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Cytotaxonomy of the genus Dichromatos Cigliano 2007 (Orthoptera, Acridoidea, Melanoplinae)

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Abstract

All four species of the genus *Dichromatos* now cytologically analyzed, including *D. montanus* and *D. corupa* (Cigliano 2007), studied in the present paper, are 2n = 21(♂)/22(♀) (FN = 23/24) with a X_1X_2Y ♂ / $X_1X_1X_2X_2$ ♀ sex-determination mechanism. The possibility that this mechanism arose from a single ancestral species from which a rapid process of speciation and dispersion took place is discussed.

Key words

sex determination, chiasma, centric fusion, chromosomes, speciation

Introduction

The genus *Eurotettix* was erected by Bruner in 1906 for *E. femoratus* and *E. minor*, from Paraguay, of which the first species was chosen as the type of the genus. In 1910 Rehn described *E. schrottkyi.* Bruner (1911) described *E. robustus*, Lieberman (1948) described *E. lilloanus* and, more recently, Assis-Pujol *et al.* (2001) published the description of three Brazilian species: *E. monnei*, *E. carbonnelli* and *E. raphaelandrearum*. Carbonell & Mesa (2006) have submitted for publication the description of two new species, *E. corupa* and *E. montanus*, and in the same paper report the sex-determining mechanism of both species as belonging to the X_1X_2Y (♂), $X_1X_1X_2X_2$ (♀) type.

Cigliano (2007), after a review of the genus *Eurotettix* taking into consideration the results of a phylogenetic analysis performed on a matrix comprising 29 adult morphological and one karyotypic character, the sex-determining mechanism, and twenty taxa — considered that the synapomorphy 'tegmina narrow at base' was inadequate justification for a group at the genus level. Thus, Cigliano (2007) divided *Eurotettix* into two independent monophyletic groups, and described the new genus *Dichromatos* for *E. corupa* Carbonell & Mesa, 2006, *E. lilloanus* Liebermann, 1948 (type species), *E. montanus* Carbonell & Mesa, 2006 and *E. schrottkyi* Rehn, 1910. Twelve species were recognized for *Eurotettix,* of which six were described as new: *E. concavus*, *E. procerus*, *E. brevicerci*, *E. similraphael*, *E. bugresensis* and *E. latus*.

The state of cytological knowledge of the species of the genus *Eurotettix* before Cigliano's (2007) review received extensive contribution from Mesa, beginning in 1962 with the study of *E. lilloanus*. Later on, Mesa *et al.* (1982) published the cytogenetic of *E. minor* and *E. schrottkyi* and of two still-undescribed new species (*Eurotettix* sp 1 collected in Minas Gerais and *Eurotettix* sp 2 collected in Aratinga, State of Rio Grande do Sul, Brazil). Carbonell & Mesa (2006) submitted two manuscripts to the journal Neotropical Entomology dealing with the description of two new species, *E. corupa* and *E. montanus*, and their sex-determining mechanism. Unfortunately, Prof. Mesa became seriously ill, and passed away before completing the revisions for the papers as suggested by the referees. Consequently these papers were never published.

Later on Cigliano (2007), knowing of these two manuscripts (referred to by her in press) took into consideration their results in her revision of the genus *Eurotettix*. At the moment, the literature registers three species studied; two of them, *E. lilloanus* and *E. schrottkyi*, share a multiple X_1X_2Y male and $X_1X_1X_2X_2$ female sex-determining mechanism. *Eurotettix minor* is the only species that has a sex-determining mechanism of neo-XY male neo-XX female and judging from its morphological characters and size, the latter being significantly smaller than its congenerics, this species probably belongs to a different genus (Mesa *et al.* 1982, Mesa *et al.* 2001).

In the present paper the karyology of *D. corupa* and *D. montanus* are described and their karyotypes discussed and compared to *D. lilloanus*, *D. schrottkyi* and *Eurotettix minor*.

Materials and Methods

Material examined.— Six males of *D. corupa* from Brazil, Santa Catarina, 12 km NW of Corupa (lat 26°23′04″S, long -49°18′12″W), 11/23/2002 and five males of *D. montanus* collected from two localities in Brazil, Mantiqueira Chain. One of these latter was at Saiqui Farm, Barreira do Piquete County and on Itajubá Road, 1 km NW of the border between São Paulo and Minas Gerais State, at 1400 m, the other in the State of Rio de Janeiro, between 1.5 to 3.5 km from Garganta do Registro to Rebouças road, at 1800 m.

For chromosomal studies, males were vivisected and their testes fixed in Carnoy 3:1 (three parts of ethyl-alcohol one of acetic acid). Permanent slides were made after softening the tissues in 45% acetic acid, drying on a hot plate and staining with lacto-acetic orcein for 3 to 5 min.

Results

Dichromatos corupa Cigliano, 2007

D. corupa has a karyotype formed by 2n=21 (♂) (FN=23) and 2n=22 (♀) (FN=24) and a sex-chromosome mechanism of the X_1X_2Y (♂) and $X_1X_1X_2X_2$ (♀) type. All the autosomes and the X_2 sex chromosome are acrocentrics, while X_1 and Y sex chromosomes are metacentrics. The autosomes comprise one pair of long chromosomes, five pairs medium size and three pairs of small chromosomes. The differences between the smallest medium size and the largest small pair are not significant and this gives rise to doubts in the identification of both groups.

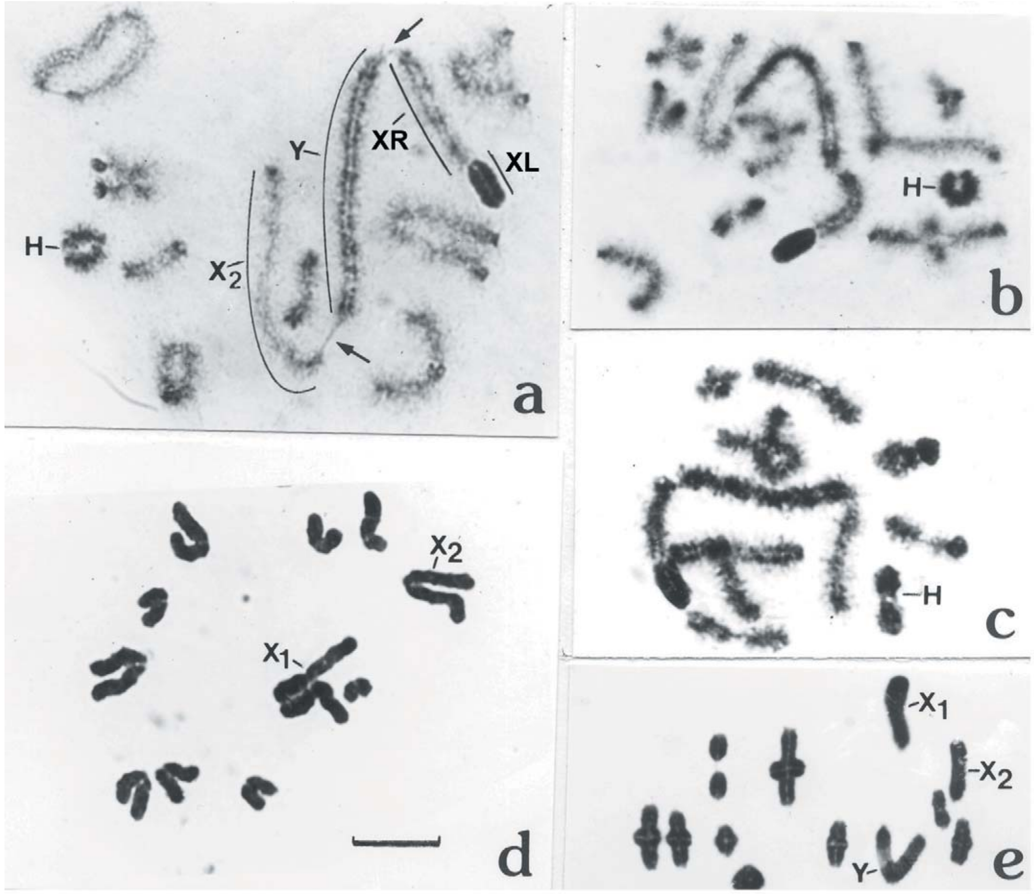

Fig. 1. Meiotic cells from male *D. corupa*. a) Early diplotene showing the sex trivalent. XLA, XR, Y and $X_2$are indicted. Arrows point to terminal associations between the sex chromosomes. b) Middle diplotene. c) Late diplotene. d) Second metaphase containing X_1 and X_2. e) First metaphase. Bar = 10µm.

During first prophase one of the small pairs (indicated by H in Fig. 1 b) appears entirely heterochromatic (positively heteropycnotic) from early diplotene (Fig. 1 a), remaining as such while the diplotene advances (Fig. 1b, c). From early diplotene the sex trivalent appears as a single succession of the three elements (Fig. 1 a), only connected by two terminal chiasmata: between X_1R and Y and X_2 and Y. From early diplotene, the Y chromosome is observed as uniformly more condensed than the X_1R arm and the X_2, but there are no differences in condensation between the arms of the metacentric neo-Y. The XL arms, originally the X chromosome, remain wholly heterochromatic (positively heteropycnotic) during first prophase. At first metaphase the sex chromosomes form a trianglular figure, with the X_1 and X_2 centromeres pushing to the same pole and the Y to the opposite one. The X_1 and Y are metacentrics, as shown in Figs 1d, e. The number of chiasmata between each of the nine bivalents is normally one, sometimes two (as observed in Figs 1a-c), frequently with interstitial localization as shown in Fig. 1a-c, e.

Dichromatos montanus Cigliano, 2007

D. montanus is 2n = 21 (♂) with nine pairs of acrocentric autosomes and a X_1X_2Y (♂), $X_1X_1X_2X_2$ (♀) complex sex-determining mechanism. The X_1 is metacentric, the Y submetacentric with arm ratio =1.55, and X_2 is acrocentric. According to their length the autosomes are grouped as two large, five medium-size and two small pairs. The Y chromosome originated by the centric fusion of a large neo-Y and a medium-size autosome. The pairing and chiasma between XR-Y and Y-X_2 are always terminal and during the first prophase the Y chromosome appears uniformly, but not extremely, isopicnotic (Fig. 2b, c). During the first metaphase the sex trio is arranged with X_1 and X_2 pointing to a pole, while the Y goes to the opposite one (Fig. 2a). In this figure, the largest bivalent of the small group, segregates before the rest of the autosomic bivalents (see arrows). Following the first anaphase (Fig. 2e), second metaphases including either X_1X_2 or Y are formed. (In Fig. 2f the group of chromosomes going to the lower side in Fig. 2e is shown with a different focus in order to visualize the X_2). A second anaphase with X_1X_2 going to each pole is shown in Fig. 2g.

Discussion

It is well known that the great majority of orthopteran species, including Ensifera and Caelifera, present a sex-chromosome determining system of the XO (♂), XX (♀) type, with the X chromosome being always univalent, acrocentric and positively heteropycnotic during the first prophase. This makes it easily recognized, contrasting with the autosomes that are usually euchromatics.

According to Mesa *et al.* (1971) about 50 neotropical species of grasshopper have a neo-XY♂, neo-XX♀; a dozen have a X_1X_2Y♂, $X_1X_1X_2X_2$♀ sex-chromosome determining mechanism. The neo-X and neo-Y are the result of a centric fusion involving the former X acrocentric chromosome with an A1 chromosome of the acrocentric autosome pair A1A2 of the standard karyotype. The neo-X is thus metacentric, with one of its arms corresponding to the ancestral X positive heteropycnotic during the first prophase, while the other arm, formed by the A1 chromosome, is isopicnotic. The unfused autosome A2 becomes the neo-Y, also isopicnotic in the first prophase.

In the few species which have been recorded with a X_1X_2Y (♂)-$X_1X_1X_2X_2$ (♀) sex-chromosome determining mechanism, a second centric fusion took place between the neo-Y and an acrocentric

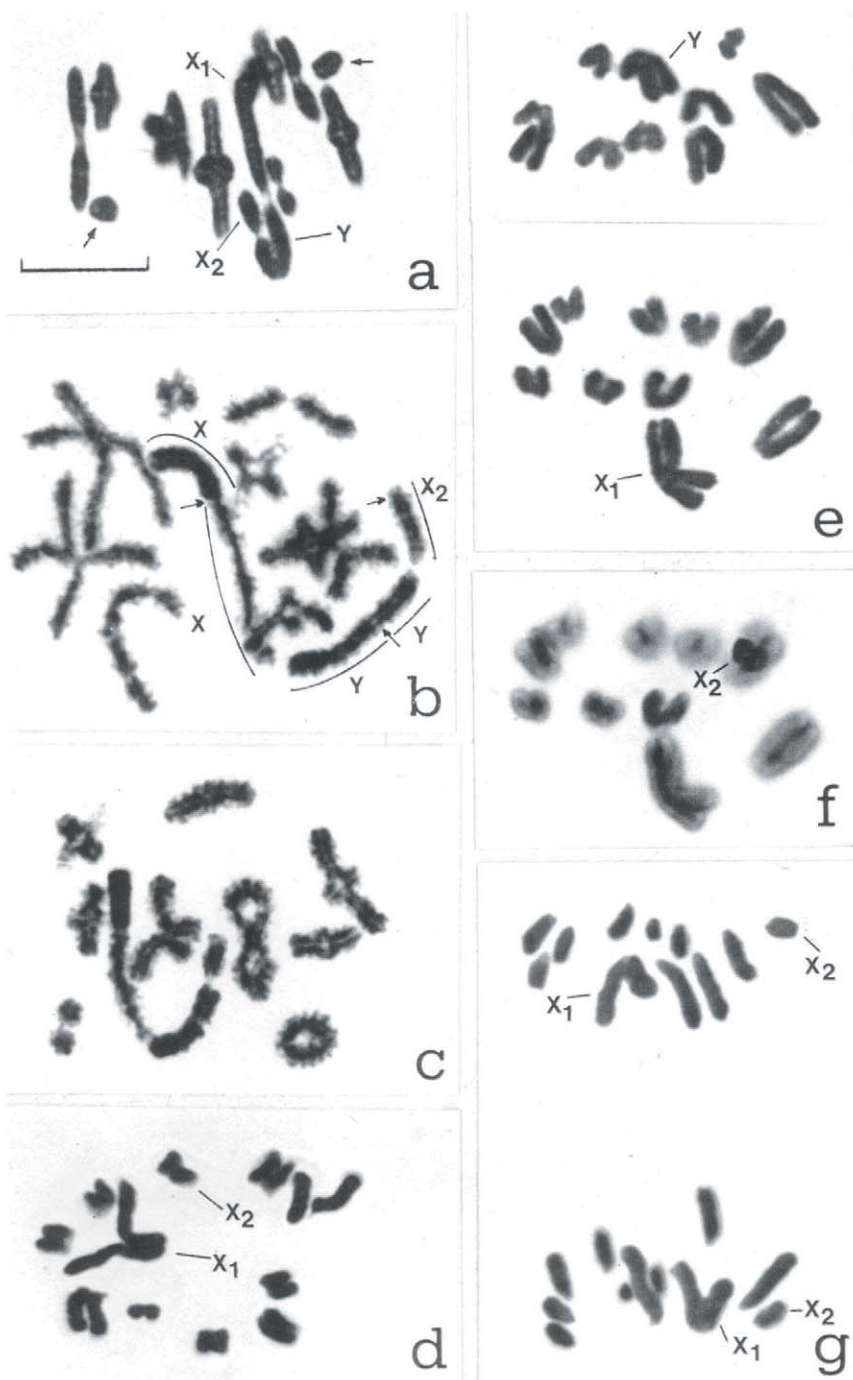

Fig. 2. Testicular meiotic cells of *D. montanus*. a) First metaphase showing the sex chromosomes. Arrows indicate precocious segregation of one of the smallest bivalents. b) Diplotene. Arrows indicate the sex chromosome centromere. c) Diakinesis. d) Second metaphase in polar view, with X_1 and X_2. e) First anaphase with Y at one pole and X_2 [focused in f] and X_1 at the opposite pole. g) Second anaphase with X_1 and X_2. Bar= 10 µm.

autosome B1 (of the B1B2 autosomic pair from the standard karyotype) of a B1B2 autosome pair; this occurred in such a way that the Y turned metacentric, while the unfused B2 autosome behaved as an acrocentric X_2, as observed in *D. corupa* and *D. montanus*. The origin of these mechanisms does not differ in principle from that already described by several authors (Mesa 1962, Mesa *et al.* 1967, Mesa *et al.* 1982, Ferreira 1975, Ferreira *et al.* 1979, Ferreira *et al.* 2006, White 1973, Fernandez-Piqueras *et al.* 1982) for other orthopteran species.

The behavior, morphology, and heteropycnosity of the autosomes that were incorporated into the new sex-chromosome system have been used as an indicator of the relative age of the mechanism. If they are paired throughout their entire lengths and do not develop any sign of heterochromatinization, they are equal in length during early prophase and exhibit interstitial chiasmata. With these signs of low divergence, it is understood that the origin of the system is very recent. After Robertsonian fusions take place (Saez 1963, White 1973), a gradual process of heterochromatinization in the exhomologues begins. This, in addition to several other mechanisms such as inversions and limitation of the crossing-over to the distal end of the exhomologue chromosomes, causes loss of homology between the exautosomes, which is characteristic of the older system.

Mesa *et al.* (2001) took into consideration the results obtained in more than 50 species with neo-XY males in several hundreds of neotropical species cytologically analyzed, and observed that approximately 10% of these species have acquired this mechanism. Three of them show a recently established neo-XY sex-determining mechanism with full Y-XR pachytene pairing: *Neuquenina fictor* (Rehn) (Mesa 1960), *Tetrixocephalus* (= *Calcitrena*) *willemsei* Guerney & Lieberman (Mesa 1973, Mesa *et al.* 1977), and *Leiotettix sanguineus* Bruner (Mesa *et al.* 1967). Sometimes the populations still have XO and neo-XY males. Six species are at the end of the neo-XY evolutionary process, with both X and Y wholly heterochromatic during the first prophase, as observed in *Atamacris diminuta* (Carbonell *et al.* 1971, Mesa *et al.* 1971, Mesa 1982), *Dichroplus vittigerum* (Blanchard) (Mesa 1973, Mesa *et al.* 1982), *Zygoclistron nasicum* (Gerstaecker) (Ferreira *et al.* 1979), *Z. falconicum* (Gertaecker) (Mesa *et al.* 1982) and *Z. trachysticum* (Rehn) (Mesa *et al.* 1982).

Intermediate stages were observed in nearly 40 other species (Mesa *et al.* 2001), among them *Eurottetix minor*. According to Mesa (1967) the second centric fusion can take place at any stage of the neo-XY differentiation. In *Leiotettix politus* the second fusion is recent, since we can find males neo-XY♂ and neo-X_1X_2Y♂ in the same populations; however, the first that gave rise to the neo-XY is very old, since the Y chromosome, which is metacentric, shows a clearly condensed arm.

According to Fernandez-Piqueras *et al.* (1982) the arguments that have been used to support the evolution of the sex-chromosome mechanism in Orthoptera are based on comparisons between largely unrelated species and, according to him, their validity remains to be demonstrated.

If the X-autosome centric fusion occurs at a more or less constant rate in the evolutionary history of the Acridoidea, it is expected that earlier fusions gave the involved species enough time to speciate, giving rise to higher taxa. Examination of the present literature shows that usually only isolated species from a genus are neo-XY (Mesa *et al*. 1982, Mesa *et al*. 2001), but there are at the moment two cases suggestive of a fusion X-A occurring in an ancestral species of the genus, from which all the others have then evolved: in the closely related genera *Zygoclistron* and *Aleuas*, all species have a neo-XY sex chromosome mechanism (Ferreira 1975, Ferreira *et al.* 1979, Mesa *et al.* 1982, Mesa *et al.* 2001). It is a debatable question which requires more investigation, whether the X-A fusion has a single origin in a common ancestral species of both genera, or if it took place twice independently, in two different ancestral species of each genus. As the behavior between XR and Y during diplotene and diakinesis are different in the species of both genera, it seems that the X-A fusion has occurred independently in two ancestral species of each genus.

According to Cigliano (2007), the new genus *Dichromatos*, which includes *D. lilloanus, D. schrottkyi, D. montanus and D. corupa*, is supported by 10 morphological synapomorphies and one synapomorphy from the karyotype, the latter having the highest Bremer support value on the tree.

The species of the genus *Eurotettix* are included in a clade supported by six synapomorphies; the clade is composed of two monophyletic groups recovered by high values of nodal support. All the known species of the genus *Dichromatos* have similar karyotypes, 2n = 21 (♂) and 2n = 22 (♀) with an X_1X_2Y (♂) and $X_1X_1X_2X_2$ (♀) mechanism of sex determination. In *D. corupa* both arms of the Y chromosome show the same degree of condensation and are slightly more condensed than X_2 and X_1R, indicating that the second fusion which originated the X_1X_2Y system, occurred a long time after the first, thus responsible for the neo-XY sex-chromosome mechanism origin that precedes it. In *D. montanus* the arms appear uniformly, but not extremely, heterochromatic, indicating a possible recent origin of the system when compared with that of *D. corupa*. In *D. lilloanus* and *D. schrottkyi* these details were not observed; however this should be seen as an indication of relative value since the staining technique used for the chromosome studies is unspecific in order to determine the nature of the chromatin.

The only species of *Eurotettix* cytologically known among the twelve recognized by Cigliano (2007) is *E. minor*. Its karyotype is formed by 2n = 22♂, 2n = 22♀ and FN = 23/24 for both male and female. The sex-determining mechanism is of the neo-XY/XX type, thus resulting from a single centric fusion involving the ancestral X chromosome and an autosome. The fused chromosome is metacentric, having one arm that corresponds to the former X, and the other to the acrocentric autosome with which it was fused. This chromosome is the neo-X. The unfused acrocentric autosome is recognized as neo-Y. This sex system represents the first step toward the origin of the $X_1X_2Y/X_1X_1X_2X_2$ (♂/♀) sex-chromosome system.

Considering that all the species belonging to the genus *Aleuas* and *Zygoclistron* are neo-XY (Mesa *et al.* 2001, Ferreira 1975, Ferreira *et al.* 1979) and the existence of $X_1X_2Y/X_1X_1X_2X_2$ (♂/♀) in all four known species of *Dichromatos*, the point of view of Fernandez-Piqueras *et al*. (1982) cannot be assured. We favor the hypothesis that in the *Dichromatos* species both fusions could be interpreted as a consequence of several similar and independent events, whose probability is so low that it should not be considered; or that the occurrence of only two fusions, X-A1and Y-B1 in the same ancestral species of the genus, is highly probable. The analysis of the cladogram, Figure 1 of Cigliano (2007), supports this hypothesis.

This can be also interpreted as a quick process of speciation and dispersion from a single $X_1X_2Y/X_1X_1X_2X_2$ (♂/♀) ancestral species. The time elapsed between the first and the second step (that is, from the neo-XY to the neo-X_1X_2Y species) was not very long since the loss of homologies between XR and Y should be much more cytologically visible.

Of the 12 species recognized so far by Cigliano (2007) as belonging to the genus *Eurotettix*, only *E. minor* from the *femoratus* group has had its karyotype studied and it exhibits a neo-XY sex-chromosome mechanism (Mesa *et al.* 1982). It shares with others, particularly morphological characteristics such as dorsal valves poorly projected laterally, and is (statistically) smaller than the remaining species. Mesa *et al.* (1982) prefer to interpret this species as belonging to a different and perhaps new genus. It is interesting to note that of all the species reviewed by Cigliano (2007), only those that exhibit a neo-X_1X_2Y sex-chromosome mechanism were incorporated into the new genus *Dichromatos*. The cytological studies of those species belonging to the genus *Eurotettix*, which according to Cigliano (2007) is an independent monophyletic group, are of extreme interest and should help to reach a better understanding of its genetic relationship with the genus *Dichromatos*. Sex-chromosome determining mechanisms more complex than those with X_1X_2Y (♂) were only found in species of crickets, where several species show significantly more complex mechanisms.

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