Chromosomes of *Bungarus caeruleus* Schneider
(Elapidae: Ophidia)

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Reference to the cytological literature indicates that the chromosomes of the Elapidae have been reported in two Elapid snakes, *Bungarus multicinctus* and *Naia naia atra* by Nakamura (1935). They are remarkable in having different karyotypes: the karyotype of *B. multicinctus* has four metacentrics and that of *N. naia atra*, ten. The fundamental chromosome number in the former is 40 and the latter, 48. The present study deals with the chromosomes of the commonly available Indian krait, *Bungarus caeruleus*, with the details of meiosis.

**Material and methods**

Testes of a number of specimens of *Bungarus caeruleus* were fixed throughout the year in Champy's fluid as modified by Nakamura (1928). According to the usual paraffin method, the material was sectioned at 10–12 micra. The sections were bleached with hydrogen peroxide and then kept in Chura's fluid (Chura 1925) for 12 hours. Newton’s gentian violet, iron-haematoxylin and Feulgen’s reagent were employed as stains. Squash preparations of the fresh material were also made for study. The drawings were made with the help of a camera lucida, at a level 12.5 cm below the microscope stage, using Leitz’s 25× ocular and 1/12 oil-immersion lens, at a magnification of 4,000 times.

**Observations**

The male diploid complement of *Bungarus caeruleus* as observed at the spermatogonial metaphase plate (Figs. 1 and 2) consists of 44 elements, which are distinguishable into macro- and micro-chromosomes. The macro-chromosomes are 24 in number and ordinarily lie scattered on the periphery of the equatorial plate. Of these 24 elements, two are V-shaped but their arms are of unequal lengths and possess sub-median spindle fibre attachment, while the remaining 22 are acrocentric, rod-shaped chromosomes of intergrading sizes. The micro-chromosomes consist of 20 rod-shaped bodies and possess terminal spindle fibre attachments. It is not possible to distinguish sex chromosomes from the autosomes. The male karyotype of *B. caeruleus*, therefore, has the formula:
Meiosis. In the leptotene nucleus (Fig. 3), the chromosomes present a meshwork of indistinct threads, which do not take up any stain whatsoever and also do not show any sign of polarization. The pairing of the homologous threads now starts and the threads become polarized. The details of the pairing process are not clearly observable. By the time the pairing is complete, the zygotene nucleus (Fig. 4) shows a tangled mass of threads which occupies only a portion of the nuclear cavity. From this polarized tangled mass, arise somewhat thicker and stouter threads and the pachytene stages sets in. At this stage (Fig. 5), the bivalents show marked condensation.
and their ends, which are clearly made out, bear heterochromatic, Feulgen-positive knobs. The process of condensation of the bivalents continues and after some time the separation of the partners in bivalents starts. It may commence proximally, distally or interstitially. The late diplotene nucleus (Fig. 6) shows 22 paired bodies, of which, 10 are quite small in size and apparently do not show any chiasmata. The remaining 12 bivalents comparatively are much bigger in size and the number of chiasmata, present in each one of them, varies to a considerable degree. Some of them show one, while the others may have two, three or even four chiasmata each. The bivalents now become greatly thickened and the nucleus passes into the diakinetic stage. The diakinetic nucleus (Fig. 7) shows 22 elements which remain scattered all over the interior of the nucleus. Although, some reduction in the number of chiasmata from diplotene to diakinesis occurs, still most of the macro-tetrads hold at least a pair of chiasmata each. No 'lampbrush' fibres are observed at any of the meiotic prophase stages.

The distribution of the chromosomes on the equatorial plate of the first metaphase recalls the typical reptilian pattern. The characteristic arrangement is clear in the polar view (Figs. 8 and 9), where 22 elements consisting of 12 macro- and ten micro-tetrads are present. Invariably the micro-tetrads lie at the interior encircled by the macro-tetrads. Most of the macro-tetrads still hold a pair of chiasmata each and appear like ring-shaped bodies, but one of them is V-shaped and obviously corresponds to two V-shaped chromosomes seen at the spermatogonial metaphase. It is impossible to spot out the sex-bivalent. At the first anaphase, the partners in all the bivalents separate normally and pass to their respective poles at the same time. In polar view (Fig. 10), two groups of chromosomes, each consisting of 22 elements, are noticed. Interkinesis appears to be completely abolished and immediately the second metaphase succeeds the first anaphase.

In polar view of the second metaphase (Figs. 11 and 12), 22 univalent chromosomes are always seen to be arranged in a rosette-like fashion. Some of the bigger elements are precociously split and, therefore, the rod-shaped chromosomes appear like V-shaped ones. Presumably, two monadial components remain attached together at the points where the centromeres are placed. Similarly, the V-shaped dyad appears as double V’s, as if, two V-shaped monads have been superimposed. As usual, 10 micro-dyads remain scattered at the centre of the spindle and 12 macro-dyads lie on the periphery of the equatorial plate. The staining capacity of the chromosomes of the second metaphase is poor as compared to those of the first metaphase chromosomes. As the second anaphase gets under way, each dyad is separated into two monadial chromosomes. Only one type of spermatids are produced. The details of the spermatid nucleus could not be followed because of the fact that the chromosomes are extremely small in size (Fig. 13) and quite often show a strong tendency to becoming
clumped together.

Figs. 14-19. 14 and 15, serial arrangements of the spermatogonial chromosomes corresponding to Figs. 1 and 2, respectively. 16 and 17, serial arrangements of the first metaphase bivalents corresponding to Figs. 8 and 9, respectively. 18 and 19, serial arrangements of the second metaphase chromosomes corresponding to Figs. 14 and 15, respectively.

Discussion

From the family Elapidae, karyotypes of two snakes (Bungarus multicinctus and Naia naia atra) have been studied so far by Nakamura (1935). For B. multicinctus Nakamura describes 36 chromosomes, which consist of four V-shaped, 18 rod-shaped and 14 dot-shaped bodies. The karyotype of B. caeruleus differs from that of B. multicinctus, in that it possesses four rod-shaped and six dot-shaped chromosomes extra and two V-shaped metacentrics fewer. The loss of two metacentrics and the simultaneous incorporation of four rod-shaped chromosomes in the karyotype of B. caeruleus can be explained in the light of Robertson's law (Robertson 1916), but it is difficult to explain the increase in the number of dot-shaped chromosomes. Another point, that draws the attention of the present author, is the fundamental chromosome number, which in the snakes has been considered by Nakamura (1935) to be 46, at least in the families Colubridae and Viperidae. As Nakamura classifies B. multicinctus under the sub-family Elapinae of the family Colubridae (classification according to
Boulenger 1896), it is pertinent to apply these findings to *B. caeruleus* as well, although, according to the more recent classification by Smith (1943), *Bungarus* is placed under the family Elapidae. No doubt, the results obtained by the present author for *B. caeruleus* can be well reconciled with Nakamura’s formulations, but no explanation is offered by Nakamura himself for the karyotype of *B. multicinctus*, where the fundamental chromosome number is forty and, which thereby, falls short of his own formulations.

The karyotype of *B. caeruleus* also differs from the karyotype of *Naia naia atra* because the fundamental chromosome number in the former is 46 and the latter, 48.

When compared to the different karyotypes found in the family Colubridae, the karyotype of *B. caeruleus* appears much similar to those of *Macropisthodon* and *Dinodon* (Nakamura 1935), as all these snakes possess two V-shaped metacentrics and many rod-shaped acrocentric chromosomes. But the fundamental chromosome number is different.

The karyotype of *B. caeruleus* also resembles the karyotypes of the members of the family Geckonidae and of certain Agamid lizards (*Sitana ponticerina*, Makino and Asana 1948). A parallel case of the occurrence of two V-shaped chromosomes is offered by *Gerrhonotus*—Anguidae (Matthey 1931, 1949).

**Summary**

1. The male diploid chromosome complement of *B. caeruleus* shows 44 chromosomes which consist of 2 V-shaped, 22 rod-shaped and 20 dot-shaped elements.
2. The fundamental chromosome number is 46.
3. This karyotype differs markedly from the karyotype of *B. multicinctus* described by Nakamura (1935).
4. Meiosis is normal and the male is the homogametic sex.

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