CHROMOSOME STUDIES IN INDIAN DIPLOPODA (MYRIAPODA): II
A NOTE ON THE OCCURRENCE OF TRANSLOCATION IN *AULACOBOLUS EXCELLENS*

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ABSTRACT

The chromosome cytology of male meiosis of a millipede species, *Aulacobolus excellens* (Silvestri) (Pachybolidae: Diplopoda) has been studied using air-drying technique. The paper reports the occurrence of a translocation complex involving autosomal bivalents and the sex-bivalent (XY) in Diplopoda. The role of translocation polymorphism in natural populations is stressed.

INTRODUCTION

In recent years there has been a significant growth of interest in Diplopod cytogenetics. The introduction of air-drying[1], acetic-saline-Giemsa (ASG)[2] and Giemsa C-banding[3] techniques to study diplopod chromosomes has renewed interest in the chromosome cytology of Diplopoda. The literature on the cytology of Diplopoda has been reviewed recently[4]. This paper presents an account of male meiosis of a south Indian Diplopoda, *Aulacobolus excellens*, by the application of air-drying technique. The development of a translocation complex in the course of male meiosis of this species is also presented here.

MATERIALS AND METHODS

Males of *Aulacobolus excellens* (Silvestri) (family Pachybolidae) constituted the material for the present cytological studies. The specimens were collected from Alagarkovil hilly tracts, near Madurai, south India, during the monsoon season.

Adult males were injected with 0.5 ml of 0.05% colchicine and after 5 hr, testes were dissected in normal saline. Testes follicles were pretreated separately with one of the hypotonic solutions[5], such as 0.125 M potassium chloride (KCl) and 0.016 M sodium citrate (Na citrate) for 1 hr at room temperature. The use of 0.125 M KCl was found suitable for a fairly good preparation of meiotic prophase chromosomes, while 0.016 M Na citrate gave the best results in the preparation of mitotic as well as meiotic metaphase chromosomes.

Air-drying methods[6] were employed as in the previous studies[1]. Pretreated testes follicles were thoroughly minced in hypotonic solution for 15 min and centrifuged at 800–1000 r.p.m. for 5 min each time. The supernatant was discarded and the cell button was resuspended in the hypotonic solution twice, followed by centrifugation. Later the cellular material was fixed in 5 ml of freshly prepared fixative (3 methanol:1 glacial acetic acid) for 30 min at room temperature. After recentrifugation and removing the supernatant twice, the cells were resuspended in 1 ml of fresh fixative. A few drops of cell suspension were dropped on each of the alcohol cleaned slides wetted with ice-cold distilled water using a Pasteur pipette. The slides were dried on a histological slide warmer at 60°C for 2 min and stained in 2% Giemsa working solution for 5 min at room temperature. Finally, the slides were rinsed in distilled water and air/heat-dried and mounted in Euparal.

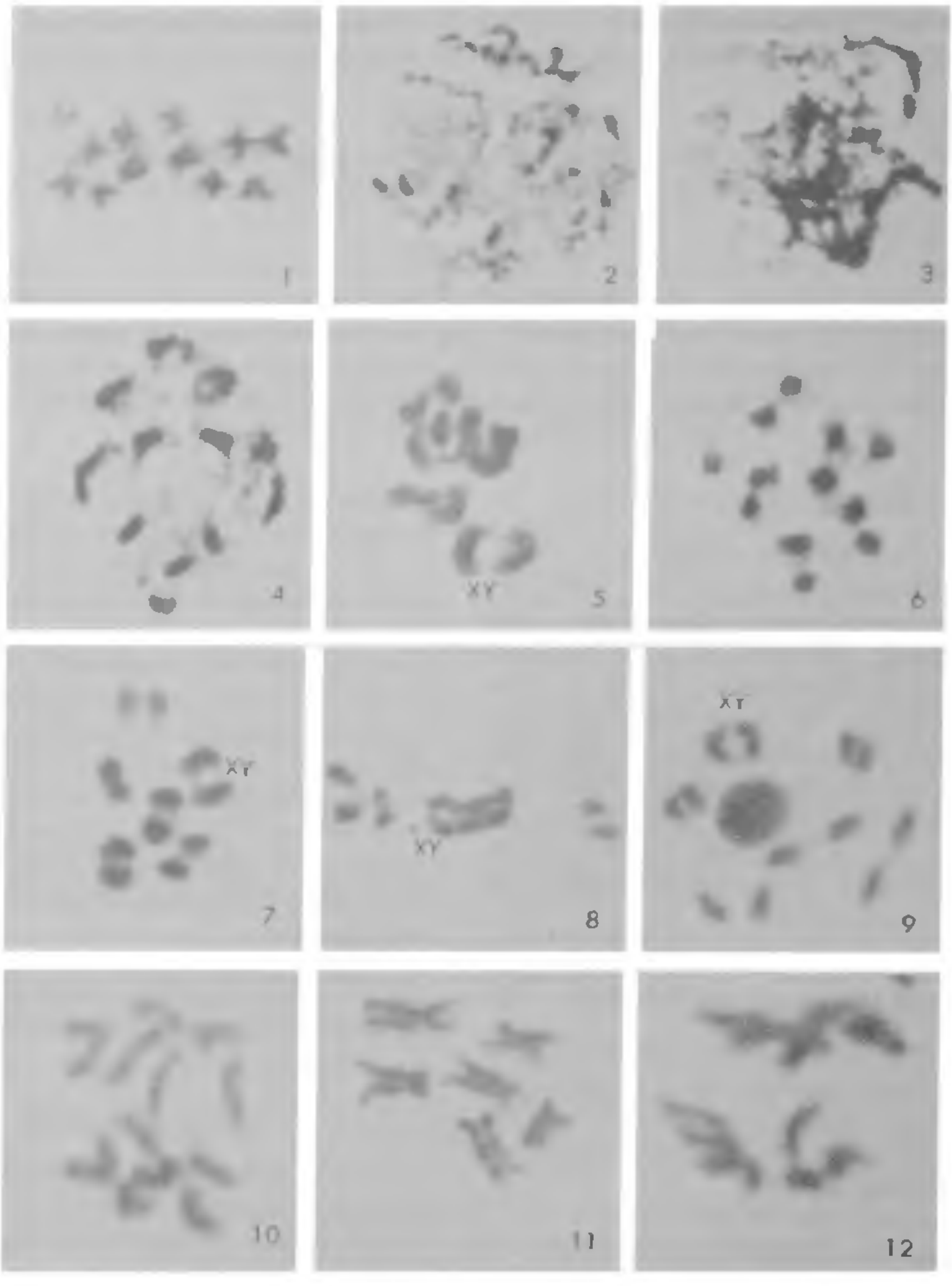
Photomicrographs were made on 35 mm NP 22 ORWO (ASA 125) panchromatic film using a Carl Zeiss microscope with a dark green filter.

OBSERVATIONS

The diploid chromosome number ($2n$) as revealed by somatic metaphase is 12 (figure 1). The male is heterogametic with an XY type of sex-mechanism.

The leptotene (figure 2) chromosomes appear as unpaired slender chromatin strands with numerous chromomeres along their length. Zygotene 'bouquet' (figure 3) shows paired homologous chromosomes with several chromomeres and dark-staining heterochromatic knobs. At mid-prophase (figure 4) 12 dark-staining knobs have been observed, whose number corresponds to the diploid number of this species. A late diakinetic configuration (figure 5) shows the bivalents at various levels of terminalization of chiasmata. The presence of non-staining/lightly staining gaps has been characteristically observed in the pre-metaphase bivalents (figure 6).

At metaphase I (figure 7), both autosomal and sex-



Figures 1–12. Photomicrographs of air-dired, Giemsa-stained, chromosome preparations showing the somatic metaphase as well as the course of male meiosis in *Aulacobolus excellens*. **1.** Somatic metaphase (2n-12). **2.** Leptotene nucleus **3.** Zygotene 'bouquet' **4.** A mid-prophase nucleus. **5.** A late diakinetic configuration. **6.** Premetaphase bivalents showing non-staining gaps. **7.** Metaphase I, showing dumb-bell shaped bivalents. **8.** Metaphase I, showing a translocation chain comprising 2 autosomal bivalents and the sex-bivalent (XY). **9.** Late metaphase I. **10.** Anaphase I (side view). **11.** Metaphase II. **12.** Anaphase II (side view).

bivalents appear to be dumb-bell shaped. At late metaphase I, the presence of 3 rod-shaped and 3 ring-shaped bivalents has been observed. The ring-shaped sex-bivalent (XY) is conspicuous by its large size (figure 9). The occurrence of a translocation chain comprising two autosomal bivalents and the large sex-bivalent (XY) is highly significant (figure 8).

Meiosis I is reductional (figure 10) while the second division is equational, for all the chromosomes. Thus in metaphase II (figure 11), 6 half-bivalents/dyads are

seen and in each one of them the chromatid separation is clearly visible. During anaphase II (figure 12), the sister chromatids separate and the ensuing daughter chromosomes occupy the opposite poles.

DISCUSSION

A. excellens has the lowest diploid number of chromosomes ($2n = 12$) among the species of the family Pachybolidae thus far studied. All the chromosomes appear to be submetacentric. Meiotic prophase chromosomes exhibit the phenomenon of heteropycnosis, which is characterized by pycnotic condensation of the chromatin at one end of each thread. The number of pycnotic knobs corresponds to the total number of chromosomes of this species, which is probably an indication of the fact that condensation and coiling of the chromosomes begin from one end of each chromosome. The phenomenon of heteropycnosis which was earlier reported in a species of millipede, *Thyropygus sp*[7], is by no means confined to diplopod meiosis alone. In a still more exaggerated form it occurs in Orthoptera[8], Pentatomid Hemiptera[9] and Odonata[10].

The premetaphase bivalents of *A. excellens* are usually characterized by the presence of non-staining gaps or despiralized zones. Such non-staining gaps have been reported in the premetaphase bivalents of *A. levissimus* and *A. thurstoni*[4]. Brogger[11] attributed the occurrence of chromatid gap to a folding defect due to a protein change. The interpretation was supported by the observations of Brinkley and Shaw[12] and Scheid and Traut[13]. Such folding error may be due to either a change in the DNA molecule or in the packing protein.

The sex-chromosomes (XY) seem to be almost indistinguishable from the autosomal bivalents. Further, the X and Y chromosomes exhibit minimal size difference, eventhough these are the largest in a given complement. This observation agrees with Ohno *et al*[14], who postulated on the basis of his studies on sex-chromosomes of a variety of placental mammals that X and Y chromosomes have evolved from a homologous pair of autosomes. Since no studies on the chromosome cytology of female of any diplopod species have been carried out by anyone so far due to difficulties in the preparation of chromosomes, the identification of their sex-chromosomes cannot be regarded as absolutely certain.

Occurrence of a translocation chain of 6 chromosomes, comprising 4 autosomes and 2 sex-chromosomes ($X Y$) during first meiotic metaphase of *A. excellens* is one of the significant observations of the present investigation. Such a translocation complex was not reported so far in Diplopoda. Instances of natural polymorphisms involving (reciprocal) translocations are known in a few special cases, such as *Periplaneta americana* and *Blaberus discoidalis* (cockroaches)[15–18]. A few species of plants belonging to the genera *Oenothera, Gayophytum, Rhoeo, Isotoma* and *Hypersicum* are 'fixed heterozygotes' for translocations[19–23]. Piza[24–29] reported translocations in various Brazilian scorpions (species of *Tityus* and *Isometrus*), and his observations are especially interesting because certain individuals which are heterozygotes for several translocations have revealed the formation of multiple rings of chromosomes at meiosis, comparable to those of the plant *Oenothera*. Imai *et al*[30], reported 11 instances of translocations in 9 species of Australian ants. In general, mutual translocations seem to diminish the fecundity of the heterozygotes too much to establish themselves in natural populations or to provide a basis of speciation.

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ANNOUNCEMENTS

SYMPOSIUM ON INSECTICIDAL PLANTS AND CONTROL OF ENVIRONMENTAL POLLUTION

Symposium on Insecticidal Plants and Control of Environmental Pollution is being organised at the Department of Botany, Bharathidasan University, Trichy from **9 to11 January 1986** with a view to bringing together all workers from various disciplines who are interested in the study of insecticidal plants and control of environmental pollution. It is expected, that the interactions and cross fertilizations of ideas arising from the symposium and the informal discussions would help to promote a better understanding of pest control agents of plant origin.

Scientists who are interested in participating in this Symposium are requested to contact Prof. G. R. Rao (Convener of the Symposium), Department of Botany, Bharathidasan University, Trichy 620 023.

INTERNATIONAL WINTER SCHOOL ON DIRECT METHODS

An International Winter School on Direct Methods has been scheduled for **9–19 December, 1985** to be held at Madras. Those interested in knowing more details about the Winter School may please contact Dr S. Parthasarathy, Convener, International Winter School, Department of Crystallography and Biophysics, University of Madras, Madras 600 025.

SYMPOSIUM ON CRYSTAL GROWTH

A Symposium on Crystal Growth is being organized during **29–31 January, 1986** by The Materials Science Committee, Board of Research in Nuclear Sciences, Department of Atomic Engergy, Government of India. Details can be ascertained from Dr B. Ghosh, Convener, Symposium on Crystal Growth, Technical Physics & Prototype Engineering Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085.