

THE CYTOGENETICS OF *Abracris flavolineata* (ORTHOPTERA, CAELIFERA, OMMATOLAMPINAE, ABRACRINI)

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ABSTRACT

The mitotic (neuroblastic) and meiotic (testicular) chromosomes of a population from Rio Claro of *Abracris flavolineata* were analysed by standard (Giemsa) and differential (C banding, Mild N, Strong N and silver) staining. All of the chromosomes in the karyotype ($2n \text{ ♂} = 23$ and $2n \text{ ♀} = 24$) differed in morphology from those of the basic karyotype of the superfamily Acridoidea, all of which are acrocentric. The chromosomes of pairs L1 - L3, M4 - M6 and M8 were subtelocentric, those of pairs M7 and S9 were submetacentric, and those of S10 and S11 metacentric. The sex chromosome was subtelocentric. C banding showed that the small arms of all chromosomes except those of pair M7 originated from the addition of chromosomal material made up of constitutive chromatin. The submetacentric morphology of pair M7 originated from a pericentric inversion. Silver staining did not reveal any region with the characteristics of a NOR but permitted the visualization of points similar to Cd bands in the centromeric regions, which were interpreted to be kinetochore plates. N banding stained the proximal region of the long arms of the chromosomes of pair M8, which was considered to be one of the nucleolar organizer regions of *A. flavolineata*.

INTRODUCTION

Until 1981, *Abracris flavolineata* was known as *Osmilia violacea*. Roberts and Carbonell (1981), in a taxonomic revision of the genus *Abracris* and of other related genera (*Omalotettix*, *Orthoscapheus* and *Jodacris*), considered *Osmilia* to be congeneric with *Abracris* and the single species existing in the old genus as *flavolineata*, thus maintaining the designation given by Geer (1773), who first described the species as *Acrydium flavolineatum*.

The superfamily Acridoidea is cytologically characterized by great karyotypic stability. The families Pneumoridae, Xyronotydae, Trigonopterigidae, Charilaidae, Lentulidae, Paulinidae, Romaleidae and Acrididae have a basic karyotype of $2n = 23$ (δ) and 24 (φ) acrocentric chromosomes and a XO-XX type chromosomal mechanism of sex determination (White, 1973). Approximately two thirds of the species belonging to the superfamily Acridoidea have karyotypes similar to those described above. The karyotypic stability assumed to exist for this species is not manifested when results obtained by more refined techniques are compared. Wide variability is detected when the amount of DNA present in spermatid nuclei (John and Hewitt, 1966; Wilmore and Brown, 1975; Gosálvez *et al.*, 1980) and the presence and distribution of C bands (King and John, 1980; Santos *et al.*, 1983; John *et al.*, 1985; Cabrero and Camacho, 1986) are compared among related species having similar karyotypes.

Of 19 species of Abracrini that have been studied cytologically (Mesa *et al.*, 1982), 13 (68%) have secondary karyotypes, whereas this phenomenon is observed in only 33% of the species in the superfamily Acridoidea. The chromosome mechanisms which have been detected and identified as being responsible for the appearance of these derived karyotypes are: a) centric fusion - six species, 32%; b) centric fusions followed by pericentric inversions - four species, 21%; and c) increase of the short arm of some chromosomes - three species, 15%. The karyologic structures of the species belonging to the tribe Abracrini permit us to characterize some of the genera: *Siltaces* ($2n \delta = 19$, 2 metacentric pairs and all the others acrocentric), *Eujivarus* ($2n \delta = 19$, 2 metacentric pairs and all the others acrocentric), and *Jodacris* ($2n \delta = 19$, all pairs being acrocentric), but does not permit phylogenetic grouping (Ferreira *et al.*, 1980).

In the present study, we investigated the chromosomes of embryonic (neuroblasts) and testicular cells of *A. flavolineata* using colchicine treatment, hypotonia and C-banding, Mild N, Strong N and silver staining techniques, which permit a better characterization of the chromosomes, of their variability and of the mechanisms responsible for variation.

MATERIAL AND METHODS

Nine adult males and 64 embryos (33 males and 31 females) were studied. Males were collected directly in nature and the embryos were obtained from eggs laid by females captured in nature and held in captivity until oviposition. Males and females were collected at the Forest Park "Navarro de Andrade" located near the city of Rio Claro, State of São Paulo, Brazil.

Mitotic cytological preparations were obtained from embryo neuroblasts by the technique of Webb *et al.* (1978), and meiotic preparations from testes according to standard techniques of cell suspension in 45% acetic acid. C bands were obtained by the technique of Sumner (1972), Mild N and Strong N bands by the technique of Hägele (1979), and NOR staining was performed by the technique of Howell and Black (1980).

RESULTS

Standard staining

The diploid number detected for the species was $2n (\delta) = 23$ and $2n (\varphi) = 24$ and the chromosome mechanism of sex determination was of the XO (δ) - XX (φ) type.

Examination of neuroblast mitotic metaphase preparations permitted organizing the chromosomes into three distinct groups according to size (Figure 1): large (L1 - L3), medium (M4 - M8) and small (S9 - S11). The chromosomes belonging to the L and M groups were subtelocentric, except for M7 which was submetacentric. The chromosomes of pair S9 were submetacentric and those of pairs S10 and S11, metacentric. Chromosome X was subtelocentric and similar in size to the chromosomes of pair L2 (Table I).

Table I - Mean length of the long and short arms of *Abracris flavolineata* chromosomes.

Chromosome pairs	Long arm	Short arm	Arm ratio	Centromere position
01	1.8	0.3	6.0	st
02	1.6	0.3	5.3	st
03	1.5	0.3	5.0	st
04	1.3	0.2	6.5	st
05	1.1	0.2	5.5	st
06	1.0	0.2	5.0	st
07	0.7	0.3	2.3	sm
08	0.7	0.2	3.5	st
09	0.4	0.2	2.0	sm
10	0.3	0.2	1.5	m
11	0.2	0.2	1.0	m
X	1.6	0.3	5.3	st

st, subtelocentric; sm, submetacentric; m, metacentric.

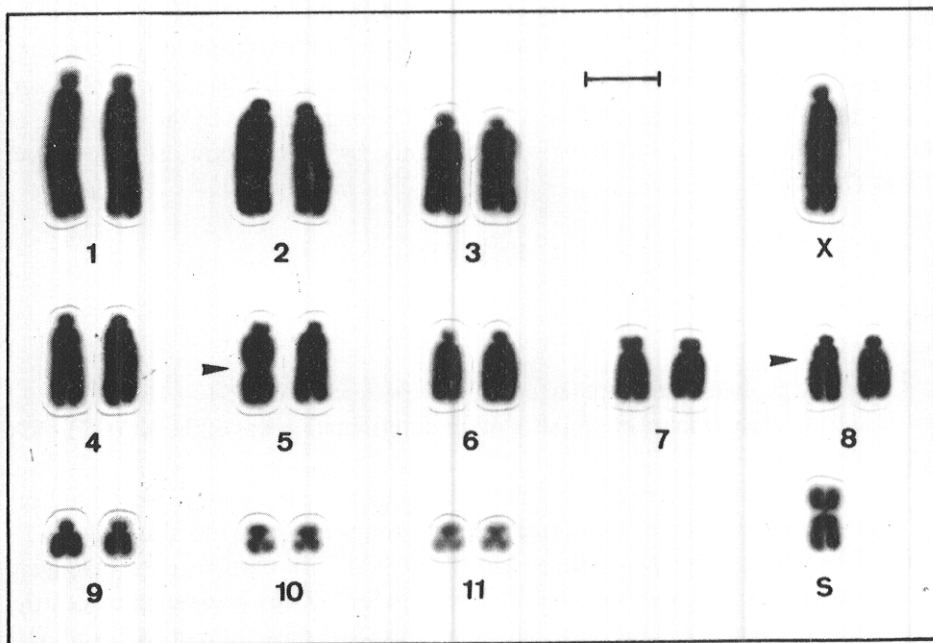


Figure 1 - Karyotype of an *Abracris flavolineata* male ($2n = 22 + XO + S$) with standard staining. A supernumerary (S) chromosome and a secondary constriction in one of the homologues of pairs 5 and 8 (arrows) can be seen.

A metacentric supernumerary chromosome of a size similar to that of pairs M6 - M7 was detected in three males and eight females from a single ootheca. In the karyotype of a smale specimen with supernumerary chromosomes there were secondary constrictions in the middle region of the long arm of one chromosome of pair M5 and in the proximal region of the long arm of one of the homologues of pair M8. These constrictions were not observed in metaphases from the other specimens analyzed.

At prophase I, the chromosomes become organized into 11 bivalents, side by side with the sex chromosome. At diplotene (Figure 2A), most bivalents exhibited proximal heteropycnotic blocks and chromosome X was totally heteropycnotic positive. In the bivalent formed by the L1 pair, these heteropycnotic positive blocks were apparently separated from the remainder of the bivalent by a marked elastic constriction. At metaphase I, these blocks were no longer visible and chromosome X became heteropycnotic negative.

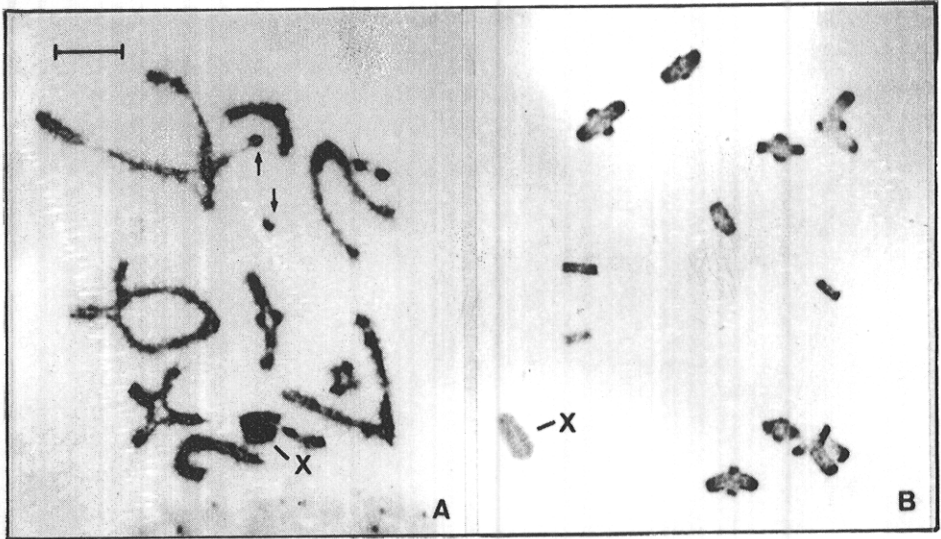


Figure 2 - Meiotic karyotypes of an *Abracris flavolineata* male ($2n = 11 \text{ II} + \text{XO}$). A, Early diplotene, with standard staining; bivalent 1 shows a proximal heteropycnotic positive block separated from the chromosomes by a marked constriction (arrows); B, metaphase I, with Strong N treatment, showing proximal and distal staining in some chromosomes.

C bands

Neuroblast metaphases submitted to C-banding showed constitutive heterochromatin in the pericentromeric region of all chromosomes in the complement (Figure 3A). In the subtelocentric chromosomes of pairs L1 - L3, M4 - M6 and M8, in the submetacentrics of pair S9 and in the metacentrics of pairs S10 and S11, heterochromatin extended throughout the short arm of the chromosomes. The submetacentric chromosomes of pair M7 did not show heterochromatic short arms.

Chromosome X showed a fully heterochromatic short arm, whereas the supernumerary showed no staining (Figure 3B).

NORs

Silver staining did not reveal any structures that could be interpreted to be NORs, but deeply staining points appeared in the centromeric region of all chromosomes (Figure 4).

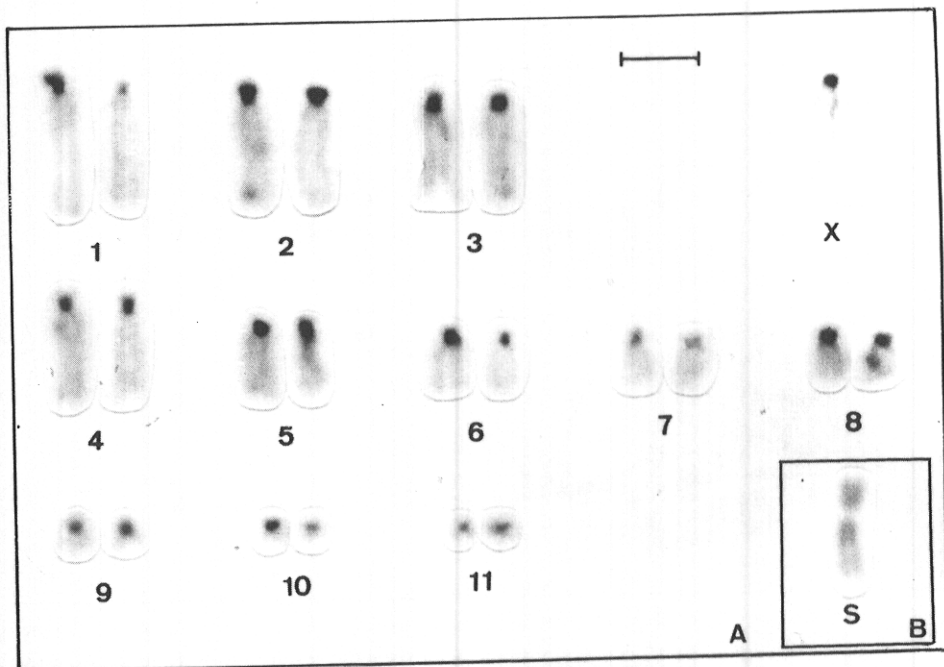


Figure 3 - C-banding pattern in *Abracris flavolineata*. A, Male specimen ($2n = 22 + XO$); B, the supernumerary (S) does not show a C band.

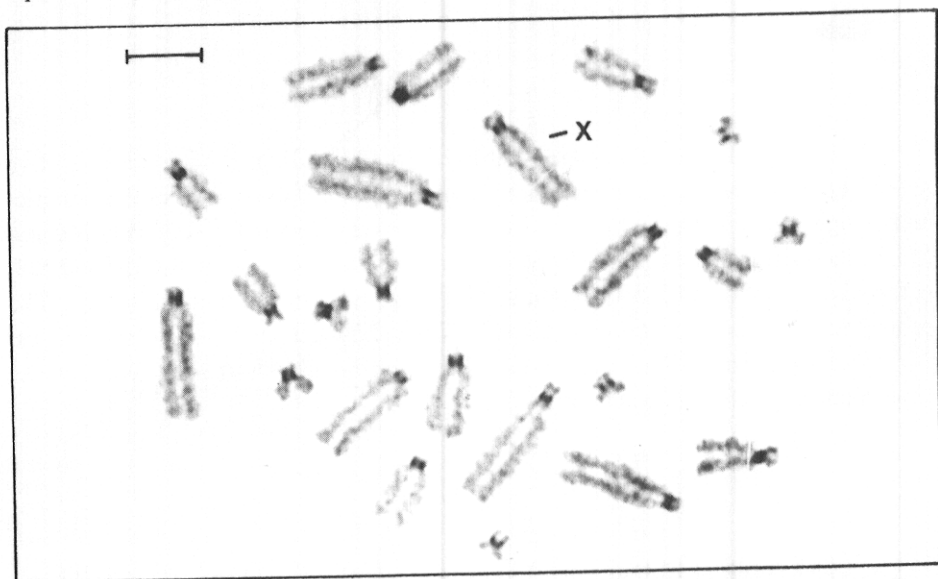


Figure 4 - Mitotic metaphase of an *Abracris flavolineata* male ($2n = 22 + XO$) with silver staining. The centromeric region of the chromosome shows punctiform staining.

Mild and Strong N bands

The Mild N banding technique showed the presence of a characteristic proximal band on the long arm of the chromosomes in pair M8. The short arms of all chromosomes in the complement appeared to be well stained, but staining was less marked than for pair M8. Trace staining was also present in the telomeric region of most chromosomes except for pairs S9, S10 and S11, and in the interstitial region of the long arm of chromosome X.

The proximal staining of the long arm of the M8 pair persisted when the Strong N technique was used (Figure 5). The trace staining obtained with the Mild N technique sometimes persisted, and the telomeric staining, when persisting, was punctiform.

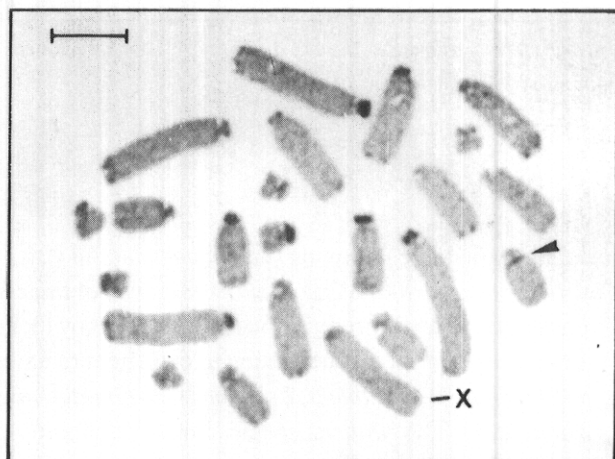


Figure 5 - Strong N - banding pattern in a male specimen ($2n = 22 + XO$) of *Abracris flavolineata*. The arrow indicates the presence of bands on chromosome 8. The short arms and the telomeric regions of some chromosomes appear to be more deeply stained.

In initial diplotene preparations submitted to the Mild N banding technique, most autosomal bivalents were stained in the proximal regions and chromosome X appeared to be deeply stained (Figure 6A). As diplotene progressed, the staining in the proximal regions could be visualized only in some bivalents even when TCA treatment was intensified. Chromosome X was poorly defined in morphology, with no trace staining. At metaphase I, the chromosomes were not stained.

In initial diplotene preparations treated with the Strong N variant, the staining pattern was similar to that obtained with the Mild N technique (Figure 6B). At metaphase I, however, most bivalents were stained in proximal and distal regions, whereas chromosome X showed trace staining in the proximal region (Figure 2B).

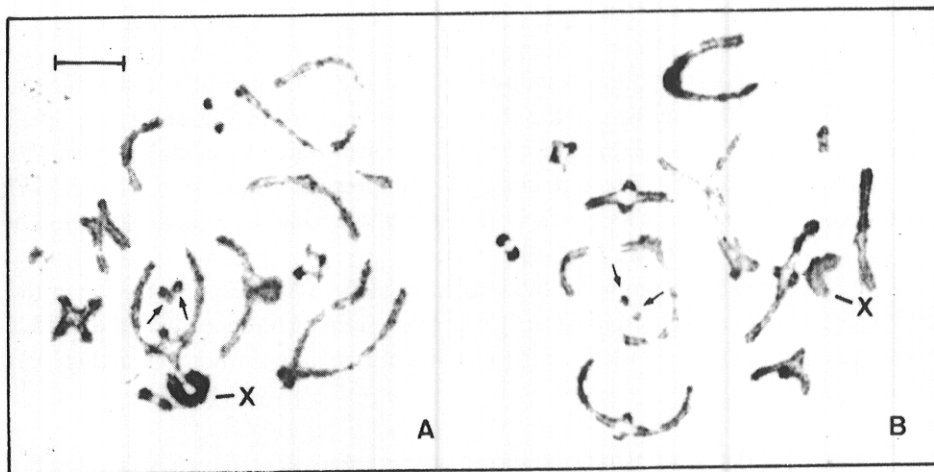


Figure 6 - Early diplotene cells of an *Abracris flavolineata* male ($2n = 11 \text{ II} + \text{XO}$). A, Mild N treatment; B, Strong N treatment. The arrows indicate staining on bivalent 1.

DISCUSSION

Most of the cytological data available concerning the tribe Abracrini (Piza, 1945; Ferreira *et al.*, 1980; Carbonell *et al.*, 1980; Mesa *et al.*, 1982) was obtained through interpretation of metaphase I from testis preparations fixed in Carnoy (3:1) and stained with lacto-acetic orcein. Nineteen species belonging to eight genera have been studied to date (*Abracris*, 3 - *Eujivarus*, 5 - *Eusitalces*, 2 - *Jodacris*, 3 - *Omalotettix*, 1 - *Psiloscutus*, 3 - *Sitalces*, 1 - *Xiphiola*, 1). The *Abracris* species initially denoted *A. meridionalis*, *A. caeruleipennis* and *Abracris* sp. A. (Ferreira *et al.*, 1980) were later identified as *Abracris dilecta*; *Jodacris* sp. A and *Jodacris* sp. C. as *Jodacris ferrugineus* and *Jodacris* sp. C. as *Jodacris ferrugineus*, and *Jodacris* sp. B. as *Jodacris chapadensis* (Mesa *et al.*, 1982).

While in the superfamily Acridoidea the diploid chromosome number varies from $2n \text{ ♂} = 8$ in *Dichroplus silveiraguidoi* (Acrididae - Melanoplinae) (Saez, 1956a,b,c) to $2n \text{ ♂} = 25$ in *Conometopus sulcaticollis* (Ommexechidae) (Mesa, 1963), in the tribe Abracrini (Ferreira *et al.*, 1980) this variation is limited to $2n \text{ ♂} = 19$ (genera *Jodacris* and *Sitalces*) and $2n \text{ ♂} = 23$, which is the basic number for the superfamily (*Xiphiola*, *Psiloscutus*, *Abracris* and *Eusitalces*). In the genus *Sitalces*, the only known species is *volxemi*, in which the reduction of chromosome number to 19 was due to two independent centric fusions. In the genus *Jodacris*, all of the three known species have 19 acrocentric chromosomes and, as was possibly also the case

for *Dichroplus pratensis* (Mesa, 1956), two centric fusions initially occurred and were followed by two centric inversions that reestablished the subterminal position of the centromeres.

Four of the five known species in the genus *Eujivarus* have a secondary karyotype as a consequence of an ancient centric fusion that seems to have become fixed in the genus (Ferreira *et al.*, 1980).

Most of the *Abracris* species studied thus far have a meiosis of the orthodox type, a sex determination system of the XO (♂) - XX (♀) type, and a sex chromosomes which is always acrocentric and heteropycnotic positive during prophase I (Ferreira *et al.*, 1980; Mesa *et al.*, 1982).

The morphology of *A. flavolineata* chromosomes obtained from neuroblasts was significantly different from that of the remaining Acridoidea species, whose chromosomes have usually been reported to be all acrocentric. In the population of Rio Claro *A. flavolineata* population all chromosomes have two arms and their morphology varies from subtelocentric to metacentric. These differences may originate from two of four mechanisms which are responsible for the specific and interspecific chromosome variability detected in eukaryotes: a) structural rearrangements which invariably cause modifications in the gene order of euchromatic components. These changes alter the interrelationships between the linkage groups of structural genes, with a consequent reduction, restriction, increase or channeling of effective gene exchange between groups. b) Addition or loss of chromosomal material, including changes in the amount of constitutive heterochromatin as well as euchromatin.

The similarity in the dimensions of the small arms of most chromosomes in the complement suggests that the typical karyotype of *A. flavolineata* originated from the addition of chromosomal material of a constitutive heterochromatin nature (except for pair 7), as shown by the results obtained with neuroblast cells submitted to C banding. The hypothesis of the occurrence of structural rearrangements (pericentric inversions) seems unlikely, since it would be necessary to assume the occurrence of breaks near the same chromosomal region of all karyotypic elements. If we admit the occurrence of an addition of chromosomal material to account for the morphological change in all elements, except for those of pair M7, we are forced to assume that this addition was of a similar level in all chromosomes, a fact that may possibly strain the limit of change that a species may withstand. The addition of this material in equivalent amounts transformed the large and medium chromosomes into subtelocentric and the small ones into metacentrics.

The chromosomes of pair M7 are submetacentric and their shorter arms are longer than the remaining ones in the M groups and than those of the S group. This difference is more marked when the short arms of the M7 pair are compared with the

remaining chromosomes of the same group (M4 - M8). When we add the fact that the short arms of pair M7 are not fully heterochromatic, these data lead us to propose the hypothesis that their origin was linked to a pericentric inversion.

Few reports are available in the literature about the occurrence of structural rearrangements involving all chromosomes in the complement. According to Cabrero and Camacho (1986), the short arms of most chromosomes of *Chorthippus vagans* originated from pericentric inversions. However, the short arm of pair 4 of *C. vagans* is fully heterochromatic, possibly being the only one that originated from the addition of constitutive heterochromatin.

The amount of constitute heterochromatin revealed by C banding varies widely when interspecific (King and John, 1980; Santos *et al.*, 1983; Cella, 1988) and intraspecific (Shaw, 1976; Rees *et al.*, 1978) comparisons are made. Perhaps the most striking example is that encountered in the species complex of the genus *Caledia* (King and John, 1980). Three of the chromosomal races of *Caledia captiva* have the same 2C DNA content, but the percentage of C-positive material varies widely among races, sometimes reaching two-fold values. Among the three Abracrini species studied by Cella (1988), *A. flavolineata* was the richest in C-positive material.

The C-banding pattern obtained for mitotic chromosomes seems to coincide with that of the heteropycnotic positive proximal blocks visualized by standard staining in diplotene autosomal bivalents. However, the X chromosome, which is heteropycnotic positive throughout meiotic prophase, shows constitutive heterochromatin only in the pericentromeric region and along the short arm in mitotic metaphases, demonstrating the facultative heterochromatic nature of the remaining chromosome. Similar cases in terms of the nature of the chromatin present in chromosome X have been reported for many Acrididae species (King and John, 1980; Santos *et al.*, 1983; John *et al.*, 1985; Cabrero and Camacho, 1986).

The silver staining technique used in the present study did not reveal any region that could be identified as a NOR, not even those that in one specimen were interpreted to be secondary constrictions (middle region of the long arm of pair M5 and proximal region of the long arm of pair M8). The NORs may also be located in the centromeric regions (Di Bernardino *et al.*, 1979), but the silver-stained regions obtained in *A. flavolineata* did not present the characteristics of NORs, since they were present in the centromeric regions of all chromosomes and exhibited a morphology very similar to that of Cd bands (Eiberg, 1974).

According to Virkki (1983), the silver staining techniques permit the visualization of ribonucleoproteins and perhaps of some acid proteins. As a consequence, the nuclear matrix, the axial chromosomal skeleton, the synaptonemal complex, the kinetocore plates, the centromeric regions, the sex chromosomes during meiosis and the nucleic acids are stained when silver ion is used for impregnation

(Black and Ansley, 1964; Quack and Noel, 1977; Noel *et al.*, 1978; Fletcher, 1979; Hernandez-Verdum *et al.*, 1980; Virkki, 1983; Earnshaw and Laemmli, 1984).

The staining pattern obtained for *A. flavolineata* was similar to that reported by King and John (1980) using C banding for all chromosomes of *Perala viridis*, for chromosomes 1 and 3 of *Ailopus thalassinus*, for the Neo Y chromosome of *Stenacatanops angustifrons*, for chromosomes 1 and 2 of *Rectitropis sp 1.*, for chromosomes 1, 2, 3, 5 and 6 of *Valanga irregularis*, and for chromosome 1 of *Oedalus australis*. These stained regions were also interpreted to be structures related to the centromere and probably correspond to the kinetochore plates.

Among Orthoptera, Grillidae yielded the most satisfactory results when their chromosomes were submitted to silver staining (Allen and Cave, 1972; Lima de Faria *et al.*, 1973; Cave and Allen, 1974; Czaker, 1978; Satya-Prakash and Pathak, 1984). However, only in a few Acrididae species was it possible to identify the NOR-bearing chromosomes (Gosálvez *et al.*, 1981; Rufas and Gosálves, 1982; Cabrero *et al.*, 1986; Camacho *et al.*, 1986; Croft and Jones, 1986). In a careful study of neuroblast chromosomes from eight acridian species, Cella (1988) was able to identify the NORs in only three species. According to Goodpasture and Bloom (1975), this may be due to the fact that ribosomal genes are arranged in small groups of difficult identification because of the lack of sensitivity of the technique used.

The only stained region having the characteristics of N bands in *A. flavolineata* chromosomes was that of the proximal region of the long arm of the chromosomes of pair 8. According to Matsui *et al.* (1986), the N band marks two of the non-histone proteins that show great affinity for rDNA.

The location of this band coincides with the NOR described by Cella (1988) for *Schistocerca flavofasciata* in the proximal region of the chromosomes of pair 8. Since N-banding reveals NOR-associated proteins, we assume that part of the NORs of *A. flavolineata* may also be located on the chromosomes of pair 8.

The traces of telomeric staining observed both in mitotic and meiotic chromosome submitted to N banding seem to show the occurrence of a particular chromatin which is not always revealed by C banding. According to Sen and Gilbert (1988), the chromatin present in the telomeric regions differs in DNA composition and organization when compared to the DNA of the remainder of the chromosome, probably because of its functions, such as initiating homologue pairing from the ends attached to the nuclear envelope.

Of the three species of the genus *Abracris* studied cytologically, *A. dilecta* is the only one that maintains the basic karyotype of the superfamily Acridoidea ($2n \text{ ♂} = 23$, $2n \text{ ♀} = 24$), with all of its chromosomes being acrocentric. *Abracris sp. A.* ($2n \text{ ♂} = 21$, $2n \text{ ♀} = 22$) has a pair of metacentric chromosomes which originated from

a centric fusion between two of its medium-sized chromosomes, and all of the chromosomes of *A. flavolineata* ($2n \sigma = 23$, $2n \varphi = 24$) are of derived morphology.

The pronounced elastic constriction observed in the bivalent formed by the chromosomes of pair 1 has also been observed in *Omalotettix obliquus* (Carbonell *et al.*, 1980) and in several other species of the tribe Abracrini.

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RESUMO

Os cromossomos mitóticos (neuroblásticos) e meióticos (testiculares) de *Abracris flavolineata* foram analisados usando-se técnicas de coloração convencional (Giemsa) e diferencial (bandas C, "Mild N", "Strong N" e impregnação pelo íon prata). Todos os cromossomos do cariótipo ($2n \sigma = 23$ e $2n \varphi = 24$) possuem morfologias diferentes daqueles que representam o cariótipo básico da superfamília Acridoidea e que são acrocêntricos. Os cromossomos dos pares G1 - G3, M4 - M6 e M8 são subtelocêntricos, os dos pares M7 e P9 são submetacêntricos e os dos P10 e P11 são metacêntricos. O cromossomo sexual é subtelocêntrico. Os resultados obtidos com o emprego da técnica de bandas C mostram que os braços pequenos de todos os cromossomos, com exceção daqueles do par M7, foram originados por adição de material cromossômico de natureza heterocromática constitutiva. A morfologia submetacêntrica do par M7 tem sua origem ligada a uma inversão pericêntrica. A técnica da impregnação pela prata não marcou nenhuma região que exibisse características de RON, mas evidenciou pontos nas regiões centroméricas, semelhantes às bandas Cd, e que foram interpretados como placas cinetocóricas. As técnicas de bandas N marcaram as regiões proximais dos braços longos dos cromossomos do par M8, que foram consideradas como sendo uma das regiões organizadoras de nucléolos de *A. flavolineata*.

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