Cytological Investigations on some Indian Diplopoda (Myriapoda)

THE class Myriapoda has received scant attention from cytologists, the order Diplopoda even less. Among the earlier contributions to this field of study, mention may be made of Oettinger[1], Sokoloff[2] and Bessiere[3]. Barring a recent contribution to the cytology of three South Indian species of Diplopoda by Natarajan[4], no attention has been devoted to chromosome studies in this group.

This note presents the chromosomal cytology of eight South Indian species of Diplopoda investigated for the first time; these were collected from different parts of the state of Mysore. The scheme of classification adopted here is based on the investigation of Attems[5].

Three species of the family Harpagophoridae (*Harpurostreptus* sp., *Ktenostreptus* sp. and *Thyropygus* sp.) reveal different diploid chromosome numbers of twelve, twenty and twenty-four respectively. The autosomal heteropycnosis in *Thyropygus* sp., regular 'Bouquet' formation in *Ktenostreptus* sp. in early meiotic prophase and tetraploidy in *Harpurostreptus* sp. are some of the significant observations made in the course of this investigation. *Chondromorpha mammifera* Attems is a member of the family Strongylosomidae. The diploid chromosome number is fourteen. Very extensive endopolyploidy has been observed in this form. *Strongylosoma* sp., a member of the same family, exhibits the diploid chromosome number of twenty-four.

The other three forms investigated are *Arthrosphaera zebraica* and two unidentified species of the genus *Arthrosphaera* belonging to the family Sphaerotheridae. They are commonly called pill-millipedes. They present the highest diploid chromosome numbers for the group: twenty-six, thirty and thirty respectively.

In addition to the variation in chromosome numbers, the cytology of diplopods offers many points of interest. In all the species studied all the chromosomes are acrocentrics. The sex-chromosome mechanism is of the XY type. The prereductional meiotic division and succession are a few of the characteristics of the sex-chromosomes. The occurrence of single chromatid bridges in the meiotic anaphase of one of these (*Arthrosphaera zebraica*) is a significant observation.

It is too early to evaluate the importance of cytological data based on the numerical relationships of the chromosomes of a few species, although it indicates the probable existence of a cytological basis for taxonomic purposes. It is hoped that the completion of the cytological analysis of large numbers of species will afford a clearer perspective of the evolutionary history and phylogeny of this ancient group.

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B. N. CHOWDAIAH

Department of Zoology,
Central College,
Bangalore.

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Ribonucleic Acid-storage Inclusions of Freshwater Sponge Archaeocytes

THE presence of RNA in the lenticular, metachromatic inclusions of freshwater sponge archaeocytes has been recently commented on by Ruthmann[1]. Typically, the RNA occurs in conjunction with a basic protein to form a combined structure of characteristic appearance (*RS* in Fig. 1).

In attempts to determine the ultrastructural organization of the RNA–protein complex, by means of RNase digestion techniques, Ruthmann was unable to determine conclusively whether the RNA was directly bound to fibrillar protein or was carried on ribosomes presumed present in the complex.

We have been examining the structure and functions of the archaeocyte inclusions of the Australian freshwater sponge, *Ephydatia multidentata*, with techniques very similar to Ruthmann's and, in general, have come to similar conclusions. However, we have also used an additional technique the results of which strongly suggest that a large proportion of RNA, if not all of it, is carried on ribosomes which are attached to the fibrillar protein of the inclusions. In this technique electron-microscope sections were prepared from archaeocyte inclusions fixed in veronal acetate buffered with 1 per cent osmium tetroxide (*p*H 7·3) for 2 h, followed by 1 per cent potassium permanganate solution for 30 min and treated with 2 per cent uranyl nitrate. Definite leaching out occurred in the ribonucleoprotein 'shell' material of the inclusions which assumed the appearance shown in Fig. 2, where the darker nucleoprotein region can be seen to be perforated with many small 'holes' the diameter of which is in the order of 40 mμ. Their arrangement is orderly and in some regions there is evidence of a lamination reminiscent of membranes or fibrils. Since potassium permanganate solutions are known to remove ribosomes from the endoplasmic reticulum, we consider that a similar event has occurred here, and the section may be interpreted to mean that the spaces and laminations have been caused to appear by the removal of ribosomes from the fibrillar protein matrix. Neither we, nor Ruthmann, have obtained so definite a picture with RNase-treated material, although a faint suggestion of it has sometimes been seen.

Ruthmann has also speculated on the source of deoxyribonucleotides for chromosomal replication during the initial phase of histogenesis. Many workers[3–6] have noted

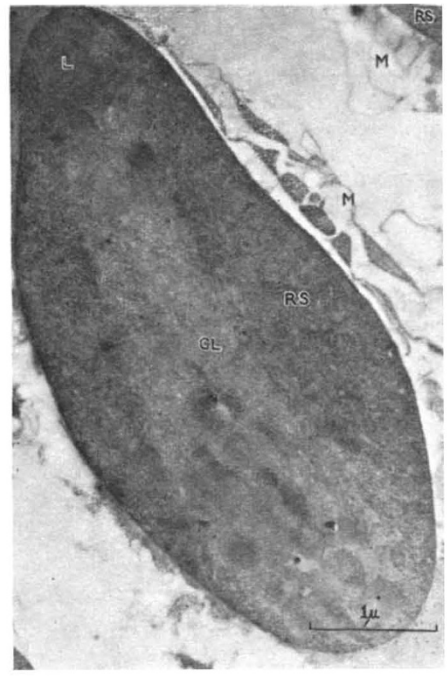

Fig. 1. Electron micrograph of an archaeocyte lenticular granule within the gemmule. Veronal acetate buffered 1 per cent osmium tetroxide, 2 h, 0·1 per cent uranyl acetate in absolute methanol, 1 h; *in toto*. *RS*, Ribonucleoprotein 'shell' material; *GL*, inner region of the granule composed mainly of acidophilic proteins and glycoprotein; *M*, phospholipid membrane surrounding granule; *L*, lipid droplet