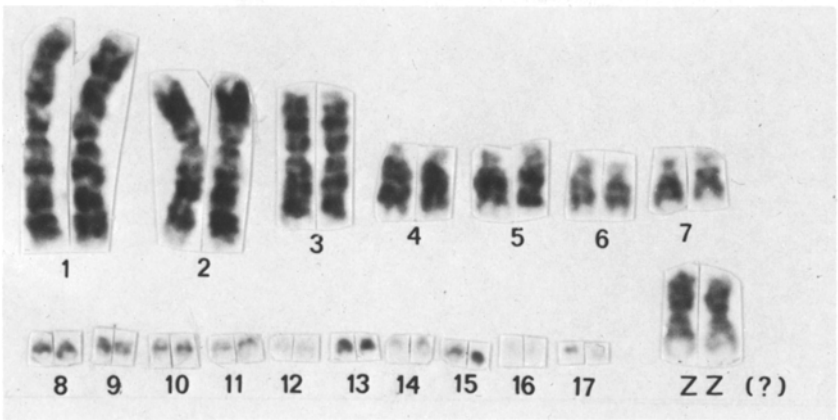

Fig. 9. G-banded karyotype of *Liasis olivaceus*

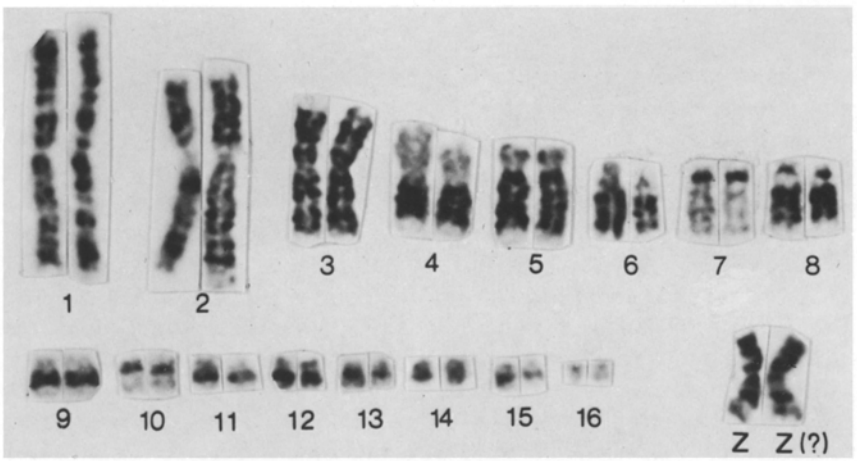

Fig. 10. G-banded karyotype of *Sanzinia madagascarensis*

is homologous with chromosome 7 in *E. obsoleta* except for the presence of a pericentric inversion. The smallest 9 pairs of chromosomes and the short arm of chromosome 6 of *E. subocularis* appear to match the smallest 10 pairs in *E. obsoleta*. The W chromosome of *E. subocularis* is much larger than both the Z and W chromosomes found in other species of *Elaphe* (Baker et al., 1972). The Z chromosome of *E. subocularis* differs in size and morphology from that of *E. obsoleta*. This difference may be the result of an inversion.

Boid G-Bands. The three *Liasis* species studied all have similar G-band patterns (2n = 36, Figs. 7–9). Chromosome 1 and the long arm of chromosome 2 in the *Liasis* species appear homologous to the same chromosomes in the *Elaphe*

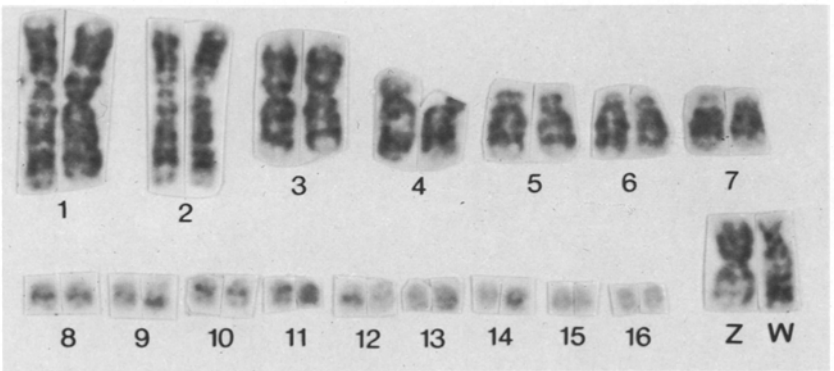

Fig. 11. G.banded karyotype of *Acrantophis dumereli*, note the pericentric inversion difference in the sex chromosome pair

genome. The short arm of chromosome 2 cannot be readily matched. No other readily apparent homologies were noted between *Liasis* and the colubrids studied.

The G-band pattern of *Sanzinia* (2n=34, Fig. 10) differs from the other species examined due principally to the presence of large amounts of heterochromatin in the form of whole-arm additions (Fig. 3a). This addition of G-band negative, heterochromatic, arms and a reduction in the number of microchromosomes from 20 to 16 obscures the relationship of these chromosomes to those of other boids examined. It is obvious, however, that the major portions of the first three chromosome pairs are directly homologous to the corresponding elements in *Liasis* and *Acrantophis*. The long arm of chromosome 4 has the same G-band pattern as chromosome 7 in *Liasis*. Likewise, chromosome 5 of *Sanzinia* is homologous to chromosome 5 of *Liasis*, chromosome 6 to chromosome 5 of *Liasis;* chromosome 7 may be homologous to one of the microchromosomes of *Liasis*, and chromosome 8 to chromosome 6 of *Liasis*. The presumed Z chromosome of *Sanzinia* appears to be comparable to that of *Liasis* and *Acrantophis*. In place of two of the pairs of microchromosomes which occur in *Liasis*, one finds a single pair of macrochromosomes which is predominantly heterochromatic in *Sanzinia*. The G-band pattern of *Acrantophis dumereli* (2n= 34, Fig. 11) is like that of *Liasis* and the karyotype differs from it only in the number of microchromosomes. A distinct W chromosome is present which is approximately the same size as the Z but is acrocentric. Since most of our samples were from unsexed live specimens it is possible that sex chromosomes may occur in other boids as well.

Sex Chromosome Morphology. The W chromosome of *E. subocularis* is much larger than the Z rather than the reverse as reported by Baker et al. (1971). The W chromosome of *Acrantophis* is similar in size to the Z but has a more subterminal position of the centromere. The Z chromosomes of the two *Elaphe* species are very different in morphology and banding patterns (Fig. 6d). The differences may result from an inversion. The chromosomes of *Liasis* and *Sanzi-*

nia which may be homologous to the Z in *Acrantophis* are all apparently the same in banding pattern.

Discussion

Two-thirds of the approximately 150 species of snakes karyotyped to date possess a diploid number of 36 and a complement which includes 16 macrochromosomes and 20 microchromosomes. Such a karyotype is found in many colubrids, boids and crotalids, as well as in other families and has been regarded as an ancestral condition in snakes (Beçak and Beçak, 1969). Such an idea reflects a simplistic approach that predicts an ancestral role for karyotypes found to be the most prevalent in a group. Our data from G and C-banding studies lends little support to this assumption. They also refute the assumed lack of chromosomal variation between genera which other authors have claimed from an analysis of conventional chromosome preparations. As an alternative we suggest that a karyotype which possesses a higher diploid number and a larger number of acrocentrics without a distinct separation of micro and macrochromosomes may well be a more realistic candidate for the ancestral condition.

Our study has demonstrated the presence of several mechanisms of chromosomal change in snakes not detected by studies prior to the advent of banding technology. The first of these involves an interconversion between micro- and macrochromosome categories. This may well have occurred in two of our cases. Thus, from the combined C and G-banding data for *Sanzinia* (2n=34; 18M+16M) and *Acrantophis* (2n=34; 16M+18M) it appears that a new macrochromosome (no. 7 in Fig. 3a C-banded and Fig. 10 G-banded) has been formed by the addition of a large block of G-band negative heterochromatin to a microchromosome. It is interesting to note that one microchromosome in *Sanzinia* (no. 10 in Figs. 3 and 10) also shows a heterochromatic long arm that is G-band negative, C-band positive.

The addition of heterochromatin to microchromosomes may be partially responsible for the radical differences in the karyotypic morphology between New World natricine snakes (2n=36 with few or no microchromosomes) and their Old World counterparts and other colubrids (most with 2n=36; 16M+20M). Baker et al. (1972) hypothesized that the natricine karyotype was derived from what they considered a primitive snake karyotype (2n=36; 16M+20M) by a series of unequal translocations. From chromosome banding details (Fig. 3b), however, we conclude that the karyotype of the New World natricine snake *Thamnophis* may have more plausibly originated as a result of fusion coupled with the addition of heterochromatin.

The G-band and C-band analysis of *Sanzinia* demonstrates unambiguously that micro- and macrochromosomes are interconvertible. A comparable argument explains the unusual nature of the karyotype in the natricine *Thamnophis*. Both cases suggest that the material of the microchromosomes may be conserved to a far greater extent than was assumed by earlier workers.

While our data suggest a change from microchromosomes to macrochromosomes it does not exclude the possibility that the reverse change may not also

occur. Thus, a genetic system containing both micro- and macrochromosomes offers an effective mechanism to reduce the amount of crossing over between genes of a specific linkage group maintained as a microchromosome compared to that in an equivalent chromosome complement lacking a distinct separation into micro and macro elements. It is possible, therefore, that the occurrence of both categories of chromosomes in turtles, snakes and birds may represent independently derived conditions. Significantly, many turtles (Stock, 1972), and some snakes, saurian reptiles and birds lack the division into micro and macro categories; also, microchromosomes are rare in fish, amphibians, crocodiles and mammals.

Apart from converting microchromosomes into macrochromosomes there are other instances of heterochromatic addition in the evolution of snakes. For example, in *Sanzinia* chromosome 4 possesses an entirely heterochromatic short arm which is not seen in other boids.

A second mechanism of chromosomal change suggested by our study is to be found in *Elaphe subocularis*. The second chromosome of this species is distinguishable from that of *Elaphe obsoleta* in two respects. First its centromere is terminal not median. Second, it has a large block of heterochromatin proximal to the centromeric heterochromatin and this additional block gives a distinctive staining reaction since it is lighter than the centromeric heterochromatin and gives a swollen appearance relative to the rest of the chromosome (Fig. 1a). When G-band patterns are compared it appears that there has been a relocation of the centromere without accompanying alteration of the G-band pattern (Fig. 6a) but with the presence of an associated heterochromatic block. Whether this implies a three break arrangement equivalent to that demonstrated by Rothfels and Freeman for *Twinnia* (1966) is not clear.

Baker et al. (1971) regarded the differences between the karyotypes of *Elaphe subocularis* and *E. obsoleta* as due to fission ($2n=36$ to $2n=40$) coupled with pericentric inversion. With banding details we find that a fusion and inversion model is equally plausible. We are presently studying other close relatives of *E. subocularis* from Mexico and the morphological and chromosomal characteristics of this group may provide critical information on the status of the genus *Elaphe* and the species groups that make it up.

Another type of chromosome change detected by banding pattern analysis involves the sex chromosomes. From conventional preparations sex chromosomes have been found to be the most variable element in the serpent genome.

Most reports concerning sex chromosome differentiation in snakes have relied on differences in length and centromere position. Our finding of a heteromorphic W chromosome in *Acrantophis* is the first report of sex chromosomes in a boid snake. G-banding comparisons indicate that a pericentric inversion may have played a significant part in the differentiation of the sex chromosomes of *Acrantophis* which are similar in length. Other examples of sex chromosome differentiation could have been missed due to lack of morphological differences between the Z and W chromosomes.

Ray-Chaudhuri and Singh (1972) reported autoradiographic data for the boid *Eryx johni* which suggests that the presumed sex chromosomes are in a primitive state of differentiation and lack asynchrony in their DNA replicating pattern. Such a lack of asynchrony, however, cannot be an absolute indicator

of a lack of differentiated sex chromosomes, since heterochromatin need not be early or late replicating. Additionally this may not be the first step in forming a sex chromosome. Further C-band analysis of other boid species is necessary before a definitive answer can be obtained.

Of particular interest is the fact that the large heterochromatic W in *Elaphe subocularis,* which is largely C-band positive, also exhibits a very distinct and clear G-banding pattern. The striking difference between the Z chromosomes of the two *Elaphe* species (Fig. 6d) may either indicate a more distant relationship for these than the present taxonomic arrangement suggests or else imply that the Z chromosomes of snakes may not be as conservative as are the X chromosomes of mammals (Pathak and Stock, 1974).

Within the family Boidae the species of pythons examined by us all appear to share a closely related G-band pattern. Two species we have included in *Liasis,* namely *L. boeleni* and *L. amethystinus,* have been allocated to the genus *Python* by McDowell (1975). We feel such a separation of *L. boeleni* and *L. amethystinus* from *L. papuanus* deserves reconsideration in view of the close agreement of the G and C-banding patterns of the species examined. Some of the elements of the boid genera *Sanzinia* and *Acrantophis* from Madagascar show similarities to elements of the *Liasis* genome despite the fact that they are distinctly separated in C-banding pattern. Portions of these elements may have been conserved throughout the Family. Examination of chromosomal banding patterns in *Python* and other boid genera is needed to better understand the evolutionary relationships of this large group of snakes.

A marked lack of G-band homology has been demonstrated between the classes of amphibians, reptiles and birds (Stock and Mengden, 1975). In the present study we find that even in snake species which share a diploid number of 36, G-band patterns are not broadly homologous between families. The common occurrence of $2n=36$ in snakes with very different karyotypic details parallels the condition seen in some mammalian groups where a constant diploid number persists despite marked differences in karyotypic details. The genus *Peromyscus* with its many species, all of which possess 48 chromosomes, is a good example, since the arm number of this genus ranges from 56 to 96. Other related genera of rodents (Mascarello et al., 1974) do not conserve a specific diploid number. The mechanism which so vigorously selects for a specific number of linkage groups in some genera and not others remains to be elucidated.

Acknowledgements. We wish to acknowledge Louis DiSabato, Josef Laszlo, Tommy Logan and John Werler for their assistance in collecting samples from specimens in their charge. We thank Drs. Max King, George Miklos and Professor B. John for critically reading the manuscript. We are indebted to Dr. T.C. Hsu, M.D. Anderson Hospital and Tumor Research Institute and the Houston Zoological Gardens for providing support, equipment and space during various stages of the investigation.

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Received and accepted December 13, 1979 by B. John
Ready for press March 2, 1980