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Abstract

Scaphura nigra has a unique chromosome complement among approximately 100 species studied so far belonging to the subfamily Phaneropterinae. It is formed by 2n ($\male$) = 26 and a FN = 29 and derived from the ancestral karyotype of the group 2n ($\male$) = 31, FN = 31, by means of two centric fusions and one tandem fusion. The first between the X chromosome and a medium-sized autosome giving rise to a neo-XY sex chromosome mechanism of recent origin, and the second between two acrocentric ones, the bigger and a medium size, that gave rise to a large submetacentric element whose length is very uncommon in the subfamily. This process has created a bimodal karyotype that contrasts with the majority in this group, whose chromosomes usually can be arranged in a decreasing order of size. A third rearrangement incorporating the chromatin of a medium-sized autosome to the bigger one, explains the reduction observed in the number of chromosomes and the enlarged size of the submetacentric elements. These features demonstrate the effectiveness of chromosome number, their morphology and the change of the sex mechanism as useful tools for taxonomy.

Key words

karyotype, sex mechanism, centric fusion, Phaneropterinae

Introduction

The present paper is a study of the karyotype of *Scaphura nigra* (Thunberg, 1824), a species that is polymorphic and a Batesian mimic of wasps, exhibiting three different color patterns in the adult stage; it mimics species of the genus *Pepsis* and *Entypus* (Pompilidae) and *Polistes* (Vespidae) (Del Claro 1991).

The genus *Scaphura* was erected by Kirby (1825), based on *Gryllus nigra* (Thunberg, 1824). For revision see Eades & Otte (2008): they list eleven species for the genus (with some synonyms), six of them described in the first half of the 19th century [*S. denuda* (1836), *S. edwarsi* (1828), S. *elegans* (1838), *S. lefebrei* (1835), *S. nigra* (1824) and *S. obscurata* (1813)], four in the second half, [*S. conspurcata*, *S. fasciata*, *S. infuscata* (1878) and *S. marginata* (1869)] and a single species in the 20th century [*S. argentina* (1931)].

The localities mentioned for each species are imprecise. Seven species are reported from Brasil, two from Argentina, one from Misiones, another from Buenos Aires and one from South America (?), Demerara. Imprecise localities, poor descriptions and the presence of color polymorphisms make identification of the specimens doubtful. The identification of the specimens here studied as *S. nigra* is consequentially provisional, until future studies elucidate the species of the genus with more accuracy.

We aim to describe the karyotype of *S. nigra*, one that is unique among species belonging to the Phaneropterinae and seems to be an excellent character for species identification.

Materials and methods

Several living specimens of brown and black color were obtained: (Fig. 1a) 3rd instar nymph, body size 8 to 10 mm; (Fig. 1b) 5th instar nymph, body size 19 to 22 mm; (Fig. 2a) adult female, body size 34 mm; (Fig. 2b) adult male, body size 32 mm. All specimens were collected by Alejo Mesa and Carmem Silvia Fontanetti during January 2004 at the Estação Biológica de Boracéia – Boraceia Reserve (lat 45°52′18.38″W, long 23°35′35.80″S 648 m) Serra do Mar, near Salesópolis county, state of São Paulo, 100 kms distant from São Paulo city and at Saiqui farm Fazenda Saiqui Minas Gerais State, Mantiqueira Mts (lat 45°12′32.94″W, long 22°33′26.60″S 1155 m), Piquete county. Five males were vivisected in the field and their testes fixed in a mixture of 3 parts 100% ethyl alcohol and 1 part acetic acid. In the laboratory, 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

Specimens of brown and black color were studied and no differences observed in their karyotypes. The diploid number of chromosomes in *S. nigra*, as seen at spermatogonial metaphase, is 2n = 26 (FN = 29) in both brown and black specimens (Fig. 3a-f). There are three submetacentric chromosomes in the males, two of which are autosomes, with their longer arm being at least three times the length of the shorter; one is the X element that is the biggest of the karyotype. The remaining 23 are acrocentrics, including the Y that is the biggest among them. In the primary spermatocytes, 12 pairs of autosomal bivalents are formed (Fig. 3a-d). They can be divided according to length into three groups: one large pair formed by the submetacentrics' elements, a group of medium sized pairs in which there is one that is slightly longer than the other two, and eight small pairs (Fig. 3f) of approximately the same size, being impossible to individualize them from each other. The large autosomal submetacentric pair has, in the majority of diplotene-stage cells, a single distal chiasma at each chromosomal end, as shown in Fig. 3c,d. In a few nuclei, one of the chiasmata is subterminal, as in Fig. 3a,b, while only one occurs between the acrocentric ones.

The sex-determining mechanism is of the neo-XY male type. The neo-X is submetacentric, with XL being at least three times the lengths of XR. The neo-Y is acrocentric, being slightly bigger than the biggest autosome pair.

In the prophase of first meiotic division, the large majority of diplotene stages show XR and Y in contact at the distal end (Fig. 3a,b). Less frequently, a subterminal chiasma occurs (Fig. 3c), and rarely an interstitial one (Fig. 3d). During first meiotic division, the X and Y, in spite of the kinds of pairing involving XR and Y,

neo-XY sex chromosome mechanism does not differ in principle from that already described by McClung (1902, 1905, 1917), White (1951, 1953), Dave (1965), White *et al.* (1967), Fernandez-Piqueras *et al.* (1982), Ferreira (1969, 1976), Messina *et al.* (1975), Alicata *et al.* (1974), and Hewitt (1979). There was a centric fusion involving a long acrocentric X chromosome, the biggest one of the set, which is totally heteropic-notic during the first prophase; the biggest of the second group was formed by acrocentric autosomes, giving rise to the biggest element of the set that becomes submetacentric and is now recognized as neo-X. Its bigger arm, the former X, is now called XL and the shorter, the ex-autosome of XR. The unfused autosome has become the neo-Y chromosome.

In spite of the fact that we used conventional stain, no progressive heterochromatinization was observed in the neo-Y, indicating that this mechanism has been acquired recently and contrasting with what happened in the

remain together until early anaphase, at which time they segregate and move to opposite poles, giving rise to two kinds of secondary spermatocytes with neo-X or neo-Y, but with the same chromosome number.

Discussion

The Phaneropterinae are insects belonging to the family Tettigoniidae, comprised of a large number of species with wings mimicking leaves. They are easily recognized by the lack of a lateral groove in the first and second tarsal segments and also because the females exhibit a very short upcurved ovipositor. They are found all around the world, but are particularly common in tropical and subtropical regions.

From a cytological point of view, species of Phaneropterinae are the best studied among all Tettigonioidea. To the present, the karyology of approximately 100 species is known. But despite the first cytological studies having appeared at the beginning of the 20th century, little progress has been made compared with other orthopteran groups. The majority of published papers make reference to the description of chromosome number, morphology, behavior and the sex-determination mechanisms during meiosis, using conventional methods of staining. The paper of Warchalowska-Śliwa (1998) reports the results obtained by an extensive bibliographic revision of 400 species, subspecies, and chromosome races of 15 subfamilies of Tettigoniidae that have been studied karyologically, of which 70 belong to the subfamily Phaneropterinae: that, plus the thirteen papers published by Ferreira & Mesa (2007), constitute the main part of what is relevant and available at the moment.

Thus far *S. nigra* is the only species among the 14 described for the genus that has been cytogenetically studied. The origin of its

neo-X system of certain orthopterans (Sáez 1963; White 1973a, 1973b, 1973c; Ferreira 1975; Ferreira & Mesa 1979; Mesa 1973; Mesa & Mesa 1967) that are at the end of the neo-XY evolutionary process, with both X and Y wholly heterochromatic during first prophase. According to Fernández-Piqueras *et al.* (1982) the argument supporting this is based on a comparison between largely unrelated species, so its validity remains to be demonstrated.

Mesa *et al.* (2001), based on analysis of more than 50 species of neotropical grasshoppers with a neo-XY mechanism, suggest that species acquiring such mechanisms are finally extinct at the end of a relatively short evolutionary process, since there is no accumulation in the number of species with old mechanisms and no higher taxon than genus which has all its species with neo-XY mechanisms of a single origin. In *S. nigra* there is an equality in lengths between the neo-Y and the XR: during the first meiotic division they are paired through their total lengths, do not develop any sign of heterochromatinization and exhibit the presence of interstitial chiasmata, which led us to place the occurrence of this sex mechanism in the early stages of evolution, as observed in *Yorkiela picta* (White *et al.* 1967), *Polichene parvicauda*, *Caedicia marginata* (Ferreira 1969) and others.

Variability in chromosome number in the subfamily Phaneropterinae is from 2n ($\male$) = 16 to 2n ($\male$) = 33 (Pearson 1929, Dave 1965, Ferreira 1969, Ferreira & Mesa 2007, Cineros-Barrios *et al.* 1990); this is by far the largest variablity among subfamilies of tettigonioids. Species with 2n ($\male$) = 32, 31, 30, 29, 28, 27, 25, 23, 21, 20, 19, 17, 16 chromosomes have been described by several authors from all around the world (see the revision of Warchalowska-Śliwa 1998). The most common karyotype is formed by 2n ($\male$) = 31, FN = 31 — it was observed in approximately 50% of species studied and considered by Ferreira (1977) as ancestral for the majority of
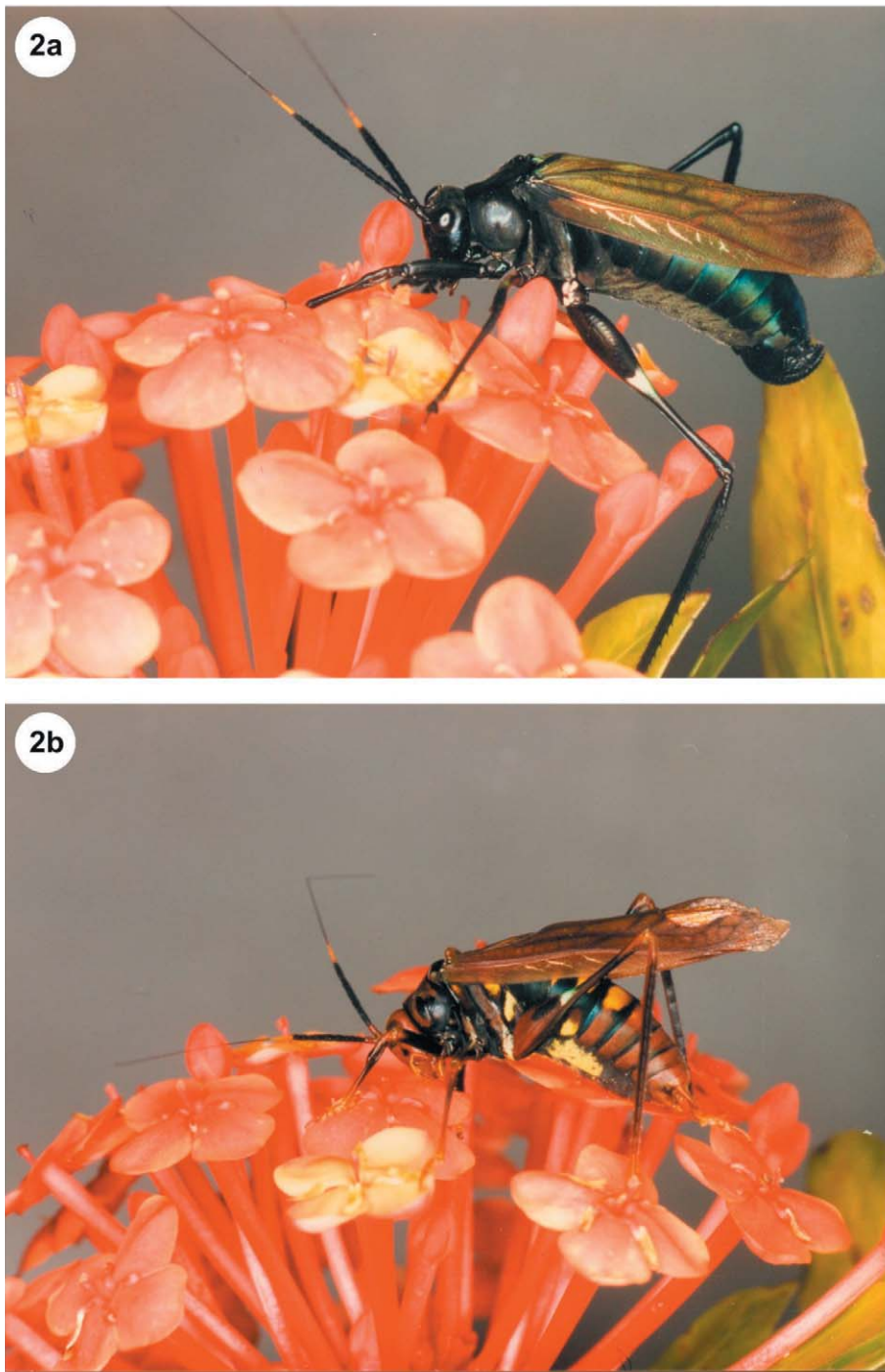
Fig. 2. Female and male of *S. nigra*, feeding on *Ixora brevifolia* (Rubiaceae) flowers. For color version, see Plate II.

S. nigra has, beyond the X-autosome fusion, a second one, involving a long acrocentric autosome (the third in size among the medium-sized group), giving rise to a pair of submetacentric chromosomes that is the largest of the complement. The event that was responsible for the loss of a chromosome pair as an independent unit has created a karyotype with a bimodality chromosome size that is uncommon in tettigoniids, since generally they can be ordered in a decreasing order of size, with the X being always the largest chromosome of complement, even when the species has a XO sex mechanism.

A tandem fusion was responsible for the integration of the chromatin of a medium-sized autosome with the submetacentric pair, enlarging its size and reducing the chromosome number from 2n ($\male$) =31 to 26. Reduction in the number of chromosome arms by the integration of chromatin in the Phaneropterinae, giving origin to a very large pair of chromosomes comprising a substantial amount of whole-autosomal chromatin, was already observed by Ferreira (1969), Mesa & Ferreira (1977), and by Cineros-Barrios *et al.* (1990) in three species of the genus *Dichopetala*. These share as common features a high number of chiasmata, well out of the normal if compared with other chromosomes in Tettigoniidae, and a delayed chromosome movement to the poles in anaphase. In *S. nigra* the number of chiasmata never exceeds two and all the bivalents have a synchronized movement to the pole.

In spite of being derived from the basic karyotype of the subfamily Phaneropterinae (2n ($\male$) = 31, FN = 31), *S. nigra* has a unique chromosome complement, since it is not shared with any of the almost 100 species studied so far, and is consequently a good tool for taxonomy purposes.

The same karyotypic particularities are observed in specimens from Boracéia Reserve (São Paulo) and Mantiqueira Mts (Minas Gerais); the presence of a relatively recent centric fusion involving the X chromosome and a medium-sized autosome, giving origin to a sex mechanism of neo-XY type, together with an autosome-autosome fusion and a process of chromatin integration, all are excellent characters for species identification.

species of Phaneropterinae, from which all the others were derived by means of several mechanisms of chromosomal rearrangement (Ferreira & Mesa 2007). Species with 2n ($\male$)=21, FN=21 (Ferreira 1969, Ferreira & Mesa 2007, Dasgupta 1961) cannot be explained as originating from living species with 2n ($\male$)=31, FN=31, since a strong reduction in number and arms should be admitted and there is no evidence at the moment that supports this point of view. This strong reduction probably took place a long time ago, giving rise to a second ancestral karyotype for Phaneropterinae with 2n ($\male$)=21, FN = 21, from which then evolved the chromosomal complement of species with 2n ($\male$)=20, 19 and 16 over the last 250 million years, during which the Tettigoniidae have been an independent lineage.

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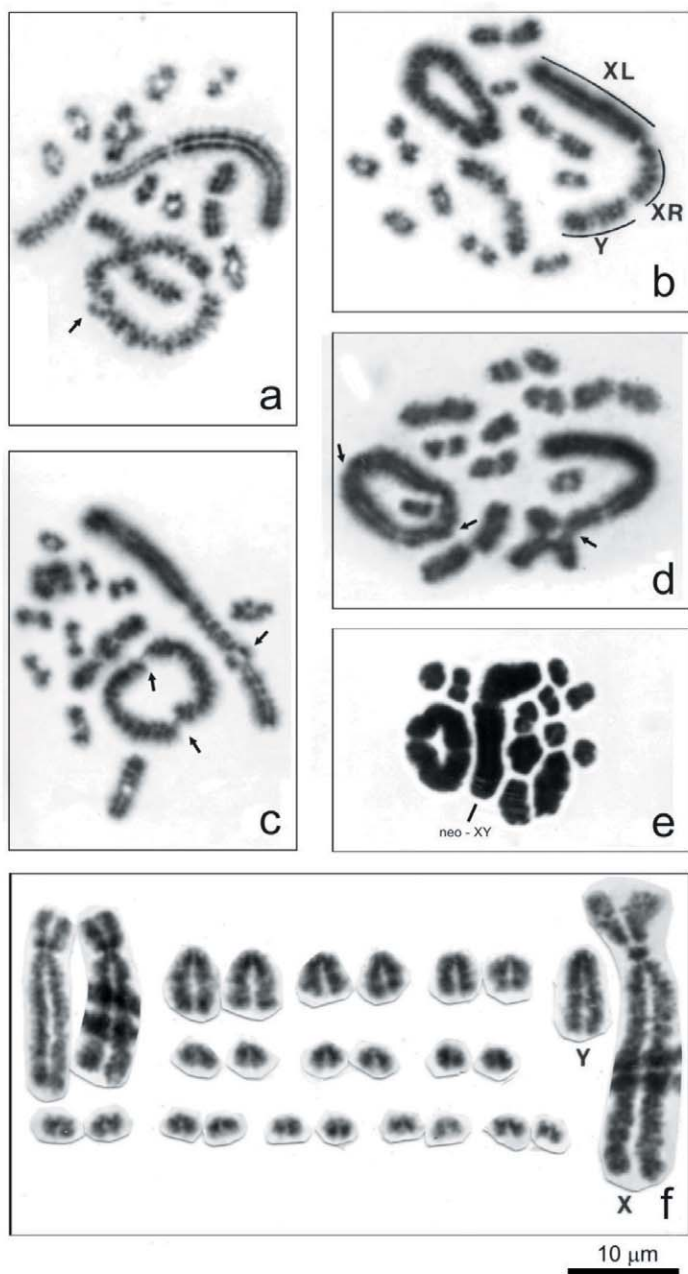
Fig. 3. *S. nigra* primary spermatocytes. a,b) Showing diplotene stage cells with the large autosomal pair having a subterminal chiasma and the XR and Y in contact at the distal end. c,d) Diplotene stages, nucleus with a single chiasma at each end of the large submetacentric pair. c) XR and Y exhibit a subterminal chiasma and in d) a rarely interstitial one. e) Metaphase I with a typical behavior and orientation of the sex elements (neo-XY). f) Karyotype of *S. nigra*, the large autosomal submetacentric pair are placed at the extreme left side and the neo X and the Y at the right. Arrow = chiasma.

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