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Research Article

B-chromosomes in two Brazilian populations of *Dendropsophus nanus* (Anura, Hylidae)

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Abstract

We report on the presence of B-chromosomes in two populations of *Dendropsophus nanus* (= *Hyla nana* Boulenger, 1889) from São Paulo State, Brazil. Such chromosomes were observed in 4 out of 43 specimens (9.3%) and in 9 out of 15 specimens (60%) from the municipalities of Nova Aliança and Botucatu, respectively. The karyotype 2n = 30 + 1B found in *D. nanus* was similar to that of other species with 2n = 30 chromosomes, except for the presence of an additional small telocentric chromosome. In one specimen from Botucatu, cells with one to three extra chromosomes were observed. These B-chromosomes appeared as univalent in meiosis I and did not bear a nucleolar organizer region or exhibit constitutive heterochromatin.

Key words: Anura, b-chromosome, cytogenetics, *Dendropsophus*, supernumerary chromosome.

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Introduction

B-Chromosomes are extra chromosomes that occur in animals and plants and are generally considered dispensable for normal development, since they have no apparent function (Jones and Rees, 1982). B-chromosomes have been found in approximately 15% of living species (Beukeboom, 1994), and described in 26 species of salamanders and frogs (Green, 2004). As a rule, carriers of these chromosomes are phenotypically indistinguishable from those individuals without them (Clark and Wall, 1996). B-chromosomes bear no similarity to the autosomes, are inherited according to a non-Mendelian pattern, and occur as univalents in meiosis (Jones and Rees, 1982; Green, 1991, 2004).

The number of B-chromosomes can vary among populations of the same species, among individuals in a population and among cells in an individual. In the latter case, this variation results from anaphase lag, with subsequent elimination of B-chromosomes from some cells or tissues, or, alternatively, it is caused by mitotic non-disjunction, with sister chromatids migrating to the same pole (Clark and Wall, 1996).

Among vertebrates, one of the largest variations in the number of B-chromosomes has been described in *Leiopelma hochstetteri*, a frog endemic to New Zealand, in which individuals with up to 16 of these chromosomes have been observed (Green, 1988). This variation has been attributed to mitotic non-disjunction resulting from instability during cell division.

In Anura, B-chromosomes have been described in 10 species belonging to six families: Hylidae (*Acris crepitans*, *Hypsiboas* sp. aff. *circundata*) (Nur and Nevo, 1969; Baldissera *et al*., 1993), Leiopelmatidae (*Leiopelma hochstetteri*) (Green *et al*., 1987, 1993; Green, 1988, 2004; Sharbel *et al*., 1998), Discoglossidae (*Discoglossus pictus*) (Schmid *et al*., 1987), Pelobatidae (*Scaphiopus hammondi*) (Green, 1991), Ranidae (*Amolops liangshanensis*, *Rana everetti*, *R. temporaria*) (Ullerich, 1967; Schmid, 1978; Wu and Zhao, 1985; Kuramoto, 1989; Belcheva and Sofianidou, 1990), and Leptodactylidae (*Megaelosia massarti, Gastrotheca espeletia*) (Rosa *et al*., 2003; Schmid *et al*., 2002). Anuran B-chromosomes vary in size, in the amount of heterochromatin and repeated DNA sequences, and in number. Most B-chromosomes in anurans are derived from the A set of chromosomes in the corresponding species and have apparently undergone cumulative evolutionary changes since their formation (Green, 2004).

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Herein we describe the presence of B-chromosomes in specimens of *Dendropsophus nanus* (= *Hyla nana* Boulenger, 1889) from two populations collected at different sites in southeastern Brazil.

Materials and Methods

Forty-three specimens (40 males and 3 females) of *D. nanus* were collected in Nova Aliança (22°11'S, 49°42'W), São Paulo State (SP), Brazil, from February through May 1998 and from October 1998 through May 1999, and 15 specimens (all males) were collected in Botucatu (22°53'S, 48°26'W), SP, Brazil, in November 2000.

The specimens were identified according to Medeiros *et al.* (2003), and were deposited in the "Prof. Adão José Cardoso" Museum of Natural History (ZUEC), of Universidade Estadual de Campinas, SP, Brazil, and in the Zoology Department (DSJRP) of Universidade Estadual Paulista, São José do Rio Preto, SP, Brazil, under the accession numbers ZUEC 11405 to 11412, 11416 to 11418, 11647 to 11677, 12242 to 12272 and DZSJRP 1111. The frogs were collected with the permission of the Brazilian Institute for the Environment and for Natural Renewable Resources (Instituto Brasileiro do Meio Ambiente e Recursos Renováveis - IBAMA - Proc. 02001.008876/01-83).

Chromosome preparations were obtained from intestinal and testicular cell suspensions, as described by Schmid (1978) and Schmid *et al*. (1979) and analyzed after routine staining with a 10% Giemsa solution, C-banding (King, 1980), Ag-NOR staining (Howell and Black, 1980) and fluorescence *in situ* hybridization (FISH) (Viegas-Péquignot, 1992) with an rDNA probe. The FISH probe consisted of a recombinant HM123 plasmid containing a fragment of *Xenopus laevis* rDNA (Meunier-Rotival *et al*., 1979), which was biotin-labelled by nick translation reaction using a BioNick^{TM} Labeling System (Invitrogen). The probe was detected using goat anti-biotin and fluorescein anti-goat IgG (Vector Laboratories). Analysis was done with an Olympus BX60 photomicroscope. The chromosomal nomenclature regarding the position of centromere followed the classification proposed by Green and Sessions (1991).

Results

The karyotype of 39 specimens of *D. nanus* from Nova Aliança and of five specimens from Botucatu showed 2n = 30 chromosomes (Figure 1A), as previously described for a population from Nova Aliança (Medeiros *et al*., 2003). However, in four males from Nova Aliança (ZUEC 11673, 11651, 11652 and DZSJRP 1111) and nine males from Botucatu (ZUEC 12242, 12245, 12246, 12247, 12249, 12251, 12254, 12256 and 12261), the karyotype was 2n = 31 chromosomes (Table 1). This karyotype differed

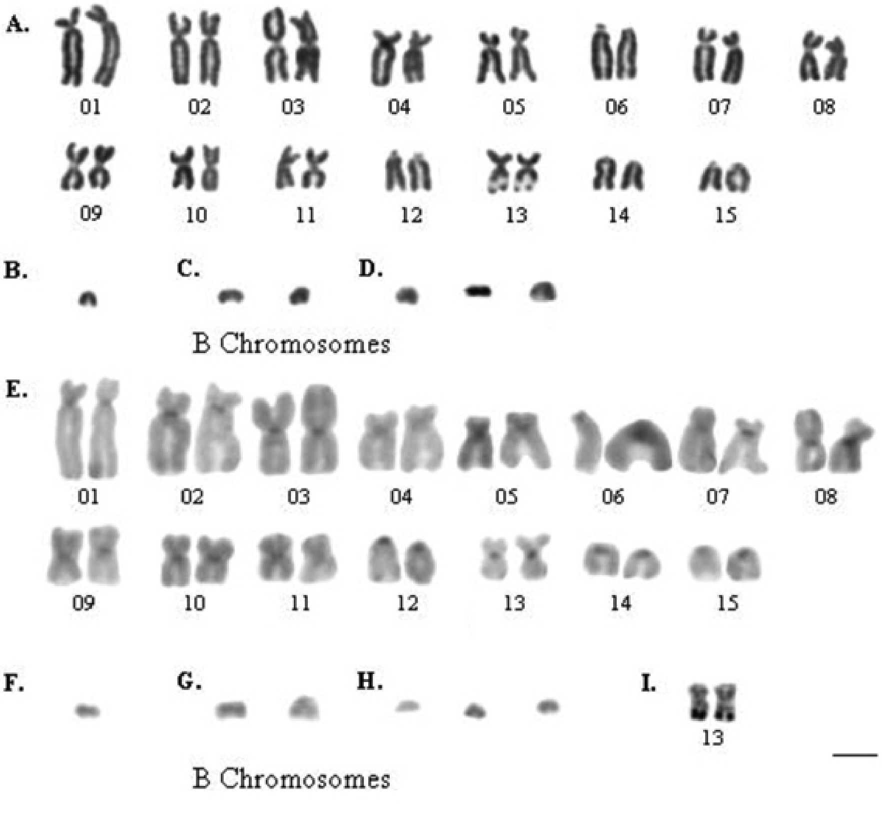

Figure 1 - Karyotype of *D. nanus* after Giemsa staining (A) and C-banding (E). One, two or three small telocentric B-chromosomes were observed in metaphases from some specimens after Giemsa staining (B-D) and C-banding (F-H). Ag-NORs were detected on chromosome pair 13 (I). Bar = 5 μm.

from that with 2n = 30 by the presence of an extra small telocentric chromosome (Figure 1B). In one specimen from Botucatu, we observed cells with 2n = 30 + 1B, 30 + 2B and 30 + 3B chromosomes (Figure 1B-D; Table 1). These additional chromosomes were all of the same size and morphology.

The small extra chromosome was observed in meiosis I metaphases that had 15 bivalents and one, two or three small univalents (Figure 2A-C). Hence, the B-chromosomes did not pair with the A-chromosomes. Individuals with 2n = 30 chromosomes had 15 bivalents, and no univalents were observed.

The two populations of *D. nanus* shared a common C-banding pattern characterized by small amounts of heterochromatin in the pericentromeric region of all chromosomes (Figure 1E), except for the extra chromosomes (Figure 1F-H), in which no C-bands were observed. These chromosomes did not bear a NOR, as demonstrated by Ag-NOR staining and/or FISH in four specimens with 2n = 30 + 1B chromosomes from Nova Aliança and in all specimens from Botucatu with 2n = 30 + 1B and 2n = 30 + 1 - 3B. Chromosome pair 13 was identified as the NOR-bearing chromosome in all specimens analyzed (Figures 1I and 3A, B).

Discussion

The extra chromosomes found in specimens of *D. nanus* with 2n = 31 and in the specimen from Botucatu that had cells with 2n = 31 to 2n = 33 chromosomes can be considered as B-chromosomes, since they showed some of the characteristics usually attributed to these chromosomes. This small extra element, which differs morphologically from autosomes, always occurs as a univalent in cells in metaphase I (reviewed in Jones and Rees, 1982).

Previous descriptions of the karyotype of *D. nanus* from other populations (Rabello, 1970; Bogart, 1973; Skuk and Langone, 1992) did not mention the presence of B-chromosomes.

Since individuals carrying B-chromosomes are generally indistinguishable from those without these chromosomes, the conclusion is that B-chromosomes must not carry genes with important phenotypic effects. However, the maintenance of B-chromosomes in certain populations has been taken as indicative of their role in conferring some advantage to transcriptional activity or to the genetic variability of the species, as pointed out by Belcheva and Sofianidou (1990), when describing B-chromosomes in *Rana temporaria*. Indeed, several studies have shown that some B-chromosome genes are expressed (Green, 1990; Jones, 1995; Covert, 1998; Camacho, 2000; Green, 2004), and their phenotypic influence may be dependent on the number of these chromosomes present in the cell, although this has not yet been documented for amphibians (Green, 1991). The deleterious effect can be a decrease in fertility (Hewitt *et al*., 1987) or an abnormal meiosis (Parker *et al*., 1981). An interesting case of B-chromosomes in amphibians involves *Leiopelma hochstetteri* (Anura), which may have up to 16 extra chromosomes. In this species, the B-chromosomes in the lampbrush state have small lateral loops indicating transcriptional activity, although lower than that of autosomes (Green *et al*., 1987; Green, 1988).

Table 1 - Museum accession numbers of specimens, sex, (locality of) collection site, number of metaphases analyzed and number of B-chromosomes per cell in *Dendropsophus nanus*.

Specimens (Museum accession number)	Sex	Collection site in the State of São Paulo	Number of metaphases analyzed		Number of metaphases with B-chromosomes		Number of B-chromosomes/cell
			Intestine	Testis	Intestine	Testis	
ZUEC 11651	M	Nova Aliança	19	17	11	12	1
ZUEC 11652	M	Nova Aliança	23	11	14	7	1
ZUEC 11673	M	Nova Aliança	12	8	5	6	1
DZSJRP 1111	M	Nova Aliança	14	12	8	6	1
ZUEC 12242	M	Botucatu	28	39	9	10	1
					6	9	2
					5	12	3
ZUEC 12245	M	Botucatu	24	6	18	9	1
ZUEC 12246	M	Botucatu	14	12	12	8	1
ZUEC 12247	M	Botucatu	8	5	5	3	1
ZUEC 12249	M	Botucatu	22	7	17	5	1
ZUEC 12251	M	Botucatu	12	10	8	8	1
ZUEC 12254	M	Botucatu	18	12	13	9	1
ZUEC 12256	M	Botucatu	28	14	23	9	1
ZUEC 12261	M	Botucatu	13	8	7	6	1

ZUEC = Museu de História Natural “Professor Adão José Cardoso”, Universidade Estadual de Campinas, SP, Brazil.
DZSJRP = Departamento de Zoologia, Universidade Estadual Paulista, São José do Rio Preto, SP, Brazil.

The B-chromosomes of *D. nanus* did not bear a NOR, as demonstrated by silver staining and FISH). Ribosomal genes are rarely present on B-chromosomes, and in Anura they have been detected only in *Scaphiopus hammondi* (Green, 1988) and *Gastrotheca espeletia* (Schmid *et al.*, 2002). In the former species, the NOR contributed to increase the number of nucleoli in the cell, indicating that B-chromosomes can carry functional genes of great importance (Green, 1991).

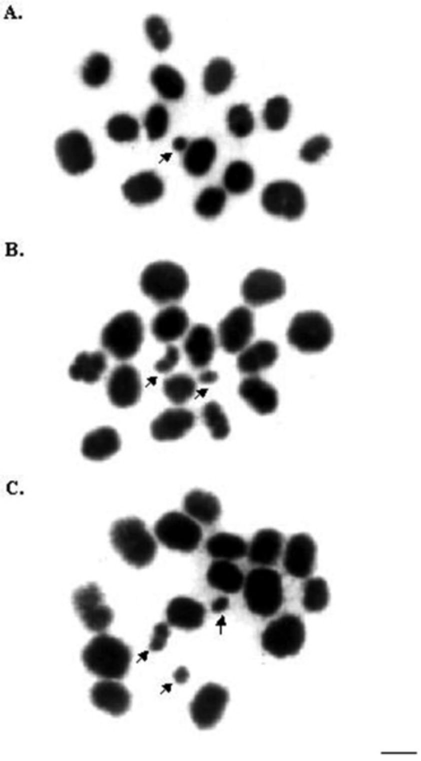

Figure 2 - *D. nanus* metaphases I with 15 bivalents and one (A), two (B) and three (C) small univalent B-chromosomes, after Giemsa staining. Bar = 5 μm.

Various mechanisms have been proposed to explain the origin and maintenance of B-chromosomes. Traditionally, these chromosomes are believed to be derived from autosomes (Jones and Rees, 1982). Since pairing or chiasmata between B-chromosomes and A-chromosomes are not observed in meiosis, it is probable that the original homology between them was rapidly lost. Modifications in the structure and in the pairing behavior during meiosis would prevent association with the ancestral A-chromosomes (Camacho *et al*., 2000). Interestingly, in the anuran *Leiopelma hochstetteri,* the B-chromosomes are most probably derived from the sex chromosome (Green, 2004), a conclusion supported by the sequence homology between the B-chromosomes and the univalent W chromosome (Sharbel *et al*., 1998; Green, 2004).

Heterochromatization is a common process in the differentiation of B-chromosomes, since many are completely heterochromatic in numerous species (Venere *et al*., 1999). Although the heterochromatic condition of B-chromosomes is not a general pattern, these chromosomes may originate from centromeric fragments, but this hypothesis has received little support (Green *et al.*, 1987; Camacho *et al.*, 2000). This process of B-chromosome differentiation is not applicable to *D. nanus*, since in this species the B-chromosomes showed no heterochromatic band, and hence their derivation must have involved different mechanisms.

Another possibility to explain the origin of B-chromosomes is that they derive from A-chromosomes of closely related species through interspecific hybridization, as suggested for hybrid species of the fish *Poecilia formosa* (Schartl *et al*., 1995) and of the wasp genus *Nasonia* (McAllister and Werren, 1997). *Dendropsophus sanborni* Schmidt, 1944, is morphologically (Cei, 1980; Medeiros *et al.*, 2003) and ecologically (Rossa-Ferres and Jim, 2001) very similar to *D. nanus*, and both belong to the same intrageneric group (Frost, 2004). Considering their great similarity and syntopic occurrence in several localities

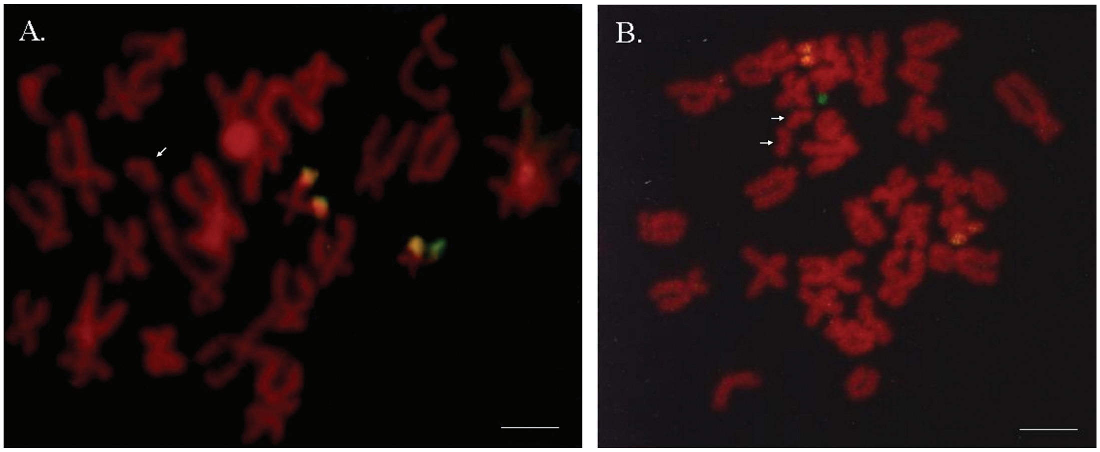

Figure 3 - *D. nanus* mitotic metaphases with one (A) and two (B) B-chromosomes (arrows) after fluorescence *in situ* hybridization to *Xenopus laevis* rDNA probe. No FISH signals were detected on the B-chromosomes. Bar = 5 μm.

(Langone and Basso, 1987), we cannot rule out the hypothesis of interspecific hybridization in the origin of *D. nanus* karyotypes with B-chromosomes.

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