Genetics and Molecular Biology, 34, 2, 214-219 (2011)

www.sbg.org.br

Research Article

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

Marília de França Rocha[1], Natoniel Franklin de Melo[2] and Maria José de Souza[3]

[1]*Laboratório de Biodiversidade e Genética de Insetos, Instituto de Ciências Biológicas, Universidade de Pernambuco, Recife, PE, Brazil.*
[2]*Empresa Brasileira de Pesquisa Agropecuária, Petrolina, PE, Brazil.*
[3]*Departamento de Genética, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, PE, Brazil.*

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_3/DA/DAPI staining revealed CMA_3-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 M_6 and S_9 of *O. rufipes* and M_6 and M_7 of *E. fusiformsi*. The rDNA sites revealed by FISH coincided with the number and position of the active NORs detected by $AgNO_3$ 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.

Received: September 11, 2010; Accepted: January 19, 2011.

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 (Amedgnato, 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) – Abracrini (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-

Send correspondence to Maria José de Souza. Departamento de Genética, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego S/N, Cidade Universitária, 50732-970 Recife, PE, Brazil. E-mail: mjslopes.ufpe@yahoo.com.br.

cluding satellite or ribosomal DNA (rDNA) (Rodríguez Iñigo *et al.*, 1993, 1996; Loreto *et al.*, 2008). Since, in contrast to silver nitrate ($AgNO_3$) 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 (CMA_3 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 CMA_3/DA/DAPI and $AgNO_3$ 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 anti-biotin (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* hydridization (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 acrotelocentric and were classified according to size into two large pairs (L_1-L_2), six medium sized pairs (M_3-M_8) including the X chromosome, and three small pairs (S_9-S_{11}). In contrast, *E. fusiformis* had a diploid number of 2n = 21 and an XO sex determination mechanism. The L_1 pair was submetacentric and the other chromosomes were acrotelocentric. There were two large pairs (L_1-L_2), five medium sized pairs (M_3-M_7) including the X chromosome, and three small pairs (S_8-S_{10}) (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. CMA_3/DA/DAPI staining revealed CMA_3-positive blocks on four bivalents of *O. rufipes*, including one interstitial block on L_2, proximal and telomeric blocks on M_5, and pericentromeric blocks on M_6 and S_9 (Figure 2b). The CMA_3-positive blocks of the L_2 and M_5 bivalents were not detected by C-banding. *Eujivarus fusiformis* had two medium sized chromosomes (M_6 and

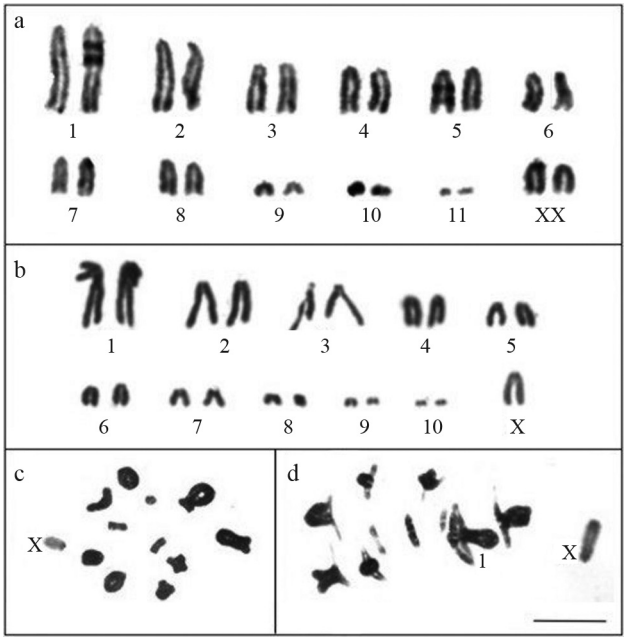

Figure 1 - Conventional staining of *O. rufipes* (a,c) and *E. fusiformis* (b,d) cells. (a) and (b), karyotypes mounted based on female mitotic metaphase chromosomes and male anaphase I chromosomes, respectively; c, diakinesis; d, metaphase I. Note the submetacentric chromosome L_1 (b,d). Bar = 5 μm.

M_7) with CMA_3-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 M_6 and S_9 of *O. rufipes* and in M_6 and M_7 of *E. fusiformis.* At both sites, the NORs coincided with CMA_3-positive blocks. Additionally, the rDNA sites detected by FISH coincided with the results of $AgNO_3$ staining in both species (Figure 3a-d). Table 1 summarizes the data obtained with conventional staining, C-banding, base-specific fluorochromes, $AgNO_3$ 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 Abracrini (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 Abracrini (Ommatolampinae, Acrididae).

Orthoscapheus rufipes had a basic karyotype (23, XO) consisting of acrotelocentric 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 Abracrini 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*

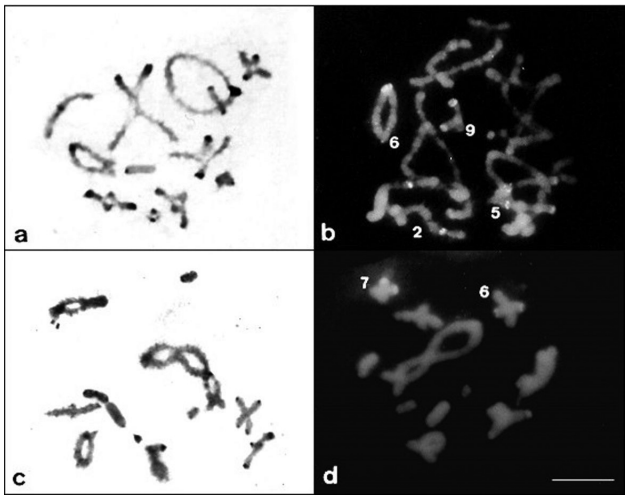

Figure 2 - Distribution pattern and characterization of CH by C-banding (a,c) and CMA_3/DA staining (b,d) in diplotenes of *O. rufipes* (a,b) and *E. fusiformis* (c,d). Note the chromosomes with CMA_3-positive blocks in (b) and (d). Bar = 5 μm.

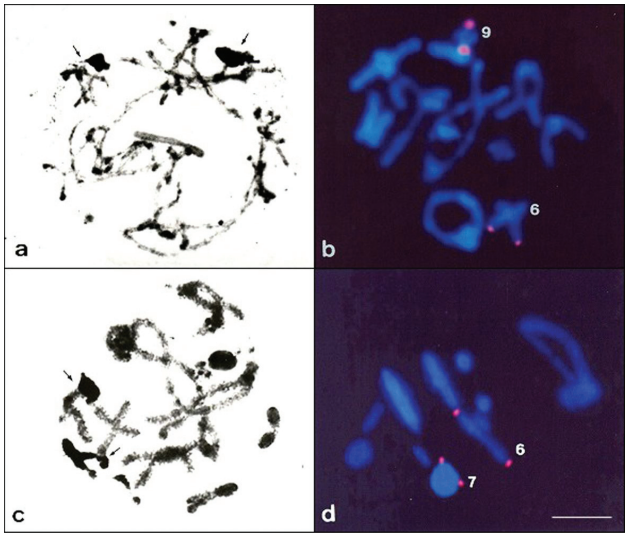

Figure 3 - Nucleolar organizer regions detected by silver nitrate staining (arrows) and fluorescent *in situ* hybridization (FISH) in meiotic cells of *O. rufipes* (a,b) and *E. fusiformis* (c,d). Bar = 5 μm.

Table 1 - Chromosomal data for *Orthoscapheus rufipes* and *Eujivarus fusiformis*, showing the diploid number and sex determination system, the distribution and classification of CH based on C-banding and staining with base-specific fluorochromes, and the occurrence of NORs detected by $AgNO_3$ and FISH.

Species	Karyotype	CB +	CMA_3^+	DAPI	NORs/FISH
O. rufipes	23,XOM 24,XXF	Pc	*L_2 I; *M_5 Px, Tel, M_6 Pc S_9 Pc	0	M_6 Pc S_9 Pc
E. fusiformis	21,XOM 22,XXF	Pc	M_6 Pc M_7 Pc	0	M_6 Pc M_7 Pc

CB+ = presence of C bands; * = constitutive heterochromatin not detected by C-banding; 0 = absence of staining; I = interstitial blocks; NORs = nucleolar organizer regions; Pc = pericentromeric blocks; Px = proximal blocks; Tel = telomeric blocks. M = male. F = female.

Table 2 - Chromosome number, sex mechanism and chromosome morphology in representatives of the tribe Abracrini (Ommatolampinae-Acrididae).

Species	Diploid number	Sex mechanism	Chromosome morphology	Reference
Abracris dilecta	23,24	XO, XX	ac	2, 3
Abracris flavolineata	23,24	XO, XX	mt, sm, st	2, 3, 5
Abracris sp.	21,22	XO, XX	ac, mt	2, 3
Eujivarus fusiformis	21,22	XO, XX	ac, mt	2, 3, 6
Eujivarus vittatus	23,24	XO, XX	ac	2, 3
Eujivarus n.sp. A	21,22	XO, XX	ac, mt	2, 3
Eujivarus n.sp. B	21,22	XO, XX	ac, mt	2, 3
Eujivarus n.sp. C	21,22	XO, XX	ac, mt	2, 3
Eusitalces vulneratus	23,24	XO, XX	ac	2, 3
Eusitalces sp. A	23,24	XO, XX	ac	2, 3
Jodacris f. ferrugineus	19,20	XO, XX	ac	2, 3
Jodacris chapadensis	19,20	XO, XX	ac	2, 3
Jodacris furcillata	19,20	XO, XX	ac	2, 3
Ommalotettix obliquum	21,22	XO, XX	ac, sm, sa	1, 3
Orthoscapheus rufipes	23,24	XO, XX	ac	6
Psilocirtus bolivianus	23,24	XO, XX	ac, sa	2, 3
Psilocirtus olivaceus	23,24	XO, XX	ac, sa	2, 3
Psilocirtus sp. A	23,24	XO, XX	ac, sa	2, 3
Roppacris griseipes	23,24	XO, XX	ac	4
Sitalces volxemi	19,20	XO, XX	ac, mt	2, 3
Xiphiola borellii	23,24	XO, XX	ac	3

References: 1: Carbonell *et al.* (1980); 2: Ferreira *et al.* (1980); 3: Mesa *et al.* (1982); 4: Mesa and Fontanetti (1983); 5: Cella and Ferreira (1991); 6: present study. ac = acrotelocentric; mt = metacentric; sa = subacrocentric; sm = submetacentric; st = subtelocentric.

(Arana *et al.*, 1987), *Leptysma argentina* (Colombo, 1993) and *Dichroplus pratensis* (Bidau and Martí, 2002).

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 M_7 (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 Abracrini.

Differences in the base composition of CH (detected by CMA_3/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 CMA_3-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*, CMA_3-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 CMA_3 staining and restricted to NORs (pairs M_6 and S_9), and (3) CH detected by CMA_3 staining, but not by C-banding, and unrelated to NORs (pairs L_2 and M_5). 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 S_9, as observed in *O. rufipes*, has also been described for most Neotropical species studied so far, especially those of the subfamilies Leptysminae (Rocha *et al.*, 2004) and Gomphocerinae (Loreto *et al.*, 2008). According to Loreto *et al.* (2008), bivalent S_9 probably represents the ancestral location of rDNA sites in Neotropical gomphocerine species since this pattern was identified in five species analyzed (*Rhammmatocerus brasiliensis*, *R. brunneri*, *R. palustris*, *R. pictus* and *Amblytropidia* sp.). Ribosomal DNA sites on S_9 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* (L_2) and *Radacridium mariajoseae* (X) (Rocha *et al.*, 1997), *Xyleus angulatus* (L_3, M_4 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* (M_8) (Rocha *et al.*, 2004), or in interstitial regions, as described in *Schistocerca pallens* (M_5) and *S. flavofasciata* (M_5 and M_6) (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 Abracrini are necessary to improve our understanding of the evolutionary patterns within this group.

Acknowledgments

The authors thank Dr. Carlos S. Carbonell (University of Montevideo, Uruguay), for taxonomic identification of the species studied, Dr. Marcelo Guerra (Department of Botany, UFPE) for providing access to the Laboratory of Plant Cytogenetics and for allowing us to do the FISH experiments, and Francisca Tavares de Lira for technical assistance. We also thank Dr. Neide Santos (Department of Genetics, UFPE) for critically reading the manuscript. This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), Brazil.

References

Amedgnato C (1974) Les genres d'acridiens néotropicaux, leur classification par familles, sous-familles et tribus. Acrida 3:193-204.

Arana P, Santos JL and Henriques-Gil N (1987) Interference relationships in grasshopper reciprocal translocation heterozygotes. Heredity 59:85-93.

Bidau CJ and Martí DA (2002) Geographic distribution of Robertsonian fusions in *Dichroplus pratensis* (Melanoplinae, Acrididae): The central-marginal hypothesis reanalyzed. Cytogenet Genome Res 96:66-74.

Bugrov AG (1996) Karyotypes of the short-horned orthopteran insects (Orthoptera, Caelifera) from Russia, Kazakhstan, Central Asia, and the Caucasus. Folia Biol (Krakow) 44:15-25.

Cabrero J and Camacho JPM (1986) Cytogenetics studies in gomphocerine grasshoppers II. Chromosomal location of active nucleolar organizing regions. Can J Genet Cytol 28:540-544.

Cabrero J and Camacho JPM (2008) Location and expression of ribosomal RNA genes in grasshoppers: Abundance of silent and cryptic loci. Chromosome Res 16:595-607.

Camacho JPM, Cabrero JM, Viseras E, López-León MD, Navas-Castillo J and Alche JD (1991) G banding in two species of grasshopper and its relationship to C, N, and fluorescence banding techniques. Genome 34:638-643.

Carbonell CS (1977) Origin, evolution and distribution of the neotropical acridomorph fauna (Orthoptera): A preliminary hypothesis. Rev Soc Ent Argentina 36:153-175.

Carbonell CS, Mesa A and Conduta VL (1980) Systematics and karyotype of the grasshopper genera *Ommalotettix* and *Abracris*. Proc Acad Nat Sci Philadelphia 132:279-284.

Cella DM and Ferreira A (1991) The cytogenetics of *Abracris flavolineata* (Orthoptera, Caelifera, Ommatolampinae, Abracrini). Rev Bras Genet 14:315-329.

Colombo PC (1993) Chromosome polymorphisms and natural selection in *Leptysma argentina* (Orthoptera). II. Gametic phase disequilibrium and differential adult male viability. Heredity 71:295-299.

Ferreira A, Mesa A and Carbonell CS (1980) The chromosomes of neotropical short-horned grasshoppers of the tribe Abracrini (Orthoptera, Acridoidea, Acrididae, Ommatolampinae). Naturalia 5:89-95.

John B, King M, Schweizer D and Mendelak M (1985) Equilocality of heterochromatin distribution and heterogeneity in acridid grasshoppers. Chromosoma 91:185-200.

King M and John B (1980) Regularities and restrictions governing C-band variation in acridoid grasshoppers. Chromosoma 76:123-150.

López-León MD, Cabrero JM and Camacho JPM (1999) Unusually high amount of inactive ribosomal DNA in the grasshopper *Stauroderus scalaris*. Chromosome Res 7:83-88.

Loreto V and Souza MJ (2000) Karyotype, constitutive heterochromatin and nucleolar organizer regions (NORs) in *Belosacris coccineipes* (Acrididae, Leptysminae). Genet Mol Biol 23:575-579.

Loreto V, Stadtler E, Melo NF and Souza MJ (2005) A comparative cytogenetic analysis between the grasshopper species *Chromacris nuptialis* and *C. speciosa* (Romaleidae): Constitutive heterochromatin variability and rDNA sites. Genetica 125:253-260.

Loreto V, López-León MD, Camacho JPM and Souza MJ (2008) Comparative analysis of rDNA location in five Neotropical gomphocerine grasshopper species. Genetica 132:95-101.

Mesa A, Ferreira A and Carbonell CS (1982) Cariologia de los Acridoideos neotropicales: Estado actual de su conocimiento y nuevas contribuciones. Annls Soc Ent Fr (N S) 18:507-526.

Mesa A and Fontanetti CS (1983) Karyotypes of nine Brazilian species of acridids (Orthoptera, Acridoidea). Rev Bras Genet 6:295-305.

Moscone EA, Matzke MA and Matzke AJM (1996) The use of combined FISH/GISH in conjunction with DAPI counterstaining to identify chromosomes containing transgene inserts in amphidiploid tobacco. Chromosoma 105:231-236.

Pereira LG and Souza MJ (2000) Nature and distribution of constitutive heterochromatin and NOR location in the grasshopper *Phaeoparia megacephala* (Romaleidae, Orthoptera). Cytobios 103:111-119.

Rocha MF, Souza MJ and Tashiro T (1997) Karyotype variability in the genus *Radacridium* (Orthoptera, Romaleidae, Romaleinae). Cytologia 62:53-60.

Rocha MF, Souza MJ and Moura RC (2004) Karyotype analysis, constitutive heterochromatin and NOR distribution in five grasshopper species of the subfamily Leptysminae (Acrididae). Caryologia 57:107-116.

Rodríguez Iñigo E, Bella JL and Garcia de La Vega C (1993) Heterochromatin differentiation between two species of the genus *Dociostaurus* (Orthoptera, Acrididae). Heredity 70:458-465.

Rodríguez Iñigo E, Fernández-Calvin B, Capel J and Garcia de La Vega C (1996) Equilocality and heterogeneity of constitutive heterochromatin: *In situ* localization of two families of highly repetitive DNA in *Dociostaurus* (Orthoptera). Heredity 76:70-76.

Rufas JS, Esponda P and Gosálvez J (1985) NOR and nucleolus in the spermatogenesis of acridoid grasshoppers. Genetica 66:139-144.

Rufas JS, Giménez-Abian J, Suja JA and Garcia de La Vega C (1987) Chromosome organization in meiosis revealed by light microscope analysis of silver-stained cores. Genome 29:706-712.

Santos JL, Arana P and Giraldez R (1983) Chromosome C-banding patterns in Spanish Acridoidea. Genetica 61:65-74.

Schweizer D, Mendelak M, White MJD and Contreras N (1983) Cytogenetics of the parthenogenetic grasshopper *Warramaba virgo* and its bisexual relatives. X. Patterns of fluorescent banding. Chromosoma 88:227-236.

Souza MJ and Melo FN (2007) Chromosome study in *Schistocerca* (Orthoptera, Acrididae, Cyrtacanthacridinae): Karyotypes and distribution patterns of constitutive heterochromatin and nucleolus organizer regions (NORs). Genet Mol Biol 30:54-59.

Souza MJ, Rufas JS and Orellana J (1998) Constitutive heterochromatin, NOR location and FISH in the grasshopper *Xyleus angulatus* (Romaleidae). Caryologia 51:73-80.

Souza MJ, Haver ORP and Melo FN (2003) Karyotype, C- and fluorescence banding patterns NOR location and FISH in the grasshopper *Xestotrachelus robustus* (Romaleidae). Caryologia 56:261-267.

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75:304-306.

Sumner AT (1990) Chromosome Banding. 1st edition. Unwin Hyman Ltd., London, 434 pp.

Unfried I and Gruendler P (1990) Nucleotide sequence of the 5.8S and 25S rRNA genes and of the internal transcribed spacers from *Arabidopsis thaliana*. Nucleic Acids Res 18:4011.

Unfried I, Stocker U and Gruendler P (1989) Nucleotide sequence of the 18S rRNA gene from *Arabidopsis thaliana*. Nucleic Acids Res 17:7513.

Associate Editor: Yatiyo Yonenaga-Yassuda