Karyotypic characterization by mitosis, meiosis and C-banding of *Eriopis connexa* Mulsant (Coccinellidae: Coleoptera: Polyphaga), a predator of insect pests

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Eriopis connexa presents a chromosome number of $2n = 18 + XX$ for most females analyzed and a meioformula of $n = 9 + Xy_p$ for all males. A small metacentric B chromosome restricted to females occurred in 10 % of our sample and, when submitted to C-banding, it was shown to be almost completely euchromatic. Chromosome pairs 2 and 3 had satellites and probably contained the nucleolar organizer regions (NORs). C-band analysis also revealed that the constitutive heterochromatin was localized in the centromeres of all chromosomes in the complement.

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Since the end of the last century, many Coccinellids have been found to be efficient predators of aphids, cochineals and lepidopteran eggs (DE BACH 1964; GORDON, 1985). They have been used efficiently in Pest Management Programs and there is great interest in more detailed studies (SMITH and REYNOLDS 1966; DE BACH 1964; LEVINS and WILSON 1980). While there are large number of ladybug species native to Brazil, few studies about this group are available. The Order Coleoptera comprises approximately 350,000 described species (LAWRENCE 1982), but cytologic information about the families are highly fragmented, and covers some 3000 species (SMITH and VIRKKI 1978; SHARMA et al. 1980; SERRANO and YADAV 1984; PETITPIERRE et al. 1988; GILL et al. 1990; GÁLIAN and MOORE 1994). The chromosome numbers vary widely ranging from $2n = 4$ in *Chalcolepidius zonatus* (FERREIRA et al. 1984) to $2n = 69$ in *Ditomus capito* (SERRANO 1981). There is certainly a need to study a larger number of species for a better understanding of this variability (FERREIRA and MESA 1977; MARTINS 1994). The basic karyotype of Coleoptera, probably the ancestral one, has been reported to consist of nine pairs of autosomes and the X and y_p sex chromosomes, which associate in a "parachute" configuration during metaphase I, with chromosome X being relatively much larger than chromosome y, which is a very small chromosome (SMITH 1950).

Cytogenetic analyses of Coleoptera have been mostly performed during male meiosis because of the difficulty in obtaining mitotic metaphase chromosomes (PETITPIERRE 1996). In the family Coccinellidae, few analyses have been made, but available data have shown that the basic karyotype $n = 9 + Xy_p$ described for Coleoptera is the most frequently (SMITH and VIRKKI 1978). No cytogenetic analyses have been performed thus far on any species of the genus *Eriopis*.

We describe here the number and morphology of mitotic metaphase chromosomes, evaluate their behavior during meiosis, and describe the distribution of heterochromatin in the genome of *Eriopis connexa*.

MATERIAL AND METHODS

Specimens of *Eriopis connexa* were collected on the Campus of the Universidade Federal de Viçosa, Minas Gerais, Brazil, where they occur naturally, and were reared in the laboratory for reproduction. Adults and larvae were fed with aphids and their eggs were separated from the adults, hatching on average after 5 days. Fifteen larvae in the prepupal stage were used to obtain mitotic metaphase chromosomes. Twenty five adult males were used for analysis of meiosis. Ten mitotic metaphases per individual were analyzed, on average, during the larval phase.

Cytogenetic analysis of mitotic metaphase chromosomes was performed according to the method of IMAI et al. (1988). Cerebral ganglions were dissected from prepupal larvae into hypotonic solution-colchicine (1 % sodium citrate plus 0.005 % colchicine) and left in this solution for 1:30 hours. After this time, each ganglion was transferred to a slide and

several drops of fixative 1 (4:3:3, water: ethanol:acetic acid) were added. The ganglion was dissociated with a pair of dissecting needles and two drops of fixative 2 (1:1, ethanol:acetic acid) were added. Three drops of fixative 3 (100 % acetic acid) were then added and 24 hours later the slides were stained with Giemsa in Sorensen buffer (0.06 M buffer, pH 6.8, at the proportion of 1 ml Giemsa:30 ml buffer) for 10 minutes. The chromosomes were classified according to the nomenclature of LEVAN et al. (1964). For meiotic analysis, males testes were removed in Ringer and slides were prepared by the method of IMAI et al. (1988) without using colchicine.

C-Banding

C-banding was performed by the technique of SUMNER (1972), modified by POMPOLO and TAKAHASHI (1990). The slides were submitted to the following treatments: a) hydrolysis with 0.2 N HCl at room temperature for 4 minutes; b) a quick wash in distilled water and incubation with 5 % barium hydroxide in a water bath at 60°C for 8 minutes; c) a wash in 0.2 N HCl for about 30 seconds; d) incubation with 2xSSC solution (0.03 mol/L sodium citrate and 0.3 mol/L sodium chloride, pH 7.0) at 60°C for 10 minutes; e) Giemsa staining (2 ml Giemsa: 30 ml 0.06 M Sorensen buffer, pH 6.8) for 50 minutes.

RESULTS

Mitotic chromosomes of 12 *Eriopis connexa* females were analyzed by standard staining. Eight of these females presented a diploid number of $2n = 20$ and four showed a small metacentric B-chromosome in all cells, resulting in a karyotype of the $2n = 18 + XX +$ B type (Fig. 1 and 2A, B and C). The autosomes of this species were grouped into 4 metacentric (M) pairs, 4 submetacentric (SM) pairs and 1 subtelocentric (ST) pair. The X chromosome was of the M type. Pairs 2 and 3 presented secondary constrictions located on the short arms of the chromosomes. C-banding analysis revealed that constitutive heterochromatin is located in the centromeric region of all chromosomes in the complement. The chromosome B, in turn, was almost completely euchromatic (Fig. 2E).

The three male larvae evaluated for mitosis presented $2n = 18 + Xy$, with a very small y chromosome (Fig. 2D). The 25 adult males evaluated for meiosis had a chromosome number of $n = 9 + Xy_p$, with all prophase I stages being visible. More in-

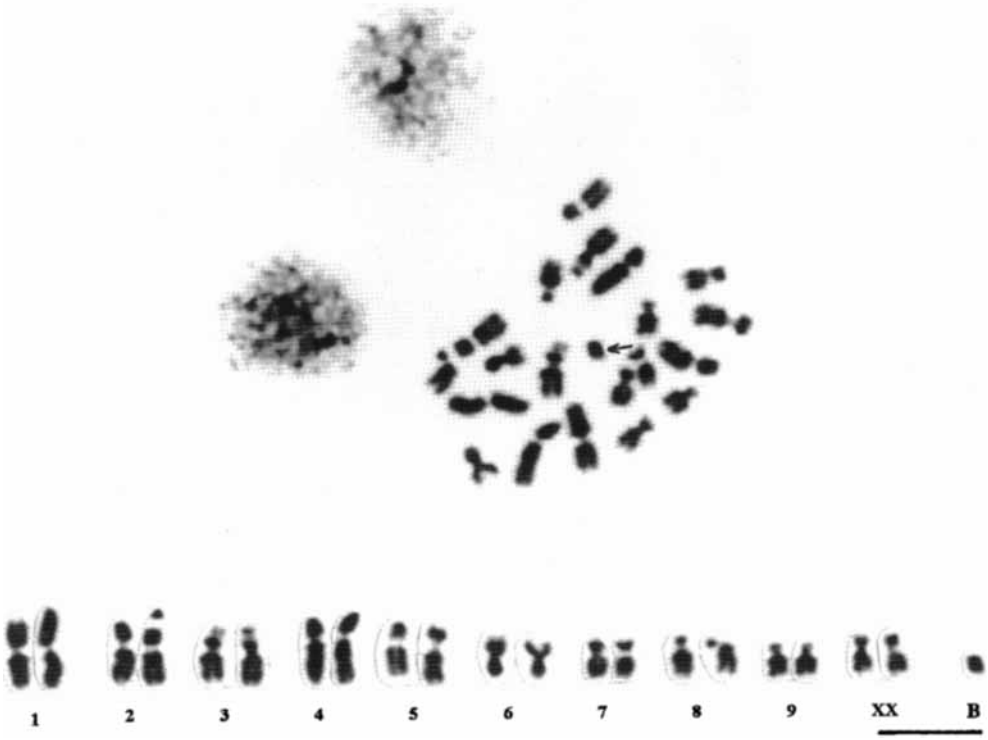

Fig. 1. Metaphase and karyotype of an *E. connexa* female with $2n = 18 + XX + 1B$. The arrow indicates the B chromosome. Bar = 5 μm.

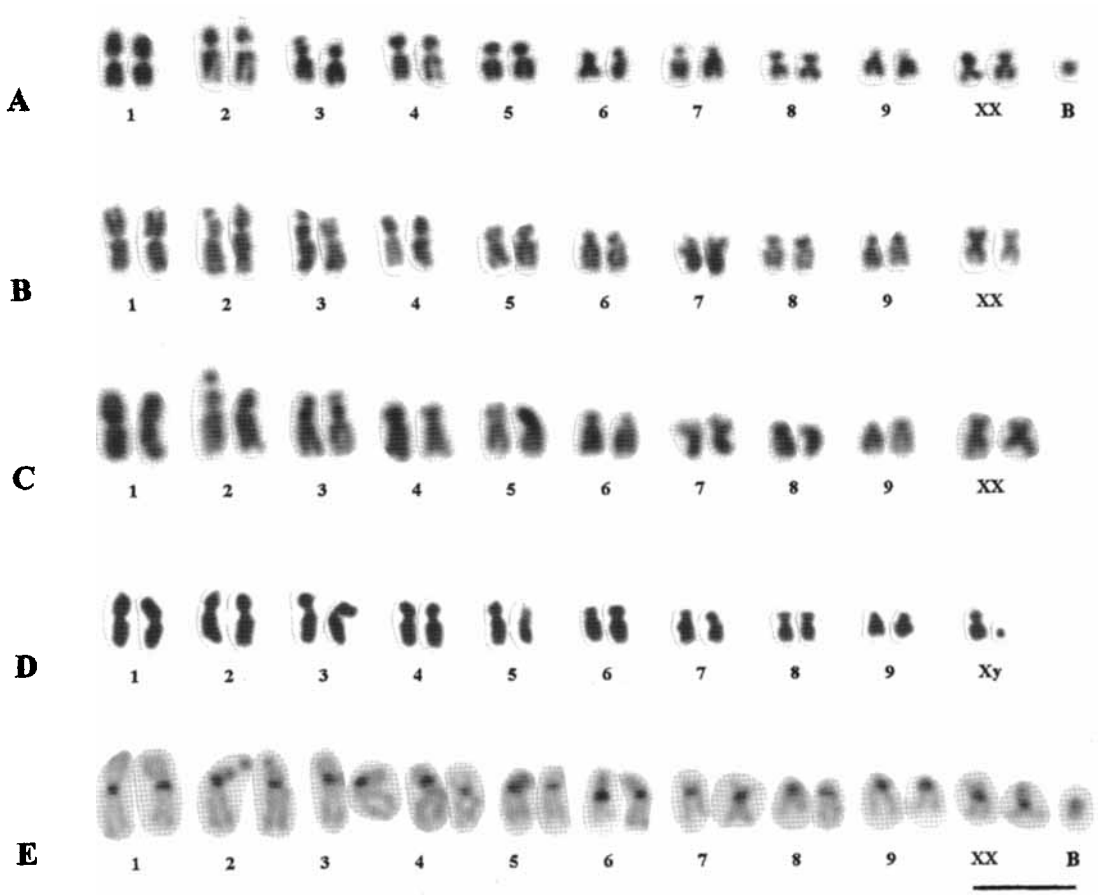

Fig. 2A–E. Karyotypes of various *E. connexa* individuals. **A** Karyotype of a female with 2n + XX + 1B. **B and C** Females with 2n = 18 + XX. **D** Male with 2n + 18 + Xy. **E** C band in a female with an almost completely euchromatic B-chromosome. Bar = 5 μm.

tensely stained regions (heteropycnotic) were observed in leptotene (Fig. 3A), while they were less intensely stained by C banding (Fig. 5A). A larger heterochromatic region and other smaller heterochromatic regions were observed during zygotene both by standard staining (Fig. 3B and C) and by C banding (Fig. 5B). At pachytene, the bivalents were individualized and it was possible to visualize the chromomeres (Fig. 3D). Chiasmata and some bivalents were observed at diplotene (Fig. 3E), and at diakinesis the bivalents were more condensed and uniformly distributed (Fig. 3F and G).

Metaphase I was characterized by the presence of 9 bivalents and by an associated sex pair forming a "parachute" figure in all cells evaluated (Fig. 4A and B). At anaphase I, chromosome segregation was normal (Fig. 4C).

DISCUSSION

Eriopis connexa presented a chromosome number of 2n = 18 + XX for most females analyzed and a meiotic formula of $n = 9 + Xy_p$ for males. The chromosome number and the "parachute" configuration during metaphase I agree with the descriptions for most Coleoptera species, probably representing the typical (ancestral) karyotype, especially in the Polyphaga suborder (SMITH 1950; SMITH and VIRKKI 1978).

Chromosome pairs 2 and 3 presented satellites and probably contained the nucleolar organizer regions (NORs). The chromosomes presenting these regions are frequently seen associated with the nucleoli during prophase (GUERRA 1988).

A small-sized supernumerary chromosome restricted to females occurred in 10 % of our sample. When submitted to C-banding it presented staining (an intermediate positive block) in a small region of the chromosome, with the remainder being euchromatic.

B chromosomes are frequently fully heterochromatic (JONES and REES 1982; JONES 1995). However, some euchromatic B chromosomes have been reported, e.g., in *Allium fava* (VOSA 1973), *Najas marina* (VIINIKKA 1975) and *Allium schoenoprasum*

(STEVENS and BOUGOURD 1994). In some populations of *Allium ericetorum* a B chromosome occurred and no correlation between B chromosomes and particular C-banding patterns was observed (WETSCHNIG 1995).

The function and composition of B chromosomes is still a controversial question. Reports on the occurrence of B chromosomes in the lizard *Nothobachia ablephara* showed the Bs were not clearly distinguishable from the autossomes in Giemsa-stained metaphases and C-banding, but showed late replication after R-banding. One widespread heterochromatin feature is its late-replication, the Bs could be a specific class of heterochromatin undetected by routine C-banding procedures (PELLEGRINO et al. 1999).

Recently, SILVA and YONENAGA-YASSUDA (1998) reported a conspicuous heterogeneity of size, morphology, constitutive heterochromatin patterns and localization of telomeric sequences of B chromosomes

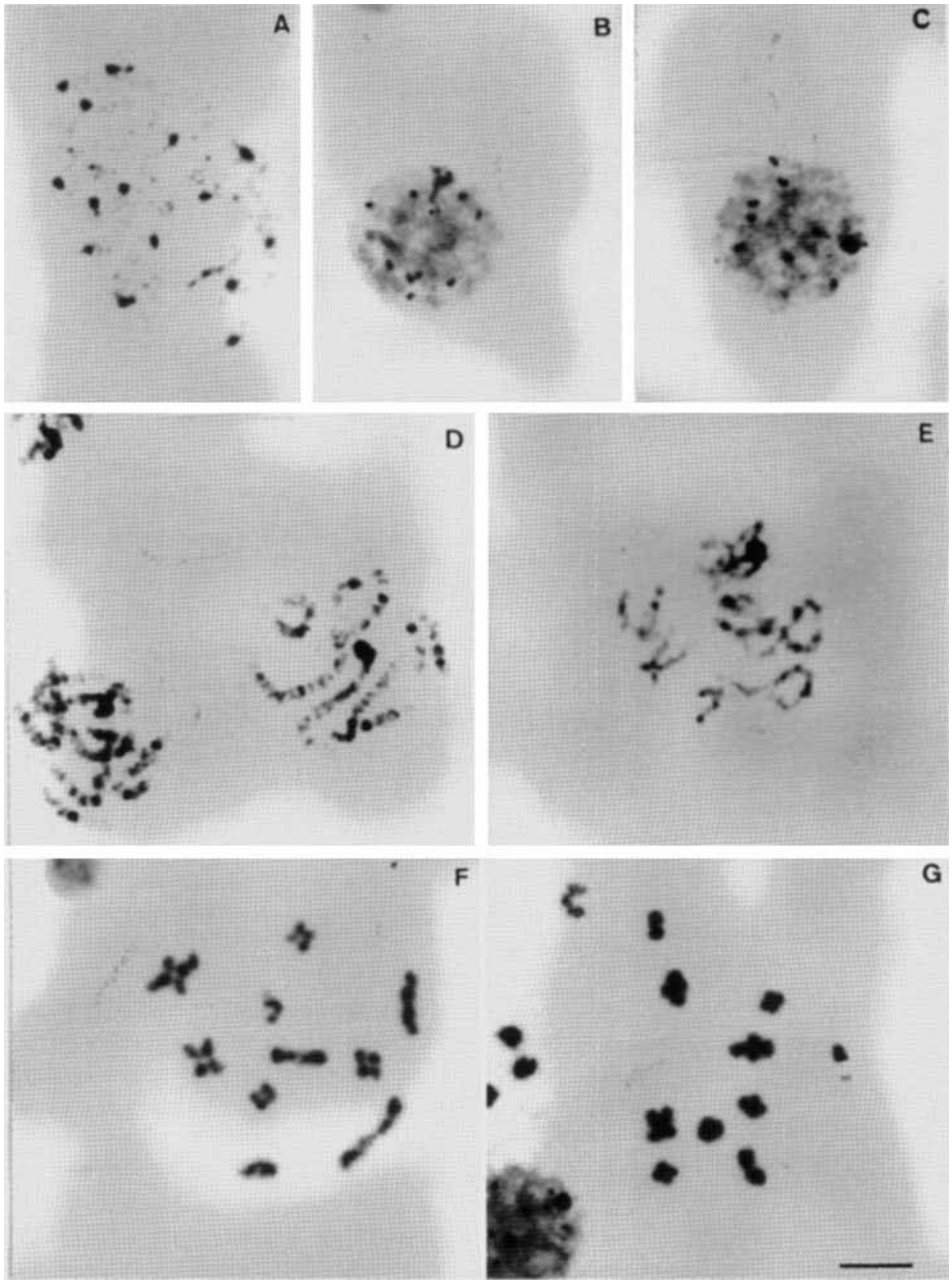

Fig. 3A–G. Meiosis of an *E. connexa* male. **A** Leptotene. **B and C** Zygotene. **D** Pachytene. **E** Diplotene. **F and G** Diakinesis. Bar = 5 μm.

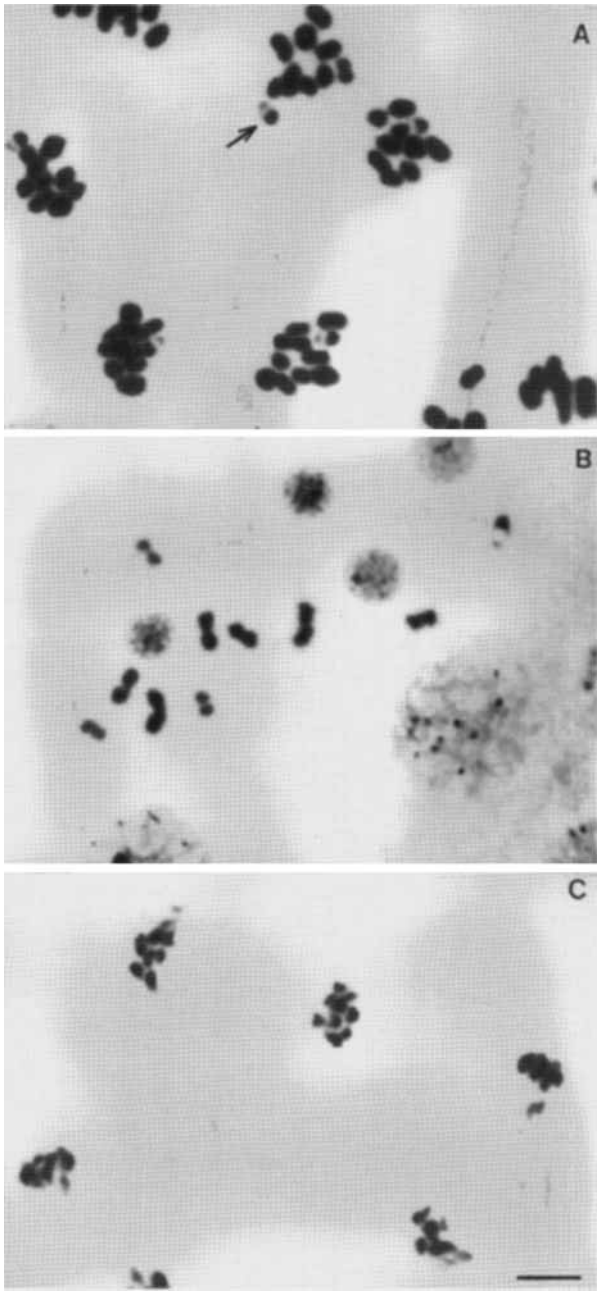

Fig. 4A–C. Meiosis of an *E. connexa* male. **A and B** Metaphase I of a male. **C** Anaphase I. The arrow indicates "parachute"-shaped sex chromosomes. Bar = 5 μm.

for the rodent *Nectomys*, which allowed them to suggest differences in the composition of these chromosomes.

Different types of repetitive sequences of DNA from a B chromosomes have been characterized by molecular analyses, in some species of plants (rye *Secale cereale* HOUBEN et al. 1996; daisy *Brachycome dichromosomatica* JOHN et al. 1991; LEACH et al. 1995; among others). In animals the parasitic wasp *Nasonia vitripennis*, presents males that carry a B chromosome, called PSR (paternal sex ratio), which causes the compaction and subsequent loss of the paternal chromosomes in fertilized eggs. Three families of related tandemly repetitive DNAs (PSR2, PSR18, PSR22) were shown to be present only on the PSR chromosome (EICKBUSH et al. 1992). A species specific satellite DNA family (pSsP216) of *Drosophila subsilvestris* appears predominantly in B chromosomes. The pSsP216 family consists of tandemly arranged 216 bp repetitive units (GUTKNECHT et al. 1995).

According to SMITH and VIRKKI (1978), supernumerary chromosomes have been reported in approximately 50 species and subspecies of Coleoptera (suborder Polyphaga), in which they are restricted to only a few individuals, occurring in small numbers.

A correlation between frequency of B-chromosomes and sex proportion has been reported for *Exochomus quadripulsatus*, and probable factors influencing the sex proportion have been suggested to occur, affecting the frequency of B chromosomes in different populations (HENDERSON 1988).

B-chromosomes restricted to one sex have also been reported in fish, e.g., *Astyanax scabripinnis*, which presented a B chromosome limited to males (STANGE and ALMEIDA-TOLEDO 1993). Also a casual relationship has been reported to occur in fish between sex proportion and presence of B-chromosomes (VICENTE et al., 1996).

The hypothesis that explains the absence of supernumerary chromosomes in a given sex may be the existence of a mechanism of elimination of these chromosomes in the somatic tissues of these individuals. The elimination of B-chromosomes in somatic tissues has been reported, as observed in grasshoppers (LOPEZ-LEON et al. 1991) and fish (KOHNO et al. 1986; NAKAI and KOHNO 1987). However, the causes of these mechanisms are still unclear.

C-band analysis revealed that the constitutive heterochromatin of *Eriopis connexa* was localized in the centromeres of all chromosomes in the complement. Evaluation of meiosis by C-banding revealed the presence of small chromocenters which were more numerous in leptotene than at the end of zygotene. These chromocenters increased in size and decreased in number, with the presence of a much larger heterochromatic region suggesting that the pairing of homologous chromosomes had occurred. The larger heterochromatic region is due to the association of the sex chromosomes. Reductions in the number of chromocenters at the beginning of prophase I (leptotene) have also been described for *Epilachna paenulata* (Coccinellidae) (DRETS et al. 1983).

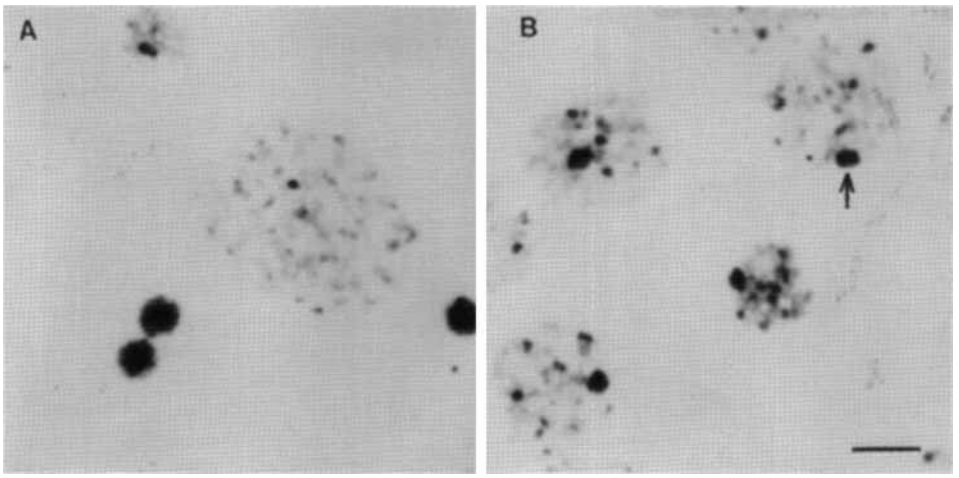

Fig. 5A and B. C-band of an *E. connexa* male. **A** Leptotene. **B** Zygotene. The arrow indicates the association of sex chromosomes after C-banding. Bar = 5 μm.

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