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# Evolution of Karyotypes in Snakes

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*Abstract.* Karyotype analysis and morphometric measurement of the chromosomes of 17 species of snakes have been done. Chromosomes of different species so far worked out in each family have been compared using quantitative methods to derive chromosomal affinities between species of different taxonomic categories. The following conclusions have been drawn: (i) It is suggested that the retention of *Xenopeltidae* as a separate family is unnecessary and the only species *Xenopeltis unicolor* referred to in that group should be included in the family *Boidae*. (ii) The subfamilies, *Boinae* and *Pythoninae* cannot be distinguished chromosomally. (iii) On the basis of chromosomal similarities, the cytologically known species of *Colubridae* have been put into 13 different groupings which do not always correspond to the views of the present day colubrid taxonomists. (iv) In *Hydrophiidae*, speciation seems to have occurred through changes in the 4th pair of autosomes and sex chromosomes in general and the *W* chromosome in particular. Evidences are presented to show that fission and inversion have played an important role in bringing about the structural rearrangements in this group. (v) Family *Viperidae* according to taxonomists is divided into two subfamilies. Both the subfamilies are chromosomally very similar.

## Introduction

A species can only be defined by a whole array of morphological, physiological and behavioural features. Overemphasis on a few morphological characters has led to a certain amount of ambiguity and confusion in the delineation of species in different groups of animals. It is expected that the karyotypes, like other species specific characters, will provide us with an additional means for assessing the evolutionary patterns and pathways that have led to natural group assemblages. White (1970) has emphasised the role of chromosomal rearrangements in generating genetic isolating mechanism which is a prerequisite for speciation. In the dipterous genera, *Drosophila, Chironomus, Anopheles, Sciara* and several genera of *Simuliidae* even the most closely related species usually differ in respect of the banding sequence in the giant polytene chromosomes in ways which are clearly due to structural rearrangements of the chromosomes that have arisen in the course of phylogeny. But exceptions are also found, for example. *Drosophila mulleri, D. aldrichi* and *D. wheeleri* show exactly the same sequences of bands in their polytene chromosomes (Wasserman, 1962) and they have been designated as homosequential by Carson, Clayton, and Stalker

(1967). Here speciation cannot be explained on the basis of obvious chromosomal rearrangements. In groups other than *Diptera*, chromosome analysis of related species can reveal detectable differences only when the chromosomal changes are of a gross nature. In spite of this limitation, extensive karyological studies in different groups of mammals, with the help of modern techniques have helped the cytologist in understanding the role of chromosomal rearrangements in speciation and also in assessing the phylogenetic relationship and evolutionary trends within and between different taxa with a good deal of success (Matthey, 1966; Nadler, 1966; Chu and Bender, 1962; Wurster and Benirschke, 1968; Nadler, 1969; Gropp, 1969). Similar attempts have also yielded significant results in birds (Ray-Chaudhuri, Sharma, and Ray-Chaudhuri, 1969; Ray-Chaudhuri, in press). A preliminary attempt has also been made in snakes amongst reptiles (Beçak and Beçak, 1969).

A study of DNA content per nucleus estimated by microspectrophotometric measurements as well as by measurements of the total chromosomal area of a complement shows that the reptiles can be divided into two distinct groups: 1. *Serpentes*, having similar DNA content per nucleus as in birds and 2. *Crocodilia* and *Chelonia*, containing nearly 80% of DNA per nucleus found in mammals (Atkin, Mattinson, Beçak, and Ohno, 1965). These results have been taken to measure a closer kinship between snakes and birds on the one hand and *Crocodilia*, *Chelonia* and mammals on the other.

Among reptiles, snakes exhibit a narrow range of variation in their karyotypes. There is a preponderance of species having 36 chromosomes. There is also a distinct bimodality between macro and microchromosomes, generally 8 pairs belong to the former group and 10 pairs to the latter. All the 8 pairs of macrochromosomes can often be described as marker chromosomes in the sense that these can be individually identified. Consequently snakes are quite suitable for cytotaxonomical studies, The discovery of *W* chromatin in the interphase nuclei of snakes. asynchrony in the replicating pattern of *W* chromosome and various stages of differentiation between *Z* and *W* chromosomes according to the evolutionary status of the families (Ray-Chaudhuri, Singh, and Sharma, 1970, 1971) have provided the cytotaxonomist with additional data which can be utilized for the study of the mechanism of karyotype evolution in snakes.

In the present report the karyotype analyses of 17 cytologically unknown species of snakes are presented alongwith a discussion of the manner of chromosome evolution in snakes and the possible evolutionary relationships within and between taxonomic groups. We have taken into consideration in our cytotaxonomic discussion all the published work on snake chromosomes using modern technique (see Table 1, p. 206).

## Materials and Methods

The different species of Indian snakes utilized for karyological analysis in the present communication have been identified by the Zoological Survey of India. They are *Eryx conicus* (Schneider) belonging to the family *Boidae; Ptyas mucosus* (Linn.), *Coluber fasciolatus* (Shaw), *Natrix stolata* (Linn.), *Lycodon aulicus* (Linn.), *Cerberus rhynchops* (Schneider), *Gerardia prevostiana* (Eydoux and *Gervais*), *Boiga forsteni* (Dum and Bib.) and *Boiga trigonata* Schneider to the family *Colubridae; Naja naja naja* (Linn.) and *Naja naja kaouthia* Lesson to *Elapidae; Echis carinatus* (Schneider) to *Viperidae* and *Hydrophis spiralis* (Shaw), *Hydrophis ornatus ornatus* (Gray), *Hydrophis cyanocinctus* (Daudin), *Hydrophis fasciatus fasciatus* (Schneider) and *Microcephalophis gracilis* (Shaw) to *Hydrophiidae.*

The chromosome analysis has been done from colchicinized marrow of ribs, spleen, and short term leucocyte culture. For leucocyte culture blood is drawn directly from the heart of living snakes without anaesthesizing them with the help of a heparinized syringe under aseptic condition and transferred either to sterilised universal container or to centrifuge tube, depending on the quantity of the blood taken. After withdrawal of the blood, the snakes are injected intraperitonially with 0.25 ml colcemid/kg body weight and sacrificed after 4 hours of the injection for chromosome preparation. Generally, bone marrow and spleen in snakes have very few dividing plates. Our experience suggests that the frequency of dividing plates becomes significantly higher when chromosome preparations are made from the same tissues after withdrawal of blood from the heart and sacrificing the animal 4 hours after colcemid injection.

The procedure adopted for short term leucocyte culture is the same as described by Ray-Chaudhuri, Singh, and Sharma (1970), and Singh, Sharma and Ray-Chaudhuri (1970b). Slides are prepared by the air-drying technique and stained in carbol fuchsin. The *W* chromatin has been studied in the interphase nuclei of brain, kidney, leucocyte culture, liver, spleen, intestine and ovary after directly fixing them in aceto-alcohol (1:3) without any pretreatment. These slides are also prepared by following air-drying procedure. Feulgen, pyronin *Y*-methyl green and carbol fuchsin stains are used.

The autoradiographic study is carried out by using $^3$H-TdR 1 μc/ml of the leucocyte culture of specific activity 20 c/mM (The Radiochemical Centre, Amersham, England) and $^3$H-uridine 1 μc/ml of specific activity 5.4 c/mM (Bhabha Atomic Research Centre, Trombay, India). The cultures are given 4, 5, 6, 8 and 10 hours continuous treatment of $^3$H-TdR prior to harvesting for the study of the labelling behaviour with particular reference to the sex chromosomes at the end of the *S* period. Colcemid (0.015 μgm/ml) is given 1–4 hours before harvesting.

In order to see the DNA replicating pattern of *W* chromatin and its activity at transcriptional level, $^3$H-TdR (1 μc/ml) and $^3$H-uridine (1 μc/ml) respectively are used for 15 minutes and cultures are directly fixed in aceto-alcohol without hypotonic pretreatment. In order to trace the condensation of the *W* chromosome in interphase nuclei through prometaphase, cultures have been treated with $^3$H-TdR (1 μc/ml) for 8 hours and directly fixed in aceto-alcohol without any pretreatment. Kodak AR 10 stripping film is used. The slides are exposed for 15–20 days at 5° C and developed for 4 minutes in Kodak D 19b developer and fixed in Kodak acid fixing salt solution with hardner for 6 minutes. The procedure of Bianchi, Lima-de-Faria and Jaworska (1964) has been used for removal of grains with slight modification.

Photomicrographs are taken with the help of a Carl Zeiss Photomicroscope using a planachromatic oil immersion objective at an initial magnification of ×400.

Chromosome grouping is done mainly on the basis of the system proposed by Levan *et al.* (1964). Because of the minute size and similarity in the centromeric position, all the microchromosomes have been arranged separately in decreasing order of size. The morphometric analysis of the karyotypes of each species studied by us has been done from enlarged photomicrographs of 5 plates. In order to facilitate the comparison of one species with the other, relative length ($L^R$) and centromeric index ($I^C$) are taken as the parameters. The relative length is expressed as percent of the total chromosome length of the male haploid set, excluding the microchromosomes. This value in female cells is obtained by subtracting the $W$ chromosome and adding the measurement of another $Z$ before dividing the total chromosome length by 2.

In those cases where measurements are done from male plates only, the $L^R$ of the $W$ is calculated by taking the mean of the total length of the $W$ chromosome from five female plates and dividing it by the mean total length of the haploid set of the five male plates and multiplying with 100. The relative lengths and centromeric indices thus calculated are corrected to their nearest integers and included in the tables in the following manner. When the $L^R$ and $I^C$ of a particular chromosome are 21.3 and 36.6 respectively the values are expressed as 21/37 in the tables meaning thereby that the $L^R$ is 21.0 and $I^C$ is 37.0.

In order to be more exact in morphometric analysis, it is essential to include the microchromosomes in the measurements of the total haploid length. Although it is not difficult to include them but the morphometric measurements which are available to us from other investigators are invariably made by excluding the microchromosomes; we are therefore compelled to omit them in our measurements also. In the majority of the cases the number of microchromosomes in different species is almost the same and therefore by omitting them in calculating the $L^R$%, we have excluded more or less equal amount of chromosome material in most of the species. Thus the error introduced is not very serious for our present purpose. In those cases where no morphometric measurements are given by the authors, the $L^R$% and $I^C$% are calculated by measuring the chromosomes with the help of dial caliper from the single plate available to us in their published papers.

## Results

### Family: *Boidae*

*1. Eryx conicus.* The analyses of 100 good metaphase plates from different somatic tissues of 2 male and 2 female specimens have revealed 34 as the diploid number consisting of 16 macro and 18 microchromosomes (Fig. 1). The sex chromosomes are morphologically indistinguishable in both male and female plates. The first 4 pairs of macrochromosomes have their centromeres in the median region ($m$) which are individually distinguishable in every plate, and the remaining 4 pairs in the terminal region ($t$). There is a sharp distinction in size between the macro and microchromosomes and the latter also appear to have their centromeres in the terminal region.

The interphase nuclei of brain, kidney, leucocytes in culture, liver, spleen and intestinal epithelium, directly fixed in aceto-alcohol have not shown any sexual dimorphism. Autoradiographic studies after 6, 8 and 10 hours *in vitro* treatment of $^3$H-TdR have also not shown any asyn-

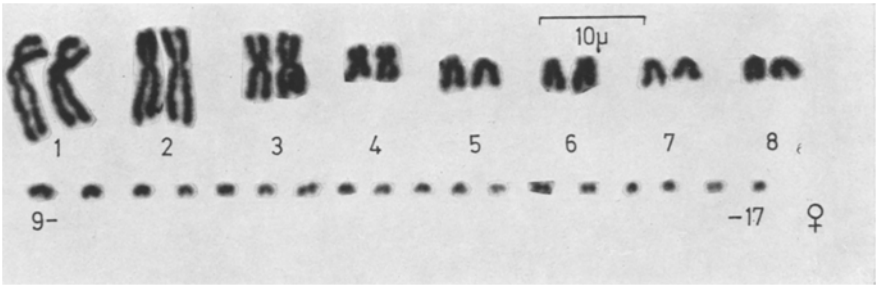


Fig. 1. Female karyotype of *Eryx conicus* ($2n = 34$) from marrow of ribs. Sex-chromosome heteromorphism is absent

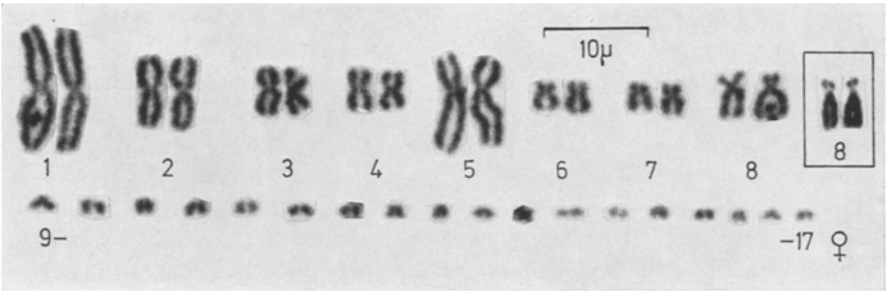


Fig. 2. Female karyotype of *Ptyas mucosus* ($2n = 34$) from spleen. No sex-chromosome heteromorphism. The 8th pair of chromosomes from another plate shown in the inset have a secondary constriction near the primary one in the short arm of both the chromosomes

chrony in the DNA replication pattern of the macrochromosomes in either of the sexes.

**Family: *Colubridae***

*1. Ptyas mucosus.* Four males and 2 females have been used which yielded 255 metaphase spreads from various tissues and all of them invariably show 16 macro and 18 microchromosomes (Fig. 2). Four pairs of macrochromosomes have their centromeres in the median, 3 pairs in the submedian and one pair in the subterminal region. The 8th pair of macrochromosomes has secondary constrictions in their short arms near the centromere in majority of the plates from both sexes (Fig. 2, inset). Chromosome pairs No. 1, 2, 5 and 8 are identifiable in all metaphase plates. The eighteen microchromosomes in this species also form a distinct size class. No heteromorphism of the chromosomes in either of the sexes is detectable in this species.

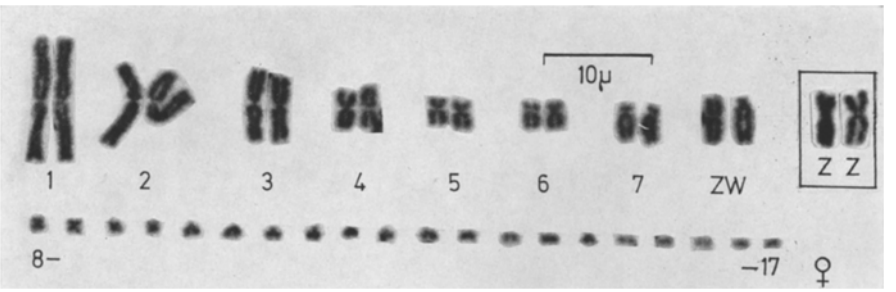


Fig. 3. Female karyotype of *Coluber fasciolatus* ($2n = 36$) from marrow of ribs. The heteromorphic *ZW* sex chromosomes are of similar size. *ZZ* of the males are shown in the inset

The interphase nuclei from spleen, brain, kidney, leucocytes in culture, liver and intestinal epithelium have not revealed any characteristic chromocentre in either of the sexes and the autoradiographic analysis failed to show any asynchrony in the replicating pattern of any macrochromosome pair.

*2. Coluber fasciolatus.* The chromosome analysis has been carried out in 2 male and 1 female specimens. Two hundred metaphase plates have been obtained from spleen and bone marrow which show 16 macro and 20 microchromosomes in all the plates with very few exceptions (Fig. 3). The difference in the size between the smallest macro and the largest microchromosomes is very pronounced. In the female plates, the chromosomes of one of the pairs are heteromorphic in all the cells. One of the members of the heteromorphic pair with its median centromere is similar in size and morphology to one of the homomorphic pairs in the male plates and is identified as the *Z* chromosome. The other member, almost similar in size but with a subterminal centromere is the *W* chromosome. Chromosome pairs no. 1, 2, 3, 4, 7, *Z* and *W* can be considered as 'marker' chromosomes. Four pairs of macroautosomes have their centromeres in the median region, two pairs in the submedian region and one pair in the subterminal region. The microchromosomes appear to have their centromeres at the terminal region except a few which have them in a median region.

Interphase nuclei of brain, kidney, cultured leucocytes and liver of the female have a distinct heteropycnotic body which is absent in similar tissues of the male. This body has been termed as *W* chromatin (Ray-Chaudhuri, Singh, and Sharma, 1970). Autoradiographic studies have not been carried out in this species.

*3. Natrix stolata.* The chromosomes from 134 metaphase spreads obtained from 4 males and 2 females revealed fourteen macro and

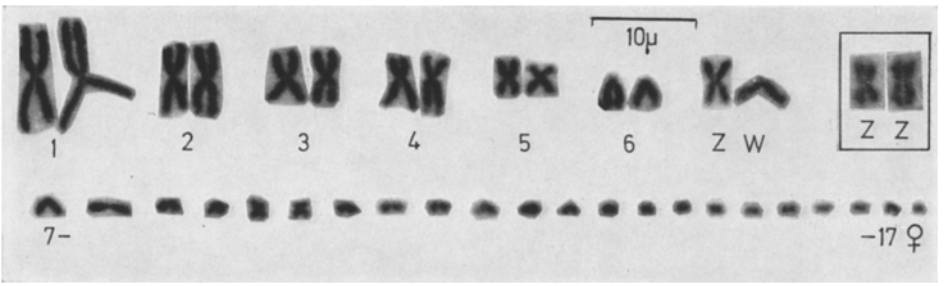


Fig. 4. Female karyotype of *Natrix stolata* ($2n = 36$) from marrow of ribs. The *ZW* sex chromosomes are unequal in size. *ZZ* in the males are shown in the inset. Note the secondary constriction at the distal end of the long arm of chromosome pair no. 4

22 microchromosomes in all the plates (Fig. 4). The macrochromosomes can be classified into two different groups. Pair nos. 1–5 having their centromeres in the median region belong to the first group. The *Z* chromosome also belongs to this group. There is an achromatic gap in the distal region of the long arm of the 4th pair but occasionally the gap is seen in one of the homologues only. Pair no. 6 alone having subterminal centromeres, constitutes the 2nd group. All the microchromosomes which form a distinct size class, appear to have terminal centromeres except two which have them in a median region. In some of the metaphase plates major spirals are distinctly visible in all the chromosomes including the microchromosomes.

The interphase nuclei of brain, kidney, liver and spleen have distinct *W* chromatin body in the females only. No autoradiographic study has been done in this species.

*4. Lycodon aulicus.* Four male and two female individuals have been used for the chromosome analysis which yielded 175 well spread metaphase plates. The karyotype analysis has revealed 36 as the diploid number for the species having 16 macro and 20 microchromosomes (Fig. 5). There is a heteromorphic pair of chromosomes in the female. The smaller member of this pair is restricted to the female sex only, hence it is designated as the *Z* chromosome. Seven pairs of the macrochromosomes including *Z* have median centromeres. The 7th pair of the chromosomes have their centromeres in the terminal region whereas in the *W* it is the subterminal region. All the microchromosomes appear to have terminal centromeres. All the macrochromosomes are individually distinguishable and can be described as 'marker' chromosomes.

The observation of the interphase nuclei of brain, kidney, cultured leucocytes, liver, spleen and intestinal epithelium has revealed the

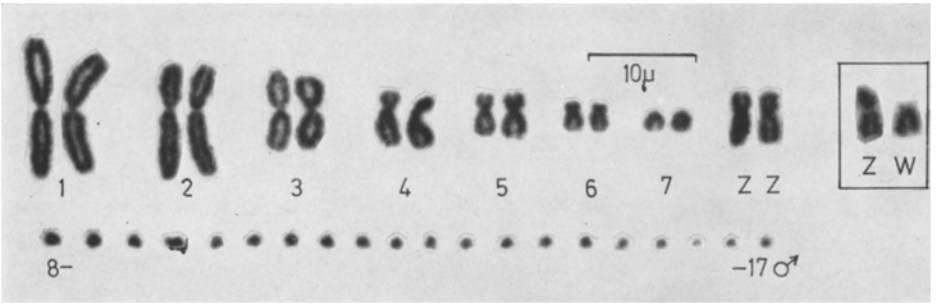


Fig. 5. Male karyotype of *Lycodon aulicus* ($2n = 36$) obtained from spermatogonial metaphase. Heteromorphic $ZW$ in the females in the inset obtained from bone-marrow cells

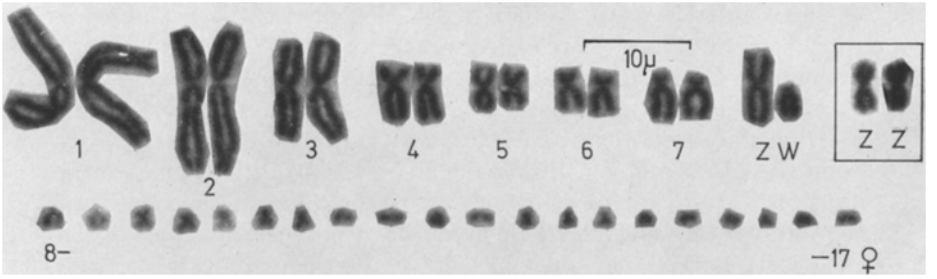


Fig. 6. Female karyotype of *Cerberus rhynchops* ($2n = 36$) from leucocyte culture showing the heteromorphic $ZW$ pair with the $ZZ$ in the males in the inset

presence of female specific conspicuous heteropycnotic $W$ chromatin body. No autoradiographic study has been carried out in this species.

*5. Cerberus rhynchops.* Three male and 4 female individuals of this species yielded 168 good metaphase spreads all having 16 macro and 20 microchromosomes (Fig. 6). There is a marked difference in the size and morphology of the $Z$ and $W$ chromosomes. The former has its centromere in the median region whereas in the $W$ it is in the subterminal region. The rest of the macrochromosomes can be classified into two distinct groups. The first group consists of 5 pairs of chromosomes (Fig. 6, *1–5*) having median centromeres, the $Z$ also belongs to this group. The second group includes two pairs of submetacentric chromosomes (Fig. 6, *6* and *7*). All the macrochromosomes are individually recognisable. Microchromosomes, forming a distinct size group, have their centromeres in the terminal and median region.

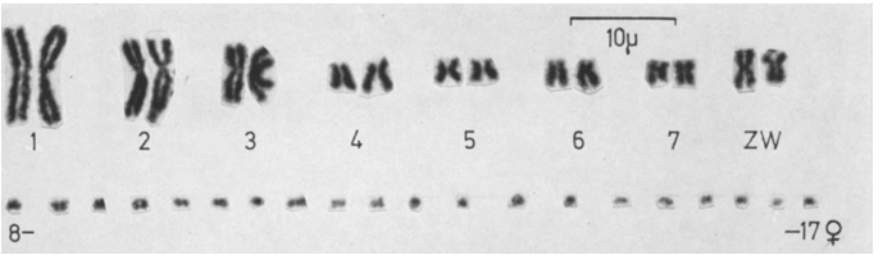


Fig. 7. Female karyotype of *Gerardia prevostiana* ($2n = 36$) from leucocyte culture showing heteromorphic sex chromosomes

The interphase nuclei of brain, kidney, leucocytes in culture and liver have shown a conspicuous *W* chromatin body in the females only. It is surprising that in two female specimens out of the 4 studied, the heteropycnotic body is very prominent but in the other two individuals in the same tissue, this body is almost totally absent. Autoradiographic studies have not been carried out in this species.

*6. Gerardia prevostiana.* The chromosome analysis has been done from 3 female individuals only and 100 metaphase plates from leucocyte culture have revealed 36 as the diploid number (Fig. 7). There is a sharp size difference between 16 macro and 20 microchromosomes. One of the pairs of the macrochromosomes is heteromorphic. Based on the analogy of other species of this family, the larger chromosome of the heteromorphic pair having a median centromere has been taken as the *Z* chromosome whereas the smaller one having the centromere in the submedian region as *W*. The rest of the macrochromosomes can be put into two groups. Pairs no. 1–4 (Fig. 7) belong to the first group which have median centromeres, and nos. 5–7, having submedian centromeres, constitute the second group. Pairs no. 1–4 and *Z* and *W* are individually recognisable. Microchromosomes, forming a distinct size class, appear to have terminal centromeres.

The interphase nuclei of brain, kidney, cultured leucocytes and liver have revealed the *W* chromatin body. Autoradiographic studies have not been carried out in this species.

*7. Boiga forsteni.* Two male and 4 female individuals of this species have been chromosomally analysed. We have obtained 300 good metaphase plates, in which there are 18 macro and 18 microchromosomes making 36 as the diploid number (Fig. 8). There is a heteromorphic

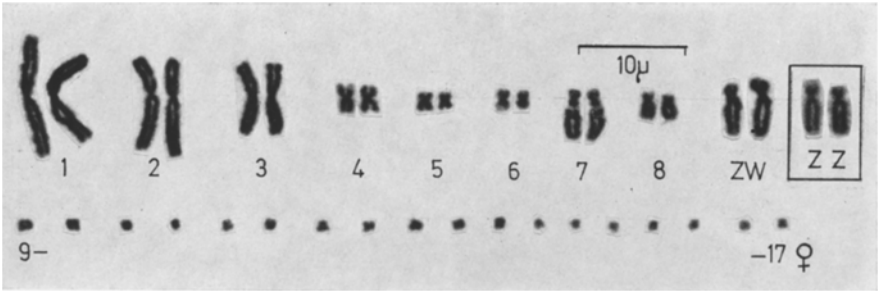


Fig. 8. Female karyotype of *Boiga forsteni* ($2n = 36$) from leucocyte culture. *ZZ* in the males shown in the inset. The *W* chromosome is larger than the *Z*. Note the conspicuous achromatic gap in the long arm of both the chromosomes of pair no. 7

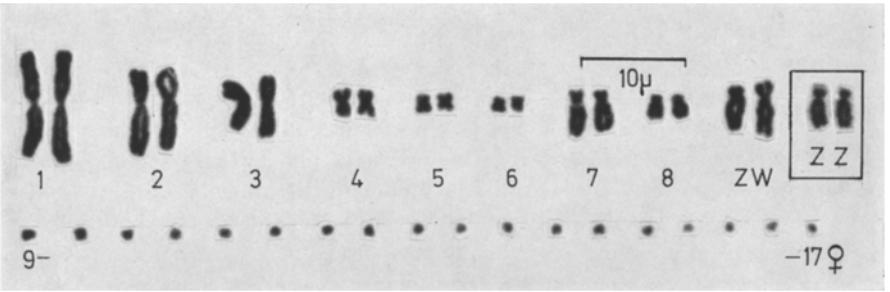


Fig. 9. Female karyotype of *Boiga trigonata* ($2n = 36$) from leucocyte culture. The *W* chromosome is larger than the *Z*. *ZZ* in the males are shown in the inset. There is an achromatic gap in the long arm of one of the chromosomes of pair no. 7

pair of chromosomes in the female plates, the smaller member of the pair with its centromere in the subterminal region is similar to one of the homomorphic pairs in the male plates and is the *Z* chromosome. The larger one, having its centromere in the submedian region, is restricted to the females only. This is the *W* chromosome, which unlike in other species of snakes is larger than the *Z*. Six pairs of the macrochromosomes (Fig. 8, *1–6*) have their centromeres in the median region and 2 pairs (*7* and *8*) in the subterminal region. The seventh pair of the autosomes show a secondary constriction very near the centromere generally in both the homologues but occasionally it is restricted to one member of the pair only or it may even be absent in both of them. In the last kind of metaphase plates *Z* can be confused with the 7th pair because of their similarity in size and centromeric position. Otherwise the *Z* is easily recognised. The *W* chromosome because of its size and centromeric position stands out quite distinct in all plates. Occasionally the long arm of *W* also shows a secondary constriction. All the macro-

chromosomes except pairs no. 5 and 6 are individually recognisable. Though the morphology of the microchromosomes which form a distinct size group is not very clear, some of the bigger microchromosomes appear to have median centromeres whereas in the others they are in the terminal region.

A conspicuous heteropycnotic *W* chromatin body in the female interphase nuclei of brain, kidney, leucocytes in culture, liver and spleen is visible.

An autoradiographic study has been carried out in this species after continuous treatment of the cultures with $^{3}$H-TdR for 5, 6, 8 and 10 hours prior to harvesting in both the sexes. No asynchrony has been observed in the replicating pattern of the macrochromosomes in the males whereas in the female the *W* finishes its replication much earlier than the other macrochromosomes (Ray-Chaudhuri and Singh, in press).

*8. Boiga trigonata.* The chromosome analysis has been done on 2 male and 2 female individuals and nearly 200 well spread metaphase plates have been obtained. The diploid number has been found to be 36 invariably in all the plates (Fig. 9). The chromosome constitution of this species is exactly similar to what has been described above for *Boiga forsteni*. The *W* is bigger than the *Z* in this species also and forms a *W* chromatin body as described in the congeneric species.

The autoradiographic study has also shown an early replicating *W* (Ray-Chaudhuri and Singh, in press).

**Family: *Elapidae***

*1. Naja naja naja.* Four males and 3 females yielded 180 metaphase plates showing 16 macro and 22 microchromosomes making 38 as the diploid number (Fig. 10). Pairs no. 5, 6 and 7 are extremely small when compared with the first four pairs and the sex chromosome (Fig. 10), but they are larger than the microchromosomes.

The 8 pairs of the macrochromosomes can be grouped into the following categories: two pairs of chromosomes having median centromeres (Fig. 10, *1* and *2*), one pair having submedian centromeres (Pair no. 3 and the sex chromosomes), one pair with subterminal centromeres (*4*) and three pairs of smaller macrochromosomes with submedian centromeres (*5–7*). All the microchromosomes have been grouped separately. Some of them appear to have median and others terminal centromeres.

Surprisingly enough, the karyotypes of males and females are exactly alike. There is no heteromorphic pair of chromosomes in either of the sexes, hence the identification of the sex chromosomes at the morphological level is not possible. The autoradiographic study from both the sexes after 6, 8 and 10 hours of continuous treatment with

$^3$H-TdR has, however, revealed that one member of a pair of macrochromosomes having submedian centromeres in females only, finishes its DNA replication much earlier than any other macrochromosome (Ray-Chaudhuri, Singh, and Sharma, 1970). This is identified as the *W* chromosome. The interphase nuclei of brain, kidney, leucocyte culture, liver, spleen, and intestinal epithelium of the females have also a conspicuous heteropycnotic body which is absent in the similar tissues of the males. Basing our argument on the replicating pattern of the *W* in various species of snakes we presume that the heteropycnotic body is the *W* chromatin. It is very difficult to distinguish the sex chromosomes from the pair no. 3, because of their similar size and centromeric position. The sex chromosomes pair can be singled out only because of the asynchronous replication pattern of the *W*. Chromosome pairs no. 1, 2 and 4 are marker chromosomes and can be unequivocally identified in all the plates.

*2. Naja naja kaouthia.* The chromosome analysis has been done in this subspecies of *Naja* from 3 male and 5 female individuals. Two hundred good metaphase spreads have been procured and the chromosome analysis revealed 38 as the diploid number. Like in the previous species, there are also 16 macro (10 bigger and 6 smaller) and 22 microchromosomes (Fig. 11). Curiously enough, in this subspecies there is a heteromorphic pair of chromosomes only in the female plates. The smaller member of this pair must be the *W* chromosome. It has its centromere in the subterminal region. The distinctly larger homologue of the pair with its submedian centromere is present in males in the form of a homomorphic pair and therefore is the *Z* chromosome. Three pairs of larger macrochromosomes have their centromeres in the median region (Fig. 11, *1–3*), one pair in the subterminal region (*4*) and 3 pairs of smaller macrochromosomes (*5–7*) in the submedian region. Microchromosomes have been grouped separately in decreasing order of size. Some of them appear to have median and some have terminal centromeres. In one of the female specimens out of the 5 studied, the *Z* and *W* chromosomes in about 60% cells were of equal size whereas in the rest the *W* was slightly smaller than the *Z*. We have no explanation to offer for this curious observation.

The characteristic female specific *W* chromatin body is present in the interphase nuclei of brain, kidney, leucocytes and liver cells. The autoradiographic studies after 6, 8 and 10 hours treatment of cultures with $^3$H-TdR have revealed a late replicating *W* chromosome (Ray-Chaudhuri and Singh, in press). It should be emphasised that *Z* and *W* chromosomes are homomorphic in *Naja naja naja* and *W* is early replicating whereas in *Naja n. kaouthia W* is quite distinct from the *Z* in its size and centromeric index and is late replicating.

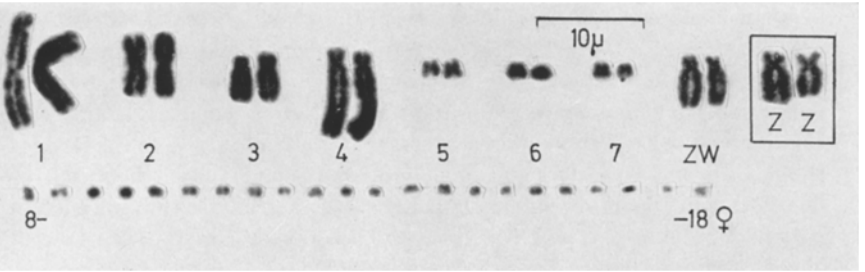


Fig. 10. Female karyotype of *Naja naja naja* ($2n = 38$) from leucocyte culture. Note that the *Z* and *W* are homomorphic. *ZZ* of a male shown in the inset

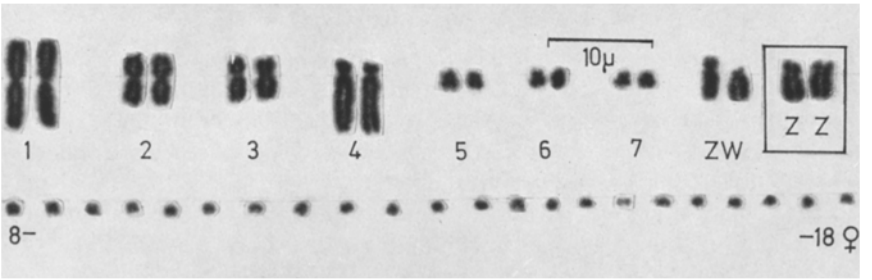


Fig. 11. Female karyotype of *Naja naja kaouthia* ($2n = 38$) from leucocyte culture. *ZW* chromosomes are heteromorphic. *ZZ* of a male shown in the inset

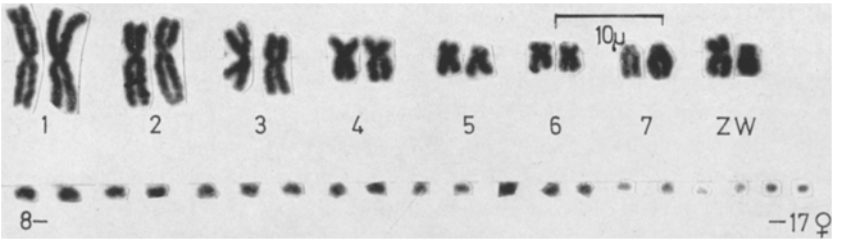


Fig. 12. Female karyotype of *Echis carinatus* ($2n = 36$) from spleen. The *Z* and *W* chromosomes are heteromorphic

**Family: *Viperidae***

*1. Echis carinatus.* We could get only two female specimens of the species from which 60 well spread metaphase plates have been obtained showing invariably 16 macro and 20 microchromosomes (Fig. 12) and

in all the plates there is a heteromorphic pair of chromosomes. Based on the analogy of the general pattern of sex chromosomes in snakes, the larger member of the heteromorphic pair having its centromere in the median region has been designated as *Z* and the smaller one with submedian centromere as *W* chromosomes. Besides the sex chromosomes, 4 pairs of macrochromosomes have their centromeres in the median region (Fig. 12, *1–4*), 2 pairs in the submedian region (*5*, *6*) and one pair (*7*) in the subterminal region. All the microchromosomes having a distinct size category, have terminal centromeres.

Since the chromosome study was done in Nagpur, Madhya Pradesh, in the field, we could not study the interphase nuclei for *W* chromatin. An autoradiographic study was therefore also impossible.

**Family: *Hydrophiidae***

*1. Hydrophis spiralis.* The chromosome analysis from 205 metaphase spreads from a single female specimen has revealed 32 as the diploid number of chromosomes for the species. The number of macro and microchromosomes are 14 and 18 respectively. The macrochromosomes have further been classified into different groups depending on their relative lengths and centromeric indices (Fig. 13). In all the plates there is a heteromorphic pair of chromosome. Due to the non-availability of males and lack of autoradiographic studies in the female, we have provisionally assumed that the larger chromosome of the heteromorphic pair, having its centromere in the median region, as *Z* and the smaller one, having its centromere in the subterminal region, as *W*. This identification of *Z* and *W* chromosome is mainly based on the analogy of the general pattern of the sex chromosome constitution in snakes. The *Z* and *W* chromosomes constitute about 8.5 and 7.8 percent of the haploid set which includes the measurements of the microchromosomes. The rest of the macrochromosomes have been put into 3 different groups. The first group consists of 4 pairs of chromosomes having their centromeres in the median region (Fig. 13, *1–4*). The *Z* chromosome also belongs to this group. The second group consists of one pair having their centromeres in the submedian region (*5*) and the third group of one pair with subterminal centromere (*6*). The *W* chromosome belongs to the third group. There is a marked difference in size between the macro and microchromosomes. All the microchromosomes appear to have their centromeres in the terminal region. All the macrochromosomes in this species are individually recognisable and can therefore be considered as marker chromosomes. The interphase nuclei of brain, kidney, leucocyte, liver and ovary have shown one characteristic *W* chromatin body (Ray-Chaudhuri, Singh, and Sharma, 1971). Autoradiography has not been done in this species.

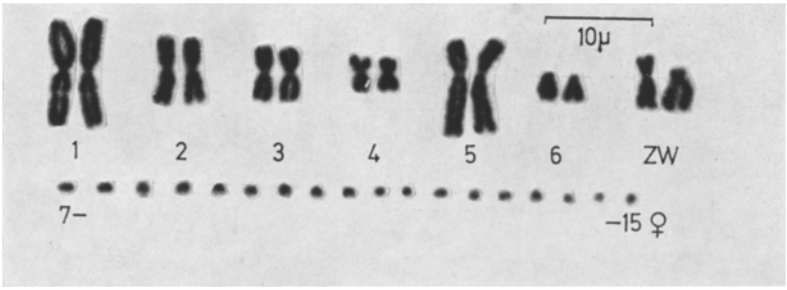


Fig. 13. Female karyotype of *Hydrophis spiralis* ($2n = 32$) from leucocyte culture. The sex chromosomes are heteromorphic

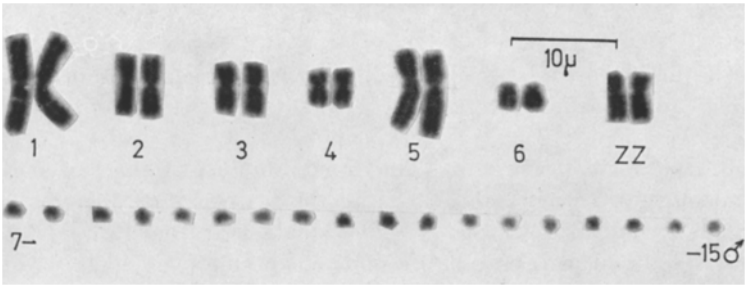


Fig. 14. Male karyotype of *Hydrophis ornatus ornatus* ($2n = 32$) from marrow of ribs showing a characteristic achromatic gap in the long arm of one of the chromosomes of pair no. 1

*2. Hydrophis ornatus ornatus.* Four males and two females of this species have been collected but unfortunately both the females died during the transportation from the sea coast to our laboratory. Chromosome analysis in this species, therefore, is restricted to the male sex only.

The diploid number of chromosomes determined from 180 metaphase spreads is 32 consisting of 14 macro and 18 microchromosomes (Fig. 14). Five pairs of the macrochromosomes have their centromeres in the median region (*1–4* and *Z*) one pair in submedian region (*5*) and one pair in subterminal region (*6*). Though no female specimen of this species has been studied, we have provisionally identified the *Z* chromosome on the basis of its centromeric position and relative length after comparing the karyotype of this species with that of *H. spiralis*. This has been possible only because all the macrochromosomes of this species are marker chromosomes and can be identified without any difficulty. One of the chromosomes of pair no. 1 (Fig. 14) has a characteristic achromatic gap in the long arm. This gap has been invariably found in

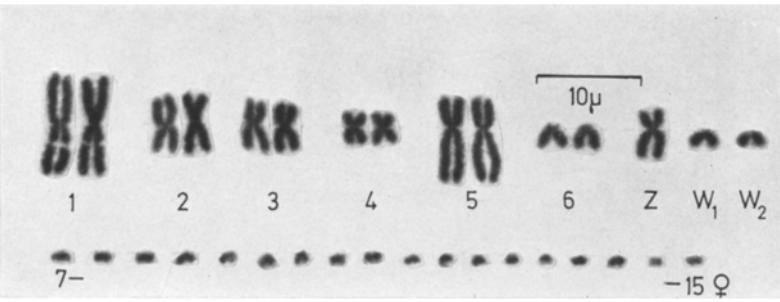


Fig. 15. Female karyotype of *Hydrophis cyanocinctus* ($2n = 33$) from marrow of ribs having multiple sex chromosomes $ZW_1W_2$; $W_1$ and $W_2$ are almost similar in size and morphology. Both the chromosomes of pair no. 1 show a conspicuous achromatic gap in the long arm

all the metaphase plates observed. All the microchromosomes of this species appear to have terminal centromeres and they form a distinct size class.

*3. Hydrophis cyanocinctus.* The diploid number of chromosomes has been found to be invariably 33 in all the plates out of 150 metaphase spreads examined from 3 female individuals, two from Kerala and one from West Bengal. The number of microchromosomes is 18 as in the previous species whereas macrochromosomes are 15 instead of 14 (Fig. 15). The odd chromosome having its centromere in the median region and comprising about 9 percent of the haploid set including the microchromosomes has been provisionally designated as the *Z* chromosome. Its identification is not unequivocal because it can be confused with the chromosomes of pair no. 3. The rest of the 14 macrochromosomes can be put into four different categories. Four pairs of macrochromosomes having their centromeres in the median region fall in the first category (Fig. 15, *1–4*). It should be pointed out that out of 3 individuals studied, in two (one from Kerala and another from West Bengal) both the chromosomes of pair no. 1 have an achromatic gap in the homologous regions of the long arm (Fig. 15, *1*) whereas in the third individual, which was also collected from Kerala, the characteristic achromatic gap is restricted to the long arm of only one of the pairs (Fig. 16, *1*). Those gaps are distinct in all the metaphases observed without any exception in all the three individuals. The third individual, heterozygous for the achromatic gap was slightly different in its body colouration from the two homozygous forms categories 2 and 3 (Fig. 15, *5* and *6*) are constituted by one pair each having their centromeres in the sub-median and subterminal region respectively. Another pair of chromosomes having its centromere at the terminal point falls in cate

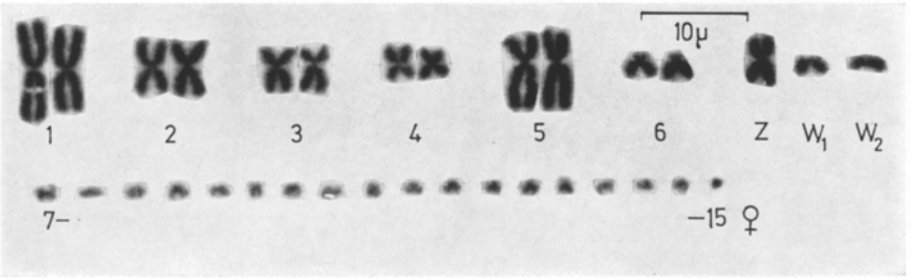


Fig. 16. Female karyotype of another individual of *H. cyanocinctus* from Kerala ($2n = 33$) from leucocyte culture. The conspicuous achromatic gap in this individual is restricted to the long arm of only one of the homologues of pair no. 1

gory 4 ($W_1$ and $W_2$). After comparing the karyotype of this species with that of *H. spiralis* and *H. ornatus ornatus* and examining the interphase nuclei where we find two *W* chromatin bodies, we have designated these chromosomes as $W_1$ and $W_2$. Because of their similarity in size and morphology it is very difficult to distinguish $W_1$ from $W_2$. All the macrochromosomes are individually recognisable. All the microchromosomes forming a distinct size class appear to have their centromeres in the terminal region.

The interphase nuclei of kidney, liver, brain and leucocyte culture have been examined and two *W* chromatin bodies have been observed (Fig. 19b). Occasionally, more than two chromocenters have also been observed, but for the relatively larger size of *W* chromatin bodies, there is no confusion regarding their identification. In this species the two *W* chromatin bodies are almost equal in size corresponding to the equal sized $W_1$ and $W_2$ chromosomes. Autoradiography has not been carried out in this species.

*4. Hydrophis fasciatus fasciatus.* The chromosome analysis has been done on only one female individual. Approximately 200 metaphases have been observed and the diploid number has been found to be 35. The number of macro and microchromosomes is 17 and 18 respectively (Fig. 17). All the microchromosomes appear to have terminal centromeres. Out of 17 macrochromosomes, there are 3 odd chromosomes whose relative lengths are 10, 6 and 3 percent of the haploid set. A comparison of its karyotype with those of *H. spiralis* and other species of the same genus studied has provided sufficient clues to designate them as $Z$,$W_1$ and $W_2$ respectively. Their centromeres are in the median region in the first two (Fig. 17, $Z$ and $W_1$) and in the submedian region in the third one ($W_2$). The rest of the chromosomes have been classified into four different groups. Three pairs (Fig. 17, *1–3*) having their centromeres

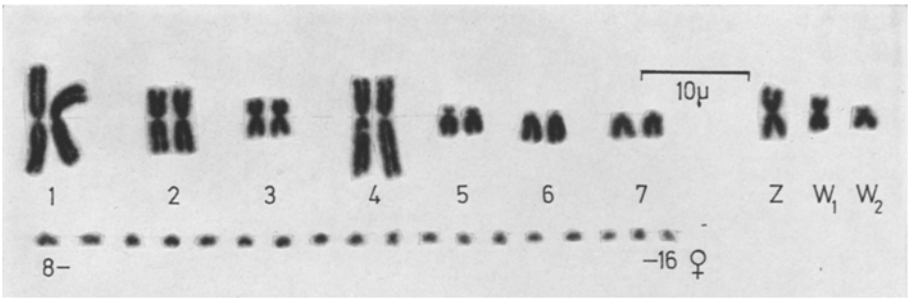


Fig. 17. Female karyotype of *Hydrophis fasciatus fasciatus* ($2n = 35$) from leucocyte culture. The 3 heteromorphic and unequal chromosomes are designated as $Z$, $W_1$ and $W_2$ in decreasing order of size

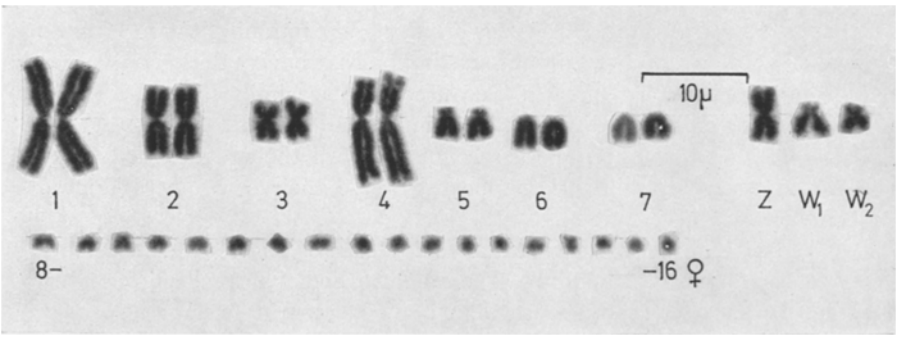


Fig. 18. Female karyotype of *Microcephalophis gracilis* ($2n = 35$) from leucocyte culture showing heteromorphic $Z$, $W_1$ and $W_2$ sex chromosomes

in the median region constitute the first group, one pair (*4*) with submedian centromeres belongs to the second group, one pair (*5*) with subterminal centromeres to the third group, and two pairs (*6*, *7*) with centromeres at the terminal point to the fourth group. All the macrochromosomes are easily distinguishable from each other and hence can be called marker chromosomes.

There are two $W$ chromatin bodies in the interphase nuclei of kidney, liver, brain and cultured leucocytes (Fig. 19a and c). Autoradiographic studies of the interphase nuclei after 8 hours treatment with $^3$H-TdR without any pretreatment have revealed a heavy concentration of grains in two localised regions (Fig. 20a) which have been found to be the $W$ chromatin bodies after removal of the grains (Fig. 20b). Occasionally, two heavily labelled bodies are found near the periphery of the nucleus (Fig. 20e). They may even fuse together to form a single body

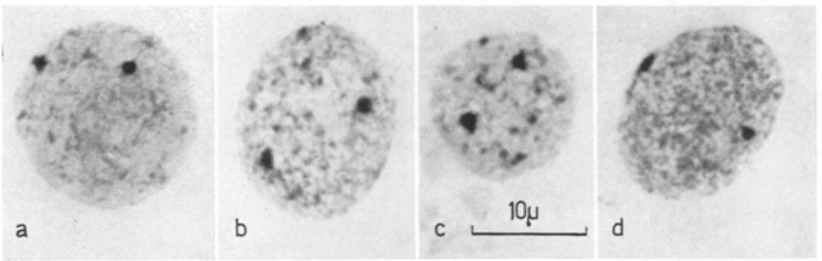


Fig. 19a—d. Interphase nuclei showing *W* chromatin bodies. a Interphase nucleus from leucocyte culture of *H. fasciatus fasciatus* female showing two *W* chromatin bodies corresponding to $W_1$ and $W_2$ chromosomes. b Interphase nucleus from leucocyte culture of *H. cyanocinctus* showing two *W* chromatin bodies equal in size corresponding to equal sized $W_1$ and $W_2$ chromosomes. c Interphase nucleus from kidney of *H. fasciatus fasciatus* showing two unequal *W* chromatin bodies. d Interphase nucleus from kidney of *Microcephalophis gracilis* showing two unequal *W* chromatin bodies

(Fig. 20f). It is quite clear, therefore, that there is a definite asynchrony in replication of the *W* chromatin bodies compared to the chromatin of the rest of the nucleus. Surprisingly enough, the allocycly exhibited by the *W* chromatin has not been detected at the chromosomal level (Ray-Chaudhuri and Singh, in press) in leucocyte cultures treated with $^3$H-TdR for 6, 8 and 10 hours continuously. Perhaps pulse labelling and more critical analysis of radioautographs in repeated cultures of a number of individuals may give more insight into the problem.

*5. Microcephalophis gracilis.* Only one female individual of this species could be collected and its chromosome studies yielded 105 metaphase spreads. Like the previous species 17 macro and 18 microchromosomes make the diploid number 35 for this species (Fig. 18). In this species also there are 3 odd macrochromosomes having their relative lengths 8, 5 and 4 percent of the haploid set. Their centromeres are in median, subterminal and submedian regions respectively. Comparison of the karyotype with that of *H. spiralis* and other species of sea snakes, and the replicating pattern of the *W* chromatin bodies have provided strong evidence to designate them as *Z*, $W_1$ and $W_2$ respectively. Out of 7 pairs of macroautosomes, 3 pairs have median centromeres (Fig. 18, *1–3*), 1 pair submedian (*4*), 1 pair terminal (*5*) and 2 pairs with centromeres at their terminal points (*6*, *7*). All the macrochromosomes including the *Z*, $W_1$ and $W_2$ are individually recognisable because of their size and morphology. All the microchromosomes appear to have terminal centromeres.

There are two *W* chromatin bodies in the interphase nuclei of kidney, liver, brain and leucocyte culture (Fig. 19d). Like the previous species

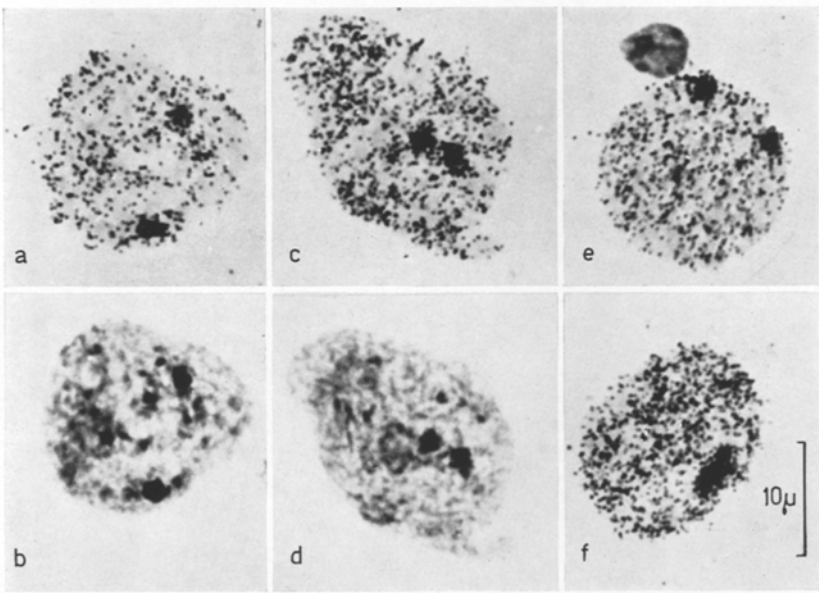


Fig. 20. a Autoradiograph of interphase nucleus from leucocyte culture of *H. fasciatus fasciatus* female after 8 hours treatment with $^3$H-TdR without any pretreatment showing heavy concentration of grains over the *W* chromatin bodies. b The same nucleus after removal of grains showing *W* chromatin bodie s corresponding to heavily labelled regions in a. c Autoradiograph of interphase nucleus from leucocyte culture of *Microcephalophis gracilis* female after 8 hours treatment with $^3$H-TdR showing heavy concentration of grains in the two *W* chromatin bodies. d The same nucleus after removal of grains. e and f Autoradiographs of interphase nuclei from leucocyte culture of *H. fasciatus fasciatus* after 8 hours treatment with $^3$H-TdR. e Two separate unequal heavily labelled *W* chromatin bodies at the periphery of the nucleus. f Single large heavily labelled body near the periphery which is assumed to be the result of the fusion of the two *W* chromatin bodies

these two *W* chromatin bodies are also unequal in size corresponding to the unequal $W_1$ and $W_2$ chromosomes. The autoradiography of interphase nuclei after 8 hours continuous treatment with $^3$H-TdR and direct fixation in aceto-alcohol without any pretreatment has revealed allocycly in the replication of *W* chromatin bodies exactly in the manner displayed by the previous species (Fig. 20 c and d). However no asynchrony has been observed at the chromosomal level after 6, 8 and 10 hours continuous treatment with $^3$H-TdR (Ray-Chaudhuri and Singh, in press). More extensive study is needed.

## Discussion

To our knowledge, about 109 species of snakes belonging to 7 different families have been chromosomally worked out so far (see Table 1, p. 206), out

of which in 27 species the chromosome analysis has been done employing mainly sectioned testis material. In such preparations, the morphology of chromosomes is obscure. Moreover, in 6 species amongst the recently studied ones, utilising modern techniques, either the preparations are poor or the karyotypes are not available to us. Morphometric data on the chromosomes of the remaining 76 species are utilized in the present cytotaxonomical analysis.

In order to compare the size and centromeric index of individual chromosomes of different species by various authors, the autosome pairs are arranged, in Tables 2–9, according to the decreasing order of size and the sex chromosomes are put in the last column. The numbering of the chromosome pairs in the karyotypes and in the Tables do not correspond because in the former the arrangment has been done according to Levan *et al.* (1964) and not to decreasing order of size. Unfortunately no standard method of arranging the karyotypes is in vogue amongst the reptilian karyologists. Under these circumstances comparison of any karyotype in whichever way it has been arranged with the data presented in our tables can only be done by estimating by visual inspection the position of a particular chromosome in a decreasing sequence of size in a karyotype.

### *Xenopeltidae*

*Xenopeltis unicolor* is the only described species of the family and was placed in a monotypic taxon by Bonaparte in 1845. Romer (1956) and Hoge (1964) have suggested that it may be placed in *Anilidae* and *Boidae* respectively. Recent authors however have not followed either allocation (see Underwood, 1967; Stimson, 1969). Cole and Dowling (1970) described the karyotype of the species and pointed out that it is identical to that of several boids. An examination of the measurements on eight species of *Boidae* and the only species of *Xenopeltidae* will reveal that the chromosomes of *Xenopeltis* are almost identical in size and centromeric position to those of the boids of the genera, *Boa*, *Epicrates*, *Eunectes* and *Python* (see measurements in Table 2). Another characteristic feature of the chromosomes of *Boidae* is the absence of recognisable sex chromosomes. Unfortunately no female specimen of *X. unicolor* has been studied but it can be presumed that when such studies are done it will show the same common characteristic of *Boidae*. In view of the above evidences from karyology, the retention of *Xenopeltidae* is unnecessary and the only species so far referred to in that group should be included in the *Boidae* as suggested by Hoge (1964) and Cole and Dowling (1970).

### *Boidae*

It is customary to divide the family into two subfamilies, *Boinae* and *Pythoninae*. *Python molurus* is the only species of *Pythoninae*

Table 1. List of cytologically worked out species of snakes and their diploid numbers

| Species | Sex studied | | 2n | Macro-chromo-somes | Micro-chromo-somes | References |
|---|---|---|---|---|---|---|
| *Boidae* | | | | | | |
| 1. *Boa constrictor amarali* | ♂ | ♀ | 36 | 16 | 20 | Beçak, Beçak and Nazareth (1962a, 1963a, b, 1966) |
| 2. *Boa constrictor constrictor* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965; Beçak, Beçak and Nazareth (1966) |
| 3. *Corallus caninus* | ♂ | ♀ | 44 | 24 | 20 | Beçak (1965); Beçak, Beçak and Nazareth (1966) |
| 4. *Epicrates cenchria crassus* | ♂ | ♀ | 36 | 16 | 20 | Beçak, Beçak and Nazareth (1966) |
| 5. *Eryx conicus* | ♂ | ♀ | 34 | 16 | 18 | Singh, Sharma and Ray-Chaudhuri (1970a) |
| *6. *Eryx jaculus* | ♂ | | 34 | 16 | 18 | Werner (1959) |
| 7. *Eryx johni johni* | ♂ | ♀ | 34 | 16 | 18 | Singh, Sharma and Ray-Chaudhuri (1968b) |
| 8. *Eunectes murinus* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965); Beçak, Beçak and Nazareth (1966) |
| 9. *Python molurus* | | ♀ | 36 | 16 | 20 | Singh, Sharma and Ray-Chaudhuri (1968b) |
| *Xenopeltidae* | | | | | | |
| 10. *Xenopeltis unicolor* | ♂ | | 36 | 16 | 20 | Cole and Dowling (1970) |
| *Colubridae* (*vide* Smith, 1943) | | | | | | |
| 11. *Boiga forsteni* | ♂ | ♀ | 36 | 18 | 18 | Singh (present study) |
| 12. *Boiga trigonata* | ♂ | ♀ | 36 | 18 | 18 | Singh (present study) |
| 13. *Cerberus rhynchops* | ♂ | ♀ | 36 | 16 | 20 | Dutt (1966); Singh, Sharma and Ray-Chaudhuri (1970a) |

Table 1 (continued)

| Species | Sex studied | 2n | Macro-chromo-somes | Micro-chromo-somes | References |
|---|---|---|---|---|---|
| 14. *Chironius bicarinatus* | ♂ ♀ | 36 | 16 | 20 | Beçak (1965); Beçak, Beçak and Nazareth (1966) |
| 15. *Clelia occipitolutea* | ♂ ♀ | 50 | 30 | 20 | Beçak (1965); Beçak, Beçak and Nazareth (1966) |
| 16. *Coluber fasciolatus* | ♂ ♀ | 36 | 16 | 20 | Singh (present study) |
| *17. *Coluber gemonensis* | ♂ | 36 | 16 | 20 | Matthey (1931) |
| 18. *Coluber viridiflavus viridiflavus* | ♂ ♀ | 36 | 16 | 20 | Kobel (1967) |
| 19. *Coronella austriaca* | ♂ | 36 | 16 | 20 | Matthey (1931); Kobel (1967) |
| *20. *Dinodon rufozonatus* | ♂ | 46 | 16 | 30 | Nakamura (1935) |
| 21. *Dryadophis bifossatus bifossatus* (*Mastigodryas bifossatus bifossatus*) | ♂ ♀ | 36 | 16 | 20 | Beçak (1965) |
| 22. *Drymarchon corais corais* | ♂ ♀ | 36 | 16 | 20 | Beçak (1965); Beçak, Beçak and Nazareth (1966) |
| 23. *Drymarchon corais couperi* | ♀ | 36 | 16 | 20 | Beçak (1965) |
| *24. *Elaphe carinata* | ♂ | 36 | 16 | 20 | Fischman, Mitra and Dowling (1968) |
| 25. *Elaphe climacophora* | ♂ ♀ | 36 | 16 | 20 | Nakamura (1929, 1935); Itoh, Sasaki and Makino (1970) |
| 26. *Elaphe longissima longissima* | ♂ ♀ | 36 | 16 | 20 | Kobel (1967) |
| *27. *Elaphe obsoleta obsoleta* | ♂ | 36 | 16 | 20 | Fischman, Mitra and Dowling (1968) |
| *28. *Elaphe obsoleta quadrivittata* | ♂ | 36 | 16 | 20 | Fischman, Mitra and Dowling (1968) |

Table 1 (continued)

| Species | Sex studied | | 2n | Macro-chromo-somes | Micro-chromo-somes | References |
|---|---|---|---|---|---|---|
| 29. *Elaphe quadrivirgata* | ♂ | ♀ | 36 | 16 | 20 | Nakamura (1927, 1928, 1935); Itoh, Sasaki, and Makino (1970) |
| 30. *Erythrolamprus aesculapii venustissimus* | ♂ | | 28 | 20 | 8 | Beçak (1969) |
| 31. *Gerardia prevostiana* | | ♀ | 36 | 16 | 20 | Singh, Sharma, and Ray-Chaudhuri (1970a) |
| 32. *Hydrodynastes bicinctus schultzi* | ♂ | | 24 | 16 | 8 | Beçak (1969) |
| 33. *Hydrodynastes gigas* | ♂ | | 24 | 16 | 8 | Beçak (1969) |
| *34. *Imantodes cenchoa* | ♂ | | 36 | ? | ? | Beçak (unpublished) |
| 35. *Liophis meliaris* | ♂ | ♀ | 28 | 20 | 8 | Beçak (1969) |
| 36. *Lycodon aulicus* | ♂ | ♀ | 36 | 16 | 20 | Bhatnagar(1961); Singh, Sharma, and Ray-Chaudhuri (1970a) |
| *37. *Macropistodon rudis carinatus* | ♂ | | 46 | 16 | 30 | Nakamura (1935) |
| *38. *Malpolon monspessulanus monspessulanus* (*Coelopeltis lacertina*) | ♂ | | 42 | 20 | 22 | Matthey (1931) |
| 39. *Natrix maura* | ♂ | ♀ | 34 | 16 | 18 | Kobel (1967) |
| *40. *Natrix maura* (*Tropidonotus viperinus*) | ♂ | | 36 | 16 | 20 | Matthey (1931) |
| *41. *Natrix natrix* (*Tropidonotus natrix*) | ♂ | | 36 | 16 | 20 | Matthey (1931) |
| 42. *Natrix natrix helvetica* | ♂ | ♀ | 34 | 16 | 18 | Kobel (1967) |
| *43. *Natrix natrix persa* | ? | ? | 34 | 16 | 18 | Kobel (1967) |
| 44. *Natrix piscator* (*Xenochrophis piscator*) | ♂ | ♀ | 42 | 18 | 24 | Singh, Sharma, and Ray-Chaudhuri (1968a, 1970a) |
| *45. *Natrix rhombifera* | ♂ | ♀ | 36 | 34 | 2 | Van Brink (1959) |
| 46. *Natrix stolata* (*Amphiesma stolata*) | ♂ | ♀ | 36 | 14 | 22 | Bhatnagar (1960a); Singh, Sharma, and Ray-Chaudhuri (1970a) |

Table 1 (continued)

| Species | Sex studied | | 2n | Macro-chromo-somes | Micro-chromo-somes | References |
|---|---|---|---|---|---|---|
| 47. *Natrix tessellata tessellata* | ♂ | | 34 | 16 | 18 | Kobel (1967) |
| *48. *Natrix tigrina* | ♂ | | 40 | 16 | 24 | Nakamura (1927, 1928) |
| 49. *Natrix vibakari* (*Amphiesma vibakari*) | | ♀ | 36 | 16 | 20 | Itoh, Sasaki and Makino (1970) |
| *50. *Oligodon arnensis* | ♂ | | 46 | 24 | 22 | Bhatnagar (1959) |
| *51. *Oligodon formosanus* (*Holarchus formosanus*) | ♂ | | 36 | 16 | 20 | Nakamura (1935) |
| 52. *Oxyrhopus petolarius* | ♂ | ♀ | 46 | 16 | 30 | Beçak (1969) |
| 53. *Philodryas aestivus aestivus* | ♂ | | 36 | 16 | 20 | Beçak (196) |
| 54. *Philodryas olfersii olfersii* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965) |
| 55. *Philodryas patagoniensis* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1969) |
| 56. *Philodryas serra* | ♂ | ♀ | 28 | 20 | 8 | Beçak (1969) |
| 57. *Pseustes sulphureus sulphureus* | | ♀ | 38 | 18 | 20 | Beçak (1969) |
| 58. *Ptyas mucosus* | ♂ | ♀ | 34 | 16 | 18 | Bhatnagar (1960a); Singh, Sharma and Ray-Chaudhuri (1970a) |
| 59. *Rhabdophis tigrinus* | ♂ | ♀ | 36 | 16 | 20 | Itoh, Sasaki, and Makino (1970) |
| 60. *Spilotes pullatus anomalepis* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965) |
| 61. *Spilotes pullatus maculatus* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965) |
| *62. *Tamnophis butleri* | ♂ | | 37 | ? | ? | Thatcher (1922) |
| *63. *Telescopus fallax* (*Tarbophis fallax*) | ♂ | | 36 | 16 | 20 | Matthey (1931) |
| 64. *Thamnodynastes pallidus nattereri* | ♂ | ♀ | 34 | 20 | 14 | Beçak (1969) |
| 65. *Thamnodynastes strigatus* | ♂ | ♀ | 32 | 18 | 14 | Beçak (1969) |
| 66. *Tomodon dorsatus* | ♂ | ♀ | 32 | 18 | 14 | Beçak (1969) |
| *67. *Xenochrophis piscator* | ♂ | | 36 and 38 | 16 and 10 | 20 and 28 | Dutt (1970) |

Table 1 (continued)

| Species | Sex studied | | 2n | Macro-chromo-somes | Micro-chromo-somes | References |
|---|---|---|---|---|---|---|
| 68. *Xenodon merremii* | ♂ | ♀ | 30 | 16 | 14 | Beçak, Beçak, Nazareth and Ohno (1964); Beçak (1965) |
| 69. *Xenodon neuwiedii* | | ♀ | 30 | 16 | 14 | Beçak (1969) |
| *70. *Zaocys nigromarginatus oshimai* | ♂ | | 36 | 16 | 20 | Nakamura (1935) |
| *Elapidae* | | | | | | |
| 71. *Bungarus caeruleus* | ♂ | ♀ | 44°/43 | 24°/23♀ | 20 | Bhatnagar (1956); Singh, Sharma and Ray-Chaudhuri (1970a, b) |
| *72. *Bungarus multi-cinctus* | ♂ | | 36 | 22 | 14 | Nakamura (1935 |
| 73. *Micrurus lemnis-catus carvalhori* | ♂ | | 42 | 22 | 20 | Beçak (1969) |
| *74. *Naja naja atra* | ♂ | | 38 | 14 | 24 | Nakamura (1935) |
| 75. *Naja naja kaouthia* | ♂ | ♀ | 38 | 16 | 22 | Singh (present study) |
| 76. *Naja naja naja* | ♂ | ♀ | 38 | 16 | 22 | Singh, Sharma and Ray-Chaudhuri (1970a) |
| *Hydrophiidae* | | | | | | |
| 77. *Enhydrina schistosa* | ♂ | ♀ | 32♂ 33♀ | 14 | 18♂ 19♀ | Singh (1972) |
| 78. *Hydrophis cyanocinctus* | | ♀ | 33 | 15 | 18 | Singh (present study) |
| 79. *Hydrophis fasciatus fasciatus* | | ♀ | 35 | 17 | 18 | Singh (present study) |
| 80. *Hydrophis ornatus ornatus* | ♂ | | 32 | 14 | 18 | Singh (present study) |
| 81. *Hydrophis spiralis* | | ♀ | 32 | 14 | 18 | Singh (present study) |
| *82. *Laticauda semi-fasciata* | ♂ | | 38 | 14 | 24 | Nakamura (1935) |
| 83. *Microcephalophis gracilis* | | ♀ | 35 | 17 | 18 | Singh (present study) |

Table 1 (continued)

| Species | Sex studied | | 2n | Macro-chromo-somes | Micro-chromo-somes | References |
|---|---|---|---|---|---|---|
| *Viperidae* | | | | | | |
| *84. *Agkistrodon acutus* | ♂ | | 36 | 16 | 20 | Nakamura (1935) |
| 85. *Agkistrodon halys* | ♂ | ♀ | 36 | 16 | 20 | Itoh, Sasaki and Makino (1970) |
| *86. *Agkistrodon halys blomhoffii* | ♂ | | 36 | 16 | 20 | Nakamura (1927, 1935) |
| 87. *Bothrops alternatus* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965) |
| 88. *Bothrops insularis* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965) |
| 89. *Bothrops jararaca* | ♂ | ♀ | 36 | 16 | 20 | Beçak, Beçak and Nazareth (1962b); Beçak (1965) |
| 90. *Bothrops jararacussu* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965) |
| 91. *Bothrops moojeni* (*Bothrops atrox*) | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965) |
| 92. *Bothrops pradoi* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965) |
| 93. *Crotalus durissus terrificus* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1965) |
| 94. *Crotalus viridis lutosus* | ♂ | | 36 | 16 | 20 | Monro (1962) |
| 95. *Crotalus viridis oreganus* | ♂ | | 36 | 16 | 20 | Monro (1962) |
| 96. *Echis carinatus* | | ♀ | 36 | 16 | 20 | Singh, Sharma and Ray-Chaudhuri (1970a) |
| 97. *Lachesis muta noctivaga* | ♂ | ♀ | 36 | 16 | 20 | Beçak (1969) |
| *98. *Trimeresurus flavoviridis* | ♂ | | 36 | ? | ? | Momma (1948); Makino and Momma (1949) |
| *99. *Trimeresurus gramineus stejnegeri* | ♂ | | 36 | 16 | 20 | Nakamura (1935) |
| *100. *Trimeresurus mucrosquammatus* | ♂ | | 36 | 16 | 20 | Nakamura (1935) |
| *101. *Trimeresurus okinavensis* | ♂ | | 36 | ? | ? | Momma (1948); Makino and Momma (1949) |
| *102. *Vipera aspis* | ♂ | ♀ | 42 | 22 | 20 | Matthey (1928, 1931) |
| 103. *Vipera aspis aspis* | ♂ | ♀ | 42 | 22 | 20 | Kobel (1963, 1967) |
| 104. *Vipera aspis zinnikeri* | | ♀ | 42 | 22 | 20 | Kobel (1967) |

Table 1 (continued)

| Species | Sex studied | 2n | Macro-chromo-somes | Micro-chromo-somes | References |
|---|---|---|---|---|---|
| 105. *Vipera berus berus* | ♂ ♀ | 36 | 16 | 20 | Kobel (1967) |
| *106. *Vipera berus sachaliensis* | ♂ | 36 | 16 | 20 | Makino and Momma (1949) |
| 107. *Vipera ursinii rakosiensis* | ♀ | 36 | 16 | 20 | Kobel (1967) |
| *Typhlopidae* | | | | | |
| *108. *Typhlops simoni* | ♂ | 32 | 16 | 16 | Werner (1959) |
| *Leptotyphlopidae* | | | | | |
| *109. *Leptotyphlops phillipsi* | ♂ | 36 | 16 | 20 | Werner (1959) |

* The species marked with an asterisk could not be utilized in the present cytotaxonomical study due to nonavailability of sufficient informations about their chromosome complements.

chromosomally known so far (Singh, Sharma, and Ray-Chaudhuri, 1968b) and the rest of the 7 known species belong to *Boinae*. When we compare the karyotype of *P. molurus* (Table 2) with the karyotypes of *Boa*, *Epicrates*, *Eunectes* and *Xenopeltis* we find a striking similarity of all the chromosomes. *Eryx johni johni* and *E. conicus* although placed in the subfamily *Boinae*, differ in their chromosome structure from other *Boinae* mentioned above. For instance, the three smaller pair of macrochromosomes, i.e. pairs no. 6, 7 and 8 in *E. conicus* and 7 and 8 in *E. johni johni* have their centromeres terminal instead of in the subterminal region found in the other species. The chromosome no. 5 of *E. johni johni* and *E. conicus* have their centromeres located more terminally than those of the other species. These differences in the chromosome structures can simply be explained on the assumption of pericentric inversions. *Corallus caninus*, the remaining known species of *Boinae* apparently differs from other *Boinae* both in chromosome number and structure. In *C. caninus*, the first 4 pairs of metacentric macrochromosomes, common in all other species, are absent and instead we find 8 pairs of chromosomes with subterminal centromeres. The data on relative lengths (Table 2) show that the combined relative lengths of 2 subtelocentrics in *C. caninus* are almost identical with the corresponding metacentric chromosomes of all other species. Chromosome pairs no. 5–8 are very similar in their relative lengths and centromeric indices in all the species. Since *C. caninus* is the only species which

Table 2. *Boidae.* In Tables 2–9 relative lengths ($L^R$) and centromeric indices are given as fractions $L^R/J^C$. (See text p. 188.)

| Species | Pairs of macrochromosomes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| *Python molurus* | 27/50 | 20/41 | 15/45 | 9/44 | 8/20 | 7/20 | 5/23 | 4/29 |
| *Boa constrictor amarali* | 26/49 | 21/42 | 16/46 | 10/49 | 9/18 | 8/14 | 6/12 | 6/14 |
| *Boa c. constrictor* | 27/47 | 21/40 | 16/45 | 9/48 | 9/21 | 7/15 | 6/8 | 5/9 |
| *Epicrates cenchria crassus* | 26/48 | 21/40 | 16/48 | 9/47 | 9/20 | 7/19 | 7/23 | 6/24 |
| *Eunectes murinus* | 25/48 | 21/40 | 16/48 | 9/47 | 9/21 | 7/21 | 6/27 | 6/30 |
| *Eryx conicus* | 26/47 | 22/40 | 16/45 | 9/44 | 8/10 | 7/0 | 6/0 | 5/0 |
| *Eryx j. johni* | 26/47 | 21/38 | 16/44 | 10/45 | 8/7 | 7/10 | 6/0 | 5/0 |
| *Corallus caninus* | 14/10 13[c]/9 | 12/10 9[c]/21 | 8/11 8[c]/19 | 5/29 4[c]/29 | 8/24 | 7/26 | 7/24 | 6/26 |
| *Xenopeltis unicolor* | 24/47 | 23/40 | 16/47 | 9/49 | 8/21 | 7/22 | 7/15 | 4/13 |

[c] Indicating fission and inversion.

has 8 pairs of subtelocentric chromosomes instead of 4 pairs of metacentrics, it is suggested that the latter is a derived species and has originated from the usual karyotype by centric fission and subsequent pericentric inversions.

"About a third of the 60 living members of the Boidae are set apart as a subfamily, the Pythons (Pythoninae). The Pythons differ from nearly all the rest (the boas, Boinae) in two skull characteristics and the habit of laying eggs instead of bringing forth the young directly. Some students doubt that this time-honoured separation is based on true relationship, and would throw nearly all of the sixty species into a single family, the Boidae. Until the matter has been studied further, it is just as well to keep the two groups apart" (Pope, 1956).

Smith (1943) stated that *Constrictor* (*Boinae*) is in many ways more closely related to *Python* (*Pythoninae*) than it is to *Eryx (Boinae).*

The karyotype of *Python molurus* (*Pythoninae*) is virtually identical to *Xenopeltis unicolor* previously placed in the family *Xenopeltidae* and *Boa constrictor constrictor, B. c. amarali, Epicrates cenchria crassus,*

*Eunectes murinus* (*Boinae*), whereas idiograms of *E. j. johni* and *E. conicus* (*Boinae*) are different from the rest of the members of the subfamily *Boinae*. The karyotype of *Corallus caninus* can be derived from the former. Thus, further studies from a cytotaxonomical point of view do not justify the retention of two separate subfamilies of *Boidae*.

### *Colubridae*

Nearly seventy-five percent of all living snakes belong to this family and they are considered to be the most successful of all snakes because of their diversified adaptive radiation. Unfortunately mutual relationship amongst the various members of the family are often obscure.

"The great family Colubridae has ever been the nightmare of the classifier of snakes. Most of the living species have always been thrown together into this unwieldy assortment, and one all important task of herpetologists has been, first, to see how many groups could reasonably be removed from it and, second, to try to split it up into good subfamilies" (Pope, 1956).

We have data on chromosome measurements of only 40 species of colubrid snakes which have been utilized here to find out, if possible, probable chromosomal similarities or otherwise in the hope of throwing some light on the classification of these snakes.

A list of chromosomally known species was forwarded simultaneously to the Directors, British Museum (Natural History) and the American Museum of Natural History for favour of providing us with the present status of the supergeneric classification of the listed species. In forwarding the classification Dr. C. J. Cole, Assistant Curator, American Museum of Natural History writes:

"It is extremely difficult or impossible to provide a supergeneric classification of the colubrids that would be acceptable and agreeable to all snake taxonomists. Colubrid taxonomy is extraordinarily difficult to handle because the evolutionary diversification is great in number of species, the characteristics for analysis or relationships are few and probably there has been a lot of evolutionary convergence. We hope that with future studies, such as with karyotypes, new evidence will emerge that will help to clarify these relationships and contribute to solving the problems associated with this complex taxonomic situation".

The two classifications are given below.

*Supergeneric Classification (American Museum of Natural History)*

| | |
|---|---|
| Family | *Colubridae* |
| Subfamily | *Xenodontinae* |
| Tribe | *Alsophiini*<br>1. *Hydrodynastes*, 2. *Philodryas*, 3. *Thamnodynastes*, 4. *Clelia* |
| Tribe | *Xenodontini*<br>5. *Erythrolamprus*, 6. *Oxyrhopus*, 7. *Xenodon*, 8. *Liophis* |

| | |
|---|---|
| Tribe | *Hydropsini*<br>9. *Tomodon* |
| Subfamily | *Colubrinae* |
| Tribe | *Colubrini*<br>10. *Ptyas*, 11. *Pseustes*, 12. *Drymarchon*, 13. *Chironius*, 14. *Coluber*, 15. *Spilotes*, 16. *Elaphe*, 17. *Coronella*, 18. *Dryadophis (Mastigodryas)* |
| Subfamily | *Natricinae* |
| Tribe | *Natricini*<br>19. *Rhabdophis*, 20. *Natrix natrix helvetica*, 21. *N. maura*, 22. *N. tessellata tessellata*, 23. *N. (Xenochrophis) piscator*, 24. *N. (Amphiesma) stolata*, 25. *N. (Amphiesma) vibakari* |
| Tribe | *Homalopsini*<br>26. *Gerardia*, 27. *Cerberus* |
| Tribe | *Boigini*<br>28. *Boiga* |

*Supergeneric Classification (British Museum, Natural History)*

| | |
|---|---|
| Infra order | *Caenophida* |
| Family | *Pseudoboidae*<br>6. *Oxyrhopus* |
| Family | *Dipsadidae* |
| Subfamily | *Lycodontinae*<br>4. *Clelia*, *Lycodon*[a] |
| Subfamily | *Xenodontinae*<br>7. *Xenodon* |
| Subfamily | *Boiginae*<br>28. *Boiga* |
| Subfamily | *Homalopsinae*<br>26. *Gerardia*, 27. *Cerberus* |
| Family | *Colubridae* |
| Subfamily | *Natricinae*<br>1. *Hydrodynastes*, 5. *Erythrolamprus*, 19. *Rhabdophis*, 23. *Xenochrophis*, 24. *Amphiesma stolata*, 25. *Amphiesma vibakari*, 22. *Natrix tessellata tessellata*, 20. *Natrix natrix helvetica*, 21. *N. maura*, 2. *Philodryas*, 9. *Tomodon*, 3. *Thamnodynastes*, 8. *Liophis* |
| Subfamily | *Colubrinae*<br>10. *Ptyas*, 11. *Pseustes*, 12. *Drymarchon*, 13. *Chironius*, 14. *Coluber*, 15. *Spilotes*, 16. *Elaphe*, 17. *Coronella*, 18. *Mastigodryas (Dryadophis)* |

As suspected by Dr. Cole, there are very few points of agreement between the American and the British system of classification. We have

[a] The genus *Lycodon* was not put in any supergeneric group by the American Museum perhaps owing to its uncertain affinities.

serially numbered the genera included under different tribes in the American classification and the same numbers were retained for the respective genera in the British system which will help to find out the similarities and differences in the two systems. While arriving at super-generic groupings through morphometric analysis of the chromosomes we encountered special difficulties with colubrids, because, unlike in other families, species groups considered to be related taxonomically either by the British or American Museum when analysed from the point of view of chromosomal similarities did not show affinities except in a few groups. Under the circumstances, the species having more or less the same number of macro- and microchromosomes were grouped together for comparing their idiograms. If now the relative lengths and centromeric indices of the corresponding macrochromosomes of the species included in the composite idiogram show sufficient homology they are considered to be mutually related. In those cases where such groupings did not show homology of their respective chromosomes, the mutual relationships between them were considered to be more remote. In certain other species groups where the karyological data indicate close relationship but have been considered by the taxonomists of both the American and British museums as distinct taxa, their viewpoints have been accepted.

The thirteen species put under the tribe *Colubrini*, subfamily *Colubrinae*, family *Colubridae* by the American museum have also been put by the British Museum in the subfamily *Colubrinae*, family *Colubridae*. An examination of the karyotypes of all the 13 species reveals that they are chromosomally very closely related. They have the same number of macro and microchromosomes which are 16 and 20 respectively. The most important structural modification is seen in *Pseustes sulphureus sulphureus* where instead of the first pair of metacentric chromosome common to all other species of the subfamily, 2 pairs of chromosomes having terminal centromeres (Table 3) are present. These two pairs of chromosomes could have been easily derived during evolution through centromeric fission of the largest pair of metacentric chromosomes. This view finds support from the data on relative lengths of the original chromosomes and those derived from them (Table 3). When we add up the relative lengths of the two telocentric chromosomes of *P. s. sulphureus* we find that the sum corresponds very closely to the relative length of the largest metacentric chromosome of the family. Other differences in chromosome structure are found in chromosome no. 5 of *Elaphe quadrivirgata*, *E. climacophora*, *E. longissima longissima* and *Coronella austriaca austriaca* where the chromosomes have terminal centromeres instead of subterminal ones present in other species. Simple pericentric inversions can be assumed to explain the differences.

Table 3. *Colubridae* I

| Species | Pairs of macrochromosomes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Z | W |
| *Ptyas mucosus* | 25/46 | 21/36 | 14/45 | 9/42 | 9/19 | 6/37 | 5/36 | 10?/40 | ? |
| *Dryadophis b. biffosatus* | 23/50 | 21/39 | 15/50 | 9/41 | 8/41 | 7/45 | 6/39 | 10/48 | 10[a]/20 |
| *Drymarchon c. corais* | 27/50 | 22/37 | 15/49 | 9/48 | 7/19 | 6/40 | 5/36 | 10/48 | *10*[a]/29 |
| *Chironius bicarinatus* | 24/50 | 22/40 | 15/50 | 9/46 | 7/34 | 7/42 | 6/27 | 10/41 | 10[a]/8 |
| *Coluber fasciolatus* | 26/48 | 21/40 | 15/47 | 8/43 | 7/19 | 6/38 | 5/36 | 13/42 | 12[a]/18 |
| *Coluber v. viridiflavus* | 25/50 | 18/35 | 15/47 | 10/40 | 8/0 | 7/39 | 6/33 | 10/40 | ? |
| *Spilotes pullatus maculatus* | 24/49 | 22/39 | 16/50 | 8/41 | 8/23 | 8/35 | 6/36 | 9/50 | 9[a]/28 |
| *Spilotus p. anomalepis* | 26/50 | 22/35 | 15/49 | 8/44 | 7/22 | 7/40 | 6/39 | 9/50 | 9[a]/27 |
| *Elaphe quadrivirgata* | 25/47 | 21/37 | 14/44 | 9/40 | 7[a]/0 | 6/25 | 5/31 | 12/39 | 9/0 |
| *Elaphe climacophora* | 26/49 | 22/33 | 14/50 | 10/41 | 6[a]/0 | 7/30 | 5/30 | 10/43 | 7/20 |
| *Elaphe l. longissima* | 25/48 | 22/37 | 15/47 | 9/46 | 7[a]/0 | 7/41 | 6/38 | 11/44 | ? |
| *Coronella a. austriaca* | 25/50 | 20/38 | 19/32 | 8/46 | 6[a]/0 | 6/38 | 5/33 | 10/39 | ? |
| *Pseustes s. sulphureus* | 12/0 12⁻/0 | 21/37 | 14/39 | 10/40 | 7/40 | 7/20 | 5/25 | 12/42 | ? |
| *Philodryas patagoniensis* | 24/50 | 20/37 | 16/46 | 9/43 | 7/27 | 6/38 | 6/22 | 12/41 | 6/0 |
| *P. o. olfersii* | 24/49 | 20/40 | 15/49 | 10/44 | 7/13 | 7/38 | 6/36 | 12/42 | 11/8 |
| *P. a. aestivus* | 23/50 | 21/43 | 15/50 | 11/45 | 7/27 | 6/27 | 6/23 | 12/39 | — |
| *Clelia occipitolutea* | 15/0 12[b]/0 | 14/0 8[b]/0 | 8/0 7[b]/0 | 4/0 3[b]/0 | 3[b]/0 3/0 | 3/ 03[b]/0 | 3/0 2[b]/0 | 12/41 | 20/0 |

[a] Indicating an inversion.
[b] Indicating fission.

The sex chromosomes in this subfamily exhibit various states of differentiation. In *Pseustes sulphureus sulphureus* (Beçak, 1969) and *Ptyas mucosus* (present study) no heteromorphic pair of chromosomes have been observed in either sex. In *Coluber fasciolatus*, *Chironius bicarinatus*, *Dryadophis bifossatus bifossatus*, *Drymarchon corais corais*, *Spilotes pullatus anomalepis* and *S. p. maculatus*, there is a heteromorphic pair of sex chromosomes in the females. In these cases, both *Z* and *W* are almost similar in size but the *Z* has its centromere in submedian region whereas in the *W* it is in the subterminal region. The difference in the morphology between the *Z* and the *W* can most simply be explained on the assumption of pericentric inversion. In *Elaphe quadrivirgata* and *E. climacophora* the *W* chromosome is slightly smaller than the *Z* and it has its centromere in the terminal and subterminal region respectively whereas in *Z* it is in the submedian region. The *Z* chromosomes of all the species in this group are more or less of similar size except in *C. fasciolatus*, *E. quadrivirgata* and *P. s. sulphureus* which are considerably larger. Similarly, the *W* chromosome in *C. fasciolatus* is the largest amongst all the species. Apart from the differences pointed out above, the karyotypes of all the species included in the Table 3 are almost identical.

In *Philodryas patagoniensis*, *P. olfersii olfersii*, *P. aestivus* and *Clelia occipitolutea* the diploid number of chromosomes is 36, constituted by 16 macro and 20 microchromosomes in the first 3 species whereas in the last named one the number is 50 constituted by 30 macro and 20 microchromosomes. The karyotypes of first 3 congeneric species are almost identical pair by pair in their relative lengths and centromeric indices (Table 3). The only difference lies in the size and morphology of the *W* chromosome. In *P. patagoniensis* the *W* chromosome is much smaller in comparison to the *Z* and has its centromere in the terminal region whereas in *P. olfersii olfersii* the *W* is almost as large as the *Z* and has a subterminal centromere. In *P. aestivus* the chromosome analysis is restricted to the male sex only and therefore the *W* chromosome is not known.

A superficial examination of the karyotype of *C. occipitolutea* does not reveal any obvious similarity with the karyotypes of the three species of *Philodryas*. For instance, in *C. occipitolutea* all the chromosomes are telocentrics except the *Z* which is submetacentric, while in *Philodryas* they have their centromeres in the median, submedian and subterminal regions. But an examination of the morphometric analysis presented in Table 3 reveals a good deal of similarity. The 14 pairs of telocentric chromosomes in *C. occipitolutea* can be presumed to have been derived, during evolution, from 7 pairs of chromosomes having median, submedian and subterminal centromeres of *Philodryas* by centromeric fission. This conclusion is substantiated from the data on

relative lengths given in Table 3 where it can be seen that the combined relative lengths of the two telocentrics are nearly equal to the corresponding chromosome with median, submedian and subterminal centromeres from which they are supposed to have been derived. The *Z* chromosomes in all the four species have their centromeres in a median region and occupy approximately 12% of the total haploid length. Another significant difference lies in the relative length of the *W* chromosome. In *C. occipitolutea* it is much larger than the *Z* and has a terminal centromere. Its relative length is 20% whereas the relative length of the *W* chromosomes in *P. patagoniensis* and *P. o. olfersii* are 6% and 11% respectively.

It may be pointed out that Beçak (1968) considered only the first 14 chromosomes of *C. occipitolutea* as macrochromosomes and the rest (36%) as microchromosomes, because of their gradual seriation. We, on the other hand, have considered the first 30 chromosomes as macrochromosomes and the rest as microchromosomes, the latter number being the same as in other species. The morphometric data given in Table 4 for this species have been calculated after measuring the individual chromosomes from a single plate available to us (Beçak, 1968). We have omitted only 20 microchromosomes instead of 36 as was done by Beçak. Therefore, the values given in the table are completely different from those of Beçak.

It is rather surprising that *Philodryas serra* (Beçak, 1969) is chromosomally completely different from the other congeneric species considered above. Its diploid number consists of 20 macro and 8 microchromosomes. It shows striking karyotypic similarities with *Erythrolamprus aesculapii venustissimus* and *Liophis meliaris* (Table 4) which also have 20 macro and 8 microchromosomes. A critical examination of the karyotype reveals that pair no. 6 of *P. serra* and pairs no. 8 and 9 of *E. aesculapii venustissimus* differ from the corresponding pairs in having terminal centromeres. This is easily explained as derivatives of pericentric inversions. The relative lengths of these chromosomes are almost similar to the relative lengths of the corresponding pairs from which they are supposed to have been derived (Table 4). Another significant difference lies in the relative lengths of the sex chromosomes. *Z* and *W* chromosomes of *P. serra* are considerably larger than the *Z* and *W* chromosomes of the other species (Table 4). Nothing is of course known about the *W* chromosome of *E. aesculapii venustissimus*. Apart from the above differences the rest of the karyotype is almost identical pair by pair which is apparent from Table 4.

It is therefore remarkable that *P. serra* is chromosomally more closely related to *Erythrolamprus* and *Liophis* than to its own congeneric species.

Table 4. *Colubridae* II

| Species | Pairs of macrochromosomes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Z | W |
| *Erythrolamprus aesculapii venustissimus* | 24/43 | 19/39 | 15/36 | 8/43 | 6/17 | 6/17 | 5/49 | 4[a]/0 | 3[a]/0 | 10/44 | ? |
| *Liophis meliaris* | 23/45 | 22/37 | 14/48 | 8/36 | 6/33 | 4/38 | 4/50 | 4/50 | 4/25 | 9/46 | 5/20 |
| *Philodryas serra* | 24/45 | 20/37 | 14/45 | 6/40 | 6/18 | 4[a]/0 | 4/29 | 3/28 | 3/50 | 15/49 | 12/10 |
| *Thamnodynastes strigatus* | 22/41 | 15/46 | 12/8 | 11/37 | 9/43 | 6/11 | 6/37 | 5/28 | — | 13/31 | 6/12 |
| *Thamnodynastes pallidus nattereri* | 23/38 | 15/47 | 12/13 | 12/32 | 4/0 4[b]/0 | 6/16 | 6/35 | 6/27 | — | 13/38 | 5/0 |
| *Tomodon dorsatus* | 21/37 | 14/41 | 11/8 | 12/23 | 10/47 | 7[a]/0 | 6/41 | 5/16 | — | 13/37 | 7/39 |

[a] Indicating an inversion.
[b] Indicating fission.

According to the classification list which we obtained from the British Museum (Natural History), all the above mentioned genera *(Philodryas, Liophis, Erythrolamprus)* have been put under subfamily *Natricinae* of the family *Colubridae*, except *Clelia* which has been placed in the subfamily *Lycodontinae* of the family *Dipsadidae*. According to the classification list of the American Museum of Natural History, all the species of *Philodryas* and *Clelia occipitolutea* have been put along with *Hydrodynastes* and *Thamnodynastes* under the subfamily *Xenodontinae* of *Colubridae* under a separate tribe *Alsophiini* whereas *Erythrolamprus* and *Liophis* under the tribe *Xenodontini* of the same subfamily.

If we compare the karyotype of *P. patagoniensis*, *P. o. olfersii*, *P. a. aestivus* and *C. occipitolutea* with those of the different species of *Colubrinae*, it will be obvious that the chromosomes of the two groups are very closely related. The relative lengths and centromeric indices given in Table 3 for the corresponding groups give a clearer indication of the same conclusion. It is therefore suggested that the genus *Philodryas* and *Clelia* may be considered for inclusion in the subfamily *Colubrinae*. The only difficulty for the suggested merger is the chromosome constitution of *Philodryas serra* published by Beçak (1960). We

think that the specimens from which the chromosome preparations were done should be reidentified or the name of the genus should be changed, because such a drastic change in the chromosomes in congeneric species in snakes is hardly ever expected.

The karyotype of *Tomodon dorsatus* is very much similar to the karyotypes of *Thamnodynastes strigatus* and *T. pallidus nattereri*. However, one metacentric chromosome of pair no. 5 is apparently missing in *T. p. nattereri* and instead two additional pairs of telocentric chromosomes are present, which can be derived from the corresponding pair by centromeric fission or *vice versa*. The combined relative lengths of the telocentric chromosomes is nearly equal to the relative length of the corresponding pair of chromosomes (Table 4). Another significant difference is found in pair no. 6 of *T. dorsatus*. It has a relative length similar to the corresponding pair of chromosomes of the other two species but its centromere is in the terminal region. This can be explained on the basis of a pericentric inversion. The morphology of the *W* chromosomes is also different in the three species. The centromere is in the subterminal region in *T. strigatus*, the terminal region in *T. p. nattereri* and the submedian region in *T. dorsatus*. Their relative lengths are 6, 5 and 7% respectively. The number of microchromosomes is 14 in all the 3 species whereas the numbers of macrochromosomes are 18, 20 and 18 respectively. Apart from these differences, the striking similarity in the karyotypes of all the three species is evident from the Table 4.

All three species have been placed by the British Museum in the subfamily *Natricinae* while the American Museum of Natural History has considered them to belong to *Xenodontinae*, but the latter authorities put *Tomodon* in the tribe *Hydropsini* while *Thamnodynastes* in another tribe, *Alsophiini*. Our chromosomal study however does not indicate sufficient difference for putting the two genera in two different tribes.

The diploid number of chromosomes of *Hydrodynastes gigas* and *H. bicinctus schultzi* is 24 constituted by 16 macro- and 8 microchromosomes. The karyotypes of these two congeneric species are almost identical pair by pair. Since chromosome analyses in these species are limited to the male sex only, the sex chromosome constitution of females is not known, however the 4th pair of chromosomes according to the size have been taken as *Z* chromosomes and plotted separately at the end. According to the American Museum of Natural History the Genus *Hydrodynastes* is related to *Thamnodynastes*, *Philodryas* and *Clelia* and has been put in the tribe *Alsophiini*. The British Museum on the other hand considers the genus to belong to the subfamily *Natricinae*. Our chromosome study shows that *Hydrodynastes* has a karyotype completely different from all others and therefore we prefer to group it separately.

*Oxyrhopus petolarius*, *Xenodon merremii* and *X. neuwiedii* have been, according to the American system, put under the tribe *Xenodontini* of the subfamily *Xenodontinae* whereas according to the British system *O. petolarius* is put separately under the family *Pseudoboidae* and *X. merremii* and *X. neuwiedii* under the subfamily *Xenodontinae* of the family *Dipsadidae*. Chromosome analysis has revealed that *Xenodon* and *Oxyrhopus* are chromosomally different from *Erythrolamprus* and *Liophis* with whom they are put under the tribe *Xenodontini*. Moreover, karyotypes of *X. merremii* and *X. neuwiedii* are almost identical, which is apparent from Table 5 whereas the karyotype of *O. petolarius* (Table 5) is completely different from *Xenodon*. In *Oxyrhopus* the diploid number of chromosome is 46 constituted by 16 macro- and 30 microchromosomes whereas in *Xenodon* $2n$ is $= 30$ constituted by 16 macro- and 14 microchromosomes. The morphology of these 16 macrochromosomes in the two groups are in no way comparable. Therefore the removal of *Oxyrhopus* from the subfamily *Xenodontinae* and its placement in a separate family *Pseudoboidae* by the British Museum appears to be justified.

*Boiga forsteni* and *B. trigonata* according to the American system of classification have been put in a separate tribe *Boigini* of the subfamily *Colubrinae* whereas according to British system, it has been given the separate status of subfamily *Boiginae* in the family *Dipsadidae*. Chromosome analysis has revealed 36 as the diploid number constituted by 18 macro and 18 microchromosomes. The *W* chromosome is larger than the *Z*. Both the congeneric species are almost identical in their karyotypes (Table 5). However, the morphology of the macrochromosomes of *Boiga* is different from all the species so far mentioned. Their karyotypes are more similar with those of the subfamily *Colubrinae* of the family *Colubridae* than with the genera put under the family *Dipsadidae* by the British Museum. Therefore its placement under a separate tribe of the subfamily *Colubrinae* by the American Museum is more justified.

*Gerardia prevostiana* and *Cerberus rhynchops* have a diploid number of 36 chromosomes consisting of 16 macro- and 20 microchromosomes. The karyotypes of these two species are almost identical (Table 5). The karyotypes of these two genera are almost identical with the karyotypes of all the species included under the subfamily *Colubrinae*. But according to the American classification they have been put under the tribe *Homalopsini* of the subfamily *Natricinae*, the chromosomes of which are totally different. According to the British system of classification they are given a status of a separate subfamily *Homalopsinae* of the family *Dipsadidae*. Other members of this family also have very little karyological similarity with these species. Chromosomally *Gerardia* and *Cerberus* belong to the subfamily *Colubrinae*.

Table 5. *Colubridae* III

| Species | Pairs of macrochromosomes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Z | W |
| *Hydrodynastes gigas* | 26/48 | 21/37 | 16/40 | 7/37 | 6/33 | 6/50 | 5/50 | 11 ?/40 | — |
| *Hydrodynastes bicinctus schultzi* | 26/46 | 22/36 | 15/47 | 9/50 | 7/43 | 6/33 | 5/19 | 9 ?/44 | — |
| *Xenodon merremii* | 26/50 | 22/38 | 15/47 | 8/46 | 7/30 | 6/35 | 5/42 | 13/41 | 7/41 |
| *Xenodon neuwiedii* | 26/46 | 21/36 | 17/50 | 7/39 | 6/13 | 5/32 | 5/49 | 13/40 | 8/35 |
| *Oxyrhopus petolarius* | 18/44 | 16/17 | 15/0 | 14/0 | 8/18 | 7/0 | 6/0 | 15/35 | 12/22 |
| *Boiga forsteni* | 23/49 | 18/40 | 13/48 | 5/50 | 3/49 | 3/50 | 9/22 | 9/21 | 12/31 |
| *Boiga trigonata* | 22/48 | 18/40 | 14/46 | 5/50 | 4/50 | 3/50 | 9/21 | 9/23 | 12/30 |
| *Gerardia prevostiana* | 23/48 | 21/41 | 15/45 | 10/43 | 8/37 | 7/30 | 7/29 | 10/41 | 6/31 |
| *Cerberus rhynchops* | 25/49 | 20/40 | 14/46 | 9/38 | 8/38 | 6/30 | 6/30 | 10/44 | 5/20 |
| *Lycodon aulicus* | 26/49 | 21/38 | 15/48 | 11/38 | 8/38 | 5/38 | 3/0 | 10/40 | 5/22 |
| *Rhabdophis tigrinus* | 26/47 | 15/50 | 15/45 | 8/45 | 15/0 | 5/0 | 4/0 | 11/40 | 11[a]/13 |

[a] Indicating an inversion.

In *Lycodon aulicus* the diploid number of chromosomes is 36 constituted by 16 macro- and 20 microchromosomes. The relative lengths and morphology of the macrochromosomes are different from other species having the same chromosome constitution (Table 5). According to the British Museum this species along with *C. occipitolutea* has been put under a separate subfamily *Lycodontinae* of family *Dipsadidae*. There is no similarity in the karyotypes of *Clelia* and *Lycodon*. We have already suggested the removal of *C. occipitolutea* from this subfamily.

*Rhabdophis tigrinus* has a diploid number of 36 chromosomes constituted by 16 macro- and 20 microchromosomes. This is again a unique species where the size and morphology of macrochromosomes is completely different from all the species discussed so far (Table 5). The *Z* and *W* chromosomes are equal in size but different in morphology. The *Z* chromosome has its centromere in the median region whereas

Table 6. *Colubridae* IV

| Species | Pairs of macrochromosomes | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Z | W |
| *Natrix maura* | 26/49 | 20/41 | 12/42 | 12/33 | 8/23 | 6/33 | 4/25 | — | — | — | 11/45 | 6/33 |
| *N. t. tesselata* | 26/46 | 20/40 | 11/40 | 11/50 | 9/33 | 7/43 | 5/40 | — | — | — | 11/45 | ? |
| *N. natrix helvetica* | 26/49 | 20/41 | 12/42 | 12/33 | 10/32 | 7/44 | 6/25 | — | — | — | 10/43 | 6/42 |
| *N. vibakari* | 26/45 | 20/36 | 15/44 | 7/0 | 8/45 | 7/33 | 5/26 | — | — | — | 11/42 | 9/16 |
| *N. stolata* | 26/47 | 17/44 | 16/46 | 13/41 | 10/44 | 7/15 | — | — | — | — | 11/45 | 10/16 |
| *N. piscator* | 28/47 | 16/50 | 15/46 | 15[a]/13 | — | — | 4/0 | 4/0 | 3/0 | 2/0 | 12/38 | 7/19 |

[a] Indicating an inversion.

the *W* in the subterminal region. Pericentric inversion appears to be a plausible mechanism to bring about the morphological difference between *Z* and *W*. This species has been put under the subfamily *Natricinae* by both the American and British Museum. However, according to the former it has been further placed in the tribe *Natricini* along with *Natrix*. But *R. tigrinus* is chromosomally completely different from them. Perhaps this may be a case of morphological convergence without sufficiently close genetic affinity.

In *Natrix maura*, *N. tessellata* and *N. natrix helvetica*, the diploid number of chromosomes is 34 constituted by 16 macro- and 18 microchromosomes. In *N. vibakari*, there are 16 macros and 20 micros. In *N. stolata* 14 macro and 22 microchromosomes make 36 as the diploid number whereas in *N. piscator* the diploid number is 42, out of which 18 are macro and 24 are microchromosomes. The karyotypes of the first four species show a good deal of similarity. Relative lengths (Table 6) and morphology of chromosomes are almost identical pair by pair excepting the 3rd and 4th pairs of *N. vibakari*. The third pair of chromosome is significantly larger in size, whereas the 4th pair is significantly smaller and has its centromere at the terminal region while in the remaining species the same pair is submetacentric. This can be explained on the assumption of the translocation of a major portion of the short arm of chromosome 4 to the 3rd and of a further pericentric inversion in the 4th pair. *W* chromosomes also show variation in size and morphology.

The karyotypes of *N. stolata* and *N. piscator* are in no way related with the karyotypes of other species of the genus. When we compare

the karyotypes of these two species we find that the first 3 pairs and the *Z* chromosomes are almost identical in their size and morphology. The 4th pairs of both the species have an achromatic gap at the distal end of the long arm, but this chromosome of *N. stolata* has its centromere in a submedian region whereas in *N. piscator* this is a subtelocentric chromosome. The relative lengths are almost the same (Table 6). This difference can be accounted on the assumption of a pericentric inversion. Pairs no. 5 and 6 are present only in *N. stolata* whereas pairs no. 7, 8, 9 and 10 are present only in *N. piscator*. Another significant difference is in the relative lengths of the *W* chromosomes, which are 10% and 7% respectively. Moreover, the number of microchromosomes is 20 in the former and 24 in the latter.

It should be pointed out that Singh, Sharma, and Ray-Chaudhuri (1968a) reported 40 as the diploid number of chromosomes in *N. piscator*. Our further studies on as many as 40 individuals of both the sexes revealed that two types of cells one with 40 and the other with 42 chromosomes are present in the same individual in this species. The counting of chromosomes has been made from spleen, bone marrow and testes, and this situation has been observed in all the tissues. In two individuals the frequency of metaphases having 40 chromosomes was found to be 60% whereas in the remaining 38 individuals majority of the cells had 42 chromosomes. This variation is concerning only the number of microchromosomes. At present we consider 42 as the diploid number for the species (Singh, Sharma, and Ray-Chaudhuri, 1970a).

All the species of *Natrix* have been put in the subfamily *Natricinae*. According to the list of classification of the British Museum the species *Natrix piscator* has been emended to *Xenochrophis piscator; Natrix stolata* to *Amphiesma stolata* and *Natrix vibakari* to *Amphiesma vibakari*.

Dutt (1970) reported that there are two populations of *Xenochrophis piscator*. In one, the diploid number of chromosomes is 36 consisting of 10 macro- and 26 microchromosomes whereas in the other the diploid number is 38 with 10 macro- and 28 microchromosomes. If the determination of the chromosome number by Dutt (1970) is correct, her results are quite at variance with that of Singh, Sharma, and Ray-Chaudhuri (1970a). Unfortunately, Dutt could not get good chromosome preparations as can be judged from her illustration (Dutt, 1970). In such preparations it is rather difficult to determine even the diploid number with accuracy.

As a matter of fact the karyotypes of *N.* (*Xenochrophis*) *piscator* and *N.* (*Amphiesma*) *stolata* are very different from other species of *Natrix*. Moreover, their karyotypes differ among themselves much more than what is usual between congeneric species. The emendation of the name of the genus therefore is quite acceptable from a cyto-

logical point of view. But the karyotypes of *N.* (*Amphiesma*) *vibakari* and *N.* (*Amphiesma*) *stolata* are also very different, the karyotype of the former being very much similar to other species of *Natrix*. It is therefore suggested that *N. vibakari* should remain as *N. vibakari*.

***Elapidae***

This large family includes roughly half of the non-marine venomous snakes. Out of 6 species which are chromosomally known so far, two, *Bungarus multicinctus* and *Naja n. atra* have been studied by Nakamura (1935) with the help of old classical techniques. Since his preparations are not adequate enough to reveal the morphology of the chromosomes, we have excluded them from the composite idiogram. Microchromosomes have not been taken into consideration while calculating the relative lengths.

In *Naja n. naja* and *Naja n. kaouthia* (this paper) the diploid number of chromosomes is 38. There are 16 macro- and 22 microchromosomes in both subspecies. The striking similarity between the karyotypes of them is quite apparent from the Table 7. All the chromosomes correspond pair by pair in their relative lengths and centromeric indices excepting pair no. 3, which has its centromere in a median region in *N. n. kaouthia* whereas in *N. n. naja* in the submedian region. It is rather surprising that *N. n. naja* which belongs to the highly evolved family *Elapidae* should have homomorphic sex chromosomes in both sexes. They have their centromeres in a submedian region and comprise approximately 11% of the haploid set. Sex chromosomes are morphologically differentiated in the subspecies *N. n. kaouthia*. The *Z* and *W* chromosomes have their centromeres in the submedian and subterminal region, respectively, and relative lengths of 10% and 7% of the haploid set respectively (Table 7). However the *W* chromosome is early replicating in *N. n. naja* and late replicating in *N. n. kaouthia* (Ray-Chaudhuri and Singh, in press). On the basis of the differentiation of the sex-chromosomes it can be postulated that *Naja n. kaouthia* is more highly evolved than *N. n. naja*. The morphological differentiation of the *W* chromosome has probably taken place by gradual elimination of genetically inert material from the *W*. The 3rd pair of chromosomes of *N. n. kaouthia* also can be derived from the similar pair of *N. naja naja* by pericentric inversion.

The karyotype of *Bungarus caeruleus* (Singh, Sharma, and Ray-Chaudhuri, 1970b) is more similar to that of *Micrurus lemniscatus carvalhori* (Beçak, 1969) than that of *Naja*. The diploid number of chromosomes is 43 in females and 44 in males in the former species whereas 42 in the males of latter. There is a multiple sex determining mechanism of $Z_1Z_1Z_2Z_2$ ♂/$Z_1Z_2W$ ♀ type in *B. caeruleus* (Singh, Sharma,

Table 7. *Elapidae*

| Species | Pairs of macrochromosomes | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $Z_1(Z)$ | $Z_2$ | W |
| *Naja n. kaouthia* | 24/48 | 14/49 | 12[a]/39 | 4/36 | 4/36 | 3/26 | 20/13 | — | — | — | 10/29 | — | 7/20 |
| *Naja n. naja* | 26/48 | 15/49 | 13/42 | 5/27 | 4/25 | 3/31 | 22/15 | — | — | — | 11/35 | — | 11/35 |
| *Bungarus caeruleus* | 14/0 | 13/0 | 12/0 | 8/0 | 7/0 | 7/0 | 6/0 | 5/0 | 4/0 | 3/0 | 12/36 | 6/0 | 23/41 |
| *Micrurus lemniscatus carvalhori* | 15/0 | 13/0 | 11/0 | 9/0 | 9/0 | 8/0 | 7/0 | 7/0 | — | 4/0 | 11/33 | 6/0 | — |

[a] Indicating an inversion.

and Ray-Chaudhuri, 1970b). Since the chromosome analysis in *M. lemniscatus* is limited to the male sex only, the sex chromosome constitution is not known. The number of microchromosomes is 20 in both species whereas there are 24 macrochromosomes in the male and 23 in the female of *B. caeruleus* and 22 in *M. lemniscatus*. In both species all macrochromosomes have their centromeres in terminal regions excepting the *Z* and *W* in the former species and one pair, probably the *Z* chromosomes, in the latter species which have their centromere in the median region. The striking similarity in the karyotype of these two species is revealed by Table 7. There is a pair of macrochromosomes in *M. lemniscatus* which is identical with the $Z_2$ of the *B. caeruleus* in its relative length and centromeric index and has been represented in the table as $Z_2$. The $Z_2$ pair of chromosomes was originally a pair of autosome of which one member in the female has been translocated to the *W* chromosomes. Therefore the $Z_2$ pair in the table indicates the pair of autosomes of *M. lemniscatus* which has become $Z_2$ in *B. caeruleus*. One pair of macroautosomes (pair no. 9) is absent in the former species but present in the latter. The relative length of this pair is 3% of the haploid set. *W* is the largest in the whole complement. It has a median centromere and occupies approximately 23% of the total haploid length in *B. caeruleus*. Nothing is known about the sex chromosome constitution in *M. lemniscatus*, which is only a matter of conjecture until females are studied. When we compare the karyotype of *B. caeruleus* or *M. lemniscatus* with that of *Naja*, we find the latter to be totally different.

***Hydrophiidae***

Since this family has been chromosomally investigated only by us, in order to be more exact, we have taken the liberty of including the

microchromosomes also in total haploid length for calculating the relative length of macrochromosomes.

The only report on the chromosomes of sea snakes is that of Nakamura (1935). He studied only the males of *Laticauda semifasciata* with the help of the classical technique and reported 38 (14 macro- and 24 microchromosomes) as diploid number. The published account of the morphology of macrochromosomes is not clear enough to be measured and compared with the karyotypes of other species of this family, and therefore the species has been excluded from the Table 8. Recently Singh (1972) has reported 32 as the diploid number in the males and 33 in the females of *Enhydrina schistosa*, thus making the sex chromosome constitution of $ZZ$ ♂/$ZW_1W_2$ ♀. Here $W$ is one of the microchromosomes. The extension of karyotype analysis to five more species included in the present paper has revealed that a multiple sex chromosome complex of the kind described in *E. schistosa* is quite widespread in the family *Hydrophiidae*. The diploid number of chromosomes in *Hydrophis spiralis* is 32, in *H. ornatus ornatus* is also 32, in *H. cyanocinctus* is 33 and in *H. fasciatus fasciatus* and *Microcephalophis gracilis* 35.

*H. spiralis* having usual $ZZ$ ♂/$ZW$ ♀ sex determining mechanism, which is most common in snakes can be taken as a species having a standard karyotype from which the rest of the karyotypes of *Hydrophiidae* studied so far can be derived. The karyotype of *H. ornatus ornatus* is identical with the karyotype of *H. spiralis* which is quite evident from Table 8. Both of them have 32 as the diploid number of chromosomes constituted by 14 macro- and 18 microchromosomes. We do not know about the $W$ chromosome of the former species because our chromosome analysis has been confined to the male sex only. Since all the macrochromosomes in this species are individually distinguishable, it has been possible for us to identify the $Z$ chromosomes after comparing the karyotype with that of *H. spiralis*.

When we compare the karyotype of *H. cyanocinctus* with that of *H. spiralis* we find that the number of microchromosomes is 18 in both the species whereas the macrochromosomes are 15 and 14 respectively. Excepting the sex chromosomes the karyotype of both species is almost identical. The main difference between these two congeneric species lies in their sex chromosome constitution. In *H. spiralis* the $W$ chromosome is considerably big occupying approximately 8% of the haploid set. It has its centromere in the subterminal region. The sex chromosome constitution of *H. cyanocinctus* is $ZZ$ ♂/$ZW_1W_2$ ♀. The $W_1$ and $W_2$ chromosomes, having terminal centromeres, occupy approximately 3% and 2% of the total haploid length. Another difference between *H. spiralis* and *H. cyanocinctus* lies in pair no. 1. There is a characteristic achromatic

Table 8. *Hydrophiidae*

| Species | Pairs of macrochromosomes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Z | $W_1$ | $W_2$ | Micro |
| *Hydrophis spiralis* | 20/49 | 18/35 | 11/44 | 9/45 | 6/45 | 4/22 | 8/39 | 8/21 | — | 14/0 |
| *Hydrophis o. ornatus* | 21/50 | 18/37 | 12/47 | 10/46 | 7/46 | 5/25 | 9/39 | — | — | 18/0 |
| *Enhydrina schistosa* | 19/49 | 15/39 | 11/48 | 9/44 | 7/45 | 5/30 | 8/44 | 4/0 | 2[b]/0 | 19/0 |
| *Hydrophis cyanocinctus* | 20/50 | 17/36 | 11/47 | 10/45 | 6/44 | 5/24 | 9/40 | 3/0 | 2[b]/0 | 16/0 |
| *Hydrophis f. fasciatus* | 19/48 | 17/37 | 14/48 | 5/0 | 4[b]/0 | 6/43 | 4/25 | 10/47 | 6/46 | 3[c]/34 | 14/0 |
| *Microcephalophis gracilis* | 19/49 | 17/38 | 12/48 | 5/0 | 4[b]/0 | 6/43 | 5/23 | 8/49 | 5/22 | 4[c]/35 | 15/0 |

[b] Indicating fission.
[c] Indicating fission and inversion.

gap in the homologous region of both the chromosomes in the latter which is absent in the former (see Fig. 15).

Karyotypes of *H. fasciatus fasciatus* and *Microcephalophis gracilis* differ from *H. spiralis*, *H. ornatus ornatus*, *Enhydrina schistosa* and *H. cyanocinctus*. In the latter four species the 4th chromosome pair, comprising approximately 9% of the haploid set have their centromeres in the median region. This chromosome is absent in the former two species and replaced by two pairs of chromosomes having terminal centromeres and measuring about 5% and 4% of the haploid set in both *H. f. fasciatus* and *M. gracilis* (Table 8). If we add the relative lengths of both the chromosomes having terminal centromeres of *H. f. fasciatus* and *M. gracilis* the value comes to about 9% which corresponds to the relative length of the 4th metacentric chromosome of the remaining four species, which clearly indicates centromeric fission or fusion as the reason for the karyotypic difference.

*H. f. fasciatus* and *Microcephalophis gracilis* also differ from *H. spiralis* in their sex chromosome constitution. They have a sex determining mechanism of $ZZ♂/ZW_1W_2♀$. Their $Z$ chromosomes are mediocentric and therefore similar to the conservative $Z$ of other species. The $W_1$ and $W_2$ chromosomes in *H. f. fasciatus* have their centromeres in the median and subterminal regions and measure about 6% and 3% respectively of the haploid set (Table 8). The $W_1$ and $W_2$ in *M. gracilis* on the other

hand, have subterminal and submedian centromeres and measure about 5% and 4% respectively. The karyotype of *H. f. fasciatus* is identical with that of *M. gracilis* excepting the size and morphology of $W_1$ and $W_2$ as mentioned above.

The karyotype of *E. schistosa* is almost identical with the karyotype of *H. spiralis* excepting in the structure of the sex chromosomes. As a matter of fact there is a striking similarity in the karyotypes of all the 6 species belonging to 3 different genera namely *Enhydrina, Hydrophis* and *Microcephalophis* belonging to the family *Hydrophiidae.*

We have suggested that the multiple *W*'s have originated by centromeric fission of the original *W* which is evident from $W_1$ and $W_2$ of *E. schistosa* and *H. cyanocinctus* which have terminal centromeres. In *H. fasciatus fasciatus* and *M. graciolis* $W_1$ and $W_2$ have further undergone inversion giving rise to median and subterminal centromeres in the former and subterminal and submedian in the latter (Table 8). The total relative length of microchromosomes is also more or less identical in different species. It should be pointed out that the karyotype of *H. f. fasciatus* is more closely related with the karyotype of other species of *Hydrophis.*

It is now quite apparent that in this family, speciation has occurred mainly at the expense of the 4th pair of autosomes and the sex chromosomes in general and *W* in particular. It appears that in this family fission and inversion have played an important role to bring about the structural rearrangements.

### *Viperidae*

There are 24 species of this family which are chromosomally known so far. Out of these, 8 species (see Table 1) have been studied by using the old classical techniques and therefore have been excluded from Table 9.

It is customary to divide the family *Viperidae* into two subfamilies *Viperinae* and *Crotalinae.* Out of the 6 genera (16 species) which we have included in Table 9, four genera viz., *Bothrops, Crotalus, Lachesis,* and *Agkistrodon* belong to the subfamily *Crotalinae* whereas the two genera *Vipera* and *Echis* belong to the subfamily *Viperinae.*

The diploid number of chromosomes is 36 in all the species irrespective of the subfamilies to which they belong, except in *V. aspis aspis* and *V. aspis zinnikeri* where it is 42. The number of microchromosomes is 20 and the number of macrochromosomes is 16 in all the species except in the last two species mentioned, where it is 22. The striking similarity in the karyotypes of all the species is very clear from the Table 9. The karyotypes of *V. a. aspis* and *V. a. zinnikeri* differ from the general pattern in having 6 pairs of chromosomes with submedian and subterminal

Table 9. *Viperidae*

| Species | Pairs of macrochromosomes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Z | W |
| *Bothrops jararaca* | 26/48 | 21/40 | 15/47 | 8/39 | 7/29 | 7/41 | 6/31 | 10/48 | 7/23 |
| *Bothrops moojeni* | 26/48 | 21/39 | 15/47 | 8/40 | 7/30 | 7/44 | 6/39 | 10/47 | 6/29 |
| *Bothrops alternatus* | 23/50 | 20/39 | 14/50 | 9/43 | 8/23 | 8/38 | 6/33 | 11/50 | 6/27 |
| *Bothrops jararacussu* | 26/48 | 20/38 | 15/47 | 9/38 | 8/30 | 7/39 | 6/36 | 10/47 | 6/32 |
| *Bothrops pradoi* | 26/49 | 21/38 | 15/49 | 8/46 | 8/36 | 6/46 | 6/31 | 10/48 | 6/24 |
| *Bothrops insularis* | 25/48 | 21/37 | 15/45 | 9/40 | 8/27 | 7/35 | 6/31 | 10/46 | 7/31 |
| *Crotalus durissus terrificus* | 26/49 | 21/39 | 15/49 | 9/39 | 7/28 | 7/40 | 6/31 | 10/45 | 6/31 |
| *Crotalus viridis oreganus* | 25/44 | 20/34 | 15/50 | 9/42 | 8[a]/0 | 6[a]/0 | 6[a]/0 | 10/47 | — |
| *Crotalus v. lutosus* | 25/44 | 19/41 | 13/49 | 9/46 | 8[a]/0 | 8[a]/0 | 6[a]/0 | 11/50 | — |
| *Echis carinatus* | 25/48 | 21/42 | 15/48 | 10/38 | 7/17 | 7/31 | 6/31 | 9/39 | 6/34 |
| *Lachesis muta noctivaga* | 27/40 | 18/36 | 16/45 | 8/32 | 7/18 | 6/43 | 6/43 | 10/44 | 6/22 |
| *Agkistrodon halys* | 25/46 | 22/41 | 15/43 | 9/40 | 7/20 | 6/25 | 6/22 | 9/42 | — |
| *Vipera b. berus* | 26/48 | 21/39 | 15/48 | 9/39 | 8/22 | 6/42 | 6/42 | 10/30 | 6/42 |
| *Vipera ursinii rakosiensis* | 27/45 | 22/37 | 14/47 | 7/37 | 7/21 | 6/39 | 5/32 | 11/31 | 5/35 |
| *Vipera a. aspis* | 13/27 12[c]/23 | 12/17 8[c]/16 | 8/16 8[c]/16 | 9/39 | 7/36 | 7/36 | 7/36 | 10/45 | 7/36 |
| *Vipera a. zinnikeri* | 14/24 13[c]/19 | 14/23 8[c]/16 | 7/15 6[c]/17 | 9/35 | 7/26 | 6/36 | 5/33 | 11/45 | 7/28 |

[a] Indicating an inversion.
[c] Indicating fission and inversion.

centromeres in place of the first 3 pairs of chromosomes having median centromeres common for the all other species. These 6 pairs of chromosomes in *V. a. aspis* and *V. a. zinnikeri* can be considered to have been derived, during evolution, from 3 pairs of metacentric chromosomes which are common for the remaining 14 species, by centromeric fission. The data given in Table 9 show that the combined relative lenghts of the two acrocentrics is nearly equal to the corresponding metacentric chromosome from which they are supposed to have been derived. Since out of 16 species of this family which are chromosomally known so far, in 14 the first 3 pairs of chromosomes are metacentric and only in the two species the change has taken place, it is more plausible to think that the latter have originated from the former by centromeric fission. It can further be presumed that after centric fission these 6 pairs of chromosomes in *V. a. aspis* and *V. a. zinnikeri* have undergone pericentric inversion.

In *C. viridis oreganus* and *C. v. lutosus* the chromosomes of pairs no. 5, 6 and 7 have their centromeres in the terminal region whereas the same chromosomes pairs of the others species have submedian centromeres. Their relative lengths are almost identical. Pericentric inversions may easily account for this change. The karyotype of *E. carinatus* is almost identical with the karyotypes of the rest of the species in all respect.

There is a pronounced heteromorphism between Z and W chromosomes of the females in all the species studied so far, except in *C. v. oreganus* and *C. v. lutosus* where no data are available on sex chromosomes. It is quite apparent from the Table 9 that the karyotype of the family *Viperidae* is essentially homogeneous. Both Z and W chromosomes in this family are very conservative. The Z chromosome has an almost similar morphology in the different species of the family and its relative length varies from 9% to 11% of the haploid length. The W chromosome also has a similar morphology in different species. It is also very conservative in this family in its morphology and relative length which varies from 5% to 7%.

The highly evolved family *Viperidae* is frequently divided into two groups (subfamilies) on the basis of presence (*Crotalinae*) or absence of a facial pit (*Viperinae*). This grouping does not find any support from the study of their chromosomes.

The discussions presented so far have demonstrated clearly that the chromosomes of snakes are potentially extremely suitable material for an evaluation of phylogenetic relationships within and between various taxa established through morphological studies alone. The most serious limitation is the lack of sufficient data for the purpose. The number of species studied in any group is extremely limited and

moreover quite a significant part of the data available had to be omitted because they were not suitable for the purpose. We hope that future workers when describing the karyotype of any species will publish details of the measurements of the chromosomes and their centromeric indices. We strongly believe that when sufficient data accumulate it would be possible for the cytotaxonomists to contribute significant new evidence which will help to clarify many complex taxonomic situations. Some of the recent techniques of staining constitutive heterochromatin (Arrighi and Hsu, 1971, Hsu and Arrighi, 1971) and its further modification by Drets and Shaw (1971) and others by which it has been possible to recognize the human chromosomes individually, will very soon enable the cytotaxonomist to compare the chromosome structure in much more detail than it has been hitherto possible.

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