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Sex chromosome polymorphism in a species of *Belostoma* (Belostomatidae, Heteroptera)

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A population of *Belostoma* sp. polymorphic for the sex chromosome determining system has been analyzed. The fundamental karyotype of the species is $2n = 16 = 14 + XY$ (male), and at different times individuals $2n = 17$ have been encountered in a low frequency (27% and 16%, respectively). Sex chromosome area measurements confirm that the original X chromosome of the XY system has fragmented into two unequal-sized chromosomes (X_1 and X_2). At male metaphase II, the sex univalents associate in a pseudotrivalent that can show different arrangements (in a chain, in a double-plate, or in other transitional arrangements). Their frequency varies among individuals. The present polymorphic population represents a direct evidence of a multiple sex chromosome system originating through fragmentation of the single X. The different kinds of arrangement of the three sex chromosomes at male metaphase II, and their frequency within each individual suggest that some forces are acting to achieve a double plate arrangement and a regular meiotic behaviour. The maintenance of the polymorphism during more than three years seems to indicate that the new chromosomal variant is neutral, or even could be selectively advantageous.

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It is generally accepted that multiple sex chromosome systems in Heteroptera are the result of fragmentation(s) of the X and/or Y chromosome(s) of an ancestral simple system. The holokinetic nature of heteropteran chromosomes and the achiasmatic behaviour of sex chromosomes during male meiosis are the principal facts that support this hypothesis (MANNA 1984; THOMAS 1987; UESHIMA 1979). Besides, some indirect evidences have been reported (HEIZER 1950; PAPESCHI 1994; PFALER-COLLANDER 1941; SCHRADER and HUGHES-SCHRADER 1958). In a few instances, however, multiple sex chromosome systems have been recognized as having a non-disjunctional origin (DARLINGTON 1939; SLACK 1939; UESHIMA 1967; WILSON 1909).

Any chromosome change, either autosomal or in sex chromosomes, has to go through a polymorphic phase before it becomes established in a population. In the population studies performed up to date in Heteroptera, chromosome polymorphisms have seldom been reported (MANNA 1984; UESHIMA 1979). In most instances, further samples of the population concerned have not been analyzed, and consequently, the stability and adaptative value of the chromosome change have remained unknown.

In the present work, a population of *Belostoma* sp. polymorphic for the sex chromosome system has been sampled at different times. The frequency of the different karyomorphs and the meiotic behaviour of the recently arisen chromosome system are analyzed.

Materials and methods

Totally, 33 adult males and 12 adult females of *Belostoma* sp. from Buenos Aires Province have been cytogenetically analyzed. Localities and dates of collection are the following:

El Cazador	4/84	2 males
San Pedro	10/87	1 male
Punta Lara	10/86 (sample 1)	3 males
	4/90 (sample 2)	12 males
	3/93 (sample 3)	15 males
		+12 females

Specimens were fixed in the field in 3:1 absolute ethanol: glacial acetic acid after a small abdominal incision. Gonads were dissected out and kept in

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ethanol 70 % at 4°C. Cytological preparations were obtained by squashing a piece of gonad in iron propionic haematoxylin.

Chromosome area measurements were performed on photographs of cells at diakinesis-metaphase I and metaphase II with a Kontron Bildanalyse Mini-Mop.

Results

All the specimens from El Cazador, San Pedro, and Punta Lara (sample 1) presented a diploid chromosome number of 16 (Fig. 1a) (14 + XY, male). Meiotic behaviour is similar to that already described in other species of *Belostoma*. Pairing occurs at synizesis, and at diplotene the autosomal bivalents present a ring shape; at the diffuse stage, bivalents decondense completely and very scarce heterochromatin is detected; at this stage, sex univalents are readily identifiable because of their positive heteropycnosis. At metaphase I autosomal bivalents arrange in a circle with the X and Y univalents at its center (Fig. 1e), and anaphase I is reductional for autosomes and equational for sex chromosomes. At metaphase II, the latter associate in a pseudo-bivalent, which lies again at the center of the autosomal ring (Fig. 1g).

In samples 2 and 3 from the population of Punta Lara, a diploid chromosome number of 17 was present in a few individuals (Fig. 1b). In sample 2, eight males were $2n = 16$, three were $2n = 17$, and one male could not be analyzed since it had only spermatozoa. In sample 3, thirteen males were $2n = 16$ and 2 males were $2n = 17$. From the twelve analyzed females, only four presented mitotic plates; three females were $2n = 16$ and one was $2n = 17$.

In male individuals with $2n = 17$, three sex univalents can be observed during the diffuse stage; they are associated with the nucleolus and are positively heteropycnotic (Fig. 1c). The three sex chromosomes are of different size, as can be observed at diakinesis and prometaphase I (Fig. 1d, arrows). At metaphase I the three sex univalents lie at the center of the ring of autosomal bivalents (Fig. 1f), and at metaphase II they associate in a pseudotrivalent, which also lies at the center of the autosomal ring. At this stage the three sex chromosomes can show different arrangements: in a chain (Fig. 1h), with two chromosomes side by side opposed to the third one (double-plate arrangement) (Fig. 1i), or in other transitional arrangements. The frequency of each kind of arrangement is different among individuals (Table 1). No metaphase II was observed in individual 3 of sample 2.

When sex chromosomes dispose in a chain, the median one is lagging at anaphase II (Fig. 1j,k); however, no abnormal telophase II or micronucleus has been observed. When they arrange in double-plate, the larger and smallest sex chromosomes always face the third one, from which they probably segregate at anaphase II.

Area measurements of sex chromosomes were performed on cells at diakinesis-metaphase I and at metaphase II from individuals with 16 and 17 chromosomes (Table 2). The total area of sex chromosomes does not differ significantly between cells with two and three sex chromosomes at diakinesis-metaphase I ($P = 0.80$) and at metaphase II ($P = 0.66$). When comparing relative sizes of sex chromosomes it can be observed that the X chromosome of the XY system has been replaced by two chromosomes in the multiple one (X_1 and X_2). The relative size of these two chromosomes together do not differ significantly from that of the single X either at diakinesis-metaphase I ($P = 0.93$) or at metaphase II ($P = 0.87$).

Discussion

Although the XY/XX system is the most common in Heteroptera, X0/XX and multiple sex chromo-

Table 1. Frequency of the different kinds of arrangement of the three sex chromosomes at metaphase II

Sample	Individual	No. cells	Kind of arrangement		
			chain	double-plate	others
2	1	108	66 (61%)	28 (26%)	14 (13%)
	2	127	100 (79%)	14 (11%)	13 (10%)
	3	–	–	–	–
3	1	100	67 (67%)	24 (24%)	9 (9%)
	2	73	22 (30%)	36 (49%)	15 (21%)

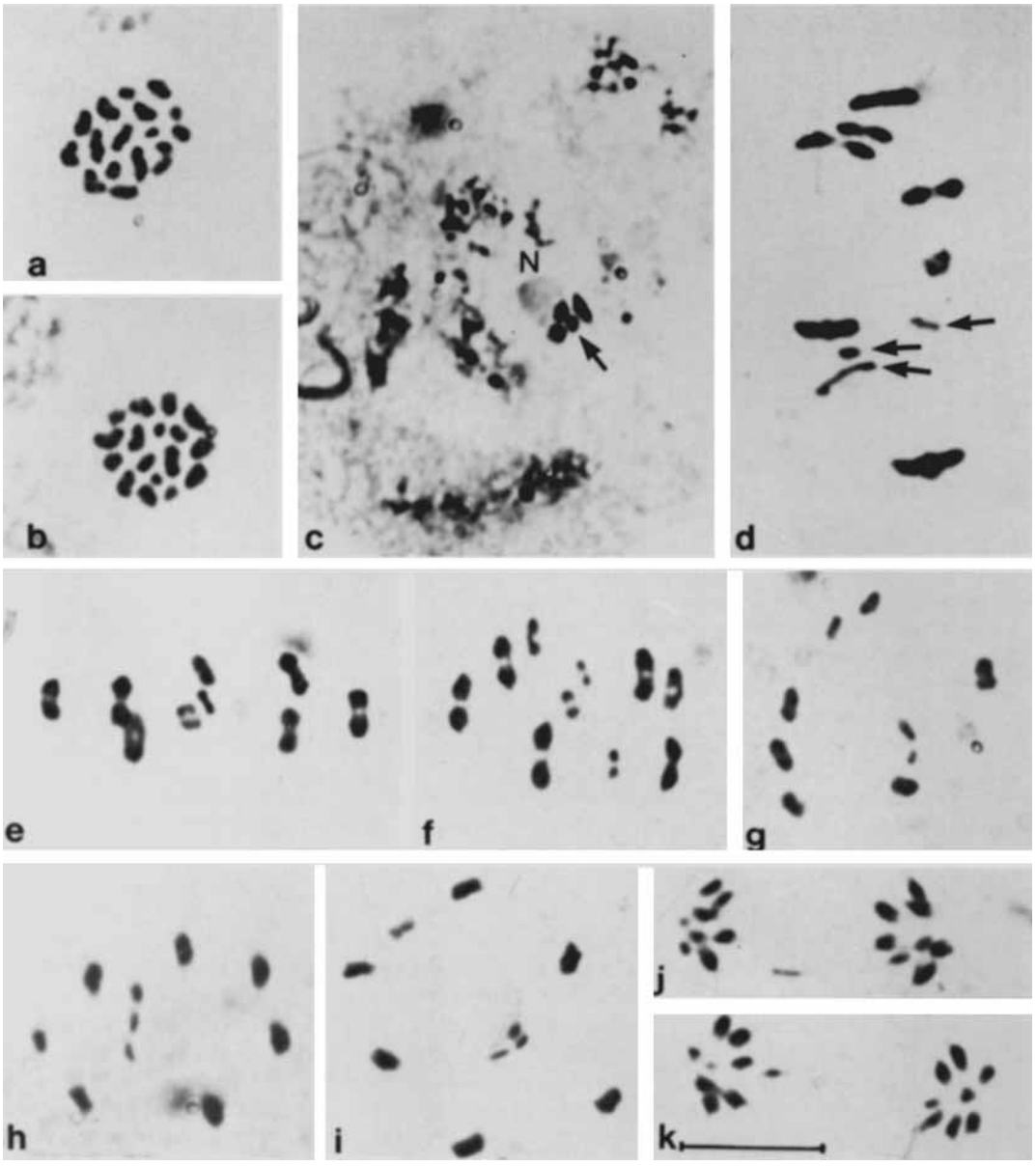

Fig. 1a–k. **a** Spermatogonial prometaphase with 2n = 16. **b** Spermatogonial prometaphase with 2n = 17. **c** Diffuse stage with the three sex univalents associated with the nucleolus (N) (arrow). **d** Diakinesis- prometaphase I; arrows show the sex univalents. **e** Metaphase I of a male XY. **f** Metaphase I of a male X_1X_2Y. **g** Metaphase II with the XY pseudobivalent. **h–i** Metaphase II with the pseudotrivalent arranged in a chain (**h**) or in a double-plate (**i**). **j–k** Anaphase II- telophase II with a lagging sex chromosome. Bar = 10 μm.

some systems (X_n0, X_nY, XY_n, X_nY_n) are also encountered (MANNA 1984; UESHIMA 1979). Many examples of intrageneric and even interspecific differences in sex chromosome systems have been reported. According to THOMAS (1987), the holokinetic nature of heteropteran chromosomes, the particular meiotic behaviour of sex chromosomes (achiasmatic in male meiosis and segregating independently from autosomes) and the lack of a strict genic balance, could account for this variability.

Table 2. Area measurements of sex chromosomes in cells at diakinesis-metaphase I (a) and at metaphase II (b) (in arbitrary units)

Total area	% of area		Total area	% of area		
	X	Y		X_1	X_2	Y
(a) Diakinesis-Metaphase I						
20.45	68	32	25.19	51	23	26
35.70	64	36	26.50	53	23	24
29.25	64	36	26.90	44	23	33
			39.00	38	26	36
			30.84	46	26	28
28.47 ± 7.65	65	35	29.69 ± 5.62	46	24	30
(b) Metaphase II						
16.27	67	33	17.93	52	22	26
17.74	70	30	18.29	40	20	39
16.83	58	42	14.33	45	24	31
13.89	60	40				
16.18 ± 1.64	64	36	16.85 ± 2.19	46	22	32

In most cases of multiple sex chromosomes, the increase in the number of sex chromosomes is not accompanied by a reduction in the number of autosomes, and this has been interpreted (keeping in mind the features already mentioned) as a fragmentation origin of the multiple sex chromosome system. As an exception, in three species of *Acantocephala* (= *Metapodius*) (Coreidae) (WILSON 1909) and in *Cimex lectularius* (Cimicidae) (DARLINGTON 1939; SLACK 1939; UESHIMA 1967) multiple sex chromosomes are true supernumeraries of non-disjunctional origin.

Some evidences do support the hypothesis of a fragmentation origin of multiple sex chromosome systems. In a population of *Oechalia pacifica* (Pentatomidae) ($2n = 10 + XY$) HEIZER (1950) found 2 out of 14 individuals with $2n = 14 + X_1X_2Y$ and $2n = 12 + X_1X_2X_3Y$, respectively. She explained the origin of the multiple sex mechanism through fragmentation of the original X, since size relationships did support this view. The sex chromosomes segregated regularly at anaphase I, and at metaphase II they associated in a chain; at anaphase II they lagged, but finally reached the poles. SCHRADER and HUGHES-SCHRADER (1958) found, in a sample of *Banasa zeteki* (Pentatomidae) ($2n = 24 + XY$), that one individual presented three sex chromosomes (probably XY_1Y_2). At metaphase II the sex chromosomes associated in a pseudotrivalent, also arranged in a chain. Finally, in *Belostoma plebejum* (Belostomatidae) ($2n = 14 + XY$), one out of 11 males was $2n = 17$, with three sex chromosomes (probably X_1X_2Y), which behaved regularly at meiosis (PAPESCHI 1994). The sex chromosomes divided equationally at anaphase I and associated in a chain at metaphase II. Although at anaphase II the median sex chromosome lagged, it finally reached the pole. In all these examples one chromosome of the simple system was replaced by two chromosomes of smaller size in the mutant individuals.

Multiple sex chromosome systems are present in approximately 11% of the species of Heteroptera, but very few sex chromosome polymorphisms have been reported. PFALER-COLLANDER (1941) described two cytological races in *Lygaeus equestris* (Lygaeidae), one with $2n = 12 + XY$ and the other $2n = 12 + XY_1Y_2$. The two Y chromosomes in the multiple system were of different size but he could not measure them. BARIK et al. (1981) found in *Lygaeus hospes* (Lygaeidae) that 7 out of 190 males presented an XY_1Y_2 system instead of the simple system XY. They described the possible presence of two types of X chromosomes and three types of Y, which were present in the population at different frequencies. *Lygaeus pandurus* had the same type of sex chromosome polymorphism, and in both species the origin of the different types of X and Y seemed to have taken place by structural changes between the X and Y elements of normal individuals (MANNA 1984). No further studies were performed on these populations, and it remains unknown whether the polymorphism stabilized or one of the sex chromosome systems became fixed.

The fundamental karyotype of *Belostoma* sp. ($2n = 14 + XY$, male) and some characteristics of male meiosis (e.g., ring-shaped bivalents at diplotene, scarce heterochromatin) are also present in two other species of the genus, namely *B. micantulum* and *B. plebejum* (PAPESCHI 1988, 1994). According to a classification of the species of *Belostoma* performed on morphological data (LAUCK 1964; ESTEVEZ pers. commun.) the three of them belong to the *plebejum* group. *Belostoma oxyurum* has a reduced chromosome complement ($2n = 6 + XY$) and the other 12 species of the genus cytogenetically studied up to date have a higher diploid number (two species are $2n = 22 + XY$, and ten species are $2n = 26 + X_1X_2Y$) (PAPESCHI 1992). It has been suggested that the multiple systems originated through fragmentation of the simple X, and the present population of *Belostoma* sp. offers the possibility of corroborating this hypothesis.

The polymorphism was detected in sample 2 and sample 3 from Punta Lara, but since the other

samples were small, it cannot be discarded that the polymorphism was already present at the time sample 1 was collected, or that it is also present in the other two populations. The frequency of individuals with multiple system is low in both samples 2 and 3 (27% and 16%, respectively), but since it is still present after some years it can be assumed that the carrier individuals have at least the same fitness as the normal ones. The chromosome rearrangement could be neutral, or even have some adaptive value.

The comparison of the area measurements of the sex chromosomes in individuals $2n = 16$ and $2n = 17$ shows that the relative size of the X_1 plus the X_2 does not differ significantly from the relative size of the single X. This indicates that the X chromosome of the simple system has fragmented into two chromosomes of unequal size, one of them a little larger than the Y chromosome.

Sex chromosomes of Heteroptera associate at metaphase II and they generally arrange themselves with the X(s) facing the pole opposed to the Y(s) (double-plate arrangement). This behaviour has been observed in most compound systems of Heteroptera, and is particularly the rule in X_1X_2Y (male) species of *Belostoma* (PAPESCHI 1992). However, when the multiple system is not characteristic of the species, as in *Oechalia pacifica*, *Banasa zeteki* and *Belostoma plebejum*, the three sex chromosomes always arrange in a chain at metaphase II; the median chromosome is lagging at anaphase II, but it is finally included in a telophase nucleus.

DARLINGTON (1939) suggested that multiple sex chromosome systems arranged in a chain would be evolutionary unstable, and there would exist a tendency to the establishment of a double plate arrangement. In the present population of *Belostoma* sp. different arrangements were observed within each individual: in a chain, in double-plate, and in other transitional arrangements. In most individuals the chain arrangement predominated (61%–79% of the cells), but in individual 2 of sample 3, 49% of the cells arranged in double-plate, and only 30% were in a chain. Keeping in mind that the mutant individual of *B. plebejum* always presented a chain arrangement, it seems probable that in the population under study a selective force towards a double-plate arrangement could be operating.

Another point to mention is what happens in females heterozygous for the fragmentation (X_1X_2X); these females will present a fragmentation trivalent during meiosis. It has been observed, in an individual of *Belostoma plebejum* heterozygous for an autosomal fusion, that the fusion behaved regularly at meiosis and no abnormal sperm was detected (PAPESCHI 1994). It is possible that in *Belostoma* sp. the heterozygous females (that in fact have been detected) show a regular meiotic behaviour. Even if their fertility were lower than that of homozygous females, their fitness could be equal or even higher if the fragmentation brings about some benefits on other components of the adaptative value.

The present population of *Belostoma* sp. represents a direct evidence of the origin of multiple sex chromosome mechanisms through fragmentation, but many questions remain to be answered. Further studies will be performed in order to get a better understanding of the mechanisms governing chromosome behaviour and karyotype evolution in Heteroptera.

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