Table I. Respiration and aerobic glycosis in *C. utilis* and *T. globosa*, as well as in the mitochondrial mutants of these species with small colonies

Strain	Qo_2	Qco_2
Candida utilis 766	40.9	2.7
Mutant 12–3	1.9	131.3
Torulopsis globosa 697	15.4	15.1
Mutant 11–3	0.7	161.1

Table II. Detection of antimetabolites synthesized by actinomycetes with the aid of *C. utilis*, *T. globosa* and their mitochondrial mutants

Inhibition of growth	Frequency (%)
Candida utilis 766 and its mutant 12–3	0.04
Only mutant 12–3	0.09
Torulopsis globosa 697 and its mutant 11–3	1.60
Only mutant 11–3	5.00

was much more difficult as compared with the induction of such mutants in the classical species *Saccharomyces cerevisiae*. Nevertheless, by using trypaflavine in the concentration 5 μg/ml as a mutagen, which was diluted in the nutrient medium containing 10% of beer wort and 0.6% of glucose, and plating suspensions of yeast cells after 24, 48, and 72 h of the exposure to the mutagen at 28 °C, a few strains of mitochondrial mutants were isolated in *Candida* and *Torulopsis*. In plating suspensions of yeast cells, we used diagnostic colour differentiation medium of NAGAI[5], containing a mixture of eosin with trypan blue. The colonies of mutants on this medium were brilliant purple, and contrasted with the normal ones, which tinted grayish violet.

Table I gives data on respiration and aerobic glycolysis in *C. utilis* and *T. globosa*, as well as in the mitochondrial mutants of these species. All these cultures grow well on mineral agar of the following composition (medium M-9): NH_4Cl – 1 g, KH_2PO_4 – 3 g, Na_2HPO_4 – 6 g, $MgSO_4$ – 0.13 g, glucose – 4 g, agar–15 g, H_2O – 1 L; pH 7.0–7.2.

In the screening of new antimetabolites synthesized by microorganisms, we used the method of agar blocks. Cultures of actinomycetes were grown on agar plates in Petri dishes containing medium No. 2 (tryptone broth –30 ml, peptone 5 g, glucose 4 g, agar 15 g, H_2O – 1 L; pH 7.0–7.2). After 7 days of growth at 28 °C, agar blocks (diameter 8 mm) were cut from agar plates, and placed on the surface of new agar plates in Petri dishes, containing medium M-9 as well as medium No. 2. On the surface of these plates were spread suspensions of the test microbes (cultures of yeasts as well as of their mitochondrial mutants). In case the growth of the test microbe was inhibited by the agar block on synthetic medium M-9, but not inhibited on the rich nutrient medium No. 2, one could infer the presence of an antimetabolite in the agar block, which was synthesized by the culture of the actinomycete under study.

2160 cultures of actinomycetes freshly isolated from various soil samples were investigated, and Table II gives information concerning synthesis of antimetabolites by these cultures, which were active against test microorganisms used by us. In accordance with the data of Table II, the employment of mitochondrial mutant of *T. globosa* 11–3 produces most interesting results. In 5% of cultures of actinomycetes used in our work, the synthesis of antimetabolites was detected with the aid of this mutant, which could not be detected with the aid of other tests used by us. Further studies have shown that cultures of actinomycetes which were active against mitochondrial mutant *T. globosa* 11–3, were inactive against *Escherichia coli* B as well as its mutant 19–8. The latter was induced by us with the aid of N-methyl-N′-nitro-N-nitrosoguanidine, and possessed increased permeability to a number of inhibitors, including actinomycin D. It is therefore possible to conclude that mitochondrial mutant of *Torulopsis globosa* 11–3 represents considerable interest in the screening for new antimetabolites synthesized by microorganisms, since it can detect products which remain unnoticed with the aid of many other tests.

ВЫВОДЫ. У дрожжей *Candida utilis* и *Torulopsis globosa*, хорошо растущих на синтетических питательных средах с минеральным азотом, были получены митохондриальные мутанты с мелкими колониями. Наиболее интересным оказался мутант *T. globosa* 11–3, который у 5% обследованных культур актиномицетов позволил обнаружить образование антиметаболитов, которые не могли быть выявлены с помощью других тестов.

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Chromosome Studies and Chromatographic Analysis of Free Amino Acids in Two Species of Coleoptera

In spite of the contributions of DUTT[1] and others, the chromosome studies of Indian Coleoptera remains incomplete. This paper reports the chromosome studies and free amino acid analysis of 2 beetles, *Gonocephalum depressum* and *Scleron* Sp., beetles belonging to family Tenebrionidae.

Materials and methods. The specimens were collected from the suburbs of Bangalore, S. India. They are found in large numbers all through the year. The chromosome studies were made on the testes material. Lacto-Aceto-orcein and Feulgen squashes were made.

For the study of free amino ocids, each of the species were starved, but allowed to take water for 48 h, in order

[1] M. K. DUTT, Curr. Sci. *22*, 278, (1955).

to eliminate food or residue from the alimentary canal. Alimentary canal, testes and ovary were homoginized separately in 80% distilled alcohol and centrifuged. The extract was dried and subjected to two dimensional paper chromatography. The haemolymph was extracted and tested for free amino acids. The following solvent systems were tried: a) butanol: Acetic acid: water: 40:7:5 (v/v/v), b) butanol: water: ethanol: 20:7:5 (v/v/v) for the first dimension and phenol: water: isopropanol: 70:25:5 (v/v/v) for the second. The spots were identified by spraying the paper with 0.25% and 0.4% ninhydrin in acetone.

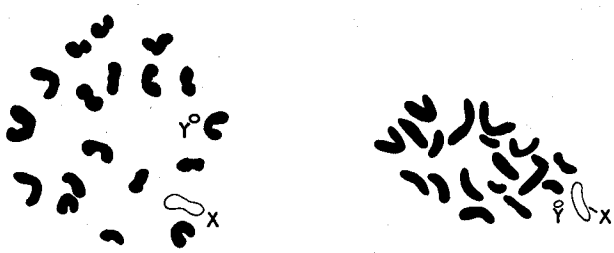

Fig. 1. A and B. Spermatogonial metaphase plates.

Observations. Gonocephalum depressum lives in moist sandy soil and feeds on dead and decaying organic matter. *Scleron* Sp. lives under the bark of trees and feeds on plant material.

20 chromosomes were counted in the spermatogonial metaphase plates of both the forms (Figures 1a and 2a). There are 18 autosomes and 2 sex-chromosomes. The autosomes fall into 2 actegories, namely the acrocentrics and mediocentrics. The mediocentrics fall into large and small mediocentric chromosomes. Table I shows the sex mechanism, the number of acrocentrics, large mediocentrics and small mediocentrics of two species.

There is a difference in the number of acrocentrics and mediocentric chromosomes in the two forms. The *X*-chromosome is acrocentric and is almost of the same size as the first pair of acrocentric autosomes. The *Y*-chromosome is small, very minute and dot like. It is always found in association with the *X*-chromosome. The sex chromosomes are positively heteropycnotic in the early prophase of meiosis. They become negatively heteropycnotic with the approach of anaphase. In the karyotype the sex

[2] T. V. RAMAKRISHNA AYYAR, *Handbook of Economic Entomology of South India* (revised edn., Government of Madras, India 1963), p. 339.

Table I. Chromosome morphology of *Gonocephalum depressum* and *Scleron* Sp.

Name of species	Acrocentrics	Mediocentrics	Mediocentrics		Sex mechanism
			Long	Small	
Gonocephalum depressum	3 Pairs	6 Pairs	4 Pairs	2 Pairs	XY-Type
Scleron Sp.	6 Pairs	3 Pairs	1 Pair	2 Pairs	XY-Type

Table II. List of free amino acids identified in the different tissues of *Gonocephalum depressum* and *Scleron* Sp.

Sl No.	Name of the amino acid	*Gonocephalum depressum*				*Scleron* Sp.			
		A	T	O	H	A	T	O	H
1.	Serine	+	+	+	+	+		+	+
2.	Alanine	+	+	+	+	+	+		+
3.	Lysine	+	+	+	+		+	+	
4.	Methionine	+	+	+	+	+	+	+	+
5.	Threonine	+				+			
6.	Arginine			+	+	+			+
7.	Cysteic acid		+	+	+			+	
8.	Citrulline		+	+	+				+
9.	Leucine		+	+			+	+	
10.	Hydroxy proline	+	+	+	+			+	+
11.	Histidine	+	+	+					
12.	Glycine		+		+	+		+	
13.	Tyrosine						+		
14.	Glutamic acid	+	+		+	+		+	+
15.	Isoleucine	+							
16.	Proline	+	+		+			+	+
17.	Aspartic acid	+	+						
18.	Valine	+	+		+				
19.	B-Alanine	+	+						
20.	Cysteine	+	+						
21.	Tryptophan	+							

+, present; A, alimentary canal; T, testes; O, ovary; H, haemolymph.

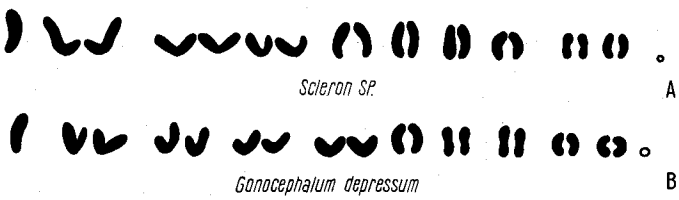

Fig. 2. A and B. Karyotype. The chromosomes are arranged according to their lengths.

chromosomes can be easily identified, as they stand out distinctly from the autosomes.

As described by SAXENA et al.[3], the presence or absence of various amino acid compounds in the whole insect and in different tissues is indicated in the Table II.

Discussion. The characteristics of organisms for animal taxonomic studies are morphological, ecological, cytological and geographical. Since morphology and physiology of organisms are determined by biochemical processes, in recent years biochemical characteristics have been taken into consideration[4]. These characteristics help to determine the systematic position of the organisms[5]. The present investigation deals with the similarities and differences in qualitative (presence/absence) distribution of the amino acids in the whole animal and in certain tissues of the animal.

The chromosome study of the two species shows that both of them possess the basic diploid number 20, characteristic of Coleoptera[6]. But they show a difference in the number of mediocentric and acrocentric chromosomes. They also show qualitative differences in the free amino acid pattern. The present cytological and biochemical investigations support the differences found in morphological and ecological characteristics.

Résumé. Le nombre des chromosomes diploïdes est de 20 dans les deux espèces de Coléoptères Ténébrionïdes *Gonocephalum depressum* et *Scleron* sp. Il y a 18 autosomes et 2 chromosomes sexuels. Ils s'observent dans les acides amino libres. Dasns le *Scleron* sp., il n'a en a que 14. La systématique de ces espèces est discutée sur la base de leurs caractères biochimiques.

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Ultrastructural Localization of Newcastle Disease Virus Surface Antigen in Infected HeLa Cells as Revealed by an Enzyme-Labelled Antibody Method

Previous studies have shown that both protein components of paramyxoviruses, the S and V antigens, are synthesized in the cytoplasm[1] and the viruses mature at the cell surface[2,3]. The S antigen has been reported to be incorporated in the nucleocapsid filaments, and the V antigen (surface antigen) in the spikes of budding particles and virions[4]. But the actual ultrastructural localization of the V antigen in infected cells before virus budding takes place has not yet been elucidated. The enzyme-labelled antibody method has recently been successfully employed for the ultrastructural localization of various cellular antigens[5]. Few papers have dealt with the application of this method in detection of viral antigens[6,7]. The purpose of this experiment was to identify the location of the Newcastle disease virus (NDV)-V antigen in HeLa cells at the early stage of infection by applying this method.

Materials and methods. Indirect enzyme-labelled antibody method was employed in this experiment. Specific antibody against V antigen was prepared by the dissociation of virus-antibody complex (HAI: 1/320)[8]. Conjugation of horseradish peroxidase (BEHRINGER und SÖHNE, Mannheim) to anti-rabbit IgG goat antibody was done with glutaraldehyde according to the method of AVRAMEAS[9]. Before use, all the antisera were absorbed with human liver powder and HeLa cell powder. Normal rabbit serum and antisera against Sendai virus (HVJ) served as control sera.

HeLa cells were infected with NDV (Miyadera strain) at a multiplicity of 10 p.f.u. per cell. Uninfected HeLa cells were used as controls. NDV-infected HeLa cells were fixed in a mixture of 0.5% glutaraldehyde and 3% paraformaldehyde in 0.05*M* phosphate buffer, pH 7.4 for 20 to 30 min at 4°C at 3, 4, 5, 7, 9, 15, and 19 h after infection. After overnight washing in several changes of PBS, the cells were incubated with specific antibody directed against NDV-V antigen for 24 h with gentle agitation, followed by washing PBS and treatment with peroxidase-labelled anti-rabbit IgG goat antibody for a further 24 h at 4°C. After washing thoroughly in several changes of PBS, the cells were refixed with phosphate buffered 2%

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