High Levels of Chromosomal Differentiation in *Euchroma gigantea* L. 1735 (Coleoptera, Buprestidae)

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Abstract

*Euchroma gigantea* was karyotypically studied using conventional staining, C-banding, silver nitrate staining and ribosomal fluorescent in situ hybridization (rDNA FISH). Broad wide autosomal polymorphism and a complex sex determination system were found in this beetle. Karyotype complements ranging from 2n = 32, X¹X²X³Y¹Y²Y³ to 2n = 24, X¹X²X³Y¹Y²Y³, were detected in the sample analyzed. Punctiform supernumerary chromosomes were present in the different karyotypes. The karyotypic evolution of Brazilian *E. gigantea* may have taken two directions, reduction in the diploid number of 2n = 36 to 24 through centric fusions or 2n = 24 to 36 due to chromosomal fissions. In addition, pericentric inversions were also involved. The complex multiple sex mechanism of this species seems to be old and well established since it is found in specimens from different populations. Small pericentromeric blocks of constitutive heterochromatin were located on the autosomes and terminal blocks were also found on some small pairs. The sex chromosomes showed larger constitutive heterochromatin blocks. Silver nitrate staining during prophase I of meiosis showed labeling of the sex chromosome chain. However, the rDNA sites could only be precisely determined by FISH, which permitted the identification of these ribosomal sites on chromosomes X¹ and X² of this species.

Key words: chromosome polymorphism, C-banding, multiple sex system, NOR, rDNA.

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Introduction

The monotypic beetle genus *Euchroma* (Buprestidae), also known as the jewel scarab beetle, is widely distributed in the Neotropics and some subspecies and varieties of this genus have been described in South America, such as *Euchroma gigantea mrazi* found in southeastern Brazil (Obenberger, 1928).

Chromosome studies involving representatives of the family Buprestidae are scarce and only about 83 species having so far been analyzed by conventional methods, corresponding to 0.55% of all species described. The Buprestidae is characterized by a wide chromosome variability, with the diploid number ranging from 2n = 12 (*Melanophila acuminata*) to 2n = 46 (*Sphenoptera scovitzi*), and sex chromosome mechanisms of the chiasmatic (neo XY, X¹Y¹, X²Y², X₃Y₃) and achiasmatic type (Xyp, XO, Xy`) (Smith and Virkki, 1978; Mesa and Fontanetti, 1984; Karagyan and Kuznetsova, 2000; Karagyan, 2001; Karagyan et al., 2004). Various karyotypes have been described in *E. gigantea*, such as 2n = 26, Xy in specimens from Panama (Nichols, 1910), 2n = 24, X¹X²X¹Y¹Y²Y³ and 2n = 26, X¹X²X¹Y¹Y² in specimens from the Brazilian state of São Paulo. In addition to chromosome polymorphism, this species presents the largest number of supernumerary chromosomes (16, 20 and 32) reported for the Coleoptera (Mesa and Fontanetti, 1984).

Most coleopteran species possess a simple sex mechanism, with only 3.4% of the species analyzed presenting a multiple sex mechanism. According to Virkki (1984), the Xyp sex system may not be the ancestral mechanism in this order, although it is apparently the most successful, being present in most species analyzed. Various families representative of the suborders Adephaga and Polyphaga present derived mechanisms such as XO or multiple sex systems. These mechanisms may have resulted from translocations between the X¹ and Y¹ chromosomes and autosomes (Virkki, 1984). Examples of multiple systems have been
described in the Tenebrionidae, Cincindelidae, Curculionidae and Chrysomelidae (Wahrman et al., 1973; Panzera et al., 1983; Postiglioni et al., 1987; Galián et al., 1995; Vitturi et al., 1996).

Autosomal polymorphisms involving chromosome fusions and dissociations (Smith and Virkki, 1978; Boyce et al., 1994), as well as the presence of supernumerary chromosomes (Smith and Virkki, 1978; Virkki and Santiago-Blay, 1993; Mesa and Fontanetti, 1984; Serrano et al., 1998), have been reported for different coleopteran species.

The aim of the research described in this paper was to analyze the meiotic and mitotic chromosomes of *E. gigantea* using conventional staining, C-banding, silver nitrate staining and fluorescent in situ hybridization (FISH) with a rDNA probe. Comparison of the results with data in the literature permitted a better chromosome characterization and the elaboration of a hypothesis on the karyotype evolution of this species.

**Material and Methods**

We analyzed 19 *Euchroma gigantea* L. 1735 specimens, the sample being made up of 15 males collected at the Igarassú Charles Darwin Ecological Refuge in the northeastern Brazilian state of Pernambuco (7°50’3” S, 34°54’23” W) and two males and two females collected from the Atlantic Rainforest in the Recife Zoo-Botanical Park in Pernambuco (8°3’14” S, 34°52’52” W). The beetles were and the testes and ovaries dissected out and fixed in Carnoy’s solution (ethanol:acetic acid 3:1 v/v). Cytological preparations were obtained using the classical testicular and ovarian follicles squashing method and the chromosomes were stained with 2% (w) lacto-acetic orcein. The method of Sumner (1972) was used for C-banding and silver nitrate staining and fluorescent in situ hybridization (FISH) with a rDNA probe. Comparison of the results with data in the literature permitted a better chromosome characterization and the elaboration of a hypothesis on the karyotype evolution of this species.

**Results**

Chromosome analysis of *E. gigantea* showed the presence of a multiple sex determination mechanism of the *X1X2X3Y1Y2Y3* type in males and wide autosomal polymorphism. Five or six punctiform supernumerary chromosomes were also observed in the different karyotypes (Figure 1b, d). Two of the 15 specimens collected in Igarassú presented a 2n = 36, *X1X2X3Y1Y2Y3* karyotype in which all the autosomes were acrocentric (Figure 1a), 10 specimens showed a 2n = 34, *X1X2X3Y1Y2Y3* karyotype with one submetacentric and 13 acrocentric autosomal pairs (Figure 1b) while the other three specimens had a 2n = 32, *X1X2X3Y1Y2Y3* karyotype with two submetacentric and 11 acrocentric autosomal pairs (Figure 1d). In addition, a heterozygous karyotype complement with 2n = 33 was observed in one specimen from the Recife population (Figure 1c), while the other specimens from this locality presented a 2n = 34, *X1X2X3Y1Y2Y3* karyotype. The X chromosomes were submetacentric, the Y1 and Y2 chromosomes were metacentric and the Y3 chromosome was acrocentric (Figure 2b, c). The sex mechanism was complex and chiasmatic. During metaphase I, the sex chromosomes were connected by distal chiasmata and formed an alternate chain with a sequence of *X1Y1X2Y2X3Y3* (Figure 2a).

In *E. gigantea*, constitutive heterochromatin (CH) was located in the pericentromeric region of all autosomes. In addition, some small pairs contained terminal CH blocks (Figure 3a). Chromosomes *X1* and *X3* possessed centromeric CH, and the long arm of chromosome *X1* was almost completely heterochromatic. In the case of the *X2* chromosome, CH was only present in the terminal region of the short arm. Chromosome *Y1* showed an almost completely heterochromatic short arm, in addition to an interstitial block on the long arm. The *Y2* presented pericentromeric CH extending along the two chromosome arms, and *Y3* possessed pericentromeric CH on the short arm (Figures 3a and 3b).
Silver nitrate staining of cells at the beginning of prophase I revealed labeling of the sex chromosome chain (Figure 3c). Analysis of spermatogonial metaphases by FISH using a ribosomal probe demonstrated the presence of two rDNA sites on different chromosomes. During metaphase I the sites were found to be associated with the sex chain, more precisely with chromosomes X1 and X2 (Figures 3d and 3e).

Discussion

Comparison between the few Buprestidae species studied cytologically clearly shows the chromosome heterogeneity of this family, with the chromosome number ranging from 2n = 12 to 2n = 46 represented by different karyotypes: 12, Xyp; 14, neoXY; 16, Xyp; 18, neoXY; 18, Xyp; 20, Xyp; 20, XY; 20, Xyp; 20, neoXY; 21, X0; 22, Xyp; 24, neoXY; 24, X1X2X3Y1Y2Y3; 26, XY; 26, neoXY; 26, X1X2X3Y1Y2; 40, XY; and 38–46 (Asana et al., 1942; Smith and Virkki, 1978; Mesa and Fontanetti, 1984; Karagyan and Kuznetsova, 2000; Karagyan, 2001). The karyotype variation found among E. gigantea specimens collected in northeastern Brazil enlarge the numerical variation described for the Buprestidae and demonstrates the presence of a wide autosomal polymorphism in this species. On the other hand, the karyotype variation observed among specimens from the southeastern region (Mesa and Fontanetti, 1984) and those analyzed here may indicate the occurrence of different species of Euchroma which, until now, has been considered to be a monotypic genus.

In Coleoptera, examples of chromosome polymorphisms have been observed for different species of Iphis 2n = 16 to 2n = 32, Pissodes 2n = 25 to 2n = 34, Mulsantina 2n = 12 to 2n = 20, and Chilocorus 2n = 19 to 2n = 25. This variability has been attributed to the occurrence of centric fusions, fissions and pericentric inversions (Smith and Virkki, 1978; Boyce et al., 1994). The genus Chilocorus has a basic chromosome number of 2n = 22 and is found in North America, India and Europe. For example, Chilocorus stigma populations from North America present a multiple sex determination mechanism (X1X2Y), wide chromosomal polymorphism (2n = 19 to 2n = 25) and supernumerary chromosomes. Centric fusion and fission have been proposed to explain the karyotype variation observed in this species (Smith and Virkki, 1978).

Comparative karyotype analysis between our sample of E. gigantea from Pernambuco and specimens collected in the Brazilian state of São Paulo (Mesa and Fontanetti, 1984) throws some light on the chromosome evolution of this species. The known variation in chromosome constitution suggests that the most primitive karyotype would be 2n = 36 from which successive centric fusions resulted in derived karyotypes, starting with a single homozygous fu-
sion to produce $2n = 34$, followed by two fusions to produce $2n = 32$, five fusions to produce $2n = 26$ and six fusions to produce $2n = 24$.

Centric fusions have been considered the most common rearrangement between species or chromosomal races. King (1993) has suggested that this type of karyotypic change frequently results in karyotypes with balanced polymorphism. Several species of animals, especially mammals such as the African pigmy mouse *Mus Nannomys minutoides*, presenting six cytotypes characterized by different autosome-autosome fusions (Castiglia et al., 2006), and the *Rhogoaea tumida-Rhogoaea parvula* bat complex which possesses seven different cytotypes ($2n = 30, 32, 34, 42, 44$ and $52$) which differ in regard to successive chromosomal fusions and which Baker et al. (1985) suggests correspond to different species. There are also examples in insects, such as the orthopteran *Hemideina crassidens* which has two karyotypes ($2n = 15, XO + 1B$ or $2B$; $2n = 19, XO$) resulting from two Robertsonian translocations (Morgan-Richards, 2000) and also in coleopterans *Poecilus copreus* ($2n = 43, 44$), *Platysma negrita* ($2n = 40, 41, 43, 46$), *Calathus fuscipes* ($2n = 37, 39$), *Amaru familiaris* ($2n = 33, 34$), *Harpalus serripes* ($2n = 37, 39$) and species of the genus *Timarcha* in which the diploid number varies from $2n = 18$ to $44$ (Serrano, 1981; Gómez-Zurita et al., 2004).

On the other hand, cannot affirm that the fusions seen by us are the main rearrangement involved in the chromosomal evolution of *E. gigantea*, because molecular and biogeographic studies that establish ancestral groups do not exist. Another possible alternative is the rearrangement commenced with the $2n = 24, X_1X_2X_3Y_1Y_2Y_3$ karyotype, in which autosome-sexual chromosome translocations, autosome-autosome and pericentric inversions were initially involved generating the karyotypes described by Mesa and Fontanetti (1984) with $2n = 24, X_1X_2X_3Y_1Y_2Y_3$ and a fusion producing the $2n = 26, X_1X_2X_3Y_1Y_2Y_3$ karyotype. Based on the karyotypes described in the present paper it seems that four fusions had probably occurred to produce $2n = 32, X_1X_2X_3Y_1Y_2Y_3$, five fusions to produce $2n = 34, X_1X_2X_3Y_1Y_2Y_3$, and six fissions to produce $2n = 36, X_1X_2X_3Y_1Y_2Y_3$. In addition to the rearrangements described above, we detected the occurrence of successive pericentric inversions responsible for the alteration in the proportion of chromosomal arms in the different karyotypes presented by *E. gigantea*. Karagyan et al. (2004) have pointed out that it is not yet possible to infer the ancestral karyotype for the Buprestidae, although a similarity has been proposed with the ancestral karyotype ($2n = 20, XYP$) described for the Coleoptera in general and also observed in different species of the subfamilies Chrysochroinae, Buprestinae and Agrilinae but there is as yet no concrete evidence to define the ancestral karyotype for *E. gigantea*. The different karyotypes of *E. gigantea* and the probable rearrangements involving to produce the current karyotypes found in this species is shown in Figure 4.

Mesa and Fontanetti (1984) have pointed out that successive translocations involving autosomes and sex chromosomes were responsible for the formation of the complex $3X + 3Y$ mechanism found in *E. gigantea*, which probably derived from an Xy or XY system. The presence of the $X_1X_2X_3Y_1Y_2Y_3$ sex mechanism in all karyotype compositions found in *E. gigantea* suggests that this mechanism is old and well-established in this species.

Among coleopterans, the Buprestidae is the family with the largest number of supernumerary chromosomes (Smith and Virkki, 1978; Mesa and Fontanetti, 1984). We observed 16 to 32 supernumerary chromosomes in our southeastern Brazilian *E. gigantea* sample, while Mesa and Fontanetti (1984) have reported that *E. gigantea* from this region showed the lowest diploid number ($2n = 24$ and $26$). However, in our study, the *E. gigantea* specimens from northeastern Brazil showed extensive autosomal polymorphism and also the largest diploid number ($2n = 32$ to $36$), as well as a markedly smaller number (five) of supernumerary chromosomes. Derivation from A chromosomes is the most widely accepted of the various hypotheses on the origin of B chromosomes, which could have arisen either from centromeres remaining after chromosome fusions or from amplified pericentromeric fragments (Jones and Rees, 1982; Camacho et al., 2000). Events of these types might have been involved in the origin of B chromosomes in *E. gigantea*. Colombo and Remis (1997) have presented data on the grasshopper *Sinipta dalmani* showing that B chromosomes arise from centric fragments produced from the high frequency of karyotypic rearrangements such as inversions and centric fusions.

As in other coleopteran species, the pattern of CH distribution in *E. gigantea* is predominantly pericentromeric but we found that in this species the autosomal CH blocks were small and some autosomal pairs possessed minute terminal blocks, in contrast to the sex chromosomes which were characterized by large CH blocks. Atypical patterns of heterochromatin distribution have been described in other coleopterans. Some Carabidae species, such as *Acapulcus Stenolophus teutonus, Acapulcus elegans* and *Bembidion minimum*, not only possess large CH blocks on the autosomes and have completely heterochromatic X chromosome but also show C-band heteromorphism and terminal and subterminal CH blocks (Rozek, 1992; Rozek and Rudek, 1992). For example, Vitturi et al. (1996) observed pericentromeric CH blocks in two *Blaps* species, whereas *Blaps gibba* presented an additional proximal and interstitial block on chromosome pair 1 and terminal blocks on pairs 9 and 12.

Our silver nitrate staining and FISH results for *E. gigantea* agree with the pattern observed in other coleopterans that possess a multiple sex mechanism (Galián et al., 1995; Vitturi et al., 1996; Galián and Hudson, 1999) and for which the rDNA sites are located on the sex chromosomes. In general, the species analyzed so far have shown
two hybridization signals, except for *B. gibba* which shows seven to eight signals scattered over the sex multivalent (Vitturi *et al.*, 1996). In contrast, preferential staining of the autosomes is observed in representatives of the Carabidae (Galián *et al.*, 1995; De La Rua *et al.*, 1996; Galián and Hudson, 1999; Sanchez-Gea *et al.*, 2000), Geotrupidae (Vitturi *et al.*, 1999) and Scarabaeidae (Colomba *et al.*, 2000) and which possess a simple sex mechanism.

To better understand the chromosome evolution of *E. gigantea*, studies investigating specimens from the North and Center-West region of Brazil are fundamental, in addition to the analysis of other specimens from the Southeast.
mainly employing FISH with a telomeric probe. Furthermore, a taxonomic revision of the genus including subspecies and varieties from different geographic regions, together with the available cytogenetic analysis, will be of great importance for better understanding the status of the genus *Euchroma*.

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