Comparative cytogenetic analysis of two grasshopper species of the tribe Abracrini (Ommatolampinae, Acrididae)

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Abstract

The grasshopper species Orthoscapheus rufipes and Eujivarus fusiformis were analyzed using several cytogenetic techniques. The karyotype of O. rufipes, described here for the first time, had a diploid number of 2n = 23, whereas E. fusiformis had a karyotype with 2n = 21. The two species showed the same mechanism of sex determination (XO type) but differed in chromosome morphology. Pericentromeric blocks of constitutive heterochromatin (CH) were detected in the chromosome complement of both species. CMA/DA/DAPI staining revealed CMA3-positive blocks in CH regions in four autosomal bivalents of O. rufipes and in two of E. fusiformis. The location of active NORs differed between the two species, occurring in bivalents M6 and S9 of O. rufipes and M6 and M7 of E. fusiformis. The rDNA sites revealed by FISH coincided with the number and position of the active NORs detected by AgNO3 staining. The variability in chromosomal markers accounted for the karyotype differentiation observed in the tribe Abracrini.

Key words: constitutive heterochromatin, fluorochromes, FISH, Orthoptera, ribosomal DNA.

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Introduction

The grasshopper subfamily Ommatolampinae (Acrididae) comprises nine tribes and more than 50 genera that have a wide geographic distribution, with most of them being found in North, Central and South America (Amedgno, 1974, Carbonell, 1977). Although numerous studies have dealt with species of the family Acrididae, the analysis of most Neotropical species, especially of the subfamily Ommatolampinae, has been restricted to conventional staining (Ferreira et al., 1980; Mesa et al., 1982; Mesa and Fontanetti, 1983); at least 20 species of Ommatolampinae have been studied using conventional techniques (Carbonell et al., 1980; Ferreira et al., 1980; Mesa et al., 1982; Mesa and Fontanetti, 1983; Cella and Ferreira, 1991). The use of specific techniques for chromosome identification has been applied only to the species Abracris flavolineata (Cella and Ferreira, 1991). Despite the small number of species studied so far, the data obtained indicate that this subfamily is characterized by a significant number of species (> 40%) with derived karyotypes originating from centric fusions or other rearrangements. These karyotypes are found in species of the genera Pycnosarcus and Lagidacris (17, XO) – Pycnosarcini, Bucephalacris (21, XO) – Dellini, Abracris, Eujivarus and Omalotettix (21, XO), and Jodacris and Siltaces (19, XO) – Abracrinri (Carbonell et al., 1980; Ferreira et al., 1980; Mesa et al., 1982; Mesa and Fontanetti, 1983).

Constitutive heterochromatin (CH) accounts for a significant part of the genome in grasshoppers and is characterized by a low gene density and the presence of highly repetitive sequences. Some studies have shown extensive polymorphism in this type of chromatin in grasshoppers, including variation in the location and size of the CH blocks, as well as heterogeneity in these regions. The use of base-specific fluorochromes has contributed to the characterization of CH (King and John, 1980; Santos et al., 1983; John et al., 1985), although for Neotropical grasshoppers the data obtained with such probes are limited to a few species of the families Acrididae and Romaleidae (Souza et al., 1998; Loreto and Souza, 2000; Pereira and Souza, 2000; Souza et al., 2003; Rocha et al., 2004; Loreto et al., 2005; Souza and Melo, 2007). Other techniques, such as fluorescent in situ hybridization (FISH), have identified important differences in the composition of CH that involve variable quantities of repetitive DNA sequences in these regions, in-
cluding satellite or ribosomal DNA (rDNA) (Rodríguez Iñigo et al., 1993, 1996; Loreto et al., 2008). Since, in contrast to silver nitrate (AgNO₃) staining, FISH does not depend on the presence of a transcription product it permits the identification of active or inactive rDNA sequences in the genome (López-León et al., 1999).

In this study, we used the C-banding technique and staining with base-specific fluorochromes (CMA₃ and DAPI) to examine the distribution of CH and the proportion of GC and AT base pairs in karyotypes of the grasshoppers Orthoscapheus rufipes and Eujivarus fusiformis; silver nitrate impregnation and FISH were used to determine the position and variability of the nucleolar organizer regions (NORs). The results described here improve our understanding of chromosomal organization in these species and display new light on the chromosomal phylogeny of the Ommatolampinae.

Material and Methods

Specimens of O. rufipes and E. fusiformis were collected in two rainforest areas in Pernambuco State, northeastern Brazil. Fifteen male and nine female specimens of O. rufipes and seven male and two female specimens of E. fusiformis were collected in Gurjaú forest, in the municipality of Cabo (8°17'12" S; 35°2'6" W). Ten male and eight female E. fusiformis specimens were also collected in the Dois Irmãos zoological-botanical garden, in the municipality of Recife (8°3'14" S; 34°52'52" W).

Cytological preparations were obtained from testes and ovarioles by the classic squashing technique. The ovarioles were pretreated with 0.1% colchicine for 6 h prior to preparation. The material was fixed in ethanol:acetic acid (3:1, v/v). Conventional staining was done with 2% lactoacetic orcein. C-banding, triple staining with CMA₃/DA/DAPI and AgNO₃ staining were done as described by Sumner (1972), Schweizer et al. (1983) and Rufas et al. (1987), respectively. FISH was done as described by Moscone et al. (1996) using 18S and 25S rDNA probes from Arabidopsis thaliana (Unfried et al., 1989; Unfried and Gruendler, 1990). The probes were labeled with bio-11-dUTP by nick translation (Life Technologies) and detected using rat antibiotin (Dakopatts M0743, Dako) and TRITC (tetramethylrhodamine isothiocyanate)-conjugated anti-antibiotin (Dakopatts R0270, Dako) antibodies. The preparations were counterstained with DAPI (2 μg/mL) and mounted with Vectashield H-1000 (Vector).

For fluorescent in situ hybridization (FISH), images of cells were captured with a Cytovision system coupled to an Olympus BX51 microscope. For the other techniques, the cells were photographed with a Leica microscope. The images were mounted using CorelDraw Graphics Suite 12.

Results

The karyotype of O. rufipes, described here for the first time, consisted of a diploid number of 2n = 23 and an XO sex determination mechanism for males, and 2n = 24 and XX for females. The chromosomes of O. rufipes (Figure 1a-c) were acrocentric and were classified according to size into two large pairs (L₁-L₂), six medium sized pairs (M₁-M₆) including the X chromosome, and three small pairs (S₉-S₁₁). In contrast, E. fusiformis had a diploid number of 2n = 21 and an XO sex determination mechanism. The L₁ pair was submetacentric and the other chromosomes were acrocentric. There were two large pairs (L₁-L₂), five medium sized pairs (M₁-M₅) including the X chromosome, and three small pairs (S₈-S₁₀) (Figure 1b-d). In both species, the X chromosome showed variable heteropyknotic behavior during meiotic prophase I (Figure 1c,d).

The CH blocks were located in pericentromeric regions of the chromosomes and varied in size between and within the species studied (Figure 2a,c). In O. rufipes (Figure 2a), the CH blocks were small and located on all chromosomes of the complement, whereas in E. fusiformis (Figure 2c) these blocks were very small and found only on some bivalents. CMA₃/DA/DAPI staining revealed CMA₃-positive blocks on four bivalents of O. rufipes, including one interstitial block on L₂, proximal and telomeric blocks on M₅, and pericentromeric blocks on M₆ and S₉ (Figure 2b). The CMA₃-positive blocks of the L₂ and M₅ bivalents were not detected by C-banding. Eujivarus fusiformis had two medium sized chromosomes (M₆ and
M7) with CMA3-positive blocks (Figure 2d). In both species, DAPI staining was homogenous throughout all of the chromosomes (data not shown).

Active NORs were observed during prophase I (pachytene-diplotene) in both species, and were located in bivalents M6 and S9 of O. rufipes and in M6 and M7 of E. fusiformis. At both sites, the NORs coincided with CMA3-positive blocks. Additionally, the rDNA sites detected by FISH coincided with the results of AgNO3 staining in both species (Figure 3a-d). Table 1 summarizes the data obtained with conventional staining, C-banding, base-specific fluorochromes, AgNO3 staining, and FISH for the two species studied.

Discussion

Variations in the karyotypes of grasshoppers have been reported, with the most frequent being chromosomal rearrangements such as inversions, reciprocal translocations and centric fusion/fission, in addition to variability in the pattern of CH distribution and the occurrence of extra chromosomal material. Among the ten Neotropical Acrididae subfamilies studied cytogenetically by Mesa et al. (1982), the Copiocerinae, Melanoplinae and Ommatolampinae were characterized by derived karyotypes (81.8%, 48.4% and 42.9%, respectively) that resulted mainly from centric fusions and inversions.

The karyotypes of 31 species of the subfamily Ommatolampinae are known, with 21 of them belonging to the tribe Abracrin as (Carbonell et al., 1980; Ferreira et al., 1980; Mesa et al., 1982; Mesa and Fontanetti, 1983; Cella and Ferreira, 1991; present study). Despite the small number of species studied so far, more than 40% of them show variations in diploid number, a finding that identifies this subfamily as an important group for studying chromosome evolution. Table 2 summarizes the chromosome number, sex mechanism and chromosome morphology of representatives of the tribe Abracrin (Ommatolampinae, Acrididae).

Orthoscapheus rufipes had a basic karyotype (23, XO) consisting of acrocentric chromosomes, which was similar to that widely found in the family Acrididae (Mesa et al. 1982; Santos et al., 1983; Bugrov, 1996). On the other hand, the karyotype of E. fusiformis (2n = 21, XO) is considered to be a derived karyotype among acridoid grasshoppers. Of the 21 Abracrin species listed in Table 2, 14 have derived karyotypes. These species belong to the genera Abracris and Eujivarus (21, XO, one pair of metacentric autosomes), Omalotettix (21, XO, pairs 3 and 6 submetacentric, pair 5 subacrocentric, and the remaining chromosomes acrocentric), Jodacris (19, XO, acrocentric chromosomes) and Sitalces (19, XO, two pairs of metacentric autosomes). Among the three species of Abracris studied cytologically, Abracris sp. is the only one with 2n = 21 and XO, with one metacentric pair originating from the centric fusion of two medium sized chromosomes. Abracris dilecta and A. flavolineata have 2n = 23 and XO. However, whereas A. dilecta has acrocentric chromosomes, A. flavolineata has chromosomes with two arms throughout the karyotype complement, in contrast to the basic karyotype of Acridoidea (Mesa et al., 1982; Cella and Ferreira, 1991).

An ancient fusion appears to have been involved in the phylogeny of Eujivarus since four of the five species whose karyotype has been studied show a reduction in the diploid number to 2n = 21 with XO (Ferreira et al., 1980; Mesa et al., 1982). In contrast, more recent fusions and polymorphisms in this type of chromosomal rearrangement have been observed in grasshoppers such as Cornops aquaticum (Mesa et al., 1982), Eyprepocnemis plorans
The CH of *O. rufipes* and *E. fusiformis* was preferentially located in pericentromeric regions, in agreement with descriptions for most species of the family Acrididae (King and John, 1980; Santos et al., 1983; Rocha et al., 2004; Souza and Melo, 2007). In *Abracris flavolineata*, in addition to the pericentromeric regions, CH blocks also occur on the short arms of all chromosomes, except for pair M7 (Cella and Ferreira, 1991). This divergence in the pattern of distribution suggests that rearrangements in CH, such as amplifications or losses, may contribute to karyotype evolution in the Abracrinini.

Differences in the base composition of CH (detected by CMA3/DA/DAPI staining) were observed in the species studied. In most chromosomes of both species, there was no predominance of AT or GC base pairs. However, GC-rich CMA3-positive regions were detected in some CH blocks, especially those located in NORs. This pattern has also been reported for other grasshopper species (John et al., 1985; Camacho et al., 1991; Souza et al., 1998; Loreto and Souza, 2000; Pereira and Souza, 2000; Rocha et al., 2004). In some cases, such as in *Xyleus angulatus*, *Phaeoparia megacephala* and *Cornops frenatum frenatum*, CMA3-positive blocks have been detected in all chromosomes of the complement (Souza et al., 1998; Pereira and Souza, 2000; Rocha et al., 2004). On the other hand, in most species,
GC-rich CH blocks occur on only some chromosomes of the karyotype (Schweizer et al., 1983; John et al., 1985; Loreto and Souza, 2000; Souza et al., 2003; Rocha et al., 2004; Loreto et al., 2005; Souza and Melo, 2007), including O. rufipes and E. fusiformis.

Three categories of CH were identified in O. rufipes: (1) CH detected by C-banding, (2) CH detected by C-banding and CMA3 staining and restricted to NORs (pairs M6 and S9), and (3) CH detected by CMA3 staining, but not by C-banding, and unrelated to NORs (pairs L2 and M5). According to Sumner (1990), CH is not always detectable by the C-banding technique since its visualization is related to the size of the segments, with blocks less than 10^6 or 10^7 base pairs (bp) generally not being detected by this method. The finding that the third type of CH was GC-rich but showed no functional relationship to rDNA sites suggested a different organization for these two regions.

Two rDNA sites preferentially located on medium sized autosomes were observed in O. rufipes and E. fusiformis, a pattern commonly found among representatives of Neotropical Acrididae (Rocha et al., 2004; Souza and Melo, 2007; Loreto et al., 2008). The presence of NORs on bivalent S9, as observed in O. rufipes, has also been described for most Neotropical species studied so far, especially those of the subfamilies Leptysmine (Rocha et al., 2004) and Gomphocerinae (Loreto et al., 2008). According to Loreto et al. (2008), bivalent S9 probably represents the ancestral location of rDNA sites in Neotropical gomphocerine species since this pattern was identified in five species analyzed (Rhammatocercus brasiliensis, R. brunneri, R. palustris, R. pictus and Amblytropidia sp.). Ribosomal DNA sites on S9 were also observed in five of six leptysmine species studied (Loreto and Souza, 2000; Rocha et al., 2004) and in the ommatolampine O. rufipes. Together, these findings suggest a pattern of ancestrality for Neotropical acridid grasshoppers.

In a broad investigation of the location and expression of ribosomal genes in 49 grasshopper species, Cabrero and Camacho (2008) showed that most Old World representatives have 1-3 rDNA sites and only six species had 5-10 sites. The predominant NOR locations were bivalents 2, 3, 6 and 9 and the X chromosome. In the subfamily Gomphocerinae, signals were detected mainly in bivalents 2, 3 and the X chromosome in species with 2n = 17, whereas in species with 2n = 23 most signals were restricted to bivalent 9. In Oedipodinae, NORs were generally found on bivalents 6 and 9. These data suggest that the location of rDNA sites on chromosome 9 represents an ancestral condition.

NORs located on large chromosomes or in the X chromosome have not yet been described for Brazilian species of the family Acrididae, in contrast to observations for the romaleid species Radacridium nordestinum (L3) and Radacridium mariagioseae (X) (Rocha et al., 1997), Xyleus angulatus (L3, M4 and X) (Souza et al., 1998), and some Old World acridid species (Rufas et al., 1985; Cabrero and Camacho, 1986, 2008).

Many rDNA sites are located in pericentromeric regions of medium and/or small chromosomes (Loreto and Souza, 2000; Rocha et al., 2004), as observed in the present study. However, some NORs occur in proximal regions, as observed in Stenacris xanthochlora and Tucayaca parvula (M3) (Rocha et al., 2004), or in interstitial regions, as described in Schistocerca palliens (M3) and S. flavofasciata (M5 and M6) (Souza and Melo, 2007). In the Old World grasshoppers studied by Cabrero and Camacho (2008), many rDNA sites detected by FISH were found in proximal regions (52.4%), although a significant number was observed in interstitial regions (34.9%) and a minority was distal (12.7%). In addition, about 13% of the 126 rDNA sites detected by FISH were silent.

In conclusion, this study provides the first detailed cytogenetic results for E. fusiformis and O. rufipes. The two species showed significant karyotype differentiation depending on the staining method used. Our findings suggest possible pathways of chromosome evolution in these species. However, additional cytogenetic and molecular analyses of other species of the tribe Abracriini are necessary to improve our understanding of the evolutionary patterns within this group.

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