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### **Expanding the geographic cytogenetic studies in the bush crickets *Eneoptera surinamensis* (De Geer, 1773) (Orthoptera, Gryllidae, Eneopterinae) from Brazilian Atlantic and Amazon Forest**

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The tropical bush cricket *Eneoptera surinamensis* (De Geer, 1773) shows a peculiar karyotype with  $2n = 9$  for males, and  $2n = 10$  for females, with  $X_1X_2Y♂$  sex mechanism systems whose origin remains unclear, probably arisen by a two-step chromosome rearrangements, including an X acrocentric and two pair of autosomal acrocentric chromosomes (Mesa & Bran 1964; Ferreira & Cella 2006). This sex system contrasts with the less derived  $X0♂$  found in the majority of the Orthoptera species (White 1973; Hewitt 1979).

Deviations from the Orthoptera  $X0$  sex-system to the neo-XY, and multiple sex system, such as  $X_1X_2Y♂$  and  $X_1X_20♂$  have been arising by different means of X/autosomal translocation or centric fusion (Saez 1963; White 1973; Mesa *et al.* 2001), and the ancient autosomal segments undergo a gradual evolutionary heterochromatinization process, followed by a progressive decrease in homology, becoming asynaptic (Saez & Dias 1958; Saez 1963; Mesa *et al.* 2001). According to Mesa *et al.* (2001), differences between X/autosome chromatin condensation supposedly affect its correct behavior along meiotic cycle, moreover failure in the sex chromosomes migration to daughter cells may occur in  $X_1X_2Y$  sex-system, with production of enviable gametes (White 1941; Mesa *et al.* 2001).

*Eneoptera surinamensis* have an extensive neotropical distribution (Robillard & Desutter-Grandcolas 2005), and individuals analyzed from Peru, Bolivia and Brazil (Claus 1956; Mesa & Bran 1964; Piza 1946) show asynaptic sex chromosomes, as well as some cells with incorrect sex chromosomes orientation along metaphase I to anaphase I (Piza 1946; Claus 1956; Mesa & Bran 1964).

The aim of this work was to expand the geographic sampled area on cytogenetic studies in *E. surinamensis* including individuals from Brazilian Amazonian and Atlantic Rainforest, highlighting the sex chromosomes heteropycnosis along the prophase I, and the chromosomes behavior during the metaphase I to telophase I.

We analyzed three adult males of *E. surinamensis* collected on shrubs and leaf litter at Amazonian Rainforest "Reserva Florestal Adolfo Ducke", municipality of Manaus, state of Amazonas, Brazil (08/11/2010), three at Brazilian Atlantic Forest "Parque Nacional de Monte Pascoal", municipality of Itamaraju, states of Bahia (15/07/2012) and six at Brazilian Atlantic Forest "Reserva Biológica de Duas Bocas", municipality of Cariacica, state of Espírito Santo (21/07/2012). Taxonomic determination was confirmed by examining the morphology of the phallic sclerites (Robillard & Desutter-Grandcolas 2005) and the calling song parameters, according to Miyoshi *et al.* (2007).

Cytological preparations were obtained from testes dipped into a hypotonic solution of sodium citrate (0.075M) for 5–10 min prior to fixation in Carnoy I (3 ethanol: 1 acetic acid). After at least one hour in the fixative, the cells were macerated in 45% acetic acid and squashed in a drop of 0.5% lacto-acetic orcein. We prepared ten cytological slides per individual with three to four testicular follicles per slide, and all meiotic phases were registered and photographed under a microscope Zeiss Lab.A1, and Axio Cam ERc5s.

The leptotene shows a densely stained block representing the sex chromosome  $X_1$  (Fig. 1). From zygotene to initial diplotene,  $X_1$  and Y chromosomes become individually visible in positive heteropycnosis (Figs 2–5). In these phases, the

In the first event present in all specimens examined, and also reported by Claus (1956) with individuals from municipality of Itatiba, SP, Brazil, the sex chromosomes  $X_1$  and/or  $X_2$  and/or Y positioned on the metaphase plate would be related to third event, moreover the amphitelic orientation of these chromosomes at the plate would precedes a subsequent equational division, as fairly well documented in X and B chromosomes of some species of Orthoptera (Mesa 1964; 1984; Mesa & Goñi 1987). It implies in the centromere disruption followed by a sister chromatids migration to opposite poles. When it occur in an  $X_1X_2Y$  sex system, the sister chromatids disjunction in meiosis I will results in potentially unviable gametes, with absence and/or duplication of one of the sex chromosomes, or none of them. We do not found intermediate phases between described events, since is rare found cells exactly at these stages.

The anaphase bridges found in individuals of *E. surinamensis* here analyzed were poorly documented in Orthoptera, being reported in tetrigids *Tetrix tenuicornis* (Sahlberg, 1891) from heavy metal polluted areas (Warchalowska-Sliwa *et al.* 2005). Generally, the anaphase bridges arises from chromosomal breaks and rejoining in inverted order, or telomere to telomere end fusions; or else as consequence of a fixed chromosome paracentric inversion at population (Krimbas & Powell 1992). In essence, the bivalent of a paracentric inversion in heterozygosis result in an inversion loop, and a recombination event within the loop results in dicentric chromatids stretched between the two daughter nuclei. This kind of chromosomal rearrangement is particularly interesting as creating postzygotic barriers between populations as it reduce the fecundity of heterokaryotypes (for review see Kirkpatrick & Barton 2006).

Meiotic configuration on *E. surinamensis* germ cells, such as incorrect distribution of the sex chromosomes along the first meiotic division (Piza 1946; Claus 1956; Mesa & Bran 1964), is also common at  $X_1X_2Y$  sex system of some species of Mantodea (White 1941; 1951), as well as in the acridid *Ronderosia bergii* (Stål, 1878) with neo-XY (Mesa 1962). This event added to our observations here described, such as the delay in the sex chromosomes migration along anaphase I/telophase I, as well as chromatin bridges may point out to production of unviable gametes, being related to low fertility. On the other hand, *E. surinamensis* seems to be well successful with wide geographical distribution (Eades *et al.* 2014) and high population density. Further studies are needed to relate the molecular rearrangement of chromatin on X/autosomes arranged chromosomes with meiotic anomalies in Orthoptera, as postulated by Mesa *et al.* (2001), as well as the relevance of these meiotic events in the reproductive success of the species.

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## References

- Claus, G. (1956) La formule chromosomique du Grylloidea *Eneoptera surinamensis* De Geer et le comportement des chromosomes sexuels de cette espèce au cours de la spermatogenèse. *Annales des Sciences Naturelles, Zoologie Paris*, 11 (18), 63–106.
- Eades, D.C., Otte, D., Cigliano, M.M. & Braun, H. (2014) Orthoptera Species File Online. Version 5.0/5.0. Available from: <http://Orthoptera.SpeciesFile.org> (accessed 18 May 2014)
- Ferreira, A. & Cella, D.M. (2006) Chromosome structure of *Eneoptera surinamensis* (Orthoptera, Grylloidea, Eneopterinae) as revealed by C, NOR and N banding techniques. *Chromosome Science*, 9, 47–51.
- Hewitt, G.M. (1979) Orthoptera: Grasshoppers and crickets. In: John, B. (Ed.), *Animal Cytogenetics 3. Insecta I*. Gebrüder-Borntraeger, Berlin-Stuttgart, 170 pp.
- Kirkpatrick, M. & Barton, N. (2006) Chromosome inversions, local adaptation and speciation. *Genetics*, 173, 419–434. <http://dx.doi.org/10.1534/genetics.105.047985>
- Krimbas, C.B. & Powell, J.R. (1992) *Drosophila Inversion Polymorphism*. Boca Raton: CRC press, 576 pp.
- Mesa, A. (1962) Cariología de *Dichroplus bergi* Stål (Orthoptera – Acrididae). *Revista de Agricultura*, 37 (1), 41–49.
- Mesa, A. (1964) Los cromosomas de *Pachyossa* sp. (Orthoptera, Ommexechidae). *Revista de la Sociedad Uruguaya de Entomología*, 6, 49–54.
- Mesa, A. (1984) The chromosomes of a relict species of eumastacid: *Daguerreacris tandiliae* Descamps & Liebermann 1970 (Orthoptera, Eumastacoidea, Morseinae) *Revista Brasileira de Genética*, 7 (2), 219–229.
- Mesa, A. & Bran, E.J. (1964) Acerca de los cromosomas de *Eneoptera surinamensis*. *Anais do II Congresso Latino-*

*Americano de Zoologia*, São Paulo, 1, 9–16.

- Mesa, A. & Goñi, B. (1987) Meiosis in the Japanese Gryllacridid *Anoplophilus acuticercus* Karny, 1931 (Orthoptera Saltatoria, Rhaphidophoridae): amphitelic orientation of the X and supernumerary chromosome(s). *Zoological Science*, 4, 259–264.
- Mesa, A., Fontanetti, C.S. & García-Novo, P. (2001) Does an x-autosome centric fusion in Acridoidea condemn the species to extinction? *Journal of Orthoptera Research*, 10 (2), 141–146.  
[http://dx.doi.org/10.1665/1082-6467\(2001\)010\[0141:DAXACF\]2.0.CO;2](http://dx.doi.org/10.1665/1082-6467(2001)010[0141:DAXACF]2.0.CO;2)
- Miyoshi, A.R., Zefa, E., Martins, L.P., Dias, P.G.B.S., Drehmer, C.J. & Dornelles, J.E.F. (2007) Stridulatory file and calling song of two populations of the tropical bush cricket *Eneoptera surinamensis* (Orthoptera, Gryllidae, Eneopterinae). *Iheringia, Série Zoológica*, 97 (4), 461–465.
- Piza-Jr, S.T. (1946) Uma nova modalidade de sexo-determinação no grilo Sul-Americano *Eneoptera surinamensis*. *Anais da Escola Superior de Agricultura “Luiz de Queiroz”*, Piracicaba, Brasil, 3, 69–88.
- Robillard, T. & Desutter-Grandcolas, L. (2005) A revision of Neotropical Eneopterinae crickets (Orthoptera, Grylloidea, Eneopteridae) with a phylogenetic discussion. *Insect Systematics and Evolution*, 35 (4), 411–435.  
<http://dx.doi.org/10.1163/187631204788912427>
- Saez, F.A. (1963) Gradient of the heterochromatinization in the evolution of the sexual system Neo-X-Neo-Y. *Portugaliae Acta Biologica, série A*, 7 (1–2), 111–138.
- Saez, F.A. & Diaz, A. (1958) Sistema sexual Neo X-NeoY em *Xyleus laevipes* (Orthoptera: Romaleidae). *Archivos de la Sociedad de Biología de Montevideo*, 23, 13–27.
- Warchalowska-Sliwa, E., Niklinska, M., Gorlich, A., Michailova, P. & Pyza, E. (2005) Heavy metal accumulation, heat shock protein expression and cytogenetic changes in *Tetrix tenuicornis* (L.) (Tetrigidae, Orthoptera) from polluted areas. *Environmental Pollution*, 133, 373–381.  
<http://dx.doi.org/10.1016/j.envpol.2004.05.013>
- White, M.J.D. (1941) The evolution of the sex chromosome. I. The X0 and X1X2Y mechanisms in praying mantids. *Journal of Genetics*, 42, 143–172.  
<http://dx.doi.org/10.1007/BF02982514>
- White, M.J.D. (1951) Cytogenetics of orthopteroid insects. *Advances in Genetics*, 4, 268–330.  
[http://dx.doi.org/10.1016/S0065-2660\(08\)60238-2](http://dx.doi.org/10.1016/S0065-2660(08)60238-2)
- White, M.J.D. (1973) *Animal cytology and evolution*. 3.ed. London: Cambridge University Press, 961 pp.