

MULTIPLE SEX CHROMOSOMES, AUTOSOMAL POLYMORPHISM AND A HIGH NUMBER OF S CHROMOSOMES IN *Euchroma gigantea* L. 1735 (COLEOPTERA, BUPRESTIDAE)

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ABSTRACT

The chromosomes of three specimens of the buprestid *Euchroma gigantea* were studied. They present the most complex chiasmatic sex chromosome system and the highest number of S chromosomes yet recorded in Coleoptera, as well as autosomal polymorphism. Two specimens have $X_1X_2X_3-Y_1Y_2Y_3$ and one specimen has $X_1X_2X_3-Y_1Y_2$. The number of S chromosomes is 16, 20 and 32. The mode of origin of the sex chromosome complex is discussed.

INTRODUCTION

According to Smith (1950), the ancestral karyotype of Coleoptera is $9II+Xy_p$. This formula is widespread within Polyphaga but uncommon in Adephaga where the species have evolved toward an increase in chromosome number by means of successive fissions (Smith and Virkki, 1978). A single species analyzed within Archostemata, *Micromalthus debilis* (Scott, 1936, 1941), shows a haplo ($\sigma = 10$)-diplo ($\varphi = 20$) system, and the only species of Myxophaga studied (*Ytu zeus*) shows a $2n\sigma = 9II+XY$ karyotype, though the sex chromosome behavior during PI and MI suggests a close relationship with an Xy_p system (Mesa and Fontanetti, unpublished results).

It is now widely accepted that $X-y_p$ pairing is nucleolar. Chiasmatic mechanisms are less frequent in Coleoptera and originate when autosomes are incorporated by translocation to one of the Xy_p components or to X in an XO system, after the loss of the y_p chromosome.

Karyotypes of the three specimens of *E. gigantea* collected in São Paulo State differ from one another and from the Panamanian specimens described by Nichols (1910). They show the most complicated chiasmatic sex chromosome system and the highest number of S chromosomes thus far described in Coleoptera, as well as the presence of autosomal polymorphism.

MATERIAL AND METHODS

Three male specimens of *E. gigantea* were collected in Colômbia (São Paulo, Brasil) in November, 1982. The testes were removed and fixed in Carnoy I. Semipermanent slides were prepared after leaving the testes in 45% acetic acid for a few minutes and squashing them in 1% lacto-acetic orcein. Magnification lines in the figures correspond to 5μ .

RESULTS

The karyotypes of the specimens studied are as follows: *Specimen 1*. The chromosome number is $2n = 24$, including nine pairs of autosomes and six sex chromosomes ($X_1X_2X_3-Y_1Y_2Y_3$). Among the autosomes, the five larger pairs are formed by metacentric chromosomes, three pairs are acrocentric and one pair is heteromorphic, with one chromosome acrocentric and its homologous metacentric (see Figure 1A). The specimen shows 32 S chromosomes (see Figure 3C). Chromosome Y_3 is acrocentric while the remaining five sex chromosomes are metacentric or submetacentric. During MI they form a zig-zag chain touching each other by distal chiasmata, in the sequence $X_1-Y_1-X_2-Y_2-X_3-Y_3$ (see Figure 1A) in such a way that during AI the X's go to a pole and the Y's to the opposite pole.

Specimen 2. This specimen has $2n = 24$ with a sex chromosome system similar to that of specimen 1 (Figures 3A and 3B). The autosomes can be grouped into five pairs of metacentrics, two pairs of acrocentrics and finally a tetravalent which forms a zig-zag chain with two metacentrics at the center and two acrocentrics at the ends of the chain, connected by means of distal chiasmata (see T_4 in Figure 2A and B). Twenty S chromosomes were observed in this specimen.

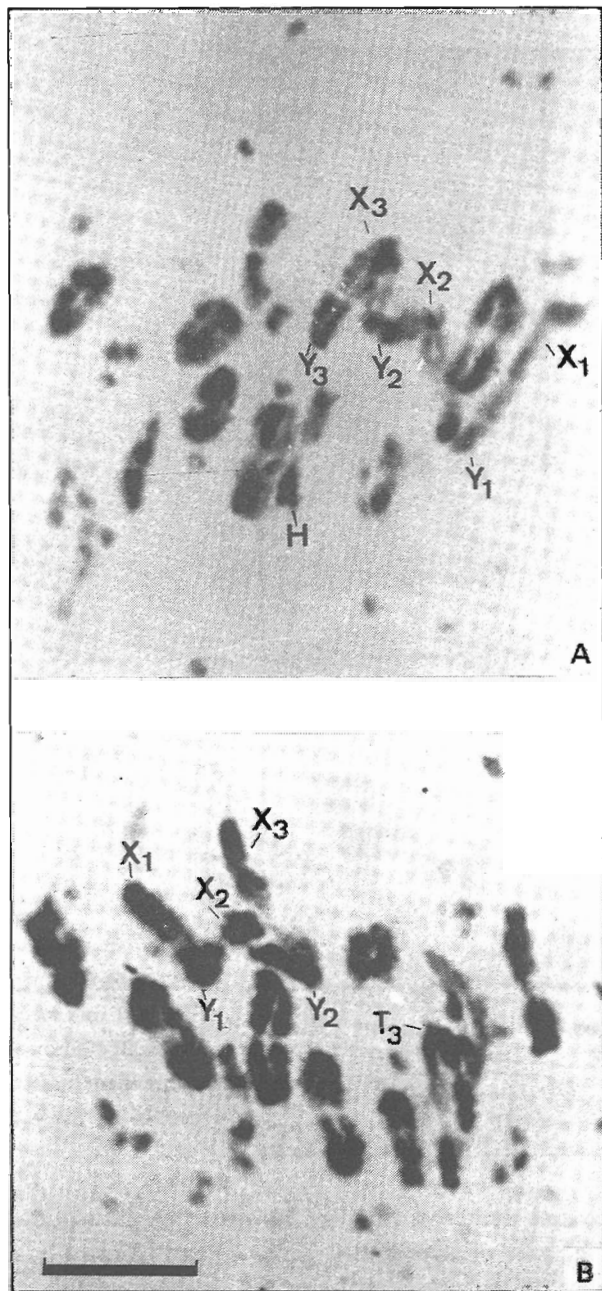


Figure 1 - *E. gigantea*.
A, First metaphase of specimen 1, H = heteromorphic. B, First metaphase of specimen 3; autosomal trivalent indicated as T₃. Bar = 5 μ .

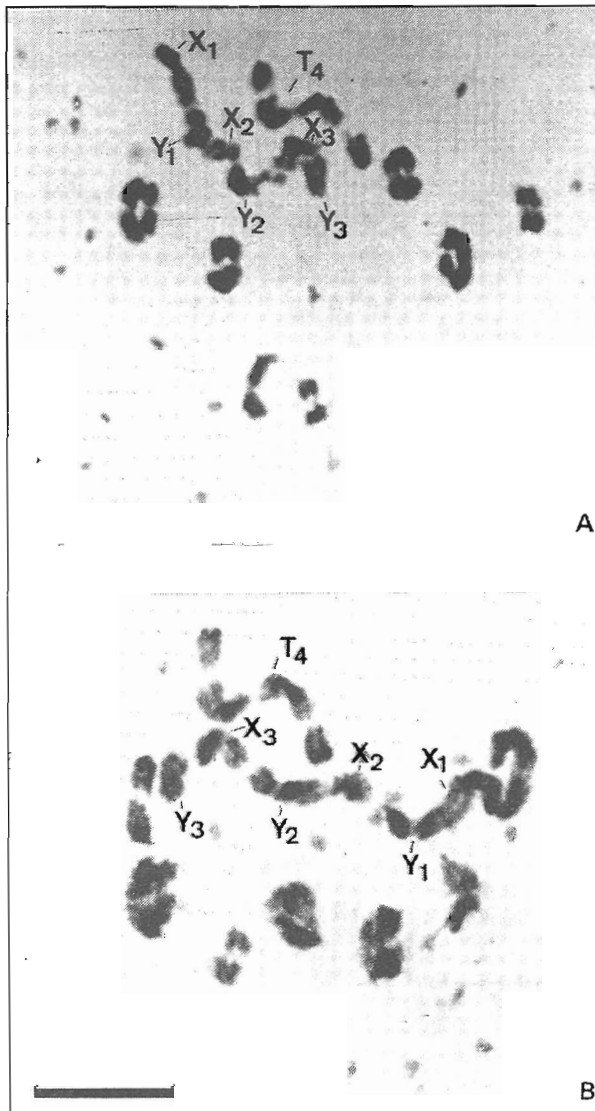


Figure 2 - *E. gigantea*.
A and B, First metaphase of specimen 2; autosomal tetraivalent indicated as T₄. Bar = 5 μ .

Specimen 3. The chromosome number is $2n = 26$. The autosomes include five pairs of metacentrics, four pairs of acrocentrics, and a trivalent formed by a metacentric in the center and two acrocentrics in the extremes of the sequence (Figure 1B). The multiple sex chromosome is formed by a chain of

five metacentric or submetacentric chromosomes in the sequence X_1 - Y_1 - X_2 - Y_2 - X_3 (see Figure 3D). This specimen shows sixteen S chromosomes.

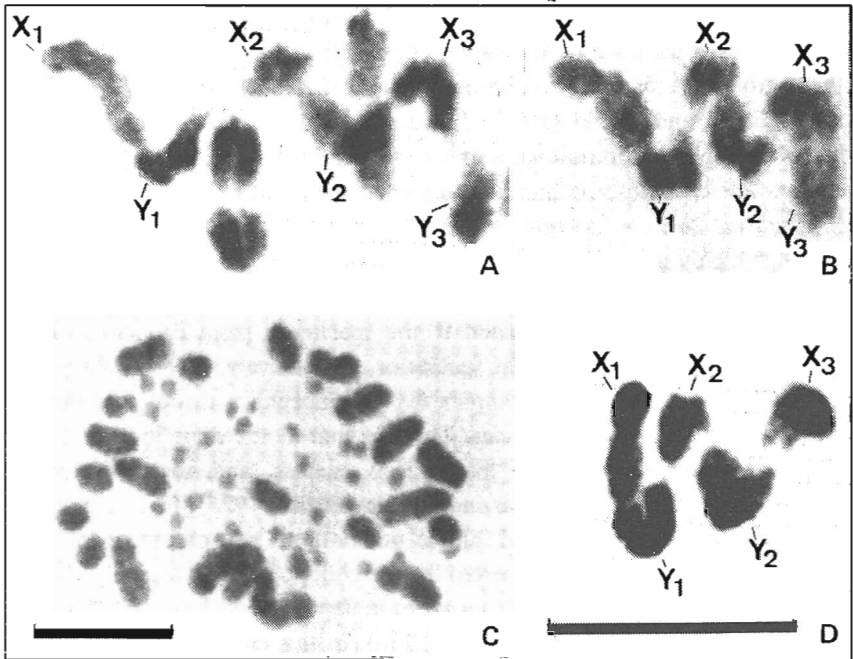


Figure 3 - *E. gigantea*. A, Sex hexavalent of specimen 1 with 32 S chromosomes; D, sex pentavalent of specimen 3 during first metaphase. Bars = 5 μ (magnification in D is the same as in A and B).

DISCUSSION

Few papers have been published on the karyotypes of buprestids (Stevens, 1905; Nichols, 1910; Asana *et al.*, 1942; Smith, 1949, 1953, 1960; Smith and Virkki, 1978). For a total of 22 species studied, the following chromosome numbers were reported: 26(3 sp.), 24(1), 22(2), 21(1), 20(8), 18(1), 16(5) and 12(1). The sex chromosome systems observed in these species were: Xy_p , Xy , XO and $neoXY$. In spite of the small number of species studied (approximately 0.1% of those described), a striking karyological heterogeneity within the family is evident.

Nichols's (1910) paper on the karyotype of *E. gigantea* from Panamá leaves some doubts about the results obtained. The author does not provide a diploid chromosome number but says that the total reduced number is 13. On the other hand, on p. 167 she says: "In Carabidae some members have an unequal pair of heterochromosomes, others, an odd chromosome. *Euchroma gigantea*, as a member of the family Buprestidae belongs in the second of these groups" and refers to Figures 21 and 22 showing a single X chromosome. Smith and Virkki (1978), however, based on Nichols's information, listed the diploid chromosome number as 26 and the sex chromosome system as Xy. The chromosome numbers obtained for the São Paulo specimens of *E. gigantea*, $2n\sigma = 24$ and 26, are close enough to the Panamá numbers, although highly polymorphic.

The notable difference in the sex chromosome system between the two localities could be explained if the specimens from Panamá and São Paulo belonged to two different species or, alternatively, if they belonged to the same species but with a complex sex polymorphism throughout the geographic distribution of the species. The fact that at the same locality in São Paulo at least two different mechanisms coexist, and that taxonomists consider the genus *Euchroma* as monotypic (Fisher, 1925), favor the second hypothesis. On the other hand, ample populational sex chromosome polymorphism has been already reported for the chilopod *Otocryptops sexspinosus* where specimens from different localities in Japan showed extremes of variation, from 2X-3Y to 5X-4Y (Ogawa, 1966). In this case, a low vagability of the species may have contributed to the cytological mosaicism.

The evolution of an Xy or XO system toward the complex 3X+3Y mechanism must have occurred by means of autosomal incorporation. The actual order of incorporation could be probably elucidated by population studies at different geographic localities, but three alternatives may be proposed in the meantime. First, the occurrence of successive translocations, each one involving the last Y or X of the growing sex chain. Another alternative is the formation of an autosomal multivalent and a single-step translocation of an X or Y from a simple Xy or XO system to one of the last chromosomes in the autosomal chain. In a third case, an intermediate process could be conceived with the formation of a relatively simple autosomal polymorphism followed by a translocation of Y with the end of the chain and then the incorporation of more autosomes by translocation with the last neo X or neo Y or the sex series.

The multiple sex chromosome system found in *E. gigantea* is the most

complex of the purely chiasmatic system so far discovered in Coleoptera. The alticid *Cyrsylus volkameriae* is the only species of beetle with a chiasmate $X_1X_2Y_1Y_2$ (Virkki, 1968) and a few species were described with X_1X_2Y systems (Smith and Virkki, 1978).

A considerable number of species show autosomal polymorphism in animal and plants. Within Coleoptera, the fact is well illustrated in Chilocorini (Smith, 1962) where the species *Chilocorus stigma* shows variability in diploid chromosome numbers from 19 to 25 in males. According to Smith, centric fusions as well as dissociation took place in this species. Similar autosomal polymorphism has been observed in the curculionid *Pissodes* (Smith and Virkki, 1978) and in the coccinellids *Brachyacantha* (Smith, 1953), *Brumus* (Smith, 1960) and *Mulsantina* (Smith, 1962).

Since three specimens from a single locality showed three different karyotypes, it is quite obvious that to fully understand the autosomal polymorphism of *E. gigantea*, future studies of its populations throughout its extensive geographic distribution will be necessary. Single Robertsonian criteria do not explain the complex relationship between chromosome number and morphology among the three specimens studied. Pericentric inversions might also be involved, making it difficult to propose any interpretations.

Supernumerary chromosomes are found at relatively high frequency in animals as well as in plants. Smith and Virkki (1978) listed approximately fifty species of beetles where S chromosomes were observed, and the highest number, 13, was found in the crysamelid *Chylocorus rubidus*.

When numerous, the S chromosomes are hard to count during MI because some of them pair and then segregate reductionally while others remain unpaired and segregate equationally. As the chromosomes are very small, it is difficult to distinguish between the two alternatives, the count being necessarily approximate. In the case of specimen 1, the total number did not offer any doubt because the count was done in a spermatogonial metaphase.

Because these supernumeraries are capable of orientation at metaphase, they obviously have a centromere, and, according to Smith and Virkki (1978), they are most probably side products of translocations between acrocentrics, or diphasic metacentrics, as described for *Chilocorus*. However, this does not seem to be the mode of origin for several species of grasshopper where we observed high frequencies of S chromosomes in spite of the fact that the ancestral karyotype ($2n = 23$) was preserved, without any evidence of centric fusions.

The numbers of S chromosomes detected here (16, 20 and 32) are probably the highest yet reported for the animal kingdom.

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RESUMO

Os cromossomos de três espécimens do buprestídeo *Euchroma gigantea* são estudados. Eles apresentam um sistema de cromossomos sexuais do tipo quiasmático muito complexo, o maior número de cromossomos S já reportados em Coleoptera e polymorfismo autossomal. Dois espécimens são $X_1X_2X_3-Y_1Y_2Y_3$ e um espécimen é $X_1X_2X_3-Y_1Y_2$. O número de cromossomos S é de 16, 20 and 32. A origem do complexo sexual cromossômico é discutido.

REFERENCES

- Asana, J.J., Makino, S. and Niyama, N. (1942). A chromosome survey of some Indian insects. IV. On the sex chromosomes of some species of beetles (Coleoptera). *Cytologia* 12: 187-205.
- Fisher, W.S. (1925). A revision of the West Indian Coleoptera of the family Buprestidae. *Proc. U.S. Nat. Mus.* 65: 1-207.
- Nichols, M.L. (1910). The spermatogenesis of *Euchroma gigantea*. *Biol. Bull.*, 19: 167-179.
- Ogawa, K. (1966). Chromosome studies in the Myriapoda. XVII. Some cytological and geographical aspects of *Octocryptops* (Chilopoda). *Proc. Jap. Soc. Syst. Zool.* 2: 1-6.
- Scott, A.C. (1936). Haploidy and aberrant spermatogenesis in a Coleopteran *Micromalthus debilis*, Le Conte. *J. Morphol.* 59: 485-515.
- Scott, A.C. (1941). Reversal of sex production in *Micromalthus*. *Biol. Bull.* 81: 420-431.
- Smith, S.G. (1949). Evolutionary changes in the sex chromosomes of Coleoptera. I. Wood borers of the genus *Agrius*. *Evolution* 3: 344-357.

- Smith, S.G. (1950). The cyto-taxonomy of Coleoptera. *Can. Entomol.* 82:58-68.
- Smith, S.G. (1953). Chromosome numbers of Coleoptera. I. *Heredity* 7:31-48.
- Smith, S.G. (1960). Chromosome numbers of Coleoptera. II. *Can. J. Genet. Cytol.* 2: 66-88.
- Smith, S.G. (1962). Cytogenetic pathways in beetle speciation. *Can. Entomol.* 94: 941-955.
- Smith, S.G. and Virkki, N. (1978). *Animal Cytogenetics*, Vol. 3, Insecta 5 – Coleoptera. Gebruder Borntraeger, Berlin-Stuttgart, pp. 366.
- Stevens, N.M. (1905). Studies in spermatogenesis with special reference to the "accessory chromosome". *Publ. Carnegie Inst.* 36:3-32.
- Virkki, N. (1968). A chiasmate sexquadrivalent in the male of an alticid beetle, *Cyrsylus volkameriae* (F.). *Can. J. Genet. Cytol.* 10: 898-907.

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