

## MEIOTIC PROPHASE IN DIPLOPODA

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### ABSTRACT

Few studies on Diplopoda chromosomes are available, with approximately 70 species investigated thus far world-wide out of a total of about 8000 species described. These studies have been mainly performed on Indian species (Oriental Zoogeographic Region) and the diploid number of the species is quite variable. The present study reports for the first time the probable sequence of prophase stages in diplopod species based on data obtained for Brazilian species, and is compared with data reported in the literature.

### INTRODUCTION

The Class Diplopoda has attracted little interest from cytologists. Only approximately 70 species have been investigated world-wide out of a total of about 8,000 species described.

The first contributions to the cytogenetics of Diplopoda were made by Oettinger (1909), Sokoloff (1914) and Bessièrè (1948). These studies, however, did not present complete meiotic figures but were mainly concerned with cell inclusions during spermatogenesis, and the references to the chromosomes of these species were not always clear. Thus, the cited studies are mainly important from a historical viewpoint.

Later studies were conducted by Natarajan (1959), Chowdaiah (1966a,b,c, 1967, 1969), Chowdaiah and Kanaka (1969, 1974, 1979) and Sharma and Handa (1974). More recently, modern techniques such as C and G, banding have been used to study Indian species (Achar and Chowdaiah, 1979, 1980; Achar, 1983a,b, 1984a,b, 1985, 1986). In 1987, Achar published an important report on chromosome evolution among Diplopoda, listing all the species studied thus far.

Most of these studies concern species from India, i.e., belonging to the Oriental Zoogeographic Region. The only study performed in another region involved 10 species of Brazilian diplopods (Neotropical Region), belonging to 4 families (Fontanetti, 1987).

The diploid number of the species studied is quite variable ( $2n = 8(?)$  to  $2n = 30$ ), and the sex determining mechanism most commonly encountered is of the XY type.

On the basis of the data reported in the literature, taken together with those obtained by the author, the probable sequence of prophase I in diplopod species is reported here for the first time. This sequence is quite peculiar and the possibility of the existence of exceptions cannot be ruled out.

## MATERIALS AND METHODS

The chromosomes of fourteen Brazilian species of diplopods were analyzed by the author. The specimens were collected in nature in different localities and during several periods of the year. The slides were prepared from adult male testes, previously fixed in Carnoy I and squashed in a drop of 45% acetic acid. Giemsa and 1% lacto-acetic orcein were used as stains. In some species, C-banding was obtained after incubation in  $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$  at  $30^\circ\text{C}$  and in 2XSSC at  $60^\circ\text{C}$ .

The species studied and the collecting localities were as follows:

- Sandalodesmus* sp - 34 km NE from Dourados, Mato Grosso do Sul
- Plusioporus setiger* (Brolemann) - Rio Claro, São Paulo
- Alloporus principes* Brolemann - Scrub of Corumbataí, São Paulo
- Gymnostreptus olivaceus* Schubart - Rio Claro, São Paulo
- G. acuticollis* Verhoeff - Biological Station of Boracéia, São Paulo
- Pseudonannolene* sp. 1 - Toca Cave, 10 km from Itirapina, São Paulo
- P.* sp. 2 - Biological Station of Boracéia, São Paulo
- P. ophiulus* Schubart - Barreiro Rico Farm, São Paulo
- P. halophila* Schubart - Alcatrazes Islands, São Paulo
- P. strinatii* Mauriès - Ressurgência das Areias Cave, Iporanga, São Paulo
- P. tricolor* Brolemann - Rio Claro, São Paulo
- Rhinocricus padbergi* Verhoeff - Rio Claro, São Paulo
- R. cachoeirensis* Schubart - Scrub of Corumbataí, São Paulo
- R.* sp - 16 km N of Jacupiranga, São Paulo.

## RESULTS AND DISCUSSION

Figure 1 illustrates the proposed prophase sequence in diplopods. The nucleus initiating the meiotic process shows highly condensed chromatin at one of the poles, from which small portions begin to individualize (Figure 1A), the delimitation of the nucleus being visible.

Leptotene presents a configuration similar to the "bouquet" formation, with part of the polarized material being quite condensed and part almost totally decondensed (Figure 1B).

At zygotene, the two different states of chromosome condensation are well visible (Figure 1C), with homologue pairing occurring in the less condensed portion (arrow in Figure 1C).

At pachytene the chromosomes still show different states of condensation and are arranged in the typical "bouquet formation" (Figure 1D). The nucleolus (n) is visible up to this phase, with two chromosomes related to it being generally observed (1, 1').

After this phase, part of the nuclear chromatin (constitutive heterochromatin, detected by C-banding) is condensed, and in most cases it is possible to count the chromosome number of the species and to observe part of the chromatin (euchromatin) in a diffuse state (Figure 1E).

Diplotene also showed part of the chromosomes more condensed than others (Figure 1F, G).

Diakinesis and premetaphases did not show any important peculiarities.

The prophase is peculiar for the following reasons: a) each bivalent is divided into two differentially condensed segments; b) the highly condensed segment is formed by constitutive heterochromatin that does not pair at first prophase; c) the euchromatin segments of the bivalents enter a diffused stage before diplotene.

The "bouquet" formation was frequently observed in these species both in the present study and in studies conducted by others, appearing to be a characteristic of the group. According to Schulz-Schaeffer (1980, p. 108), this formation is due to a certain type of polarization observed during leptotene and pachytene, whereby the chromosome ends seem to be attached to the nuclear membrane at the site where the centrosome is located in animal cells. It has been speculated that the "bouquet" formation may help join homologous chromosomes during synapsis at zygotene. Yunis and Yasmineh (1971) stated that the "bouquet" configuration is a clumping of non-homologous heterochromatin.

With respect to the diffuse state of some of the chromosomes, Sharma and Handa (1974) have reported a similar phenomenon for *Gonoplectus malayus*, whose

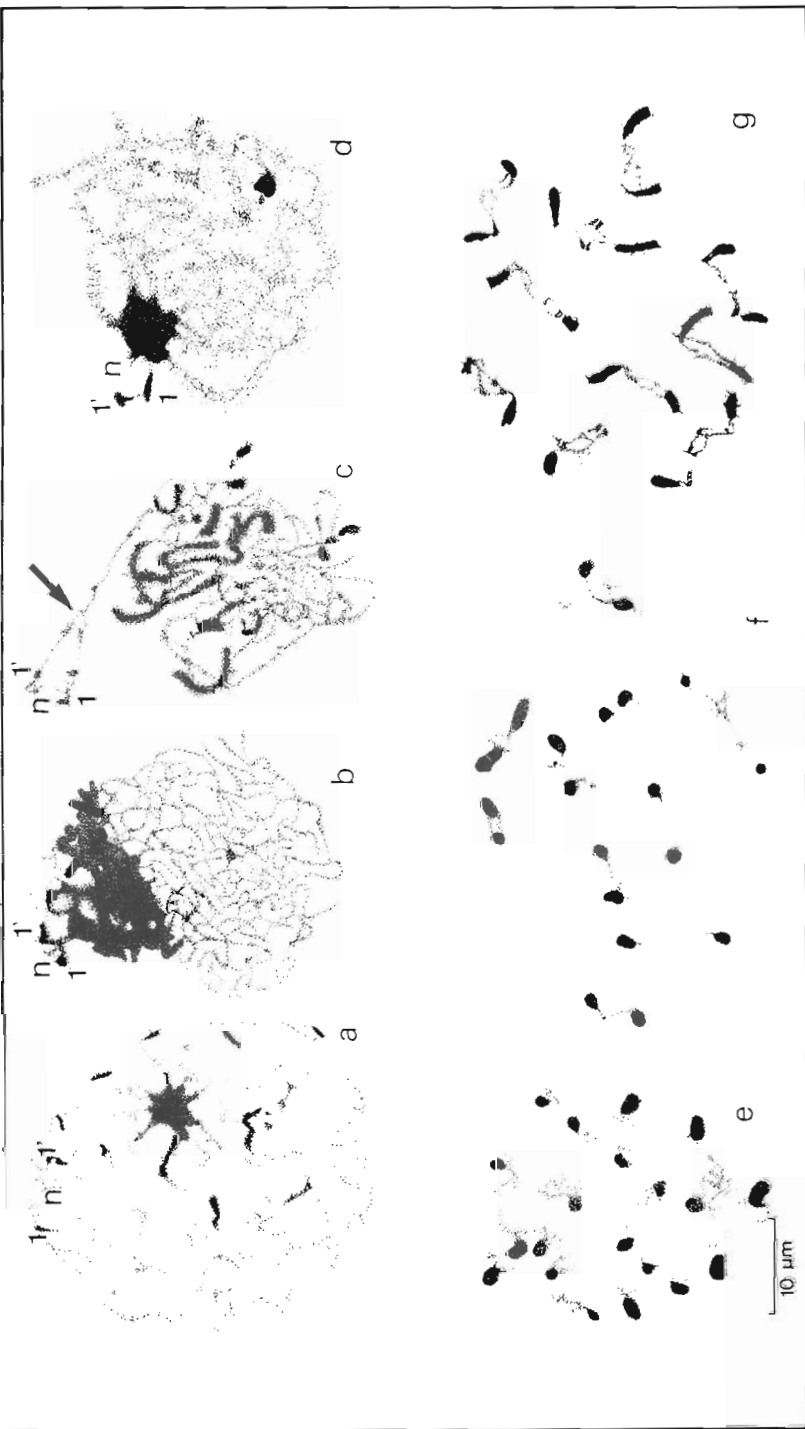


Figure 1 - Sequence proposed for the different stages of prophase I in diploids species. (A) Nucleus initiating the meiotic process; chromatin is quite condensed at one pole, and small portions start to individualize from there; (B) Leptotene; (C) Zygotene; (D) Pachytene ("bouquet" formation). In these phases, two very different condensation conditions appear in the chromosomes, the more condensed part being constitutive heterochromatin and the less condensed, euchromatin. Homologue pairing occurs in the less condensed portion (arrow). The nucleolus (n) with two chromosomes (1, 1') related to it can also be observed. (E) Euchromatin is diffuse, and constitutive heterochromatin continues to be condensed and unpaired; (F, G) Diplotene, with two different types of chromosome condensation still visible.

meiosis is interrupted after pachytene by a diffuse stage during which the chromosomes do not stain. Only a positive heteropycnotic body is left, which, according to the authors, is the sex chromosome (this is an XO species) excentrically located in the nucleus. Chowdaiah (1966b) mentioned the fact that a diffuse stage exists during the initial prophase of *Harpurostreptus* sp and *Chondromorpha mammifera* but did not discuss it in detail.

Schrader (1941) observed something similar in the hemipteran *Edessa irrorata* (Pentatomidae) which presents an extreme diffuse period preceding diakinesis.

Chowdaiah (1966b), in a study on *Thyropygus*, observed that the initial meiotic prophase is marked by the appearance of fine granular stripes that criss-cross the nucleus. In most species, there is pycnotic condensation of the chromatin located on the end of each chromosome; this condensation is equivalent to the total chromosome number and never exceeds it, suggesting that only one of the ends is heteropycnotic. This description probably corresponds to the phase observed by us and presented in Figure 1C.

The phenomenon of heteropycnosis in diplopods was also observed by Achar and Chowdaiah (1979) and by Achar (1984a,b, 1985, 1986), who commented that the number of heterochromatin blocks corresponds to the total chromosome number of each species and never exceeds it, suggesting that only one end of each chromosome becomes heteropycnotic. This characteristic of heteropycnosis starting in one of the terminal portions of each chromosome is probably due to the fact that condensation and spiralization start from one of the terminal portions of each chromosome (Achar, 1984a).

Heteropycnosis was also observed by other investigators in other groups. Corey (1938), in a study of 11 species from different orthopteran families, observed heteropycnotic elements on the proximal chromosome ends, where the chromosome binds to the spindle fiber. According to this investigator, these structures are extremely useful for chromosome orientation and behavior during meiosis. Schrader (1941) also observed this phenomenon in hemipterans belonging to the family Pentatomidae, and Das (1956) observed a similar occurrence in two Indian Odonata species.

The data obtained thus far for Brazilian species suggest that prophase I is quite long in diplopods, with the process accelerating from diplotene, since few nuclei are observed after this phase. Metaphases are rare and, in addition, many of the nuclei detected present chromosomes that are closely joined or even clumped in a single block. This is probably due to the large amounts of constitutive heterochromatin (detected by C banding), since clumping is one of its well known properties. Clumping was also observed by other investigators (Chowdaiah and Kanaka, 1969, 1974, 1979).

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## RESUMO

Poucos dados sobre os cromossomos de Diplopoda estão disponíveis. Aproximadamente 70 espécies têm sido investigadas de um total de 8000 conhecidas no mundo. A maior parte destes estudos foi feita com espécies da Índia (Região Zoogeográfica Oriental) e o número diplóide das espécies é bem variável. O atual estudo mostra pela primeira vez a seqüência provável das espécies de Diplopoda baseado em dados obtidos com espécies brasileiras, comparando-se com dados da literatura.

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