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Meiotic studies in *Lygaeus alboornatus* Blanchard 1852 (Heteroptera, Lygaeidae, Lygaeinae)

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Abstract - The subfamily Lygaeinae comprises 58 genera with about 500 species distributed world-wide. Despite the great biodiversity of the taxon, cytogenetic data of the group is scarce. To date, only 26 species belonging to 12 genera have been cytogenetically described. As it is the rule for the order Heteroptera, all the species possess holokinetic chromosomes, and a pre-reductional type of meiosis, namely at anaphase I the autosomal bivalents divide reductionally while the sex chromosomes are achiasmatic and divide equationally. Available data reveal that all the Lygaeinae are characterised by a modal diploid number of 14 and an XY/XX sex chromosome determining system. In the present study the male meiotic development of *Lygaeus alboornatus* from Argentina is analysed. The results demonstrate that the species, though sharing the basic chromosomal features from Lygaeinae, has a diploid number of 12 (10+XY), being this chromosome number the lowest reported so far for the subfamily.

Key words: Heteroptera, Lygaeinae, *Lygaeus alboornatus*, Meiosis, Holokinetic chromosomes.

INTRODUCTION

Members of the large and diverse family Lygaeidae are so-called seed bugs. Though the taxon was first recognised as a higher group by Schilling (1829) and the first and most complete early synthesis was performed by Stål (1872), it was not until 1964 when Slater (1964) provided a modern world catalogue. The Lygaeidae are probably paraphyletic, with some of the subfamilies being the sister taxa of members of other insect groups namely Bertydae, Colobathristidaae and Malcidae (Southwood and Leston 1959; Štys 1967). Consequently, the family is taxonomically difficult to characterise, and the complex relationships among its members are far to be determined (Schuh and Slater 1995). So far, 16 subfamilies comprising at least 500 genera and approximately 4000 valid species are distributed in all faunal regions both in the Old and in the New Worlds (Schuh and Slater 1995). Within Lygaeidae, the subfamily Lygaeinae represents a large taxon found world-wide with fifty-eight genera with about 500 species currently recognised. As with most lygaeid taxa, the greatest diversity is found in the tropical and subtropical regions (Schuh and Slater 1995).

Despite the wide biodiversity of the Lygaeinae, only 26 species belonging to 12 genera have been cytogenetically analysed so far (Ueshima and Ashlock 1980). As it is characteristic of Heteroptera, all the species possess holokinetic chromosomes and a pre-reductional type of meiosis: at first meiotic division the autosomal bivalents divide reductionally while the sex chromosomes are achiasmatic and divide equationally. Available data confirm that all species are characterised by the lack of a pair of *m* chromosomes; they have an XY/XX (male/female) sex chromosome determining

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system, and a modal diploid number of 14 (UESHIMA 1979). Moreover, only three species are cytogenetically exceptional by having a higher chromosome number, most probably due to fragmentation of either autosomes or the X chromosome (UESHIMA 1979): *Lygaeus simulus* (PARSHAD 1957) and *Oncopeltus famelicus* (UESHIMA 1979) possess $2n= 22$ (20+XY) chromosomes, while a diploid chromosome number of 15 ($12+X^1X^2Y$) has been reported for *Arocatus suboeneus* (UESHIMA 1979). The latter is the only species of the subfamily with a multiple sex chromosome determining system.

In the present study the male karyotype and meiotic development of *Lygaeus alboornatus* from Tornquist Park (Buenos Aires Province, Argentina) is described and discussed.

MATERIAL AND METHODS

Specimens and locality

The material included in the present study comprises 12 adult males of *Lygaeus alboornatus* collected in November 1996 and in December 2000 in Tornquist Park (Buenos Aires Province, Argentina). Three specimens were not included in the meiotic analysis since only spermatids were found in testes (Specimens 10-12, See Table 1).

Cytogenetic analysis

Immediately after capturing, all specimens were fixed in methanol:glacial acetic acid (3:1), and maintained at 4°C until dissection. Afterwards, gonads were dissected free and kept in ethanol 70% (4°C). Slides were made by the squash technique in 2% iron acetic haematoxylin following conventional procedures.

RESULTS

Lygaeus alboornatus possess a male diploid chromosome number of 12 (10+XY). An autosomal pair is easily identified due to its large size among the remaining four pairs of autosomes. The sex chromosome determining system is XY/XX, being the Y chromosome the smallest element of the complement (Fig. 1A). At zygotene the sex chromosomes X and Y are positively heteropycnotic and lie close to each other, but they are usually separated at pachytene (Fig. 1B). During the diffuse stage the autosomal bivalents decondense completely while the sex chromosomes continue positively heteropycnotic and lie either separated or close to each other (Fig. 1C). At diplotene-diakinesis the X and Y become isopycnotic and are always separated (Fig. 1D-F). At late diakinesis the Y chromosome becomes negatively heteropycnotic. At metaphase I, both the X and Y univalents orientate side-by-side at the centre of the ring formed by the five autosomal bivalents (Fig. 1G). At anaphase I, the autosomal bivalents divide reductionally while the sex chromosomes segregate equationally. Second metaphase follows immediately after telophase I without an intervening interkinesis stage. At metaphase II, the autosomes dispose again forming a ring while the X and Y chromosomes come close together and associate to form a pseudobivalent, which lies at the centre of the ring (Fig. 1H). At anaphase II, the X and Y segregate to opposite poles.

Autosomal bivalents present at least one terminal chiasma, although the largest bivalent, and less frequently other bivalents, can show two chiasmata (Fig. 1G). Cells with two ring bivalents

Table 1 – Mean chiasma frequency and percentage of cells with univalents at diakinesis-metaphase I in the specimens of *Lygaeus alboornatus* from Tornquist Park (Buenos Aires Province, Argentina).

Individual	Code	Number of cells	Mean chiasma frequency	% of cells with univalents
1	210696	5	6.20	nd
2	220696	35	6.00	14.29
3	230696	42	5.30	9.52
4	990473	20	5.70	5.00
5	990474	23	5.78	0.00
6	990475	64	5.62	9.37
7	990476	46	5.63	15.22
8	990477	46	5.60	6.52
9	990478	25	5.96	0.00

nd, frequency of cells with univalents not determined due to the reduced number of cells analysed.

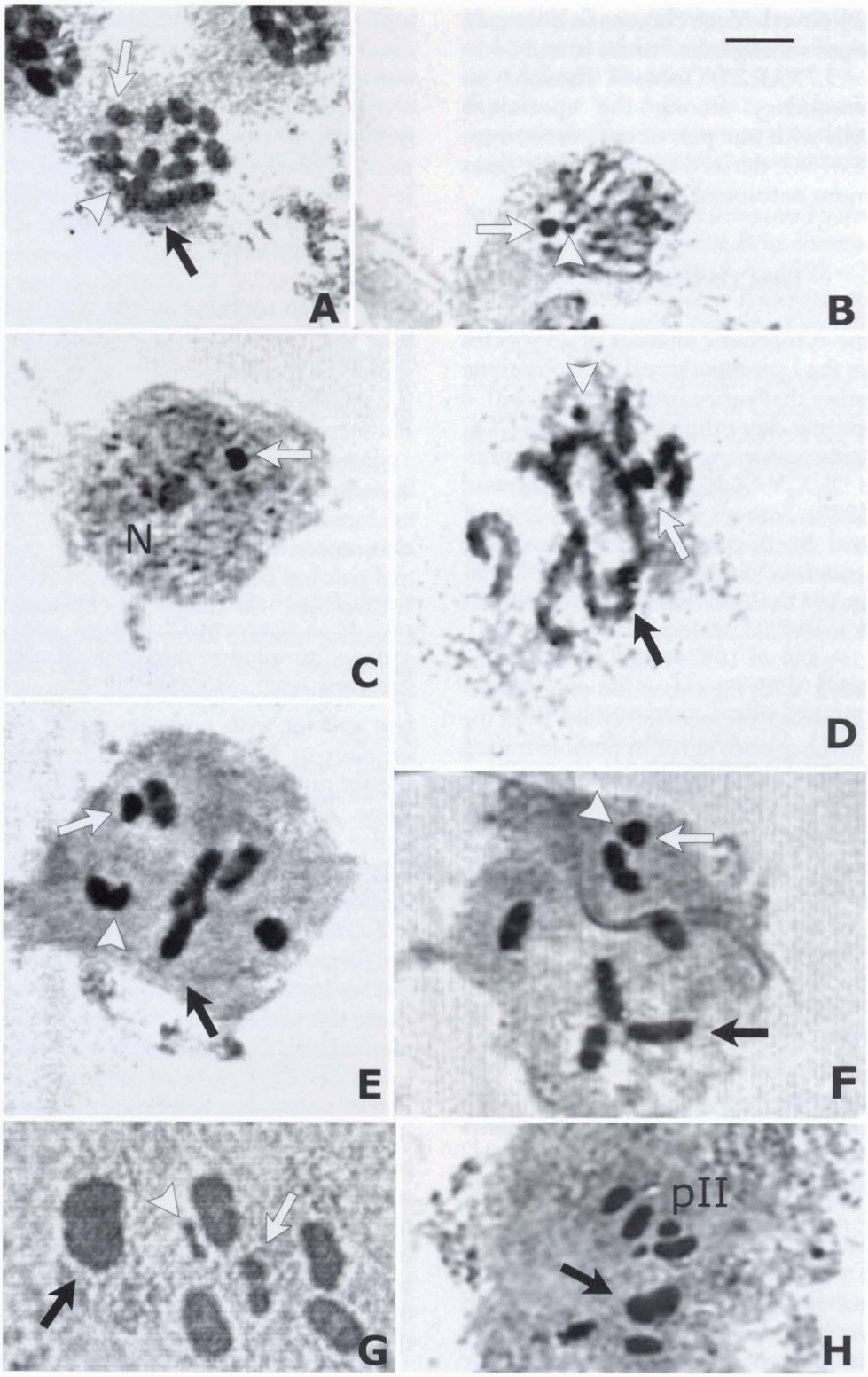

Fig. 1 – *Lygaeus alboornatus* Blanchard 1852. (A) Mitotic prometaphase; (B) Pachytene; (C) Diffuse stage; (D) Diplotene; (E) Diakinesis with the largest bivalent with one interstitial chiasma; (F) Diakinesis with the largest bivalent with a terminal chiasma; (G) Metaphase I with the largest bivalent with two chiasmata; (H) Metaphase II with X and Y chromosomes associated in a pseudobivalent. Empty arrowheads indicate Y chromosome; empty arrows indicate X chromosome; solid arrows indicate largest autosomal pair. Bar represents 10 μm.

are seldom observed. Mean chiasma frequency in cells at diakinesis-metaphase I varies from 5.33 to 6.20 (mean = 5.75 ± 0.25) (Table 1). Though with different frequency among the specimens analysed, cells with one pair of univalents were observed (8.47%), derived in most of the cases from the largest autosomal pair (57.69%).

DISCUSSION

So far, the cytogenetic analysis of 26 species belonging to the Lygaeinae reveal a chromosome diploid number that varies from 12 to 22, with a sex chromosome determining system XY/XX, except *Arocatus suboeneus* which shows a multiple system $X_1X_2Y/X_1X_1X_2X_2$ (UESHIMA and ASHLOCK 1980). Moreover, only five species of *Lygaeus* have been cytogenetically analysed, namely *L. equestris* (SCHACHOW 1932; PFALER-COLLANDER 1941), *L. kalmii kalmii* (UESHIMA and ASHLOCK 1980), *L. turcicus* (WILSON 1905), *L. simulus* (PARSHAD 1957) and *L. alboornatus* (present study). With the exception of *L. alboornatus* and *L. simulus*, cytogenetic reports from the remaining three species agree in demonstrating that they show a high homogeneity at the chromosomal level. They share a diploid chromosome number of 14, and chromosomes do not differ much in their size. The sex chromosomes are the smallest of the complement, and more or less conspicuous differences between them have been reported. *L. equestris* is an extreme example since the Y chromosome is minute (WILSON 1905; SCHACHOW 1932; PFALER-COLLANDER 1941; PARSHAD 1957; UESHIMA and ASHLOCK 1980). *L. simulus* is characterised by possessing a high chromosome number of 22, with two pairs of autosomes larger than the remaining eight pairs which do not differ much in size among themselves; the sex chromosomes X and Y are also of similar size.

UESHIMA and ASHLOCK (1980) have suggested that the modal diploid number for the subfamily Lygaeinae is 14 (12+XY). Considering this modal karyotype as the ancestral one, the chromosome complement of *L. simulus* should have originated through the fragmentation of four pairs of autosomes. Our results on *L. alboornatus* reveal that this species shows the lowest number of elements for members of Lygaeinae. Taking in account the presence of a remarkable big-sized pair of autosomes in this species, it seems highly probable that its karyotype originated from the ancestral complement through one autosomal fusion.

Though the reduced number of individuals analysed, our results on *L. alboornatus* reveals the presence of ring bivalents at diakinesis-metaphase I. It is generally accepted that most Heteroptera possess as a rule only one chiasma per bivalent (UESHIMA 1979; MANNA 1984); however, our present observations in *Lygaeus* and previous findings in other heteropteran species could indicate that the frequency of ring-shaped bivalents should be much higher than originally suggested (CAMACHO *et al.* 1985; MOLA and PAPESCHI 1993; BRESSA *et al.* 2001).

Lygaeids have relatively large chromosomes in regard to those observed in other heteropteran families. However, even with these large chromosomes, one exceptionally large autosomal pair has been reported to be present in four subfamilies. Of these, the Henestarinae and Chauliopinae are known cytologically from single species. In the Orsillinae and Blissinae, the extremely large chromosome is found in all but few species with a chromosome complement number of 14 elements while species with a higher number (usually 16) lack this chromosome. Accordingly, a fusion origin for this peculiar pair of autosomes has been proposed (UESHIMA and ASHLOCK 1980). Whether the remarkable big-sized autosomal pair we observed in *L. alboornatus* is the same that the present in the former four subfamilies remains unknown, but we know, at least, that most probably they share the same origin through a similar fusion mechanism. A detailed analysis of the chromosomes involved in the formation of this peculiar pair of autosomes will be a fundamental step in our understanding of an important biological process. Furthermore, even when this peculiar and remarkable big-sized pair of autosomes is not homologous in these five subfamilies, its formation and maintenance in the complement can be considered as a selectively neutral condition or at least, not a detrimental meiotic feature for the species.

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