SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

# Cytogenetics Studies in Brazilian Species of Pseudophyllinae (Orthoptera: Tettigoniidae): 2n(♂)=35 and FN=35 the Probable Basic and Ancestral Karyotype of the Family Tettigoniidae

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ABSTRACT - The karyotypes of five species of Brazilian Pseudophyllinae belonging to four tribes were here studied. The data available in the literature altogether with those obtained with species in here studied allowed us to infer that 2n(♂)=35 is the highest chromosome number found in the family Tettigoniidae and that it is present in species belonging to Pseudophyllinae, Zaprochilinae and in one species of Tettigoniinae. In spite of that all five species exhibit secondary karyotypes arisen surely by a mechanism of chromosomal rearrangement of centric fusion, tandem fusion and centric inversion types from those with 2n(♂)=35 and FN=35, they share some common traits. The X chromosome is submetacentric (FN=36), heteropicnotic during the first prophase, the largest of the set but its size is rather variable among the species and the sex chromosomal mechanism is of the XO( ♂ ), XX( ♀ ) type. The chromosomal rearrangements involved in the karyotype evolution of the Pseudophyllinae and its relationship with those of the family Tettigoniidae are discussed and we propose that the basic and the ancestral karyotype of the Tettigoniidae is formed by 2n(♂)=35, FN=35 and not by 2n(♂)=31, FN= 31, as usually accepted.

KEY WORDS: Chromosomal rearrangement, fusion and centric inversion, evolution

The family Tettigoniidae comprises approximately 1,155 genera and 6,521 species of which only 7.5% have been studied cytologically. Very little progress has been made so far in the chromosome survey of these insects. The main information concerning the karyotype evolution, chromosomal rearrangements, phylogenetic relationship and the structure of the chromosomes revealed by the use of C bands, Ag-NoR bands and by quantitative approaches has been made recently available. The Warchalowska´s (1998) paper is meritorius once she makes a general review of all information available at that time. The document adds new data and discusses the karyotype evolution of the subfamilies and their relationship.

The family Tettigoniidae is characterized by a variation in its chromosome number that extends over a wide range, especially in the sub-families Phaneropterinae and Tettigoniinae. The numerical variability ranges from 2n(♂)=33 to 2n(♂)=16 in the Phaneropterinae (Pearson 1929, Dave 1965, Ferreira 1969, Cisneros-Barrios *et al* 1990), whereas in the Tettigoniinae it goes from 2n(♂)=33 to 2n(♂)=15 (Ueshima *et al* 1990, Warchalovska-Śliwa1998). Despite this great variation, the family shows some traits that are commonplace and found in the majority of the species. The X chromosome is always heteropicnotic during the first prophase. It is rather variable in size among species, but always constitutes the largest unit of the karyotype. Its morphology can be acrocentric, metacentric or submetacentric. Most of species have males with an X0 sex chromosome mechanism. Only six species are at present known to have changed their X0 sex mechanism to a more complex one (Dave 1965, White, Mesa & Mesa 1967, Ferreira 1969, 1976, Messina 1975).

The family Tettigoniidae is arranged according to different authors between 14 to 24 subfamilies (Kevan 1976, 1977, 1982, Rentz 1985, 1993, Eades & Otte 2009). Presently, we will take into consideration Eades & Otte (2009) who include the Pleminiini and Pseudophyllini in the subfamily Pseudophyllinae.

The pioneer cytological work in the Pseudophyllinae is from the beginning of last century. Woolsey (1915) described the karyotype of three species of *Jamaicana*. *Jamaicana flava* (Caudell) has 2n(♂)=35 and FN=35, while *J. unicolor* (Brunner von Wattenwil) and *J. subguttata* (Walker) have 2n(♂)=33 and FN=35. Asana (1938) published the karyotype of *Satrophylia* sp. as formed also by 2n(♂)=35 and FN=35, whereas Piza (1950) reported for the first time the karyotype of *Meroncidius intermedius* (Piza) a Brazilian species formed by 2n(♂)=31 and FN=35.

According to Yadav & Yadav (1986) and Aswathanarayama & Aswath (1996), *Sathrophylia rugosa* (Thunberg) has 2n(♂)=31, FN=31 and *S. femorata* (Fabricius) 2n(♂)=35, FN=35.

In this paper, we studied the chromosomes of five species of Pseudophyllinae belonging to four tribes, discuss the chromosomal mechanism involved in the karyotype evolution of the sub-family and propose a tentative karyotype model for the family Tettigoniidae.

## Material and Methods

All species studied in here, *Bliastes viridifrons* Piza (Cocconotini), *Diophanes amazonensis* Piza (Pterophyllini), *Diophanes scaberrimus* Piza (Pterophyllini), *Leptotettix crassicerci* Piza (Leptotettigini) and *Leptotettix humaita* Piza (Leptotettigini), were collected in the vicinities of Humaita, state of Amazonas, Brazil. All specimens were dissected in the field and the testes fixed in a mixture of ethyl alcohol-acetic acid (3:1) and stored at low temperature. Squash preparations of the chromosomes were stained with 2% acetic-orcein. The meiotic first metaphase was drawn with the aid of a camera lucida. The bivalents of each species were arranged in a decreasing order of size and tentatively arranged in groups of large (L), medium size (M) and small bivalents (S). Two specimens of each species were used for the analysis of first metaphase chromosomes.

## Results

Four groups of karyotypes were found in all five species studied, but all of them shared the X0(♂) / XX(♀)sex mechanism type. The X is submetacentric, heteropicnotic during the first prophase, the larger of the set, but rather variable in size among species (Table 1).

*Diophanes scaberrimus* (Fig 1) and *L. humaita* (Fig 2) have 2n(♂)=35 and FN=36, due to the morphology of the X element that is submetacentric, while the autosomes are acrocentric. In the first prophase, the bivalents can be arranged into three groups according to their size: three large (L), seven or eight of medium size (M) and six or seven of small size (S). While there is a sharp limit among the groups L and M, the same does not happen among the groups M and S. Two chiasmata are normally seen in two bivalents, one belongs to the L group and the second to the M. The others have invariably a single one.

*Bliastes viridifrons* (Fig 3) has 2n(♂)=33 and FN=36. Its karyotype show four bivalents in the group L, five in the M and seven in the S group. The $L_1$ is submetacentric, while the others are acrocentric. Only a single pair of the M group has two chiasmata, the others have only a single one.

*Leptotettix crassicerci* (Fig 4) has 2n(♂)=31 and FN=34. Their bivalents and even the X chromosome exibit larger size when compared with the remaining species studied. The division of the bivalents by groups is only a tentative approach since they gradually decrease in size. The L, M and S groups are formed by five pairs of bivalents each. The $L_1$ is metacentric, and shows three chiasmata. The $L_2$, $L_4$, $M_2$ and $M_7$ have two chiasmata, and the remaining a single one (Fig 5).

*Diophanes amazonensis* (Fig 5) has 2n(♂)=29 and FN=34. The L group is formed by four bivalents, the M by four or five and the S by five or six. The $L_1$ and $L_2$ bivalents are formed by metacentric chromosomes and have two chiasmata, whereas the remaining are acrocentric and have one chiasma, excepting $L_4$ and $M_2$, which have two.

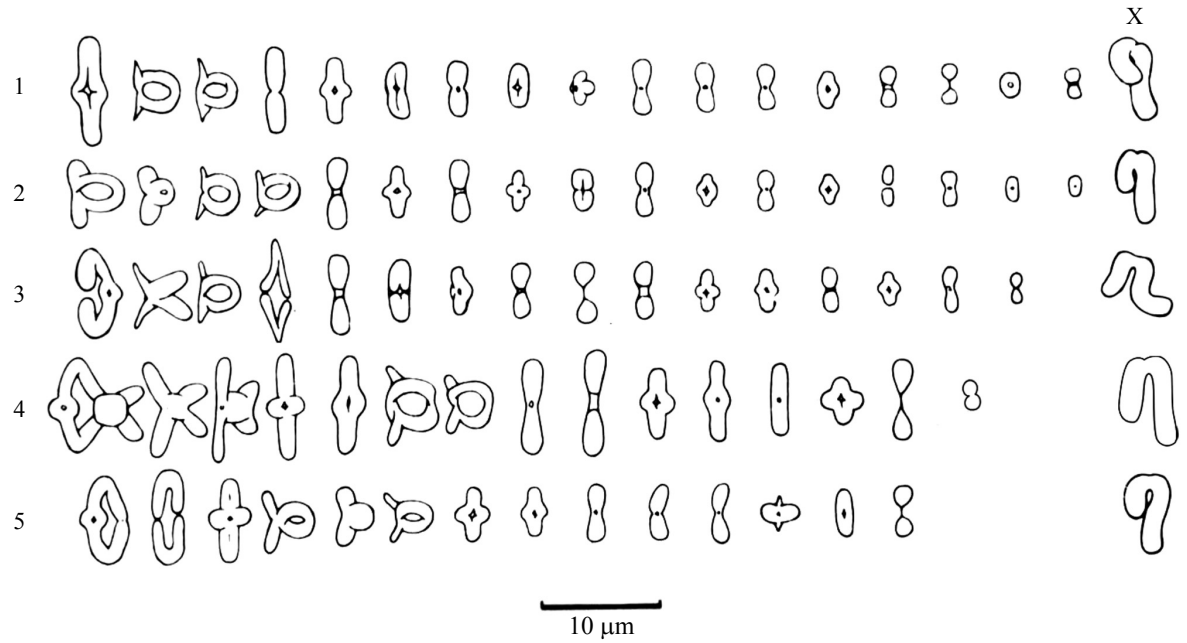
## Discussion

From the twelve species of Pseudophyllinae known cytologically, six have 2n(♂)=35 and FN=35 or 36 depending of the morphology of the X chromosome that is biarmed in two. We are not considering *Phillozelus pectinatus* studied by Aswathanarayana & Aswath (1996), since this species was not formally described. This is the highest chromosome number found in the family and it is shared by 11 out of 17 species of Zaprochilinae, an endemic Australian subfamily (Rentz & Clyne 1983, Ueshima 1993). After Mesa & Ferreira (1977) reported a chromosome number of 2n(♂)=37 for a single male of *Platydecticus angustifrons* (Chopard), an extensive taxonomic revision was undertaken by Rentz & Guerney (1985) with the description of new genera and species of South American Tettigoniinae (former Decticinae).

Now that fourteen new species of the genus *Platydecticus* have been described, as well as several species of three others related genera, it is desirable to undertake cytological studies of these species. According to these authors, Australian Tettigoniinae are unrelated to those from Africa, but they show strong relationship with the South America genera and *Neduba* from North America. While Gorochov (1988, 1995) places it in the subfamily Nedubinae, Rentz & Guerney (1985) and Rentz & Coless (1990) consider it belonging to the tribe Nedubini of the subfamily Tettigoniinae. The cytological data strengthen Rentz & Guerney (1985) and Rentz & Coless (1990) point of view since the family Tettigoniinae has a

Table 1 Chromosome number, morphology and sex determining mechanism in the five species of Tettigoniidae studied.

| Taxa | 2n♂ | NF | Sex | Morphology |
|---|---|---|---|---|
| *Diophanes scaberrimus* | 35 | 36 | XO | $L_1$-$S_{17}$ acroc; X subm. |
| *Leptotettix humaita* | 35 | 36 | XO | $L_1$-$S_{17}$ acroc; X subm. |
| *Bliastes viridifrons* | 33 | 36 | XO | L1 met; $L_2$-$S_{16}$ acr; X subm. |
| *Leptottetix crassicerci* | 31 | 34 | XO | $L_1$ met; $L_2$-$S_{14}$ acr; X subm. |
| *Diophanes amazonensis* | 29 | 34 | XO | $L_1$-$L_2$ met; $L_3$-$S_{12}$ acr ; X subm. |




Figs 1-5 First metaphase of 1) *Diophanes scaberrimus*; 2) *Leptotettix humaita*; 3) *Bliastes viridifrons*; 4) *Leptotettix crassicerci*; and 5) *Diophanes amazonensis*. For each species, the chromosomes were organized in decreasing order of size from left to right, with the X at the right end. Scale bar = 10 µm

wide numerical chromosomal variability that ranges from 2n(♂)=35 to 2n(♂)=17, according to Warchalowska-Śliwa (1998). In the six species of *Neduba* studied, the chromosome number varied from 2n(♂)=25 to 2n(♂)=22, and the FN from 28 to 23 (Ueshima & Rentz 1979).

The variability in chromosome number and morphology found among Pseudophyllinae species studied so far is not too large when compared to those seen in some subfamilies, especially in the Phaneropterinae, Tettigoniinae and Conocephalinae. It has nine karyotypes that ranged from 2n(♂)=35 to 2n(♂)=29. The basic and ancestral karyotypes were found in *J. flava* and *Sathrophyllia* sp. It is formed by 2n(♂)=35 and FN=35. A centric inversion undergoes the X morphology from acrocentric to submetacentric, giving rise to a FN and an evolutionary branch with biarmed X, like the one found in *L. humaita* and *D. scaberrimus*. *Bliastes viridifrons* with a 2n(♂)=35, FN=36, has one metacentric autosome pair surely originated by centric fusion involving acrocentric autosomes. In species with a low FN, the karyotypes could only be explained by the occurrence of tandem fusions followed by centric ones. This is the case of *L. crassicerci* - 2n(♂)=31, FN=34 - and *D. amazonensis* 2n(♂)=29, FN=34.

In the acrocentric X branch, the variability of the chromosomal number shows preponderance of tandem fusion in the origin of derived karyotypes. From seven species studied, three have karyotypes showing all the chromosomes acrocentric, but with number smaller than 35, as in the case of *J. unicolor, J. subguttata* -2n(♂)=33, FN=33 - and *S. rugosa*, 2n (♂)=31, FN=31. The only exception *is M. intermedius* with 2n(♂)=31, NF=35. The reduction in chromosomal number is associated with two pairs of metacentric autosomes, arisen surely by means of two independent centric fusions.

A similar scenario was found in Zaprochilinae (Rentz & Clyne, 1983, Ueshima 1993). From the 17 species cytologically known, 11 have 2n(♂)=35 and FN=35, three have 2n(♂)=31 and FN=31, two show 2n(♂)=31 and FN=33 and one 2n(♂)=29 and FN=36. Its basic and ancestral karyotype also has 2n(♂)=35 and FN=35, while the remaining, as observed in the Pseudophyllinae, are the result of chromosomal rearrangement.

Karyotypes with 2n(♂)=33 were also found in six species of Phaneropterinae (Pearson 1929, Cisneros-Barrios *et al* 1990), in 16 of Conocephalinae (King 1924, Hareyama 1939, Makino 1951, Warchalowska-Śliwa 1984, Ueshima & Rentz 1991) and in two of Meconomatinae (Kacker & Singh 1978). In the subfamilies Odonturinae and Austrosaginae the larger chromosome number is 2n(♂)=31 (Ueshima 1993, Warchalowska-Śliwa 1998), whereas in Meconomatinae and Hetrodinae is 2n(♂)=29 (Matthey 1948, Aswathanarayana & Aswhath 1996).

Despite of these extreme numbers -2n(♂)=35 or 2n(♂)=33- and the wide range of numerical chromosomical variability found in Tettigoniidae (Warchalowska-Śliwa 1998) as a whole, the karyotype formed by 2n(♂)=31 and FN=31 is found in the majority of the subfamilies and in 50% of the species studied so far. It is common in 12 subfamilies found all ♂ around the world and has not been observed in species of Mecopodinae and Hetrodinae (Warchalowská-Śliwa 1998) probably due to the few species studied so far. In Phaneropterinae, it is common to 50% of the species, where in Odonturini it appears in 83%, in Tettigoniinae in 37% and in 85% of the species of Austrosaginae.

Due to the prevalence of these karyotypes in terms of species, genera and its configuration, with all chromosomes being acrocentric, White (1973, 1973a, 1973b) suggested it

(2n (♂)=31 and FN=31) as the basic and ancestral character for the family Tettigoniidae. With the data now available, although still scarce and with gaps to be filled, the White suggestion does not have cytological support. To accept his hypothesis, it would be required to admit that during the process of chromosomal rearrangements that gave rise to the species with 2n(♂)=33 or 35, there was an increase in chromosome arms. Only the conjunction of a centric inversion and a centric dissociation in the same, originally acrocentric chromosome, can be responsible by the increase in arm number. While both events are frequent in the evolutionary history of orthopterans, these conjunctions are very rare. Among the grasshoppers, there is as single case where an increase in chromosome number due to dissociation seems to have occurred (Mesa 1973, Mesa & Ferreira 1977). The majority of species belonging to the family Ommexechidae have two submetacentric autosomes originated in an ancient pericentric inversion. In the Chilean species *Conometopus sulcaticollis* (Blanchard), such chromosome has split into two independent acrocentric elements.

However, 48 out of nearly 300 species of grasshoppers were observed with one, two or three autosome centric fusions and almost the same number with X-autosome fusions, some of them even with Y-autosome fusions (Mesa *et al* 1982). Ferreira (1973) and Warchalowska-Śliwa (1998) also described the importance of these mechanisms in the chromosome number and arms reduction observed in the chromosomal variability found in the Phaneropterinae and Tettigoniinae. Therefore, the cytological data available show that the basic and ancestral karyotype of the family Tettigoniidae is 2n(♂)=35 and NF=35, and that its karyotypical reorganization has occurred in two steps. In the beginning of the evolutionary history of the family it has changed to 2n(♂)=31 and FN=31 and this karyotype was probably initially fixed in most of the subfamilies. The karyotypes with 2n(♂)=35 that are circumscribed specially in the subfamilies Pseudophyllinae and Zaprochilinae and those with 2n(♂)=33 found in several families would be therefore relict karyotypes of the evolutionary history of the family.

The karyotype with 2n(♂)=31 and NF=31 is the most successful in Tettigoniidae in terms of genera and species that have inherited it. However, in the last 250 million years, probably since the Permian, when tettigoniids arouse as an independent lineage (Sharov 1968), several opportunities for the establishment of a great number of groups and a wide range of morphological and numerical chromosomal variability that is currently found in most of its subfamilies have occurred.

## References


Asana J J, Makino S, Niyama H (1938) A chromosomal survey of some Indian insects 1 Morphology of the chromosomes of eight species of the Locustidae. J Fac Sci Hokkaido Univ 6: 221-224.

Aswathanarayana N V, Aswath S K (1996) Karyology of five species of tettigoniids .(Orthoptera: Tettigoniidade). Cytologia 86: 167-176.

Cineros-Barrios R, Salinas-Moreno Y, Zuniga-Bermudes G (1990) Numeros cromosomicos de faneropterinos mexicanos I (Orthoptera, Tettigoniidae). Folia Ent Mexicana 79: 45-55.

Eades D C, Otte D (2009) Orthoptera species file online version 2.0/3.5. http://Orthoptera Species File org. Accessed in 24/07/2009.

Dave M J (1965) On unusual sex chromosomes found in two species of the Locustidae. Cytologia 30: 194-200.

Ferreira A (1969) Chromosome survey of some Australian tettigoniids (Orthoptera, Tettigonioidea): two species with neo-XY sex determining mechanism. Cytologia 34: 511-522.

Ferreira A (1976) Cytology of Brazilian Phaneropteridae (Orthoptera-Tettigonnioidea): a species with neo XY sex determining mechanism. Can J Genet Cytol 18: 79-84.

Gorochov A V (1988) In Russian. The classification and phylogeny of grasshoppers (Gryllidae-Orthoptera,Tettigonioidea), p.145-190. In Ponomarenko A (ed) The Cretaceous Biocoenotic crisis and the evolution of insects. Ed. Nauka, Moscow, 227p.

Gorochov A V (1995) System and evolution of the suborder Ensifera (Orthoptera). Russian Acad. Sci. Proc. Zool. Institute, St. Petersburg 260: Part 1: 1-224; Part 2: 1- 213. (in Russian).

Hareyama S (1939) Variation of the chromosomes number in the Locustidae. Zool Mag 51: 124-125.

Kacker R K, Singh A K (1978) Chromosomes of *Xiphidiopsis strammula* (Walker).(Orthoptera, Tettigoniidae, Meconomatinae ). Bull Zool Surv India 1: 57-59.

Kevan, D K McE (1976) Suprafamilial classification of "Orthopteroid" and related insects, applying the principles of symbolic logic. Not Lyman Ent Mus Res Lab 2: 1-31.

Kevan D K McE (1977) The higher classification of the orthopteroid insects: a general view. Mem Lymam Ent Mu Res Lab. 4: 1-27.

Kevan D K McE (1982) Orthoptera, p.252-379. In Parker S P (ed): Synopsis and classification of living organisms, vol. 2. McGraw Hill, New York, 379p.

King R L (1924) Material for demonstration of accessory chromosomes. Science 60: 362-363.

Makino S (1951) An atlas of the chromosome number in animals. Ames, Iowa State College Press 1: 113-119.

Matthey R (1948) Données nouvelles sur les chromosomes des Tettigonides et la parthénogénèse de *Saga pedo* Pallas. Rev Suisse Biol 55: 45-56.

Mesa A (1973) Los cromosomas de algunas especies de acridios y proscópidos chilenos (Orthoptera: Caelifera). V Cong Latinoam Zool 1: 150-161.

Mesa A, Ferreira A (1977a) The chromosomes of a South American species of Decticinae, *Platydecticus angustifrons* Guerney and Liebermann (Orthoptera, Tettigoniidae). Rev Bras Biol 37: 577-578.

Mesa A, Ferreira A (1977b) Cytological studies in the family Ommexechidae (Orthoptera, Acridoidea). Acrida 6: 261-271.

Mesa A, Ferreira A (1977c) The chromosomes of two species of North American tettigoniids (Orthoptera-Tettigonioidea). Ent News 88: 99-103.

Mesa A, Ferreira A, Carbonell C S (1982) Cariologia de los acridios neotropicales: estado actual de su conocimiento y nuevas contribuciones**.** Ann Soc Entomol Fr (N S) 18: 507-526.

Messina A, Ippolito S, Lombardo F (1975) Cariologia di alcune specie Europee di Phaneropterinae (Insecta, Orthoptera). Animália 2: 215-224.

Pearson N E (1929) The structure and chromosomes of three gynandromorphic katydids (*Amblycorypha*). J Morph 47: 531-553.

Piza S T (1950) Nota sobre cromossomios de alguns Orthopteros do Brasil. Ann Esc Sup Agr L Queiroz Univ São Paulo 7: 131-136.

Rentz D C F (1985) A monograph of the Tettigoniidae of Australia. I. The Tettigoninae. Camberra, Melbourne, 372p.

Rentz D C F (1993) A monograph of the Tettigoniidae of Australia, v 2. The Austrosaginae, Zaprochilinae and Phasmodinae. Melbourne, CSIRO, 380p.

Rentz D C F, Clyne D (1983) A new genus and species of pollen and nectar-feeding katydids from eastern Australia (Orthoptera, Tettigoniidae, Zaprochilinae). J Aust Entomol Soc 22: 155-160.

Rentz D C F, Colles D H (1990) A classification of the shield backed katids (Tettigoniidae) of the world, p.353-380. In Bayley J W, Rentz D C F (eds) The Tettigoniidae: biology, systematics and evolution. Springer-Verlag, Berlin, 395p.

Rentz D C F, Guerney F S (1985) Revision, key to genera South America. Entomol Scandinavia 16: 87.

Sharov AG (1968) Phylogeny of Orthopteroid insects. Akad Sc USSR (Moskow). Proc Paleont Inst 18: 1-216.

Ueshima N (1993) Karyotypes and meiosis of Phasmodinae, Zaprochillinae and Austrosaginae, p.345-358. In Rentz D C F (ed) A monograph of the Tettigoniidae of Australia, v 2. The Phasmodinae, Zaprochylinae and Austrosaginae. Melbourne, CSIRO Publication, 380p.

Ueshima N, Rentz D C F (1979) Chromosome systems in the North American Decticinae with reference to Robertsonian changes (Orthoptera: Tettigoniinae). Cytologia 44: 693-714.

Ueshima N, Rentz D C F (1990) Karyotypes and meiosis of the Australian Tettigoniidae, p.303-353. In Bayley W J, Rentz D C F (eds) The Teettigoniidae: biology, systematics and evolution. Springer-Verlag, Berlin, 395p.

Ueshima N, Rentz D C F (1991) Karyotypes and meiosis of the Australian Tettigonidae (Orthoptera). The genus *Nanodectes* Rentz (Tettigniidae). Inv Taxon 5: 33- 41.

Warchalowska-Śliwa E (1998) Karyotype characteristics of katydid Orthopterans (Ensifera, Tettigoniidae) and remarks on their evolution at different taxonomic levels. Folia Biol 4: 143-176.

White M D (1973) Animal cytology and evolution. 3rd ed. Cambridge Univ. Press, London, 961p.

White M J D (1973a) Chromosomal rearrangements in mammalian population polymorphism and speciation, p.98-129. In Chiarelle A B and Capanna E (eds) Citotaxonomy and vertebrate evolution. Academic Press, London, 783p.

White M J D (1973b) The chromosomes. Chapman and Hall, London, 169p.

White M J D, Mesa A, Mesa R (1967) Neo XY sex chromosome mechanisms in two species of Tettigonioidea (Orthoptera). Cytologia 32: 190-199.

Woolsey C J (1915) Linkage of chromosomes correlated with reduction in numbers among the species of a genus also within a species of the Locustidae. Biol Bull 28: 163-186.

Yadav J S, Yadav A S (1986) Chromosome number and sex determining mechanism in thirty species of Indian Orthoptera (Insecta). Folia Biol 34: 277- 83.

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