

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 (BHATNAGAR⁶), 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 SMITH⁷ in the fauna of the British India Volume. BOULENGER has grouped the species within the genus into 3 species groups (SMITH⁷), 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 relationship⁸.

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).
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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*-chlorophenyl carbamate (CIPC) and 2 insecticides, *N*-methyl-1-naphthyl 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 carbamates². The compounds have the same basic carbamyl moiety but differ in their substituents at the carboamino portion (R₁) and carboethoxy portion (R₂) 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 inoculated, 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 added².

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 compounds².

Table I. Structures of carbamates under study

$$\begin{array}{c} \text{O} \\ \parallel \\ \text{H} \text{---} \text{N} \text{---} \text{C} \text{---} \text{O} \text{---} \text{R}_2 \\ \diagup \\ \text{R}_1 \end{array}$$

Name	Substituent	
	R ₁	R ₂
Ethyl	H	C ₂ H ₅
IPC	Phenyl	iC ₃ H ₇
CIPC	Chlorophenyl	iC ₃ H 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).

The mutagenic effect was studied by determining the back-mutation rate from indole requiring to indole independent, the method has been reported in detail elsewhere². The poor solubility of the compounds prevented the testing of these compounds at higher levels for greater growth inhibition in a 24 h mutation assay. The results show that none of the compounds increase the back-mutation rate of *B. subtilis*. However, it was also found that in this system ethyl carbamate failed to have an effect on back-mutation rates (Table III).

The results show that the compounds studied do not have a detectable effect upon the genotype of *B. subtilis* but they do inhibit growth and cause a tendency for long chain formation such as one sees with ethyl carbamate and higher homologues of ethyl carbamate. These results are similar to the finding that Sevin induces abnormal mitotic patterns but no tumors in the roots of *Allium cepa*⁵. IPC showed no carcinogenic potential in mice and

rats, although some affect on growth was noted⁶. In another interesting study⁷, IPC and CIPC were shown to have the same tumor initiating property as ethyl carbamate but to a much lesser degree and had no effect on the incidence of lung adenomas. The results of such investigations seem to underline the fact that the many phased activities of ethyl carbamate probably involve different modes of action which may be imitated to some extent in various systems by a variety of related compounds which should be tested further⁸.

Résumé. On a étudié les effets phénotypiques et génotypiques de 2 insecticides (Sevin et Zectran) et 2 herbicides (IPC et CIPC) sur *B. subtilis*. Les composés carbamiques inhibent la croissance, induisent une tendance à la formation de longues chaînes. Ils n'ont toutefois pas un effet mutagénique sur le locus de l'indole de *B. subtilis*.

ROSALIE DEGIOVANNI-DONNELLY,
SUSANNAH M. KOLBYE
and PATRICIA D. GREEVES

Table III. Back mutation analysis

Compound	Concentration %	Mutation rate ^a	
		control	experimental
Ethyl carbamate	2.5	3.5	2.2
IPC	0.01	8.0	9.0
CIPC	0.007	17.0	11.0
Sevin	0.07	10.0	13.0
Zectran	0.01	12.0	35.0

^a Bacterium/generation $\times 10^{-9}$.

Bionetics Research Laboratories, Inc. Falls Church
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- ⁵ S. AMER, *Naturwissenschaften*, 51, 1 (1964).
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⁷ G. J. VAN ESCH, H. VAN GENDERSEN and H. H. VINK, *Br. J. Cancer* 12, 355 (1958).
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I cromosomi di alcuni *Pipidae* (Amphibia Salientia)

I *Pipidae*, la cui importanza e la cui diffusione sembrano essere state per il passato assai maggiori che oggi (HECHT¹), sono tuttora esistenti in Africa e nell'America del Sud.

Dei 3 generi africani (*Xenopus*, *Hymenochirus* e *Pseudohymenochirus*) solo *Xenopus* era stato studiato cariologicamente (MIKAMO e WITSCHT²); esso presenta 36 cromosomi, tutti meta- o submetacentrici.

Io ho studiato il corredo cromosomico di alcuni esemplari di *Hymenochirus boettgeri*: questa specie ha 24 cromosomi, suddivisibili in 9 coppie di omologhi grandi e 3 di piccoli, tutti meta- o submetacentrici (Figura 1). Tra *Xenopus* e *Hymenochirus* esistono dunque notevoli differenze cariologiche.

Circa i *Pipidi* americani, dei quali sono note 5 specie, gli AA. non sono d'accordo sul loro status tassonomico: per il passato, ogni specie costituiva un genere a se stante; DUNN³ ritiene invece che esse facciano parte di un unico genere, *Pipa*; la classificazione di DUNN è quella generalmente più seguita al giorno d'oggi.

Di queste specie, era finora cariologicamente nota solo *Pipa pipa* (WICKBOM⁴): secondo l'A., essa presenta 22 cromosomi, con 4 coppie di omologhi grandi metacentrici, 3 di omologhi grandi acrocentrici e 4 di omologhi piccoli acrocentrici.

Io ho studiato il corredo cromosomico di alcuni esemplari di *Pipa (Protopipa) parva*, gentilmente forniti dal Prof. O. A. REIG, in collaborazione col quale, in un pros-

simo futuro, conto di elaborare in modo più approfondito i dati raccolti.

Questa specie, unica tra gli Anuri finora studiati, possiede un corredo cromosomico formato esclusivamente da

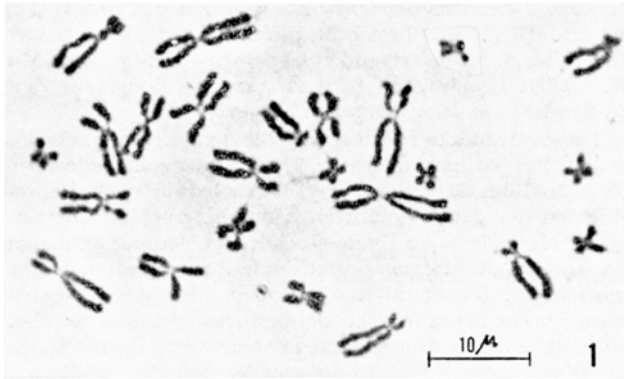


Fig. 1. Metafase intestinale di un ♂ di *Hymenochirus boettgeri*.

- ¹ M. K. HECHT, *Syst. Zool* 12, 20 (1963).
² K. MIKAMO e E. WITSCHT, *Cytogenetics* 5, 1 (1966).
³ E. R. DUNN, *Am. Mus. Novit.* 1384, 1 (1948).
⁴ T. WICKBOM, *Hereditas* 36, 363 (1950).