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Post-meiotic Splitting of Spermatid Nucleus and Beaded Tail Formation During Spermiogenesis to Eliminate DNA in Two Grasshoppers With B Chromosomes

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Abstract: The end product of meiosis in male grasshoppers is round spermatids and these will not undergo post meiotic division, under normal conditions. In two species of grasshoppers carrying B chromosomes, the post-meiotic division of the nucleus of spermatids was observed. The number of nuclear bits formed ranged between 1-4 in the elongating head region of developing spermatids during spermiogenesis. The nuclear bits were also pushed in to the tail region resulted in a beaded appearance to the tail of sperm, with five to ten number of nuclear bits in the tail region. Addition to this small, round micro-spermatids formed from the eliminated nuclear bits, remained the same without showing further development. The range of nuclear bits generated outnumbered the B chromosomes encountered in the two species of grasshoppers. The grasshopper *Neorthocris acuticeps acuticeps* had one small B and the other *Chrotogonus trachypterus* had two kinds of Bs- large and small. This unusual process of post-meiotic division of the spermatid nucleus and beaded tail formation in sperms is discussed in the light of DNA elimination during gametogenesis in males of grasshoppers.

Keywords: Post-meiosis, Nuclear bits, Micro-nuclei, Beaded tail, DNA elimination

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Introduction

Grasshoppers are ideal model organisms to study the process of Meiosis, spermatogenesis and sperm structure, because of this convenience these insects are used for teaching as well research. Studies on the meiotic process in Indian grasshoppers, initiated by Asana (1928), since then lot of work accrued on meiotic studies of grasshoppers (Singh. 2009). Spermatogenesis is highly organized process, follows a definite pattern specific to a species (White, 1954; Liu Meng-Hao *et al*., 2012). Gosalvez *et al*. (2010) identified sperms of grasshopper as good means to analyze the post-meiotic behaviour and organization of DNA within this gamete; and introduced possible analytical methods to analyze chromatin in non-orthopterans. Grasshoppers are also a good source to study occurrence of B chromosomes. These accessory chromosomes will have impact on the meiotic behaviour of a regular karyotype and spermatogenesis due to their mitotic instability or stability. In this study, impact of B chromosomes on post-meiotic process of sperm development during spermiogenesis and spermateleosis have been analyzed in two species of grasshoppers *Neorthocris acuticeps acuticeps* (Bolivar) and *Chrotogonus trachypterus* (Blanchard), carrying B chromosomes in their karyotype. In these two species of grasshoppers the process of post-meiotic development of sperms deviated from normal process is reported in this study. In the grasshopper *Neorthocris acuticeps acuticeps* occurrence of B chromosomes and their behaviour has been studied at population levels which revealed that these are mitotically instable but had normal spermatogenetic process (Channaveerappa and Ranganath, 1994; Channaveerappa, 1996).

Materials and Methods

Adult males of the grasshopper species *Neorthocris acuticep sacuticeps* (Bolivar) *and Chrotogonus trachypterus* (Blanchard) were collected from natural population at different localities of Mysuru, Bengaluru and Mandya, India. These grasshoppers were screened for presence of B chromosomes. For meiotic studies in males, the testes follicles were extirpated, treated with hypotonic solution, stained with Aceto orcein, squash preparations were made using 45% acetic acid, for air dried preparations of slides, followed Imai's (1983) technique. Slides were stained with 10% Giemsa stain. The spermatids, at different stages of sperm formation were photomicrographed using Zeiss microscope with camera attached. All the photomicrographs were taken at 100x10 magnification and images were enlarged nine times (100x10x9 is magnification of the images) and CAD drawn measurement scale is 0.1mm=100 microns.

Results

Meiosis in these grasshoppers resulted in round spermatids that further metamorphosed into sperms typical to these insects; irrespective of these two species carried B chromosomes or not (Fig. 1c, d, e). In two individuals of concern, in this work, post-meiotic development of spermiogenesis was different. In single individual of *N. a. acuticeps* (1/203) from Bengaluru population had single smaller B in two of its testes follicles (Fig. 1a) and other follicles did not had B chromosomes. In this individual, post-meiotic spermiogenesis was not normal in two of its follicles with B chromosome. The post-meiotic division of spermatid nucleus had 2- 3 fragmented components in these two follicles (Fig. 2a, b) and in other follicles the spemiogenesis was found to be normal. In *C. trachypterus* smaller and medium size Bs were recorded (Fig.1b). The post-meiotic dvelopment of spermatids had variations in all the follicles, more than 70% of spermatids had 2-3 split nuclear bodies in each (Fig. 2c). A few micro-nuclei separated (Fig. 2c) from the divided nucleus of spermatids were found scattered. Some fully developed sperms had nuclear bits accommodated in the head region but also had beaded tail due to accumulation of nuclear bits (Fig. 2d).In majority of spermatids more than two nuclear bits were found in *N. a. acuticeps*, though it had single small B chromosome. *C. trachypterus* too had more than two nuclear bits though it had two B chromosomes of different sizes. A few of the nuclear bits were large, uneven in their size resulted by division of spermatid nucleus during spermiogenesis, in both these species. In majority of spermatids, at spermateliosis phase, some nuclear bits were pushed into tail region. The number of nuclear bits in the tail varied in their numbers from 5 to 10 in both the species of grasshoppers.

Of the 300 developing spermatids scored in *N.a.acuticeps*, 240 spermatids in the spermiogenetic process deviated from normal process of spermiogenesis (Fig. 2a) and spermateleosis (Fig. 2e). In the species *C. trachypterus* more number of spermatids were available for scoring, 700 plus developing spermatids in spermiogenesis phase were scored, of these about 250 were in spermateleosis phase. The other spermatids (455 spermatids) in spermiogenesis phase showed post-meiotic nuclear division and of the 250 spermatids in

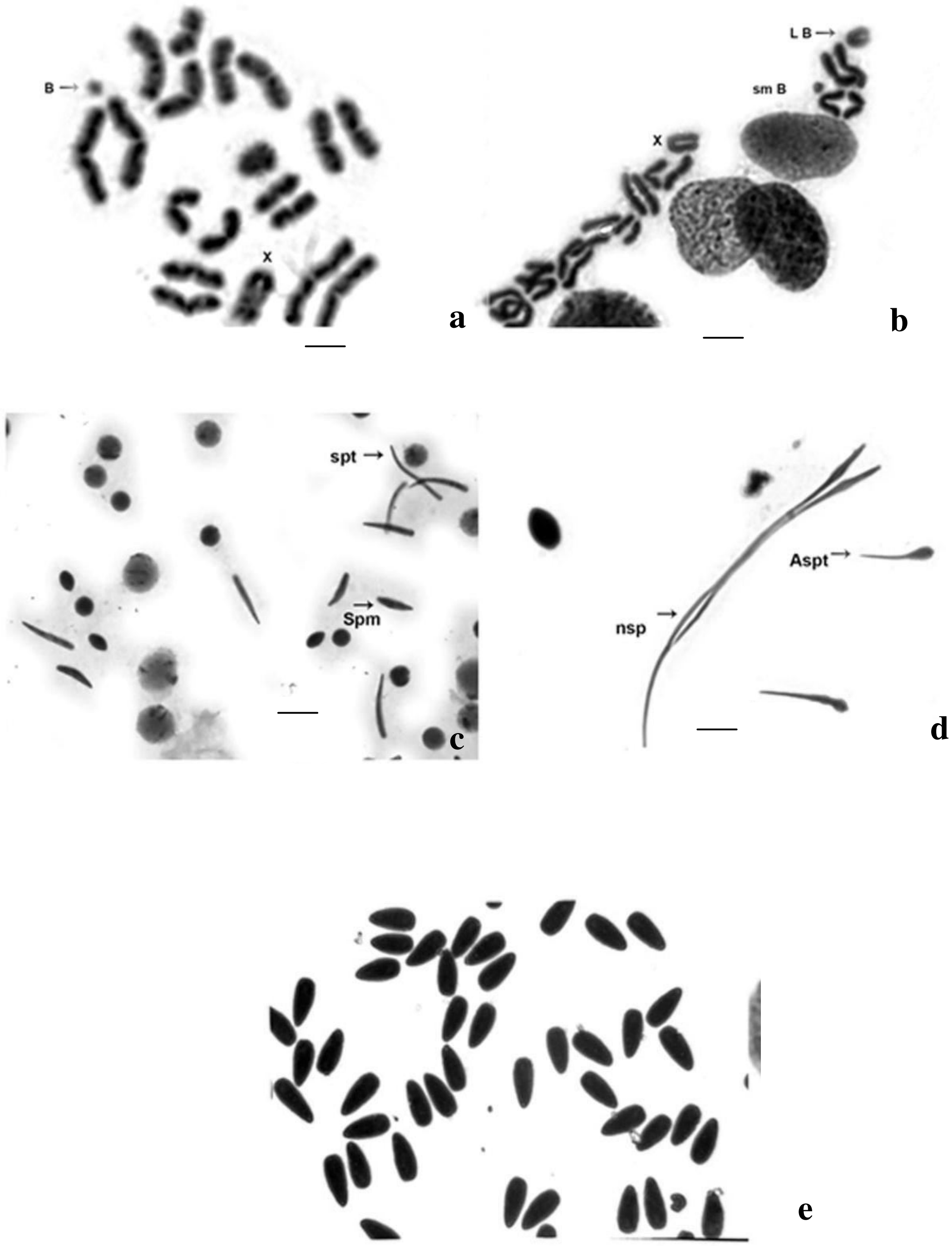

Fig. 1: (a) *N. a. acuticeps*, Anaphase 1 with B Chromosome;
(b) *C. trachypterus*, Anaphase 1 with B chromosomes, smB-small B chromosome, LB- Large B chromosome;
(c) Typical spermatids in different stages of development. Spm-spermiogenic phase, spt- spermateleosis phase;
(d) *N. a. acuticeps*. nsp-normal sperms, aspt-advanced stages of normal spermateleosis;
(e) *C. trchypterus*- Normal developing spermatids.

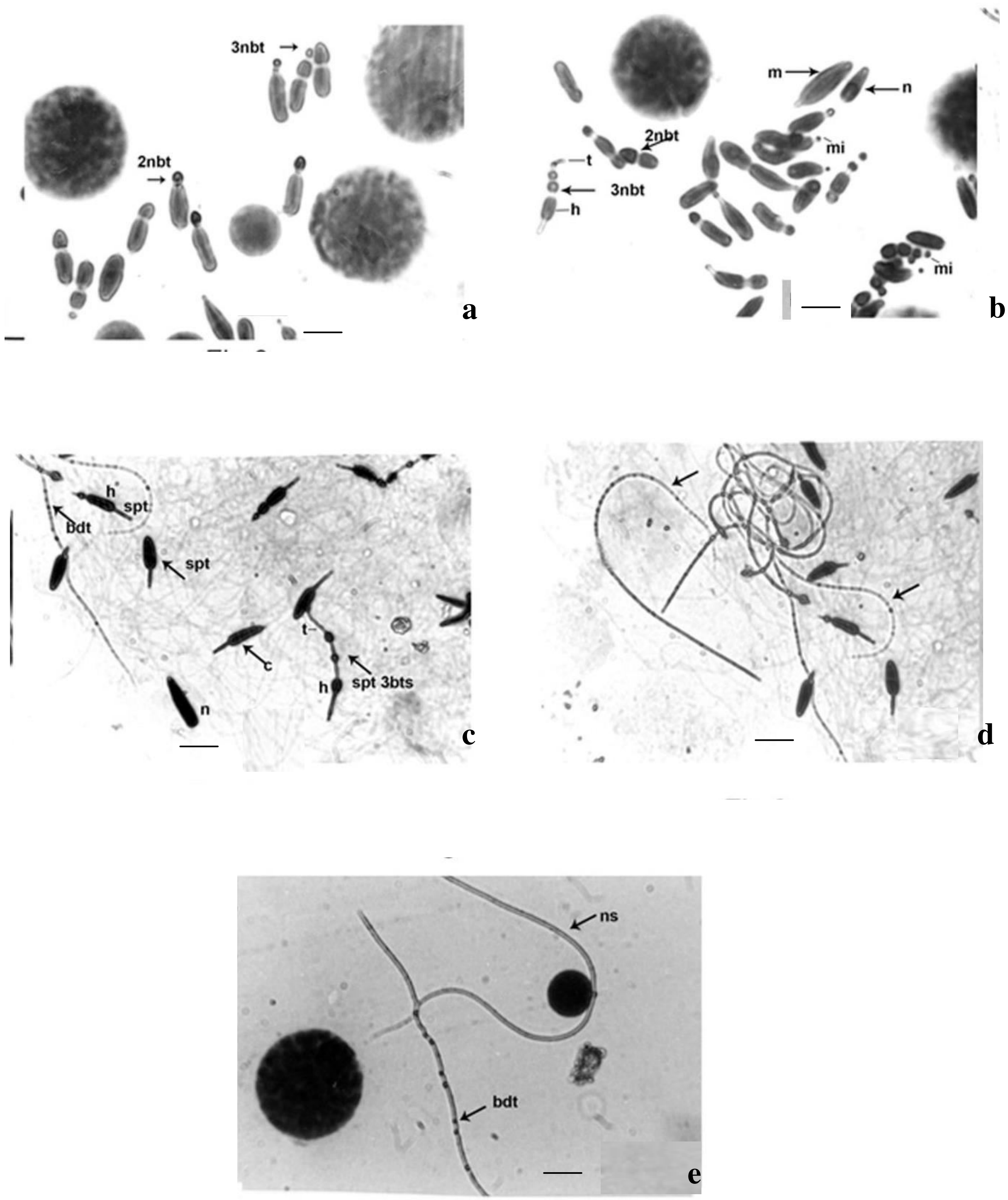

Fig. 2: (a) *N. a. acuticeps*. dividing spermatid nuclei during spermiogenesis, 2nbts-two nuclear bits, 3nbts-three nuclear bits

(b) *N. a. acuticeps*. dividing spermatid nuclei during spermiogeniesis, 2nbts-two nuclear bits, 3nbts- three nuclear bits. N-elongated normal spermatid, h-head, t-Tail, mi-micro-spermatid, M-macro- spermatid

(c) *C. trachypterus*. spermatids in different stages of spermiogenesis and spermateleosis. n-normal, bdt-beaded tail sperm, c-condensed nuclear bits spt- spermateleosis with three nuclear bits, h-head, t-tail

(d) *C. trachypterus*. bdt-beaded tail sperm with distinct head

(e) *N. a. acuticeps*. bdt-beaded tail sperm and nsp- normal sperms in the same testes follicle.

spermateleosis had nuclear bits in the head region and beaded appearance of their tail, due to serial piling up of nuclear bits. In the other species post-meiotic nuclear division and beaded tail formation were recorded (Fig. 2e) but beaded tail sperms were less in number compared to *C. trachypterus*. The normal spermiogenesis in these species takes place through spindle like modification of round sprmatids (Fig. 1e), further procede to form tailed sperms. Addition to these macro-spermatids; micro-spermatids were also encountered in the species *N. a. acuticeps*. The micro-spermatids were seen being budded off from the spermatids, some of the nuclear bits found aligned in tail forming region of developing sperms.

Discussion

Ordinarily Spermatogenesis and the proceeding phase of maturation of sperms follow a definite path in grasshoppers. The round spermatids with a compact nucleus are resulted at the end of meiosis cell division. The post-meiotic development is followed by elongation of these to form spindle like spermatids. These spermatids at the same time elongate in the direction of head and tail formation but formation of head is completed earlier followed by tail formation. Either during spermiogenesis or during spermateleosis sperm nucleus never divides under normal circumstances. This type of normal process of sperm formation was seen both in males without B chromosomes or with B chromosomes in *N. a. acuticeps* and without B chromosomes in *C. trachypterus* but for a single individual with Bs encountered, where deviations from normal spermiogenesis was seen .

Abnormal spermatids in grasshoppers are found to be due to presence of B chromosomes (Teruel *et al*., 2009) in *Eyprepocnemis plorans*. Though more than forty different kinds of B chromosomes (Bakkali *et al*., 1999) recorded in this species, not all the types of Bs induced abnormal spermatids formation. In general macro-spermatids are produced due to presence of accessory chromosome or increased ploidy, whereas micro-spermatids are resulted by eliminated B chromosomes (Nur, 1969). In some cases it is found to be due to odd and even effect of Bs (Talavera *et al*., 1990; Camacho *et al*., 2004). Influence of B chromosomes on the formation of abnormal chromatids have been recorded by many workers in different species of grasshoppers (Hewitt *et al*., 1987; Suja and Rufas, 1989; Lopez – Leon *et al*., 1992; Camacho *et al*., 2004). Gosalvez *et al*. (2010) have demonstrated that some sperm nuclei in early developmental stages had fragmented DNA organization with large haloes whereas the ones with compact nuclei had intact DNA. In some of the elongated spermatids, dispersal of DNA fragments was also observed by these workers and the fragments were found attached to central core but never encountered fragmented nuclei during spermatogenesis, as found in the present study.

B chromosomes are considered to be genomic parasites evolved as a mechanism to exist within the carrier without affecting its fitness. Some of the B chromosomes which are detrimental to the host fitness are eliminated during spermiogenes by the formation of micro-spermatids or micro-nuclei which has been recorded in Maize (Chiaverino *et al*., 2000) and Grasshoppers (Teruel *et al*., 2009). Anyway, in these cases multiple fragmentation of the spermatid nucleus in spermiogenesis were not seen. In the present study, multiple fragmentation of spermatid nucleus leading to accumulation of several nuclear bits was found and only smaller bit at the extreme end of the sperm head region was pinched off into micro-spermatid and the remaining bits of nucleus persist, irrespective of size whether those are small or large. One or two nuclear bits pushed towards the tail region of the developing spermatids which move into sperm tail along with its elongation and multiplication of these bits might have occurred several time and nuclear bits splitting to produce a beaded appearance of the sperm tail or might be, sequentially divided bits from spermatid nucleus are arranged in the sperm tail. With the complete maturation of the sperm, the sperm chromatin condenses to form a compact nucleus fusing all the nuclear bits in the head, could be implied that the nuclear DNA got reorganized. So it can be assumed that B chromosomes or fragments of DNA are eliminated through the sperm tail, as the tail cannot enter into ovum during fertilization. Programmed elimination of DNA to reorganize the genome is seen in many eukaryotes (Wang and Davis, 2014; Dmitrij and Alla, 2022), in grasshopper the extra DNA in the form of a B chromosome is eliminated at spermiogenesis phase is unique but how it has been programmed, is still a question. In *Eyprepocnemis plorans* the elimination of parasitic B in the form of micronuclei formation has been confirmed where single or two nuclear bits are recorded that are formed in micronuclei that were cut off during early spermiogenesis (Cabrero *et al*., 2017) but the present study includes extended process of spermiogenesis, micronuclei formation, reorganization of nuclear bits in to the sperm head, DNA fragments pushed in the tail region during spermateliosis and formation of a beaded tail. Though B chromosomes were recorded in 45 (45/203) individuals of *N. a. acuticeps*, only in single individual DNA elimination during spermiogenesis was noticed, other 44 individual with B chromosomes had normal process of sperm formation similar to that of insects without Bs. This study has exposed an evolutionary mechanism existing in grasshoppers, to eliminate extra chromosomal DNA, in two B chromosome carrying grasshoppers.

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