W Chromosome in the Indian Water Snake (Checkered Keel Back) *Natrix piscator* (Colubridae)

Female heterogamety has been definitely established at least in 2 families of serpents, Colubridae and Viperidae, through cytological studies (BEÇAK[1]). In all the species of the above families, whenever the chromosomes of the females have been studied, a *ZW* chromosome complex has been obtained, except in *Natrix rhombifera* reported by VAN BRINK[2] where no heterogamety was reported either in males or in females. In the Indian water snake, *N. piscator*, on the other hand, the *Z* and *W* chromosomes could be definitely identified by us in the females; and the chromosomes of both males and females of this species form the subject of this communication.

Two males and 2 females of *N. piscator* were used for the study. The slides were prepared by air drying procedure from the colchicinized marrow of ribs and stained in 1% unna blue. The centromeric positions on the chromosomes are described according to the system proposed by LEVAN et al.[3].

Slides prepared from all 4 individuals yielded nearly 100 good metaphase plates. The diploid number of chromosomes is almost invariably 40 in the males (Figure 1), as well as in the females (Figure 2). A sharp difference in size between the 5 pairs of macrochromosomes (1–4 and *ZW*, Figure 3) and 15 pairs of microchromosomes is evident from the plates. A further classification of the microchromosomes into 2 groups is possible. The first group (Figure 3, 4–9) are slightly bigger in size compared with the rest of the 11 pairs of microchromosomes. In the female plates the chromosomes of one of the pairs are heteromorphic (Figure 3) in all the cells. One of the members of the heteromorphic pair, with its centromers in the median region in the female plates, is similar to 1 of the homomorphic pairs in male plates and is the *Z* chromosome. The other member, distinctly smaller in size and having a subterminal centromere, must be the *W* chromosome. Both the *Z* and *W* chromosomes, as the case may be, can be distinguished in every metaphase plate of either sex by their size and centromeric position.

The centromeric position in the first pair of autosomes is at the median point, in the second and third pairs it is in the median region, and in the fourth pair it is in the subterminal region. The centromere in the larger microchromosomes appears to be in the terminal region, whereas in the smaller ones it is in the terminal point. All 5 pairs of macrochromosomes can be individually identified in all the cells.

Thirty-six appears to be the most common diploid chromosome number in the 70 species of snakes studied

[1] W. BEÇAK, Mammalian Chromosomes Newsletter *8*, 1, 4 (1967).
[2] J. M. VAN BRINK, Chromosoma *10*, 1 (1959).
[3] A. LEVAN, K. FREDGA and A. A. SANDBURG, Hereditas *52*, 201 (1964).

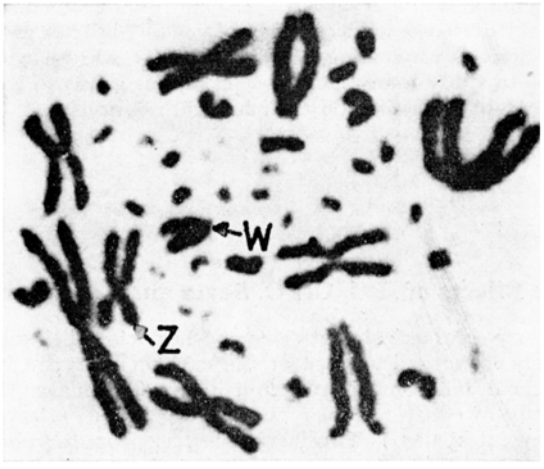


Fig. 2. A female metaphase plate of *Natrix piscator* showing heteromorphic sex chromosomes (*ZW*). × 2000.

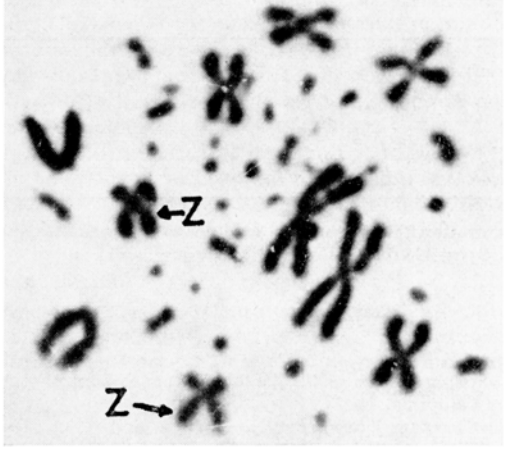


Fig. 1. Metaphase plate of somatic cell from bone marrow of *Natrix piscator* male showing homomorphic sex chromosomes (*ZZ*). × 2000.

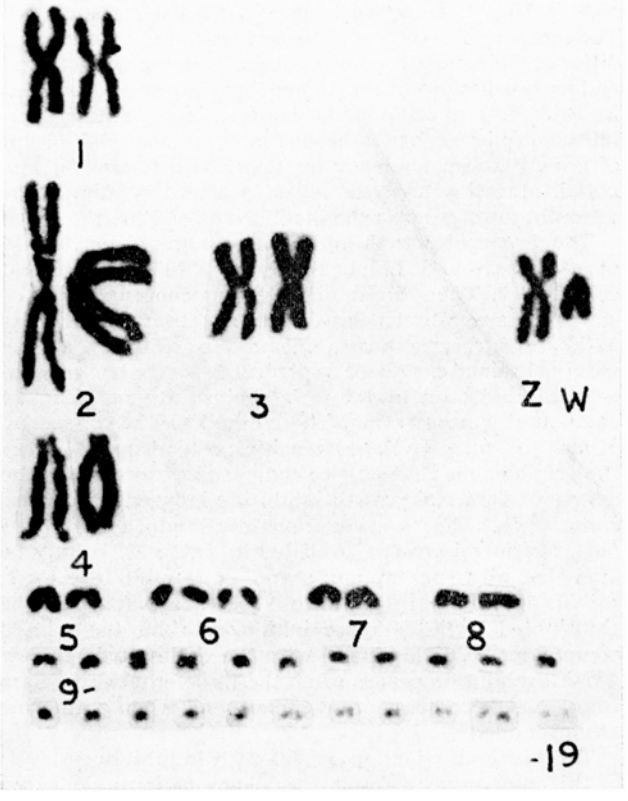


Fig. 3. Karyotype corresponding to Figure 2. × 2000.

so far. Four species of the genus *Natrix* have been studied and all but *N. tigrina* (NAKAMURA[4,5]) have 36 chromosomes. *N. stolata*, the only other Indian species studied (BHATNAGAR[6]), has also the same chromosome number. The chromosome number 40 found in the present species is exactly the same as in *N. tigrina* studied by NAKAMURA in Japan. Both the species have the same number of chromosome arms, which is 50. Any further comparison of the 2 karyotypes cannot be made because *N. tigrina* was studied by using classic techniques.

Twenty-five species of *Natrix* have been incorporated by SMITH[7] in the fauna of the British India Volume. BOULENGER has grouped the species within the genus into 3 species groups (SMITH[7]), with *N. piscator* in group II and *N. stolata* in group III. Our finding of chromosomal differences between the species of 2 species groups of *Natrix* is interesting, and therefore it will be worthwhile to study many more species of this genus to throw some light if possible on their natural relationship[8].

Zusammenfassung. Die diploide Chromosomenzahl beider Geschlechter beträgt 40: 10 Macrochromosomen und 30 Microchromosomen. Die Geschlechtschromosomen *ZZ* (♂) und *ZW* (♀) sind cytologisch erkennbar.

L. SINGH, T. SHARMA and S. P. RAY-CHAUDHURI

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 5 (India), 31 July 1967.

[4] K. NAKAMURA, Proc. imp. Acad. Tokyo *3* (1927).
[5] K. NAKAMURA, Mem. Coll. Sci. Kyoto Univ. B *4*, 1 (1928).
[6] A. N. BHATNAGAR, Caryologia *12*, 349 (1960).
[7] M. A. SMITH, *Fauna of British India* (Taylor and Francis, London 1943), vol. 3.
[8] The authors are grateful to the University Grants Commission, India, and the Ford Foundation, USA, for financial support.

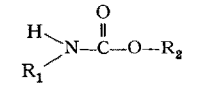
The Effects of IPC, CIPC, Sevin and Zectran on *Bacillus subtilis*

Many commerical pesticides and herbicides being used widely in agriculture are of the carbamate type. Their chemical relationship to ethyl carbamate make them worthy of study for their possible deleterious effects on biological systems. Two herbicides, isopropyl-*N*-phenyl carbamate (IPC) and its chlorine derivative isopropyl-*N*-clorophenyl carbamate (CIPC) and 2 insecticides, *N*-methyl-1-napthyl carbamate (Sevin) and methyl-*N*-dimethyl-amino 3, 5-xylyl carbamate (Zectran) were studied for their phenotypic and genotypic effects on *Bacillus subtilis* 168i⁻[1], along with several simple carbamates[2]. The compounds have the same basic carbamyl moiety but differ in their substituents at the carboamino portion (R_1) and carboethoxy portion (R_2) of their molecules, as listed in Table I. The compounds, because of their poor solubility were tested at low levels, however some inhibition of growth and a tendency for long chain formation was noted. Mutation analyses revealed that the compounds were not mutagenic at the indole locus of *B. subtilis*.

The degree of growth inhibition was scored by the use of a Bausch and Lomb Spectronic '20' photoelectric colorimeter. Tubes containing various concentrations of compounds were innoculated, incubated with aeration at 37 °C and turbidity measurements were read at 6 h. The values obtained expressed as percentage of control growth, were plotted on a probit scale against the logarithm of compound concentration. The method has been used by others to study bacterial sensitivity to drugs[3,4]. From the graph one is then able to compare and quantitate the degree of bacterial growth inhibition induced by various compounds. The concentration levels which permitted 50% of control growth are listed in Table II. It may be seen that all the compounds were more inhibitory to *B. subtilis* 168i⁻ growth than ethyl carbamate and that the chlorinated CIPC is more inhibitory than the related compound IPC. This fits in with the finding that greater growth inhibition occurs when the basic ethyl carbamate compound becomes more complex, and when a chlorine atom is added[2].

The bacteria, when grown for 24 h in inhibitory levels of the compounds showed a tendency to form elongated chains, as was reported with ethyl carbamate and related compounds[2].

Table I. Structures of carbamates under study



Name	Substituent R_1	R_2
Ethyl	H	C_2H_5
IPC	Phenyl	iC_3H_7
CIPC	Chlorophenyl	iC_3H_7
Sevin	CH_3	Napthyl
Zectran	CH_3	Dimethylamino 3,5-xylyl

Table II. Growth inhibition of *B. subtilis*

Compound	IC 50[a] %
Ethyl carbamate	2.09
IPC	> 0.025[b]
CIPC	0.005
Sevin	0.07
Zectran	0.012

[a] Concentration that permits 50% of control growth. [b] Higher dose level not possible due to insolubility of compound.

[1] Indole requirer of *B. subtilis*, obtained from S. Zamenhof, University of California.
[2] R. DEGIOVANNI-DONNELLY, S. KOLBYE and J. A. DIPAOLO, Mutation Research *4*, 543 (1967).
[3] H. J. TREFFERS, J. Bact. *72*, 108 (1956).
[4] W. P. LOCKHART and R. N. WEAVERS, J. Bact. *80*, 331 (1960).