

Variability of constitutive heterochromatin in karyotypes of representatives of the family Romaleidae (Orthoptera)

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ABSTRACT

We analyzed the C-banding patterns of 43 grasshopper specimens belonging to three species of the family Romaleidae (*Xyleus angulatus*, *Chromacris speciosa* and *Brasilacris gigas*). The three species presented uniform karyotypes in terms of chromosome number ($2n=24$, XX female) and shape (one armed) and also of pericentromeric C bands, but differed in the telomeric and interstitial C-banding patterns. A supernumerary chromosome was detected in *Xyleus angulatus*, with unstable distribution among females of the Igaracú, Pernambuco population.

INTRODUCTION

The superfamily Acridoidea has been extensively studied from a karyotypic viewpoint, and is mainly characterized by a diploid number of $2n=23$ male; $2n=24$ female and by the XO system of chromosomal sex determination (Hewitt, 1979; Mesa *et al.*, 1982).

Karyotypic data for many species of the family Acrididae have revealed the occurrence of considerable diversity of C-banding patterns among species. Use of the C-banding technique as a chromosome marker has revealed a large number of polymorphisms among populations of various species. Many of these polymorphisms are due to the variability of constitutive heterochromatin, both in the form of extra segments, and of supernumerary chromosomes (Camacho and Cabrero, 1982; Henriques-Gill *et al.*, 1984; Viseras *et al.*,

1989; Colombo, 1992). C-banding data are still quite scarce for the family Romaleidae.

MATERIAL AND METHODS

The species analyzed were collected from different areas of the State of Pernambuco (Brazil). *Xyleus angulatus* was obtained from Recife/UFPE Campus (seven specimens) and from Igaracú (18 specimens); *Chromacris speciosa* from Moreno/BR 232, Km 35 (12 specimens) and *Brasilacris gigas* from Arcoverde/BR 232, Km 230 (six specimens). All individuals were adult females. In the case of *X. angulatus*, some preparations were made from embryos at 26 days of development, obtained from eggs incubated at 30°C, laid by females collected in Igaracú.

Cytological preparations were obtained both from cell suspensions and by squashing whole ovarioles and whole embryos, according to the method of Souza (1991), using colchicine-treated material. C-banding was performed by the method of Sumner (1972).

All specimens studied have been deposited in the entomology collection of the Department of Genetics, UFPE.

RESULTS AND DISCUSSION

The karyotypes of the three species studied were uniform in terms of chromosome number ($2n=24$ female) and shape (one-armed complement). The karyotype of the *X. angulatus* consisted of three large (L1-L3), five medium (M4-M8) and three small (S9-S11) autosome pairs. Chromosome X was third in size. *B. gigas* had five large pairs, including the X (L1-L5), five medium pairs (M6-M10) and two small pairs (S11-S12). *C. speciosa* had seven large pairs, including the X (L1-L7), two medium pairs (M8-M9) and three small pairs (S10-S12).

These species all had constitutive heterochromatin (CH) in the pericentromeric region of all chromosomes. However, CH distribution in telomeric and interstitial areas differed among them.

X. angulatus - In addition to the pericentromeric bands, this species presented interstitial and/or telomeric CH in most of the chromosomes in the complement: thus, pairs 1, 2 and 3 had centromeric and interstitial C bands. Pairs 5, 7, 9 and 11 had centromeric and telomeric bands and pairs 4, 6, and 8 had centromeric, telomeric and interstitial bands (Figure 1A). Further, with the C-banding technique the proximal band of X chromosome was stained blue, differing from the remaining bands in the complement, which stained purple. Furthermore, five females, out of a sample of 18 collected in Igaracú, presented a B chromosome of the approximate size of the X, partially heterochromatic and showing only a few euchromatic bands. This chromosome was found to be numerically unstable, ranging from one to two in different female carriers (Figure 2a,b).

C. speciosa - All chromosomes in the complement presented pericentromeric CH, but varied widely in band size and position. In pairs M3, M4, M6 and M7, the heterochromatin block covered a considerable portion of the proximal part of the long arm. Pairs L1 and L2 showed telomeric C bands, and pair M9 presented a large block of interstitial C bands. Pairs M5, M6 and M8 presented narrow interstitial bands (Figure 1B).

B. gigas - This species is a monotypic genus endemic to the Northeastern Region of Brazil and its karyotype is described here for the first time. All the chromosomes of this species presented only small pericentromeric C bands, except chromosome M5

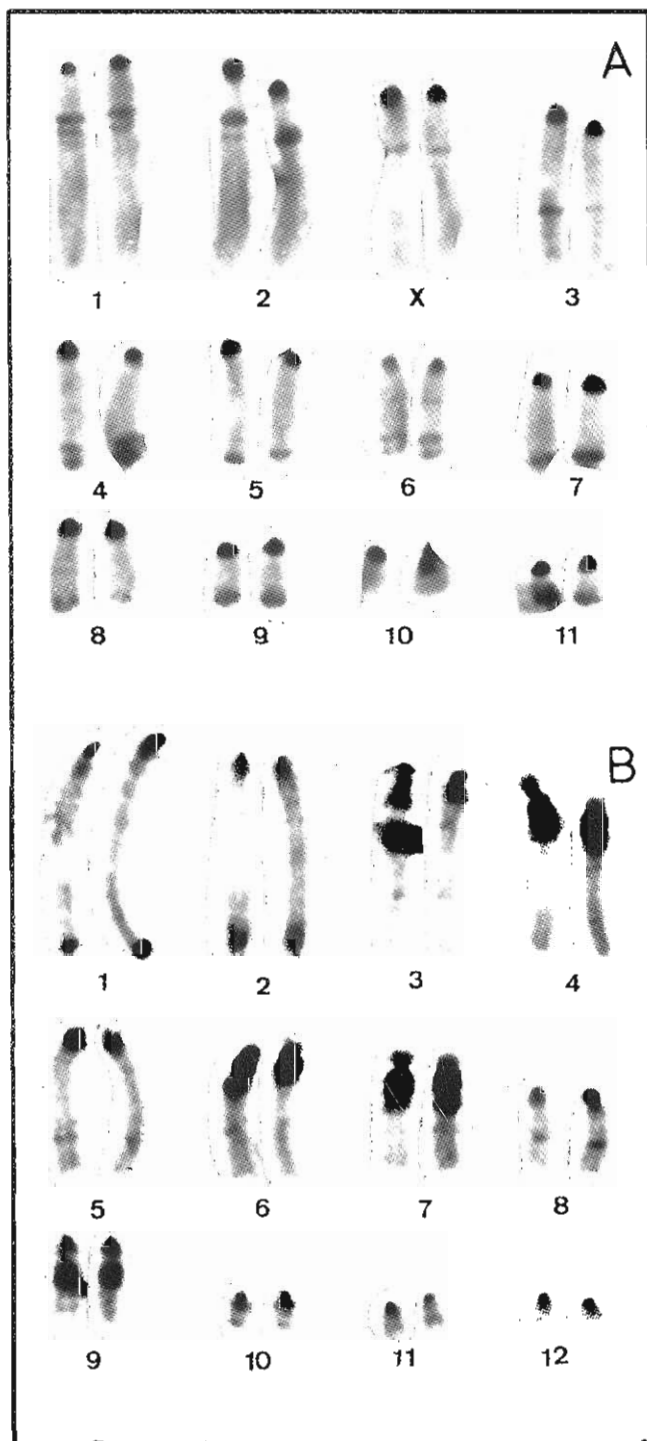


Figure 1 - C-banding in: (A) Mitotic metaphase from embryo cell of *Xyleus angulatus*, female; (B) Mitotic metaphase from ovariole cell of *Chromacris speciosa*.

which had an additional very narrow telomeric C band of difficult visualization (Figure 3).

The fact that the superfamily Acridoidea presents great uniformity in chromosome number and shape seems to suggest that the group is chromosomally conservative. However, wide interspecific variability has been detected in the



Figure 2 - Two C-banded (a,b) mitotic metaphases from ovariolar cells of *Xyleus angulatus* showing different numbers of B chromosomes. In the inset the B chromosome C-banded.

heterochromatin patterns of different groups. In addition, various species have shown karyotypic variation, both in terms of the shape of supernumerary chromosomes and extra segments, as well as in terms of polysomy, and other chromosome rearrangements (White, 1973; Hewitt, 1979; King and John, 1980; Jones and Rees, 1982; Camacho *et al.*, 1984; Viseras and Camacho, 1984).

Three species analyzed here presented an equilocal pattern of centromeric CH distribution (Heitz, 1933; John *et al.*, 1985). However, a marked interspecific variability was detected, especially in terms of interstitial and centromeric heterochromatin distribution in the chromosome complement. A large number of narrow interstitial and telomeric C bands was detected in mitotic metaphases from embryos of *X. angulatus*, probably due to the lower degree of condensation and better quality of the chromosomes of embryo cells, since these bands usually were not visible in material from adult specimens.

When defining the equilocal patterns (similar sites in non-homologous chromosomes) of constitutive heterochromatin, Heitz (1933) assumed the existence

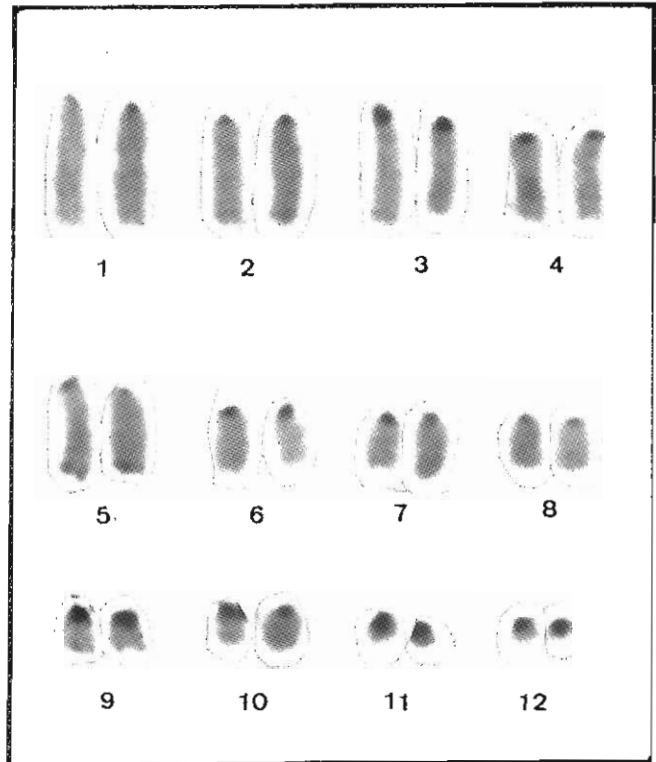


Figure 3 - C-banded karyotype of *Brasilacis gigas*, female.

of rules that might govern the distribution of heterochromatin blocks. Equilocal CH distribution is widely scattered throughout the genomes of plants and animals (Greilhuber *et al.*, 1981; Loidl, 1983; John *et al.*, 1985). Chromosome orientation in stages of narrow positioning (Rabl polarization) during meiotic interphase and prophase may permit the transfer of repetitive DNA sequences of heterochromatin among non-homologous chromosomes, which could explain the equilocal distribution of CH. According to the model proposed by Schweizer and Loidl (1987), telomeric C bands tend to occur on small arms, whereas large arms tend to present interstitial bands. Indeed, the position of broad bands on large chromosomes seems to be determined by the distance between the centromere and the telomeric band of the smallest chromosomes. According to this model, mechanical aspects, and not selective advantages, may be responsible for the position of heterochromatin bands. The positions of interstitial and telomeric C bands observed in chromosomes of *X. angulatus* embryos are compatible with the model proposed by Schweizer and Loidl (1987). The banding pattern of *B. gigas* showed relatively poor positive C-banded blocks, but is also in agreement with the prediction of the model. *C. speciosa*, however, goes against this model, due to the occurrence of telomeric bands in pairs L1 and L2, and the absence of these

bands in the telomeres of medium and small pairs. Another interesting aspect related to the distribution of C bands in this species is the similarity of the patterns of chromosomes of identical size. This was the case for chromosomes L1, L2; M4, M6, M7 and S10, S11, S12.

In the few genera studied thus far, the family Romaleidae, like the family Acrididae, is characterized as highly variable with respect to heterochromatin. For example, the presence of a B chromosome and of polymorphism for heterochromatic supernumerary segments detected in the romaleid *Zoniopoda tarsata* (Vilardi, 1986, 1988). Wide CH variability was also demonstrated in *X. angulatus* in the form of heterochromatic extra segments (Souza and Silva Filha, 1993). Further chromosome B described here for this species was almost entirely heterochromatic, as determined by C-banding, and was present at a frequency of 27.7% in one of the populations studied.

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RESUMO

Xyleus angulatus, *Chromacris speciosa* e *Brasilacris gigas* foram estudadas cromossomicamente com o uso da técnica de bandeamento C. As três espécies apresentaram cariótipos uniformes quanto ao número e forma dos cromossomos e também bandas C pericentroméricas, embora *C. speciosa* tenha apresentado blocos pericentroméricos maiores. Por outro lado, estas espécies se diferenciaram quanto ao padrão de bandas C telomérico e intersticial. *X. angulatus* apresentou um polimorfismo decorrente da presença de um cromossomo B instável, quase inteiramente heterocromático. Esse B variou em número de um a dois em células de parede de ovariolo de cinco fêmeas analisadas.

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