Genetics and Molecular Biology, 30, 1, 54-59 (2007)

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Research Article

Chromosome study in *Schistocerca* (Orthoptera-Acrididae-Cyrtacanthacridinae): Karyotypes and distribution patterns of constitutive heterochromatin and nucleolus organizer regions (NORs)

Maria José de Souza[1] and Natoniel Franklin de Melo[2]

[1]*Departamento de Genética/CCB, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil.*
[2]*Embrapa Semi-árido, Petrolina, Pernambuco, Brazil.*

Abstract

Chromosome analyses were performed in two grasshopper species of the genus *Schistocerca*, *S. pallens* and *S. flavofasciata*. Both species shared the same diploid number (2n = 23, X in males; 2n = 24, XX in females);and a conserved karyotype composed exclusively of acrocentric chromosomes, but differed in their distribution patterns of constitutive heterochromatin and nucleolus organizer regions (NORs). Constitutive heterochromatin was located in the pericentromeric region of all chromosomes in both species. *S. flavofasciata* presented an additional C-band on the distal region of the long arm of a small autosome pair (S9). Nucleolus organizer regions (NORs), revealed by silver nitrate staining (Ag-NORs), were observed on a medium autosome pair (M5) in both species. *S. pallens* presented an additional NOR-bearing autosome (M6). The same sites were labeled after FISH with an rDNA probe in *S. pallens* cells.

Key words: grasshopper, constitutive heterochromatin, nucleolus organizer regions.

Received: May 10, 2006; Accepted: September 13, 2006.

Introduction

Cyrtacanthacridinae, a subfamily of the Acrididae family, comprises around 30 genera distributed worldwide through tropical and subtropical regions. In America, it is represented by the genera *Halmenus* and *Schistocerca*. The latter has a large number of species and subspecies distributed in North, South and Central America. The most accepted hypothesis proposes that *Schistocerca* reached America from Africa (Dirsh, 1974; Carbonell, 1977).

Cytogenetic studies on the genus *Schistocerca* are scarce. Among the American species, *S. cancellata, S. flavofasciata, S. pallens* and *S. paranensis* had their diploid numbers described, 2n = 23,X in males and 2n = 24,XX in females (Mesa *et al.*, 1982). The distribution patterns of constitutive heterochromatin and of the nucleolus organizer regions (NORs), as well as chiasmata frequency, are available only for *S. gregaria* (Fox, 1973; Hagele, 1979; Rufas and Gosálvez, 1982), an Old World species. Grasshoppers, particularly species of Acrididae and Romaleidae, have a predominance of 2n = 23/24, with an X0/XX mechanism of sex determination (Mesa *et al.*, 1982; Bugrov, 1996). C-banding and Ag-NOR staining have been of great importance for inter and intra-specific characterization and karyotypic differentiation in grasshoppers (Santos *el al*., 1983, Cabrero and Camacho 1986a, 1986b; Souza and Kido, 1995; Pereira and Souza, 2000; Rocha *et al.*, 2004). Fluorochrome staining and fluorescent *in situ* hybridization (FISH) have also been used to, respectively, map AT- and GC-rich chromosome regions and locate ribosomal genes (López-León *et al.*, 1999; Santos *et al.*, 1990; Souza *et al.*, 1998; Pereira and Souza 2000; Souza *et al.*, 2003).

In this work, the karyotypes of *S. pallens* and *S. flavofasciata* were analyzed by conventional staining, C-banding and Ag-NOR. Additionally, in *S. pallens* was used the CMA_3/DA/DAPI staining to qualify the constitutive heterochromatin and FISH with a 45S rDNA probe to map the ribosomal sites.

Material and Methods

Chromosome analyses were performed in *Schistocerca pallens* and *S. flavofasciata* collected in different areas of the State of Pernambuco, Northeastern

Send correspondence to Maria José de Souza. Departamento de Genética, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Cidade Universitária, 50732-970 Recife, Pernambuco, Brazil. E-mail: mjslopes@uol.com.br.

Table 1 - Specimens of *Schistocerca pallens* and *S. flavofasciata* analyzed, collection places and geographical coordinates.

N. of specimens					
S. pallens		*S. flavofasciata*			
♂	♀	♂	♀	Collection places	Coordinates
		4	1	Recife	8°3'14" S : 34°52'52" W
4		9	3	Olinda	8°0'32" S : 34°51'19" W
6	2			Itamaracá	7°44'52" S : 34°49'32" W
15	3			Gravatá	8°12'04" S : 35°33'53" W
12	1			Bezerros	8°11'15" S : 35°48'45" W
10	4			Toritama	8°03'45" S : 36°03'45" W
9	2			Bonito	8°26'15" S : 35°41'15" W

Brazil (Table 1). Cytological preparations were obtained through squashing of testes and ovarian follicles. Mitotic embryo cells of *S. pallens* were obtained from eggs incubated at 30 °C for 13 days (Souza, 1991). Conventional chromosome analyses were performed after staining with 2% lacto acetic orcein. C-banding was obtained according to Sumner (1972) and CMA_3/DA/DAPI staining was performed according to Schweizer *et al.* (1983) with small modifications. For sequential staining ($AgNO_3$/CMA_3/DA/DAPI), the chromosomes were stained with silver nitrate, photographed, destained and stained with CMA_3/DA/DAPI. Fluorescent *in situ* hybridization (FISH) was performed according to Moscone *et al.* (1996). A probe containing a fragment of *Arabidopsis thaliana* 45S ribosomal genes (18S-5.8S-28S) (Unfried *et al.*,1989; Unfried and Gruendler 1990) was labelled with biotin-11-dUTP by nick translation (Life Technologies) and detected with TRITC (tetramethyl-rhodamine-isothiocyanate)-conjugated antibodies. The chromosome preparations were counterstained with DAPI and mounted with Vectashield H-100 (Vector). Photographs were taken with Kodak T-MAX 400 and ISO FUJI 400.

Results

S. pallens and *S. flavofasciata* presented 2n = 23, X (males) and 2n = 24,XX (females). Both species had karyotypes composed of three large (L1-L3), five medium (M4-M8), and three small (S9-S11) acrocentric chromosome pairs. The X chromosome corresponds in size to the fourth pair (Figure 1a-f).

C-banding evidenced pericentromeric constitutive heterochromatin (CH) in all chromosomes of both species (Figure 2a-d). *S. flavofasciata* presented an additional large distal heterochromatic block on pair S9. Mitotic embryo cells of *S. pallens*, with more distended chromosomes, presented interstitial CH blocks in two autosome pairs (data not shown).

In meiotic cells of *S. pallens*, CMA_3 staining highlighted the small interstitial CH blocks on bivalents M5 and M6, which are thus GC-rich (Figure 3b,c). An additional interstitial CH CMA_3-positive block was observed on M4. All chromosomes were uniformly stained by DAPI in that species. Silver nitrate staining ($AgNO_3$) revealed interstitial NORs on a medium-sized bivalent, probably M5, in thirty pachytene cells of *S. flavofasciata* (Figure 3e). Ag-NORs were interstitially located on bivalents M5 and M6 in 45 pachytene cells of *S. pallens* (Figure 3a). Fluorescent *in situ* hybridization (FISH) with a 45S rDNA probe revealed ribosomal sequences in 15 cells analyzed in each of three specimens of *S. pallens* (Figure 3d). FISH signals on M5 and M6 coincided with Ag-NORs. The sequential staining $AgNO_3$/CMA_3/DA/DAPI showed that the Ag-NOR-associated heterochromatin is CMA_3-positive (GC- rich) in this species (Figure 3a-c).

Discussion

In spite of th large number of *Schistocerca* species in the American continent, only four species had their conventional stained karyotypes described so far (Mesa *et al.*, 1982). C-banding and Ag-NOR staining have been used thoroughly in chromosome analyses of Acridoidea (Rufas *et al.*, 1985; Cabrero and Camacho 1986a; Souza and Kido 1995; Rocha *et al.*, 2004), but not in American *Schistocerca*. In *S. gregaria*, a very common Old World species, pericentromeric heterochromatic blocks were described on all chromosomes. Pairs L2 and M6 showed distal CH blocks and pair S9 presented a proximal block of low staining intensity (Hagele, 1979). Ag-NOR staining revealed two active NORs on the distal region of M6 and on the proximal region of S9 (Fox and Santos 1985), which coincided with rDNA FISH signals (Vaughn *et al.*, 1999). In the present study, *S. pallens* and *S. flavofasciata* showed similar patterns of pericentromeric CH distribution, but differed by the presence of small blocks on three medium-

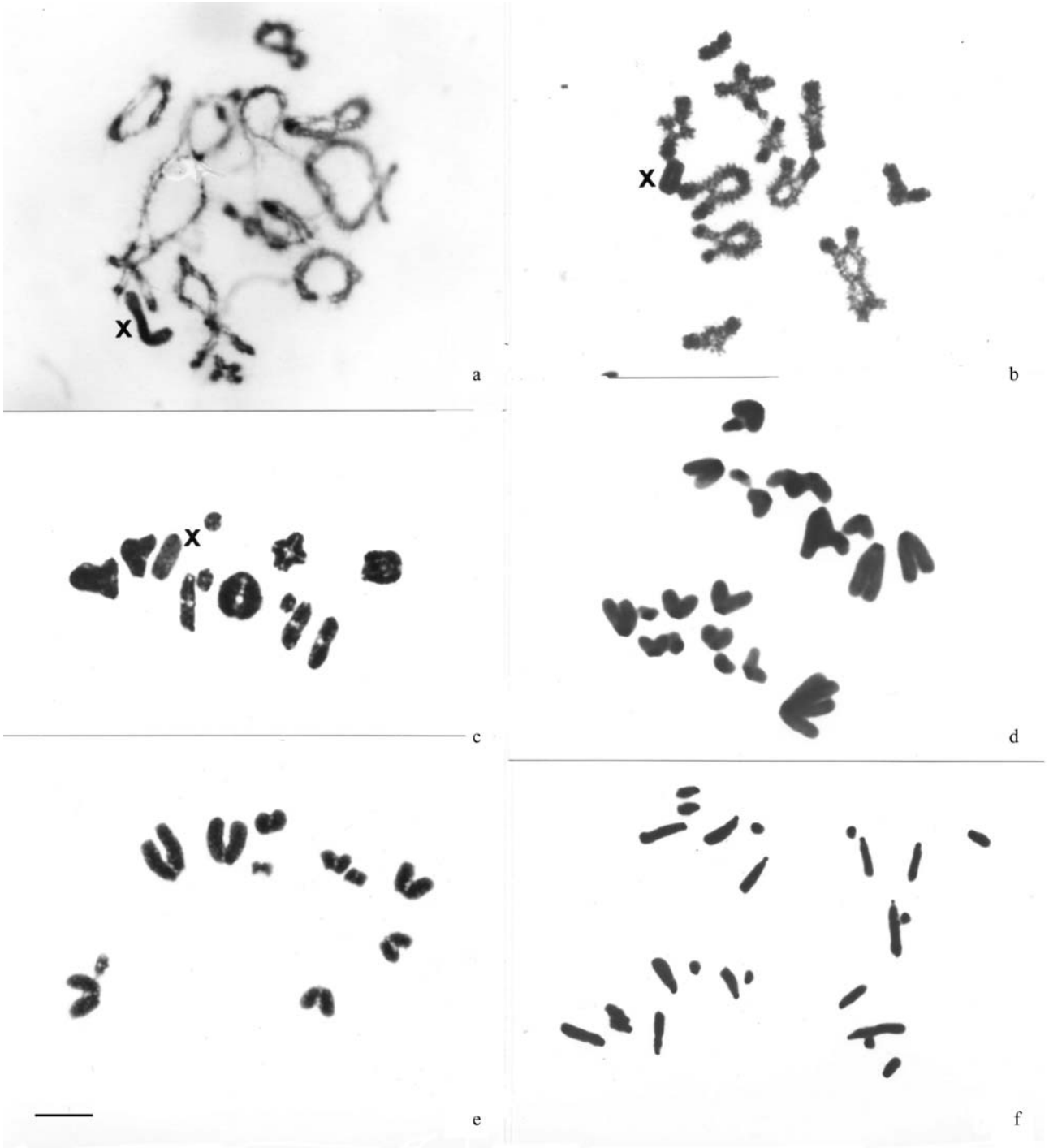

Figure 1 - Meiotic cells of *S. flavofasciata* (a,d) and *S. pallens* (b,c,e,f) after orcein staining: (a) pachytene, (b) diplotene, (c) metaphase I, (d) anaphase I, (e) metaphase II, (f) anaphase II. Bar = 10 μm.

sized pairs in *S. pallens* and by an intensely stained block at the telomere of S9 in *S. flavofasciata*. The latter represents a karyotypic marker in *S. flavofasciata* that allows the distinction between these species.

The pericentromeric CH of *S. pallens* was not entirely stained by the base-specific fluorochromes CMA_3 and DAPI, but small CMA_3-positive blocks were detected on pairs M4, M5 and M6. This indicates base composition heterogeneity of the heterochromatin, and shows that most CH in this species is not exclusively AT- or GC-rich. Species of taxonomically related Acrididae and Romaleidae (John and King 1983; John *et al*., 1985; Bella *et al*., 1993; Rodriguez-Inigo *et* al., 1993; Loreto and Souza, 2000; Rocha *et al*., 2004; Souza and Kido 1995; Pereira and Souza 2000;

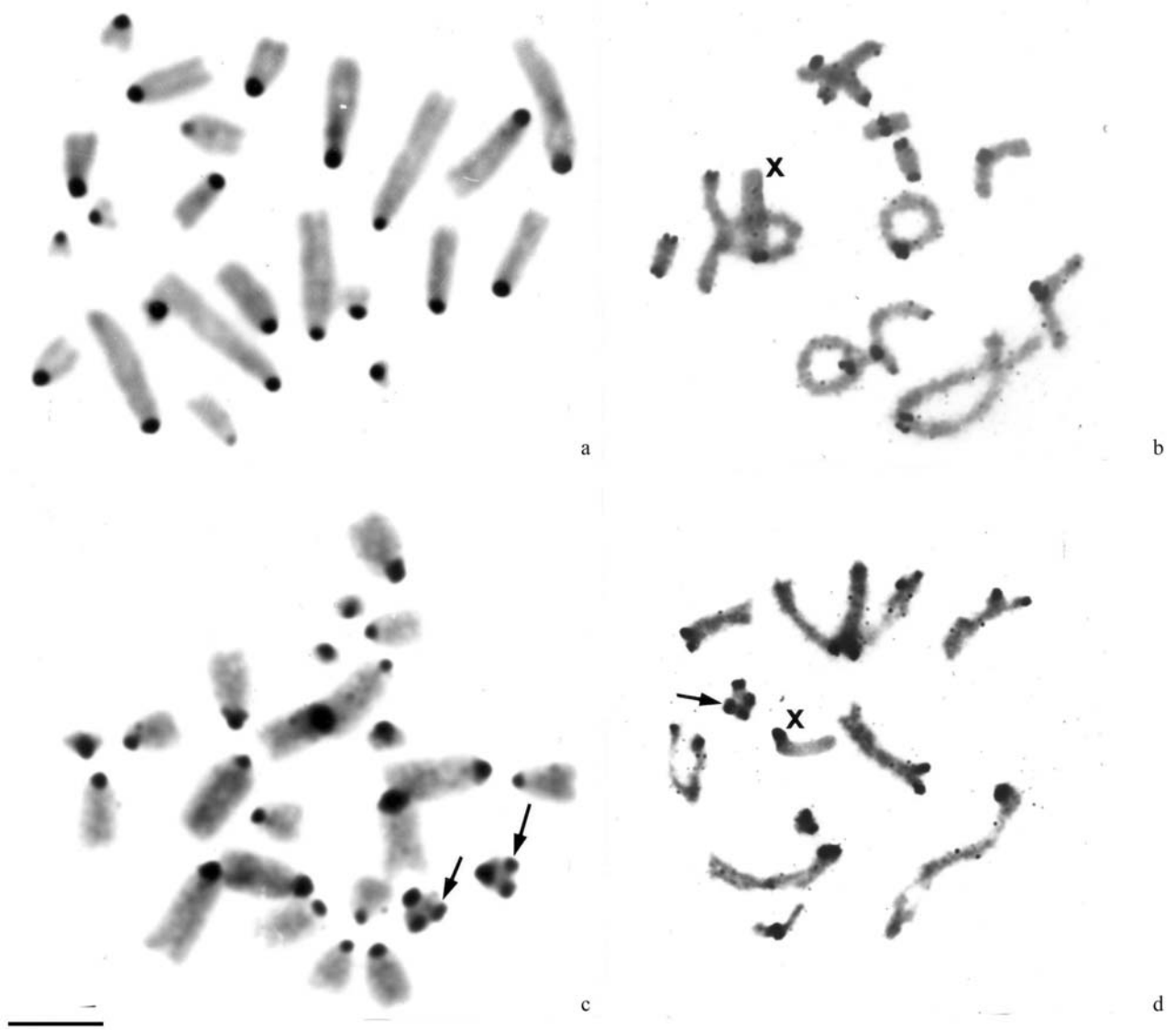

Figure 2 - C-banded mitotic and meiotic chromosomes: (a) embryonic metaphase and (b) diplotene of *S. pallens*; (c) spermatogonial metaphase and (d) pachytene of *S. flavofasciata*. Arrows in (c) and (d) point to the distal heterochromatic block on S9. Bar = 10 μm.

Souza *et al*., 2003), have very similar karyotypes, but may differ in heterochromatin distribution and base-pair composition. In the acridoids *Chorthippus brunneus* and *C. jacobsi*, the pericentromeric regions of all chromosomes showed CMA_3- positive CH blocks (Bridle *et al*., 2002). The romaleids *Xyleus angulatus, Phaeoparia megacephala* and *Xestotrachelus robustus* (Souza *et al*., 1998; Pereira and Souza, 2000; Souza *et al*., 2003) also presented CMA_3-positive CH blocks on all chromosomes. Differences in size and distribution of CH blocks have been described in several grasshopper species. *Chromacris speciosa* had large pericentromeric and telomeric CH blocks (Souza and Kido, 1995). In contrast, *C. nuptialis* predominantly showed small CH pericentromeric blocks, in addition to terminal CH blocks on pairs G1 and P9 and an interstitial block on P10 (Loreto *et. al.,* 2005). Other grasshoppers of the genera *Calliptamus, Oeidipoda, Euchorthippus* and *Radacridium* also differed in their C-banding patterns (Rocha *et al*., 1997; Santos *et al*., 1983).

The two species herein studied showed different patterns of NOR distribution. The interstitial location of Ag-NORs in *S. flavofasciata* and *S. pallens* (rDNA identified by FISH in this species), in contrast to the distal (M6) and proximal NORs (S9) of *Schistocerca gregaria* (Fox and Santos, 1985), suggest the possible occurrence of inversions including the rDNA loci in the genus.

NOR numbers and distribution can represent a good marker in the distinction between related species (Rufas *et al.*, 1985; Cabrero and Camacho, 1986b; Rocha *et al.*, 2004). In grasshoppers, chromosome localization of rDNA

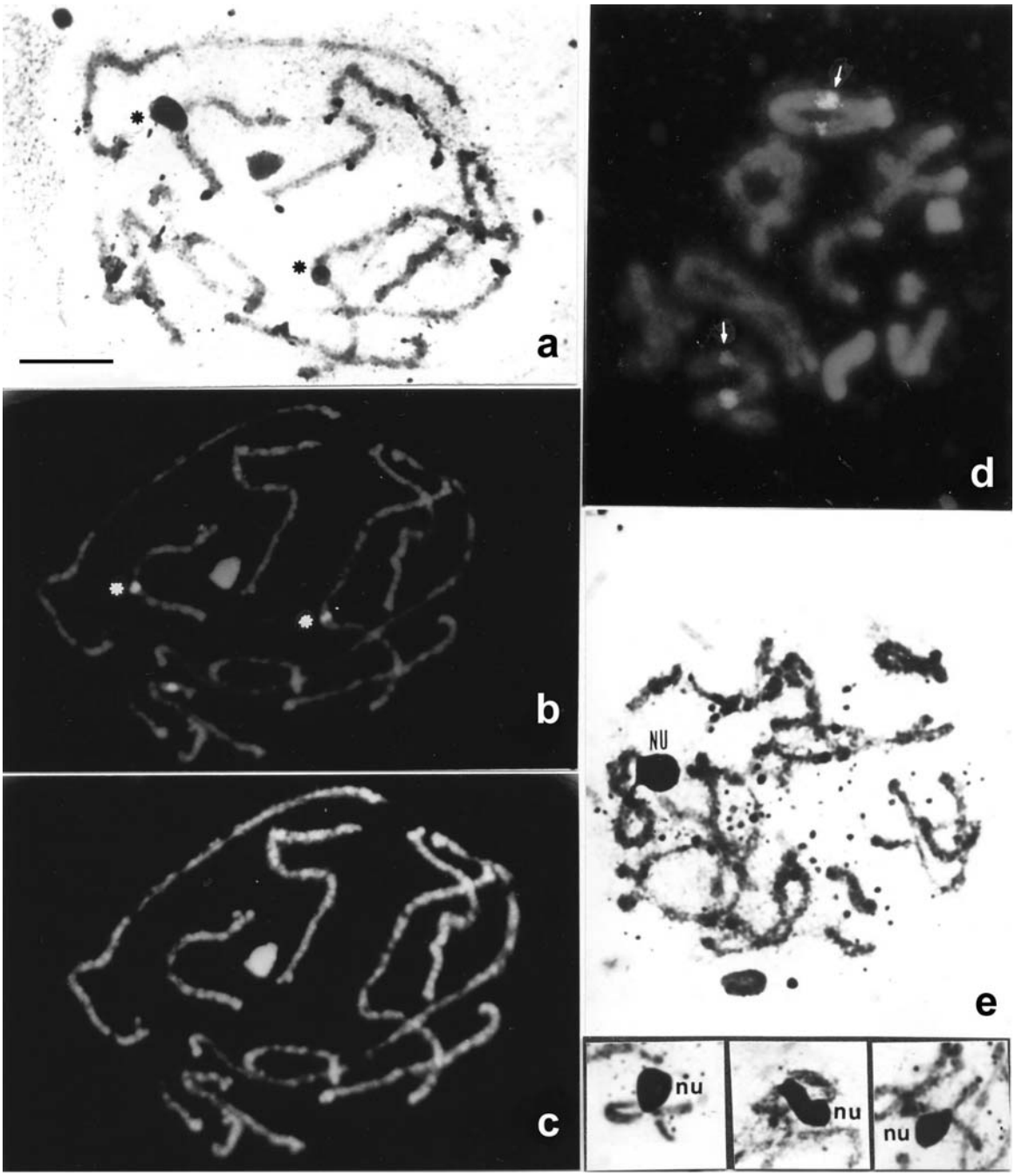

Figure 3 - (a-c) $AgNO_3$/CMA_3/DA/DAPI sequential staining and (d) FISH with a 45S rDNA probe in *S. pallens*. Asterisks indicate Ag-NORs in (a), and the CMA_3-positive block in (b); arrows point to the interstitial rDNA FISH signals on M5 and M6 in (d). (e) Ag-NORs (NU) on M5 of *S. flavofasciata*. In the inset, the Ag-NOR-bearing M5 in different cells. (a, b, c, e) pachytene; (d) diplotene. Bar = 10 µm.

genes by FISH has been performed in Acrididae (López-León *et al.*, 1999; Santos *et al.*, 1990; Vaughn *et al.*, 1999) and Romaleidae (Souza *et al.*, 1998; Souza *et al.*, 2003). Variation in the distribution of rDNA sites and/or Ag-NORs, as we observed in *S. flavofasciata* and *S. pallens*, have been found in many insect species pointing to the importance of these patterns as phylogenetic markers in the genus *Schistocerca.*

Acknowledgements

We are grateful to Dr. Carlos S. Carbonell for taxonomic identifications and for the helpful suggestions. We thank Dr. Marcelo Guerra for the use of FISH laboratory facilities. We also thank Dr. Neide Santos and Dr. Aline Alexandrino for the critical reading of the manuscript. This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and

Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE).

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Associate Editor: Yatiyo Yonenaga-Yassuda