

Evidence of B-chromosomes in the Karyotype of *Barypeithes pellucidus* Boheman 1834 (Coleoptera, Curculionidae, Entiminae) from Central Europe*

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B-chromosomes were observed in spermatogonial mitotic metaphases, meiotic metaphases I and II of *Barypeithes pellucidus* from one population in Slovakia. The number of B-chromosomes ranged from one to six per cell and they paired with the sex heterochromosomes in the first meiotic metaphase and rarely with the autosomes. In metaphase I one B-chromosome was always associated with X chromosome forming a tripartite complex. The XyB₁ was easily recognizable as a complex of three chromosomes in a parachute association. The size of the B-chromosomes was approximately the same or a little smaller than that of the y heterochromosome which was the smallest element of the regular chromosome set. Their staining intensity seems to be similar to that of the autosomes and sex chromosomes, respectively. The behaviour of B-chromosomes during mitosis and meiosis in weevils is briefly discussed.

Key words: B-chromosomes, Coleoptera, Curculionidae.

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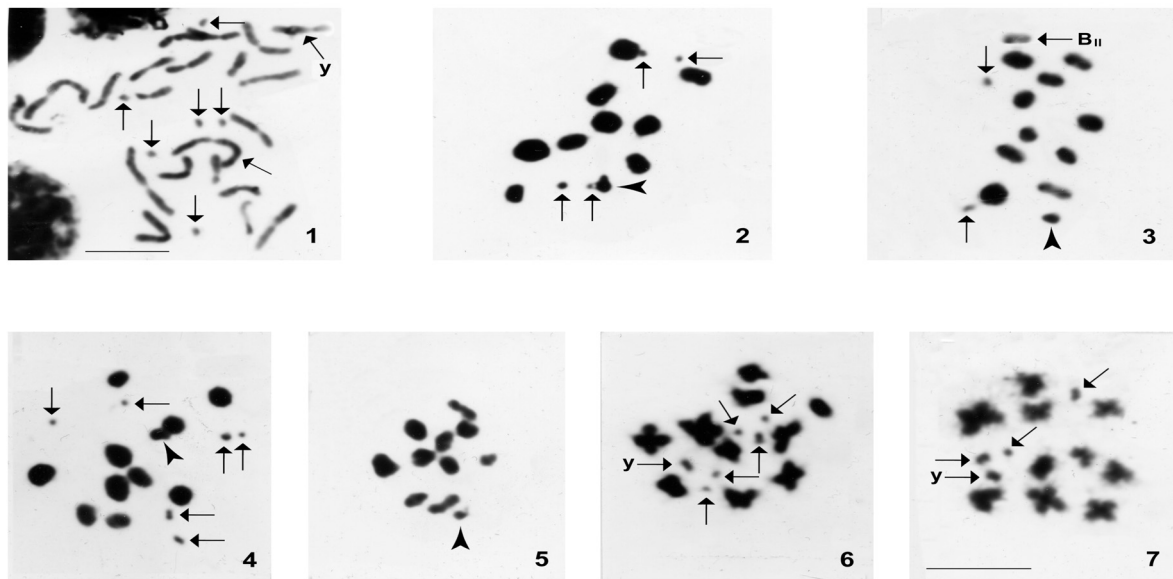
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Supernumerary (or B) chromosomes are additional chromosomes that are found in a wide variety of plants and animals (JONES & REES 1982; BEUKEBOOM 1994). B-chromosomes are nonvital (occurring in only some individuals within a species) and typically have accumulation mechanisms that increase their transmission prior, during, or following gametogenesis (NUR 1962). The origin of B-chromosomes is not clearly known. They have probably arisen from A chromosomes but follow their own evolutionary pathway. In addition, their irregular mitotic and meiotic behaviour allows them to accumulate selfishly in the germline, enabling non-Mendelian inheritance with transmission rates exceeding those of normal chromosomes (CAMACHO *et al.* 2000; PERFECTTI & WERREN 2001).

B-chromosomes were first reported in Coleoptera by STEVENS (1908) in two leaf beetle species of the genus *Diabrotica*. Since then, they have been recorded in over sixty species and subspecies of beetles belonging to eleven different families (SMITH & VIRKKI 1978; SERRANO 1981; ANGUS 1982, 1983; MESA & FONTANETTI 1984; VIDAL 1984; MADDISON 1985; SERRANO *et al.* 1986; KIDO & SAITOH 1987; NOKKALA & NOKKALA 1987, 1989a, b; VIRKKI & SANTIAGO-BLAY 1993; PROENÇA *et al.* 1999; MAFFEI *et al.* 2000; SANCHEZ-GEA *et al.* 2000). B-chromosomes were, however, recorded in four weevil species (ENNIS 1972; SMITH & BROWER 1974; DEY 1989) from among 600 curculionid species examined karyologically. The present communication has added one more species to this list.

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Figs 1-7. B chromosomes in *Barypeithes pellucidus*. Fig. 1. Mitotic plate with 6 Bs. Fig. 2. Metaphase I with 4 Bs; the association of one B with one pair of autosomes, two Bs separately and one Bs associated with X. Fig. 3 Metaphase I with 4 Bs; two joined Bs forming a bivalent and two well separated Bs. Fig. 4. Metaphase I with 6 well separated Bs. Fig. 5. Metaphase I without Bs. Fig. 6. Metaphase II with 5 Bs. Fig. 7. Metaphase II with 3 Bs. Arrows indicate B chromosomes, arrowheads indicate Xy_p . Bar = 10 μm .

Material and Methods

For the cytogenetic study, adult weevils were collected in an oak-hornbeam forest (Bratislava – Horský park, SW Slovakia) in May 2004 by sifting through leaf litter. The gonads of males were dissected and used as material for squashes. The gonads were fixed according to the method described by ROŽEK (1994) with minor modification (ROŽEK & HOLECOVÁ 2000; ROŽEK & LACHOWSKA 2001). Observations of chromosomes and photomicrographs were made using a Jenaval light microscope (C. Zeiss, Jena). The material is deposited in the Institute of Systematics and Evolution of Animals (Kraków).

Results and Discussion

The diploid male complement of *Barypeithes pellucidus* is $2n = 22 + 0-6B$ and the meioformula is $n\sigma = 10 + Xy_p + 0-6B$. *Barypeithes pellucidus* was karyologically examined by TAKENOUCI (1965) who confirmed the same autosome number and the presence of the sex bivalent forming a typical parachute. B-chromosomes were not present in the Canadian population studied by this author. According to the present observation the karyotype is composed mainly of metacentric and submetacentric autosomes with slight differences in size. The X chromosome is metacentric and is one of the longest elements while the y is the smallest

of the set with a median position of the centromere. The B-chromosomes were observed in spermatogonia and primary spermatocytes in ten of eleven males examined. In the non-B-carrying male examined, the diploid chromosome number was 22. Consequently, eleven elements ($10 + Xy_p$) were observed in metaphase I. The sex-bivalent was a bipartite complex of usual parachute-shape (Fig. 5).

In the B-carriers, one to six B chromosomes of different size could be recognize during spermatogenesis (Figs 1-4, 6-7). The Bs were of course free and did not pair with any member of the regular chromosome complement (Figs 6,7).

The association of a pair of Bs (B_{II}) bivalent and fusion Bs with X chromosome (XyB_p) were observed, and rarely an association of Bs with the regular autosomal bivalents.

The tripartite XyB_p complex was markedly visible in male metaphase I (Fig. 2). The XyB_p was quite peculiar in its configuration because it is a complex of three chromosomes of different size in a parachute association: their size was $X > B \geq y$. The occurrence of Bs in the second meiotic metaphase was quite random (0-6 B) (Figs 6, 7). A very similar meiotic behaviour of the Bs was described in the Japanese longhorn beetle *Xenicotela pardalina* (KIDO & SAITOH 1987).

According to the present state of knowledge the number of supernumerary or B-chromosomes in Coleoptera ranges from 1 to 32. Beetles occasionally show an unusually high number of Bs. In

the leaf beetle *Galerucella nymphaeae*, up to 10 Bs per cell have been encountered sometimes (NOKKALA & NOKKALA 1989b), in a Japanese ladybird *Chilocorus rubidus* up to 13 (SMITH & VIRKKI 1978). MESA & FONTANETTI (1983) published a preliminary count of 16 to 32 Bs per spermatocyte I in a population of the South American buprestid, *Euchroma gigantea*. Among weevils, B chromosomes have been recorded only in five species, viz. *Gelus californicus* (ENNIS 1972), *Sitophilus zeamais* (SMITH & BROWER 1974), *Astychus* sp., *Phytoscaphus inductus* (DEY 1989) and *Barypeithes pellucidus* (the present study). A single B-chromosome per cell was observed in Indian weevils *Astychus* sp., *Phytoscaphus inductus* having the standard curculionid chromosome formula $2n = 22, n\sigma = 10 + Xy_p$. B-chromosomes were smaller than the X and y and did not participate in Xy_p or autosomal formations (DEY 1989). ENNIS (1972) has been observed from 2 to 4 Bs per cell in *Gelus californicus* which has the lowest reported chromosome number within the Curculionoidea superfamily ($2n = 12 + X0$). In this weevil species the size and the behaviour of the Bs are similar to that of the y_p chromosome of the allied species. It has also been suggested that in *Gelus californicus*, where the sex chromosome system is $X0$, the Bs and X chromosomes are post-reductional and they are probably direct derivatives of X_p and y_p , respectively (ENNIS 1972). On the other hand, in *Sitophilus zeamais*, where the sex chromosome system is neo-XY, the B-chromosomes might have originated either from y_p or a centric fragment which was expelled from the standard karyotype during neo-XY formation. The number of Bs in this species varied usually from 3 to 6 per cell (SMITH & VIRKKI 1978). Up to six Bs could be observed in the Slovak population of *Barypeithes pellucidus* examined in the present study. The behaviour of supernumerary chromosomes was very complicated during metaphase I. Inspection of metaphase I plates showed B-chromosomes to be largely euchromatic and able to pair if more than one B was present in a cell. A similar phenomenon was described in some other beetle families, viz. Lampyridae, Cerambycidae, Chrysomelidae etc. (SMITH & MAXWELL 1953; KIDO & SAITOH 1987; NOKKALA & NOKKALA 1989a,b). The incorporation of the B chromosome to the sex bivalent that resulted in the formation of the tripartite complex designated as XyB_p was observed in Curculionidae for the first time. Within the present state of knowledge on B-chromosomes in weevils, it is difficult to comment on their genesis in *Barypeithes pellucidus*.

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