



ORIGINAL ARTICLE

# New record of B-chromosome through meiosis in the Egyptian locust *Anacridium aegyptium* (Acrididae) with indication to its origin

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**Abstract** The basic chromosome complement of the Egyptian male locust, *Anacridium aegyptium*, collected from Giza Governorate, Egypt, is  $2N = 22 + XO$ . All autosomes and sex chromosome are telocentric. The autosomes can be divided into three size-groups; three long ( $L_1$ – $L_3$ ), five medium ( $M_4$ – $M_8$ ) and three short chromosomes ( $S_9$ – $S_{11}$ ). The X-member is the longest member of all chromosomes. An extra telocentric B-chromosome was detected in two males of the collected locusts, nearly equal in size with  $S_{10}$ -member and thus the chromosome complement of these males is  $2N = 22 + XO + B$ . The origin of this extra member is discussed through its behaviour through meiosis that studied. The B-chromosome was frequently observed neighbouring the X-chromosome through most of the meiotic stages and this behaviour reinforces the present suggested hypothesis that B-chromosome was originated from X-chromosome.

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## 1. Introduction

Abdel-Haleem (1992) reported that the chromosome complement of male *Anacridium aegyptium* is composed of 23 chromosomes. He observed that autosomes and X-chromosome are more spreading through mitosis than meiosis. Thus, they observed acrocentric through mitosis stages and telocentric through meiotic ones. The chromosomes could be measured and divided into three groups, on the basis of their relative lengths, three pairs of large (L), five pairs of medium (M) and three pairs of small homologues (S). The X-chromosome is the longest member of all complement. The properties of accessory or B-chromosomes had been reviewed by Müntzing

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(1974). Nokkala and Nokkala (2004) declared that B-chromosomes are not belonging to the regular karyotype of any species of Acrididae.

Martin et al. (1996) found a decrease of mating involving males with B-chromosomes in the grasshopper *Eyprepocnemis plorans*. In the same grasshopper, Camacho et al. (1997) concluded that B-chromosome has no detectable effects on fitness. Cabrero et al. (1984) reported that the B-chromosomes in the locust *Locusta migratoria* are mitotically unstable, accumulated in the male germ-line and meiotically there is no significant differences in mean chiasma frequency between locusts with and without B-chromosomes. In this respect, the effect of B-chromosome on chiasma frequency in *L. migratoria* was studied by Viseras et al. (1988).

Bidau (1986) found a nucleolar-organising region in B-chromosome of the grasshopper *Dichroplus pratensis*. He also analyzed the meiotic behaviour of B-chromosome through meiosis. The same author (1988) investigated the effects of B-chromosomes on meiosis of the grasshoppers *Dichroplus pseudopunctulatus* and *Sinipta dalmani*. Henriques-Gil et al. (1989) noted accumulation of B-chromosomes in the gametes with X-chromosome in the examined males of *E. plorans*. Lopez-Leon et al. (1996) found that B-chromosomes were more frequent in males than females in *E. plorans*. Bugrov et al. (1999) stated that one B-chromosome in *E. plorans* has no effect on the mean chiasma frequency. They added that B-chromosomes are made up of DNA-sequences (ribosomal-DNA and a 180-bp repeat).

Abdel-Haleem (2003) studied the B-chromosome behaviour through mitotic and meiotic divisions in the grasshopper *E. plorans* and its relation with X-chromosome. Nokkala and Nokkala (2004) declared that B-chromosome behaviour in both mitosis and meiosis is without segregation and chiasmatic mechanism.

Therefore the main aims of the present work were to describe the effects of the supernumerary or B-chromosome in the meiotic division of the Egyptian locust *A. aegyptium* and to search for its origin.

## 2. Materials and methods

### 2.1. Rearing

The colony of the locust *A. aegyptium* was initiated by 20 males and females obtained from Abo-Roach, Giza Governorate, Cairo, in August 2007. The individuals were kept in cubic cage of  $30 \times 30 \times 30 \text{ cm}^3$  dimensions, at the laboratory conditions ( $28 \pm 2^\circ \text{C}$  and 65% R.H.) and were daily fed on leaves of lettuce.

### 2.2. Chromosome preparation

Meiotic cells were obtained from the testes of two adult male of *A. aegyptium* that had B-chromosome. The testes were dissected out, cleaned and placed in insect-saline solution for a half hour, fixed in 3:1 absolute ethanol:glacial acetic acid for at least 12 h at  $4^\circ \text{C}$ . Then, the fixed-testes were washed and kept in mixture of 70% ethanol (99 ml) and concentrated glycerol (1 ml) and kept at  $4^\circ \text{C}$ . Then, the squash preparations were made using 4% Giemsa stain. Fi-

nally, observations were photographed using light microscopy and printed.

### 2.3. G-banding pattern preparation

In general, G-banded-chromosomes revealed more detailed information about chromosomes than the classical karyotype. C-banding pattern preparation was made according to the technique of Webb (1976), where the colchicine-treated-meiotic cells were immersed in trypsin solution at room temperature, washed in phosphate-buffered solution followed by immersion in 4% Giemsa stain.

## 3. Results

### 3.1. The basic complement

In *A. aegyptium*, the basic chromosome complement showed 11 bivalents plus X-univalent. The autosomes and X-chromosome are telocentric. The autosomes can be divided into three groups, on the basis of their relative lengths, three pairs of large (L), five pairs of medium (M) and three pairs of small homologues (S). The X-chromosome is the longest member of all chromosomes (Fig. 1(7)).

### 3.2. The supernumerary chromosome

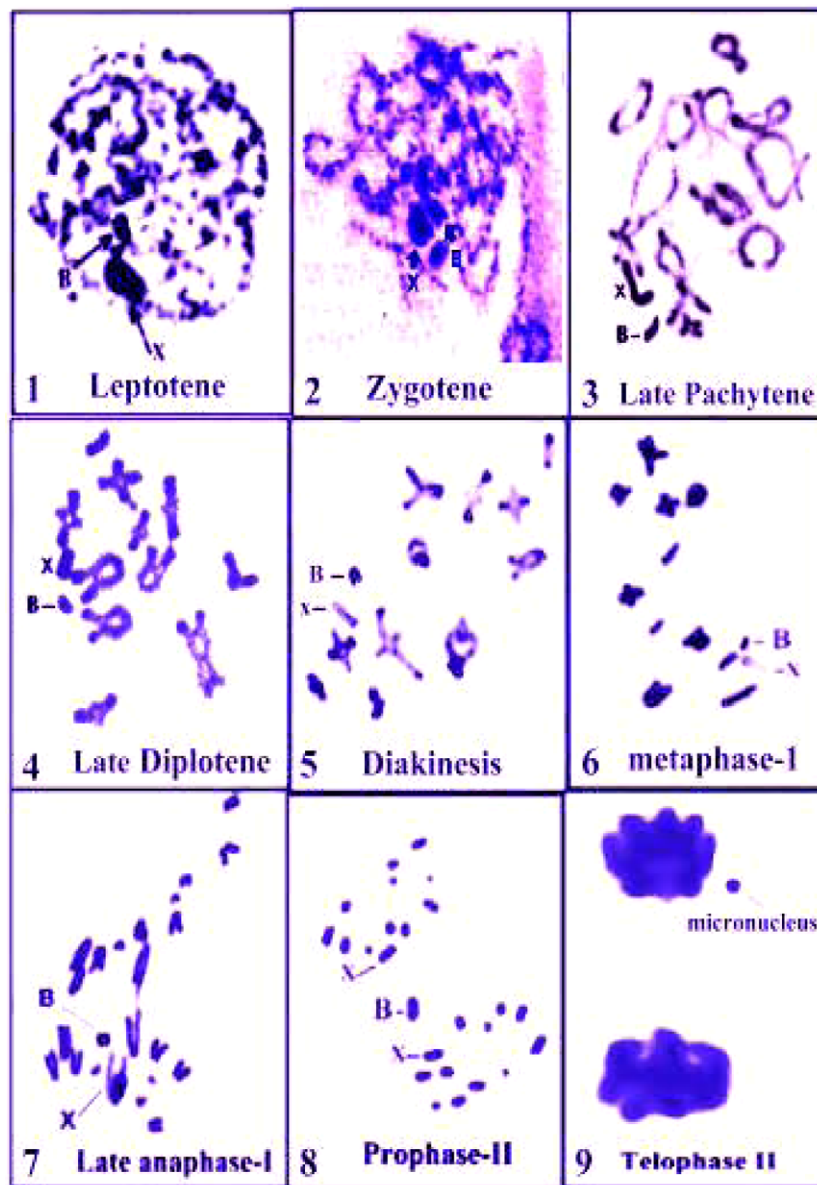
A telocentric B-chromosome was found in one male of the collected locusts *A. aegyptium*. It is small in size and its length is slightly longer than  $S_9$ -member. Thus, the chromosome complement in this male was  $2N = 22 + XO + B$  (Fig. 1(7)). In the majority of meiotic stages, B-chromosome is neighbouring the sex chromosome (Figs. 1(1–9)). Moreover, the B-chromosome behaviour through the majority of meiotic stages revealed that it is stable element. It displays a positive heteropycnosis during leptotene, zygotene and pachytene stages. In diplotene stage, the B-chromosome observed unassociated with any chromosome. In metaphase-I, both B- and X-chromosomes are unassociated and found as univalents. In late anaphase-II, the B-chromosome was the latest-moving member towards one of the two poles, in which the sex chromosome is found, in spite of its univalent form. It seems to be approached to the pole where X-chromosome found and attached to it in an abnormal manner. In late telophase-II, it is easy to see the B-chromosome near to two out of the four haploid nuclei. It shows as small micronucleus neighbouring one of the two spermatids (Fig. 1(1)).

## 4. Discussion

Bugrov et al. (1999) reported that the chromosome complement of male *E. plorans* is composed of 23 acrocentric chromosomes. The present result reinforced the diploid number of the same species ( $22 + XO$ ).

Henriques-Gil et al. (1984) classified 14 types of B-chromosomes in the grasshopper *E. plorans* according to their sizes and meiotic behaviour.

In the present work, the B-chromosome of the locust *A. aegyptium* is telocentric slightly longer than  $S_9$ -member and



**Figure 1(1-9)** Sex chromosome behaviour of *Anacridium aegyptium* through meiotic prophase-I stages, metaphase-I, anaphase-I, prophase-II and telophase-II.

is a meiotically stable element. These results support those of Hewitt (1979) who declared that B-chromosomes of grasshoppers are stable in cell division. However, Bidau (1988) studied the effects of B-chromosomes in meiosis of the grasshoppers, *D. pseudopunctulatus* and *S. dalmani* and showed an irregular meiotic behaviour (lagging at the first and second meiotic divisions and finally formation of B-chromosomes as microspermatids).

In this regard, Camacho et al. (1980) displayed two B-chromosomes in *E. plorans* that are similar in size and both are telocentric.

Webb (1976) declared that the C-banded B-chromosome in the locust *Chortoicetes terminifera* was darkly stained, but such banding was not successful in identifying the origin of B-chromosome. Webb result agrees with the Present work, especially through meiotic diakinesis stage (Fig. 1(1-9)).

In the present investigation, the meiotic behaviour of the B-chromosome demonstrated that it was frequently localized near to the univalent X-chromosome and lately reached to the pole which has X-member. This behaviour supports the study of Camacho and Cabrero (1982) who found an association between the B-segment and X-chromosome in five species of grasshoppers.

On the other hand, the effect of B-chromosome on sperm formation was investigated in the present work where large numbers of B-chromosome, as micronuclei, were observed neighbouring the poles that involved X-chromosomes.

Bidau (1986) found that the B- and X-chromosomes were associated preferentially during prophase-I of the grasshopper *D. pratensis* and this persistent X-B-association leads to both chromosome moving to the same pole through anaphase-I. The present results in *A. aegyptium* support that persistent X-B-association.

Bugrov et al. (1999) showed that the B-chromosomes of male *E. plorans* are made up of the same two DNA-sequences of X-member (ribosomal-DNA and a 180-bp repeat). This result offers a strong support for the present hypothesis about the origin of B-chromosome in *A. aegyptium*.

In this respect, Bugrov et al. (2003) analyzed the distribution of repetitive DNA of B-chromosomes of *Podisma sapporensis* (Acrididae), using meiotic chromosome microdissection followed by DOP-PCR (degenerated oligo-nucleotide primed polymerase chain reaction) and FISH analysis, and found the same two DNA-sequences of Bugrov et al. (1999).

Abdel-Haleem (2003) analyzed the behaviour of B-chromosome of male grasshopper *E. plorans* and suggested the same hypothesis that B-segment was originated from X-chromosome through mitosis and meiosis.

Furthermore, Nokkala and Nokkala (2004) analyzed the behaviour of B-chromosomes through mitosis and meiosis and found that they were without segregation and chiasmatic mechanism.

Warchaowska-Sliwa et al. (2005) studied the orthopteran *Tetrix tenuicornis*, which collected from polluted area with heavy metal in Poland, and found cytogenetic abnormalities involving anomalies in chromosome number and morphology through mitosis and meiosis, frequent presence of B-chromosome and a heterozygous deficiency in L<sub>2</sub> and M<sub>3</sub> bivalents in male as well as tetraploid, and octaploid chromosome numbers in the females.

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