Karyotype, C-and fluorescence banding pattern, NOR location and FISH study of five Scarabaeidae (Coleoptera) species

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Abstract

Meiotic chromosomes obtained from members of the coleopteran subfamilies Rutelinae and Dynastinae were studied using standard and silver nitrate staining, C-banding, base-specific fluorochromes and fluorescent in situ hybridization (FISH). The study presents detailed karyotypic descriptions of three Rutelinae species (Geniates borelli, Macraspis festiva and Pelidnota pallidipennis), and two Dynastinae species (Lygirus ebenus and Strategus surinamensis hirtus) with special emphasis on the distribution and variability of constitutive heterochromatin and the nucleolar organizer region (NOR). We found that for G. borelli, P. pallidipennis, L. ebenus and S. s. hirtus the karyotype was 2n = 20 (9II + Xy p), with G. borelli, P. pallidipennis and L. ebenus showed meta-submetacentric chromosomes which gradually decreased in size. For Macraspis festiva the karyotype was 2n = 18 (8II + Xyp). In L. ebenus we found that the NOR was located on an autosome, but in the other four species it occurred on the sex bivalents. In all five species the constitutive heterochromatin (CH) was predominantly pericentromeric while the X chromosomes were almost completely heterochromatic, although CMA3/DA/DAPI staining showed intra and interspecific variation in the bright fluorescence of the constitutive heterochromatin. The FISH technique showed rDNA sites on the X chromosome of the Rutelinae species.

Key words: karyotype, constitutive heterochromatin, NORs, rDNA sequences.

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Introduction

The coleopteran family Scarabaeidae is made up of a cosmopolitan group of approximately 2,300 genera and 27,000 species worldwide distributed with a highly conserved diploid chromosome number (2n = 20) and Xy type ‘parachute’ (XYp) sex-determining mechanism, although there is variation in chromosome morphology (Smith and Virkki, 1978; Yadav and Pillai, 1979; Colomba et al., 1996). Neotropical and Brazilian representatives of the scarabaeid subfamilies Rutelinae and Dynastinae have been extensively studied taxonomically (Endrödi, 1985; Morón et al., 1997) and it is known that more than 50% of the species from these subfamilies possess the standard karyotype, although variations in chromosome number have been observed with the chromosome number ranging from 2n = 18 to 2n = 22 in the subfamily Rutelinae (Smith and Virkki, 1978; Yadav and Pillai, 1979) and from 2n = 12 to 2n = 20 in the Dynastinae (Vidal, 1984; Martins, 1994).

Differential techniques have rarely been applied to chromosome studies of the Coleoptera, but data from the species so far analyzed have shown that the autosomal constitutive heterochromatin (CH) is preferentially located pericentromeric while the X chromosomes are almost completely heterochromatic, although CMA/D/DAPI staining showed intra and interspecific variation in the bright fluorescence of the constitutive heterochromatin. The FISH technique showed rDNA sites on the X chromosome of the Rutelinae species.

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orescence in situ hybridization (FISH) have shown that the ribosomal sites are preferentially located on the sex chromosomes (Moura et al., 2003), although in Gymnopleurus sturni and Phyllophaga (P.) aff capillata (Scarabaeidae), Trotectes intermedius (Geotrupidae), Eriopis connexa (Coccinellidae) and in 19 Zabrus species (Carabidae), the NORs are located on the autosomes (Vitturi et al., 1999; Colomba et al., 2000; Maffei et al., 2000; Sánchez-Géa et al., 2000; Moura et al., 2003).

This study presented in this paper provides detailed karyotypic descriptions of three representative Rutelinae species (Geniates borelli, Macraspis festiva and Pelidnota pallidipennis) and two representative Dynastinae species (Lygirus ebenus and Strategus surinamensis hirtus) with special emphasis on the distribution and variability of constitutive heterochromatin and NORs.

Materials and Methods

Meiotic chromosomes were obtained from Rutelinae species (Geniates borelli Camerano, 1894 (12 specimens), Macraspis festiva Burmeister, 1844 (6 specimens) and Pelidnota pallidipennis Bates, 1904 (12 specimens) and Dynastinae species (13 Lygirus ebenus De Geer, 1774 and five Strategus surinamensis hirtus Sternberg, 1910). The specimens were male beetles collected from Atlantic Forest sites situated in the northeastern Brazilian state of Pernambuco at 07°48′37″ S, 34°27′25″ W near the town of Igarassú for G. borelli, P. pallidipennis and L. ebenus and 08°0′8″, 35°1′6″ W near the town of São Lourenço da Mata for S. s. hirtus and M. festiva. Testicular follicle squashes were made in ethanol and acetic acid (3:1) fixative and the chromosomes stained with 2% lacto acetic orceína. We also performed C-banding (Sumner, 1972), silver nitrate (Rufas et al., 1987) and AT/GC base pair fluorescence staining (Schweizer et al., 1983). Fluorescent in situ hybridization (FISH) was performed as described by Moscone et al. (1996) using Arabidopsis thaliana 45S rDNA probes (Unfried et al., 1989; Unfried and Gruendler, 1990) nick translation labeled with bio-11-dUTP (Life Technologies) and detected with rat antibodion antibodies (Dakopatts M0743, Dako) and tetramethyl-rhodamine isothiocyanate (TRITC) conjugated rabbit anti-rat antibodies (Dakopatts R0270, Dako).

Results

Standard staining and C-banding

The male karyotypes of most of the species analyzed were 2n = 20 (9II + Xyp) (Figure 1a, c-f), the exception being M. festiva which had a karyotype of 2n = 18 (8II + Xyp) (Figure 1b). The chromosomes of G. borelli, P. pallidipennis and L. ebenus were meta-submetacentric and showed a gradual decrease in size. The sex-determining mechanism of all the species analyzed was of the parachute type, with a metacentric X chromosome and a diminutive Y chromosome (Figure 1a, b, e, f).

The C-banding method revealed blocks of constitutive heterochromatin in the pericentromeric region of all the autosomes of the Rutelinae species (Figure 2a-c) while for the Dynastinae species in addition to the pericentromeric blocks a terminal block was noted on a small chromosome of L. ebenus (Figure 2d) and C-banding was absent from one S. s. hirtus (Figure 2e). The X chromosomes of the five species studied were all almost completely heterochromatic and no constitutive heterochromatin blocks were detected in the y chromosome of any of the species (Figure 2b-d). Heterochromatin associations forming chromocenters between autosomal bivalents were observed in the five species analyzed, these associations being first visible during meiotic prophase and persisted until the end of the pachytene phase (Figure 2c).

Fluorochrome staining

For G. borelli CMA3/DA/DAPI staining showed small GC-rich CMA3 positive blocks, coinciding with those visualized by C-banding, in the pericentromeric region of all the chromosomes (Figure 3a), but no DAPI-positive blocks were detected in this species (Figure 3b). In
contrast, *P. pallidipennis* presented DAPI blocks similar in size and location to those detected by C-banding and the heterochromatin of this species was AT-rich except for a small CMA3-positive block detected in one of the autosomal bivalents and another detected in the sex chromosomes (Figure 3e, f). CMA3/DA staining revealed the presence of GC-rich blocks in all chromosomes of the complement except for a small bivalent, but no DAPI-positive blocks were detected in this species.

### Silver nitrate staining and FISH

Amorphous masses corresponding to nucleolar remnants were visualized by silver nitrate staining in the Xyp bivalents of *G. borelli, P. pallidipennis, M. festiva* and *S. s. hirtus* (Figure 4a, c, d, f), while in *L. ebenus* the labeling was detected on an autosomal pair (Figure 4e). These masses were visible until the end of the pachytene phase or the beginning of the diplotene phase. Silver nitrate also labeled the constitutive heterochromatin blocks (Figure 4a-c, e) and the sex chromosomes showed affinity for silver and continued to be labeled during different phases of meiosis (Figure 4b). In the three Rutelinae species, FISH of rDNA genes produced results, which coincided with those obtained by silver nitrate staining and permitted the identification of rDNA genes on the X chromosome (Figure 5a-c).

### Discussion

We found that *G. borelli, P. pallidipennis, L. ebenus* and *S. s. hirtus* had the $2n=20$ (9II + Xyp) karyotype typical of the suborder Polyphaga, but *M. festiva* karyotype of $2n=18$ (8II+ Xyp) which coincided with the karyotype of *Macraspis dichroa* (Vidal, 1984). Other species of *Macraspis* are known to have a $2n=20$ karyotype (Martins 1994) but with the Xyp-type sex-determining system changed to neoXy system. Karyotypic comparisons *M. festiva* and other species of *Macraspis* of known cytology suggests that karyotype evolution in this genus might have involved different types of chromosome rearrangements. It is possible that the reduction in the chromosome number observed in *M. festiva* might have been due to a mechanism involving pericentric inversion followed by fusion between autosomes, which would explain the occurrence of karyotypic changes in the absence of alterations in the
sex-determining system. Changes of this type have been described in the literature and are included in the five types of karyotype evolution proposed for Scarabaeidae by Yadav and Pillai (1979).

Our results show that in G. borelli, P. pallidipennis, M. festiva, L. ebenus and S. s. hirtus there was some degree of conservation in terms of the size and location of the CH blocks as well as a type of heterochromatic association in which chromocenters formed between some autosomal bivalents. It is known that the degree of ectopic pairing between heterochromatic segments that promote the formation of chromocenters varies among different coleopteran species and that this type of association seems to play an important role in nuclear organization and the segregation of meiotic chromosomes (Smith and Virkki, 1978; Drets et al., 1983).

The constitutive heterochromatin of the species analyzed by us was located on the pericentromeric region of the chromosomes, similar observations having been reported for other Scarabaeidae (Vidal and Giacomozzi, 1978; Vidal and Nocera, 1984). This pattern of distribution has been described for most coleopteran species studied by C-banding (Virkki, 1983; Rozeck and Maryanska-Nadachowska, 1991; Rozeck and Rudok, 1992), although telomeric blocks in addition to pericentromeric ones have been observed in the tenebrionid Misolampus goudoti (Juan and Petitpierre, 1989) and exclusively telomeric blocks in the carabid Bembidion minimum (Rozeck and Rudok, 1992), with extra-heterochromatic segments having been reported in the scarabaeid Bubas bison (Colomba et al., 1996).

In our study, CMA3 and DAPI staining revealed that qualitative heterogeneity in the constitutive heterochromatin of G. borelli and S. s. hirtus we found CMA3 positive blocks indicating GC-rich constitutive heterochromatin, but in Pelidnota pallidipennis we found two types of constitutive heterochromatin, a DAPI positive type
evenly distributed throughout the karyotype and a CMA₃ positive type restricted to one small block located on the sex pair (probably the X chromosome) and another small block located on one of the autosomal bivalents. Reports on the use of base-specific fluorochromes in Scarabaeidae are still scarce, but different patterns have been found in some species. For example, in Gymnopleurus sturni (Vitturi et al 1999) and Thorectes intermedius (Colomba et al 2000) GC-rich sequences were detected in all the chromosomes while Lyogenys fuscus presented AT-rich sequences in every karyotype complement studied (Moura et al., 2003).

Data regarding the location of NORs in Coleoptera have suggested that a pair of nucleolar organizer autosomes is widely distributed in this order (Virkki, 1983; Virkki et al., 1984). In representatives of the family Scarabaeidae rDNA sites are generally found on the X chromosome, although sites located on autosomes have been reported for Phyllophaga (Phyllophaga) aff capillata and Gymnopleurus sturni (Moura et al., 2003; Colomba et al., 2000). In G. borelli, P. pallidipennis, M. festiva and S. s hirtus we found that the NOR was associated with the sex bivalent, and this confirmed for G. borelli, P. pallidipennis, M. festiva by our in situ hybridization using the 45S rDNA probe.

Studies analyzing the development and segregation of the X₀p chromosome in some curculionid species have shown that, even when the NORs are autosomal, the lumen of the sex bivalent is filled from diakinesis to anaphase I with proteinaceous substances which have an affinity for silver and which probably resemble substances present in the synaptonemal complex and chromosome skeleton. It has been proposed by Virkki et al. (1990; 1991) that these substances function as an adhesive and therefore control the correct disjunction of the sex chromosomes.

In the Scarabaeidae species analyzed we found that the sex bivalent remained silver labeled after the nucleolus disappeared, suggesting that the X₀p association is not necessarily due to the presence of the NOR, but rather to the presence of argyrophilic proteins distributed within the heterochromatin of these species. Argyrophilic proteins have also been observed in the scarabaeid species Thorectes intermedius (Vitturi et al., 1999), Gymnopleurus sturni (Colomba et al., 2000), Phyllophaga (Phytalus) vestita, Phyllophaga (Phyllophaga) aff capillata and Lyogenys fuscus (Moura et al., 2003) and their presence appears not to depend on the base composition of CH, further studies being needed to establish the real biochemical composition of scarabeoid beetle heterochromatin and explain the positive reaction to silver staining.

Our results show that in the species studied by us there was a clear relationship between the NOR and the sex chromosomes, with silver staining and FISH demonstrating that NORs are preferentially located on the sex pair. Although not indicating the direct participation of the NOR in the formation of the X₀p bivalent, this relationship demonstrates the apparent conservation of the location of the rDNA sites on the X chromosomes in representatives of the family Scarabaeidae.

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