Int. J. Biol. Chem. Sci. 1(2): 151-157, 2007

ISSN 1991-8631

International Journal of Biological and Chemical Sciences

http://indexmedicus.afro.who.int/Journals/Indexj.htm

The B chromosome in the meiotic process of the African pest grasshopper *Taphronota thaelephora* STAL. (Orthoptera: Pyrgomorphidae)

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ABSTRACT

This paper presents the first record of B chromosomes in some adult male individuals of the African pest grasshopper *Taphronota thaelephora* collected from Balepa – Mbouda in the Western Province of Cameroon. Of the twenty individuals examined, thirteen had the characteristic Pyrgomorphidae karyotype of 2N= 19(18A+XO) while the remaining seven had a diploid chromosome number of 2N= 20(18A+XO+1B) indicating the presence of a single B chromosome. The B chromosome in this population was short, acrocentric in morphology and stained to the same intensity as the autosomes throughout meiosis. It did not associate with any chromosome in meiosis. In some cells it migrated to the same pole as the X-chromosome while in other cells it migrated to the pole opposite to that of the X-chromosome indicating irregular behaviour in meiosis. The presence of the B chromosome did not affect chiasma frequency in the species.

Keywords: B chromosome, Meiosis, *Taphronota thaelephora*, Chiasma frequency, Balepa-Mbouda, Cameroon.

INTRODUCTION

B chromosomes also called supernumerary or accessory chromosomes are additional large dispensable and independently segregating chromosomes (Camacho et al., 1997a). They have been found in all major groups of plants and animals and reported in more than 1300 plant species and nearly 500 animal species. They are frequent in the Orthoptera and most common in grasshoppers of the family Acrididae with acrocentric chromosomes (Camacho et al., 2000; Warchalowska-Sliwa et al., 2001). B chromosomes may be present in some individuals of a population but absent in others of the same population. They are known to originate in a number of ways including derivation from A-chromosomes. The traditional view is that B chromosomes arise from A-chromosomes (Jones and Rees, 1982) and this view is still widely accepted. Intensive investigations in recent years have revealed that B chromosomes are composed of repetitive DNA and in some cases they have specific sequences but in others all B-sequences are homologous to A-sequences in the standard chromosomes (Beukeboom, 1994). This implies that B chromosomes may be derived from standard or A-chromosomes in the same species (hence intraspecific hypothesis of B-origin). On the other hand, John et al. (1991) and Eickbush et al. (1992) have reported B-specific sequences to have homology with DNA sequences present in A-chromosomes of a closely related species indicating that these Bs may have been

derived by interspecific hybridization (hence interspecific hypothesis of B-origin). The most recent evidence from cytological and molecular studies classifies the origin of B chromosomes to be intraspecific autosomal, intraspecific sex chromosomal; interspecific autosomal and interspecific sex chromosomal (Camacho et al., 2000). Though they may probably have arisen from normal chromosomes (A chromosomes) they do not recombine with A chromosomes and therefore follow their own evolutionary pathway (Camacho et al., 1997b; Camacho et al., 2000).

B chromosome polymorphism has been shown to be highly variable and wide spread in some species such as *Eypreprocnemis plorans*. More than 40 variants, depending on their size, morphology and C-banding pattern are present in most field populations along the Mediterranean and Atlantic coast of Northern Africa (Lopez-Leon et al., 1993, Camacho et al., 1997a; Zurita et al., 1998; Bakkali et al., 1999; Bakkali and Camacho, 2004). They also show an irregular mitotic and meiotic behaviour which allows them to accumulate selfishly in the germline. It is probably for this reason that Bs show non Mendelian inheritance with transmission rates exceeding those of A-chromosomes (Camacho et al., 2000).

B chromosomes are important because they are known to have detrimental effects on the host. B chromosomes damage host fitness and possess meiotic drive and have frequently been shown to have undesirable effects on plants in which they occur (Williams and Barclay, 1968). For this reason most of them are considered as genomic parasites (Nur, 1966; Camacho et al., 1997a). B chromosomes have deleterious effects on the vigour and fertility of a population of Tetraploid hybrids between *Dactylis glomerata sp glomerata* and *D. glomerata sp lusitanica* (Williams and Barclay, 1968). Jones and Rees (1982) have summarized a broad range of mostly detrimental effects of B chromosomes in many species of plants and animals. B chromosomes are known to more frequently affect processes or characters associated with vigour, fertility and fecundity (Camacho et al, 2000).

During the cytogenetic examination of some twenty individuals from a population of *Taphronota thaelephora* in Balepa-Mbouda in the West Province of Cameroon, the presence of a B chromosome was recorded. In this paper, this B chromosome was characterized with respect to morphology, behaviour in meiosis and effect on chiasma formation.

MATERIALS AND METHODS

The twenty adult males from a natural population of *T. thaelephora* used in this study were collected in December 2004 from Balepa, a village on the outskirts of Mbouda town in the Western Province of Cameroon. On collection, the insects were immediately taken to the laboratory where they were killed with chloroform fumes and dissected for the testes. The testes were placed in a fixative (3:1 methanol: acetic acid) and stored in the refrigerator (4°C) until needed.

Chromosome smears were prepared using the Lacto-Propionic–Orcein squash technique used by Seino, (1989) with slight modifications. Two to three fixed testicular follicles were placed on a clean microscope glass slide. They were first flooded with 45% acetic acid for five minutes. This made the cells to swell. After blotting off the acid, the tissue was next flooded with one or two drops of lacto-propionic-orcein stain and macerated using the sharp pointed end of a dissecting needle. This permitted the stain to penetrate into the tissue. The preparations were then incubated at room temperature for ten to fifteen minutes while making sure that the stain did not dry off. A cover slide was then placed on the tissue, held in place with the thumb and forefinger before gently tapping with the wooden end of a dissecting needle. This helped to disperse the cells and force out excess stain. The preparation was then wrapped in a filter paper and squashed between the thumb and the top of the laboratory table. The filter paper absorbed excess stain. The edges of the cover slide were sealed with colourless nail vanish to temporarily preserve the preparation.

The chromosome smears were examined using the Fisher Laboratory microscope with a total magnification of 400X.

Chromosome numbers were determined by counting the number of chromosomes in ten cells at Metaphase-1 in each of the seven individuals examined. The

meiotic stages in Prophase-1 and Metaphase-1 were examined to determine whether the B – chromosome was associated with the A-chromosomes. Cells in Anaphase-1 were also examined to determine the movement of B-chromosome in relation to the X-chromosome. Chiasmata were counted in ten cells in Diplotene/Diakinesis of Prophase-1 in seven normal individuals randomly chosen from the population and the seven individuals collected that had B-chromosomes. The data obtained was subjected to the analysis of variance so as to determine if the differences in mean chiasma frequencies observed for the cells with and without the B-chromosome were significant.

RESULTS AND DISCUSSION

Thirteen adult male individuals of *T. thaelephora* examined had 9 bivalents and one univalent indicating a karyotype of 2N= 18+XO (Figures 1a and 2a). These individuals were considered to be normal since the karyotypes of five Pyrgomorphidae species that have been described (Sharma et al., 1974; John and King, 1983; Fossey et al., 1989; Williams and Ogunbiyi, 1995) have 19 acrocentric chromosomes and an XO sex determining mechanism in males. The other seven individuals of *T. thaelephora* examined had 9 bivalents and two univalents (Figures 1b and 2b). In Diplotene, one of the univalents was long, had a somewhat fuzzy outline and stained darker than the autosomes thereby exhibiting positive heteropycnotism while the other univalent was short and stained to the same intensity as autosomes. In Anaphase -1, the longest bivalent stained lighter than the autosomes and had a smooth outline thereby exhibiting negative heteropycnotism. According to the criteria of Seino et al. (2002), the long univalent was considered to be the X-chromosome. Therefore the short univalent was a supernumerary or B–chromosome.

The chromosome in Figure 3b revealed minute short chromosome arms indicating that the chromosomes in this species are acrocentric in morphology. Seino et al (2002) reported that all the chromosomes in the species *T. thaelephora* were acrocentric in morphology. The chromosomes smears obtained in this study confirm that the chromosomes in *T. thaelephora* are acrocentric.

The presence of the B-chromosome in this population is also not strange because supernumeraries have been recorded in some species of the Pyrgomorphidae, notably *Atractomorpha bedeli* collected from Tafowato, Japan (Sannomiya, 1973). Among animals, B-chromosomes are more likely to occur in species with karyotypes consisting of mostly acrocentric chromosomes (Palestis et al., 2004).

The B - chromosome did not associate with any chromosome in the genome during Diplotene of Prophase-1 and Metaphase-1 (Figures 1b and 2b). In some of the cells this chromosome migrated to the same pole as the X-chromosome while in other cells it migrated to the pole opposite to that of the X-chromosome. The behaviour of the B-chromosome in this population was at variance with observations in some *E. plorans* (Acrididae) and some Tettigonidae species. Bugrov et al. (1999) observed the B-chromosome in a Russian population of *E. plorans* to remain associated with the X-chromosome to the end of Anaphase-1. Warchalowska-Sliva et al. (2002) reported B-chromosomes in *Tettigonia dolichopoda maritima* collected from Korea to be positively heteropycnotic in Prophase-1 and sometimes showed the tendency to pair with the X-chromosome. It is therefore conclusive that the B-chromosome in *T. dolichopoda maritima* may have been derived from the X-chromosome while the B-chromosome in *T. thaelephora* here studied might have been derived from the autosomes (A-chromosomes). This indicated the irregular behaviour of this chromosome in meiosis which according to Camacho et al (2000) allows for selfish accumulation in the germ line of the Balepa-Mbouda population of *T. thaelephora*.

The data in Table 1 revealed that chiasma frequency in normal cells (cells without the B-chromosome) ranged from 13.0 to 15.6 while in cells with the B-chromosome, it ranged from 13.6 to 14.2. Mean chiasma frequency in normal cells (13.94 ± 1.3) was not significantly different from mean chiasma frequency in cells with the B-chromosome (13.87 ± 1.1). This indicated that the presence of the

Table 1: Mean number of chiasmata in normal cells and in cells with the B - Chromosome of *T. thaelephora*. Seven normal individuals selected randomly from the sample of thirteen and the seven individuals with the B - chromosomes were considered.

Individual	Mean number of chiasmata per cell	
	Normal cells	Cells with B-chromosome
1	15.6 ± 0.6	14.2 ± 0.9
2	14.2 ± 1.8	13.8 ± 0.9
3	13.6 ± 1.1	13.6 ± 1.5
4	13.0 ± 1.6	13.9 ± 1.0
5	14.0 ± 0.7	14.2 ± 0.7
6	13.0 ± 1.6	13.8 ± 1.6
7	13.8 ± 1.6	13.6 ± 1.5
Mean	13.4 ± 1.7	13.87 ± 1.1

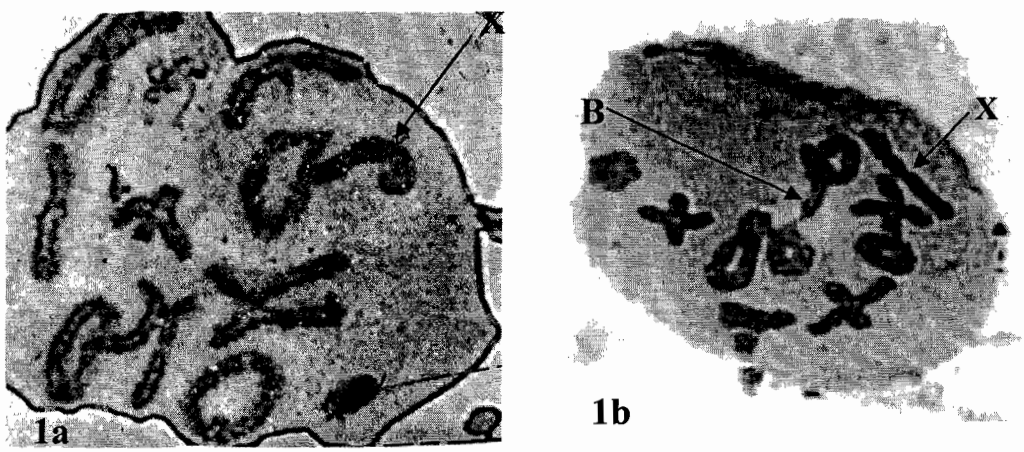

Figure 1: Diplotene in *T. thaelephora*: 1a = Normal individual, X chromosome is positively heteropycnotic, chiasma frequency = 14.0; **1b** = Individual with the B chromosome, X chromosome is positively heteropycnotic while B chromosome stains to same intensity as autosomes; chiasma frequency = 14.0.

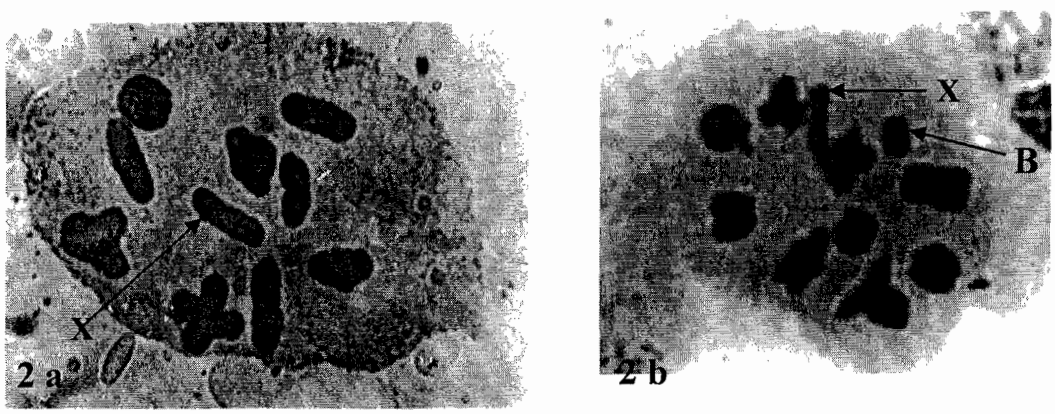

Figure 2: Metaphase-1 in *T. thaelephora*: 2a = Normal individual, X chromosome is negatively heteropycnotic; **2b** = Individual with the B chromosome, X chromosome is negatively heteropycnotic while B chromosome stains to same intensity as autosomes.

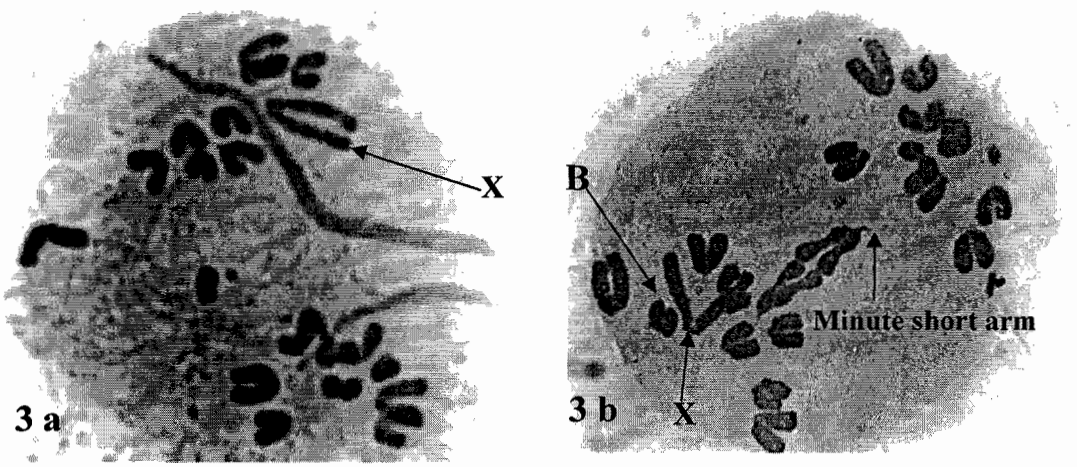

Figure 3: Anaphase-1 in *T. thaelephora*: 3a = Normal individual, X chromosome is negatively heteropycnotic; **3b** = Individual with B chromosome, X chromosome is negatively heteropycnotic while B chromosome stains to same intensity as autosomes.

B-chromosome did not affect the frequency of chiasma formation in the species *T. thaelephora*. Information is lacking on the effect of B-chromosomes on chiasma frequency in the Pyrgomorphidae. However, in the Acrididae where the effect of the B - chromosome on chiasma frequency has been extensively studied (John and Hewitt, 1965; Hewitt and John, 1970; Hewitt, 1979; Camacho et al., 1980; Lorrey et al., 1991), it has been revealed that the presence of an even number of B-chromosomes resulted in increased chiasma frequency while the presence of an odd number of B-chromosomes had no affect on chiasma frequency in *E. plorans*. Nigo et al. (1998) reported that a higher content of supernumerary heterochromatin was correlated with the formation of a lower number of chiasmata within a given chromosome complement of *Dociostaurus genei* (Orthoptera) while Grieco and Bidau (1999) observed that the presence of the B chromosome tended to increase chiasma frequency slightly in both males and females of *Metaleptea brevicomis adespersa* (Acridinae: Acrididae) collected in South Africa. Bugrov et al. (1999) investigating *E. plorans* (Charp.) did not find any evidence for a significant effect of the presence of 2B-chromosomes on either mean chiasma frequency or between cell variance. However, they observed that mean chiasma frequency increased significantly when only one B-chromosome was present.

The information recorded in this article forms a basis for investigations into the origin, polymorphism, frequency and distribution of the B chromosome in Cameroon's populations of *Taphronota thaelephora* (Stal.).

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