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Possible origins of B chromosomes in *Rineloricaria pentamaculata* (Loricariidae, Siluriformes) from the Paraná River basin

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ABSTRACT. We made a cytogenetic study of *Rineloricaria pentamaculata* from the Tauá Stream, in the Pirapó River sub-basin in Paraná State, Brazil, focused on the occurrence and origins of the B chromosomes. The diploid number varied from 2n = 56 to 2n = 59, due to the presence of 0 to 3 B microchromosomes of the acrocentric type, which were observed in 92.3% of the specimens (N = 12). These chromosomes were totally heterochromatic, with the C banding technique, and there were inter- and intraindividual numerical differences. Meiotic cells in metaphase I had 28 bivalent chromosomes and 0 to 3 univalent chromosomes. We suggest that the B microchromosomes are centric fragments originated from chromosome rearrangements.

Key words: Heterochromatin; Loricariinae; B microchromosome; Meiosis; *Rineloricaria pentamaculata*; Univalent

INTRODUCTION

Supernumerary chromosomes and/or B chromosomes are usually characterized as additional elements that are non-homologous to the standard complement, not essential to the survival of the individual and morphologically different from the A complement. Although Mendelian segregation has not been observed (Jones and Rees, 1982), the proposed origin of these chromosomes was from the standard chromosome complement of the species (Jones and Rees, 1982) or crossing between closely related species (Bataglia, 1964; McAllister and Werren, 1997; Perfectti and Werren, 2001). B chromosomes in an individual may show a parasitic, neutral or beneficial behavior (Jones and Rees, 1982).

In species of Neotropical fishes, B chromosomes have been observed in 16 families in different hydrographic basins (Carvalho et al., 2008). The first description of these chromosomes in fish was described in *Prochilodus lineatus*, cited as *Prochilodus scrofa* (Pauls and Bertollo, 1983). The greatest number of species with supernumerary chromosomes occurs in Characiformes distributed in 31 species from six different families. Among the Siluriformes, 21 species belonging to the families Calichthyidae, Heptapteridae, Pimelodidae, Loricariidae, and Trichomycteridae were reported to possess B chromosomes (Carvalho et al., 2008). In loricariids, B chromosomes were found only in the Hypoptopomatinae and *Hisonotus leucofrenatus* subfamilies, cited as *Microlepidogaster leucofrenatus* (Andreata et al., 1993) and in the Loricariinae (*Loricaria* sp and *L. prolixa*; Scavone and Júlio-Jr, 1994).

Besides possessing B chromosomes, the Loricariinae subfamily has shown great karyotypic variability ranging from 2n = 36 chromosomes in *Rineloricaria latirostris* (Giuliano-Caetano, 1998) to 2n = 74 chromosomes in *Sturisoma* cf. *nigrirostrum* (Artoni and Bertollo, 2001). Although *Rineloricaria* shows 64 valid species (Froese and Pauly, 2009) few cytogenetic studies have been conducted on this genus. The species analyzed from this genus have shown a great karyotypic diversity with diploid numbers ranging from 2n = 36 in *R. latirostris* (Giuliano-Caetano, 1998) to 2n = 70 in *Rineloricaria* n. sp (Alves et al., 2003). Chromosomal rearrangements of the Robertsonian type were considered to be the main mechanism for karyotypic evolution in this group.

The present study reports the first description of B chromosomes in the *Rineloricaria* genus and analyzes their behavior and distribution in mitotic and meiotic cells with the objective of understanding the mechanisms involved in its probable origin and evolution.

MATERIAL AND METHODS

Thirteen specimens of *R. pentamaculata* from the Tauá Stream (sub-basin of the Pirapó River and Paraná River basin, Brazil) were analyzed. The mitotic chromosomes were obtained from kidney cells (Bertollo et al., 1978). Constitutive heterochromatin was obtained by the Sumner (1972) technique. Meiotic chromosomes were obtained from male gonads according to Kligerman and Bloom (1977).

RESULTS AND DISCUSSION

The cytogenetic study of *R. pentamaculata* from the Tauá Stream showed diploid chromosome numbers ranging from 2n = 56 to 2n = 59. This variation was due to the presence

of 0 to 3 extra small chromosomes of the acrocentric type (Figure 1a, b, c). The occurrence of these chromosomes was detected in 12 of 13 specimens (92.3%), showing a high intrapopulation frequency. However, among the 4 populations of this species examined (Porto FE, Portela-Castro ALB and Martins-Santos IC, unpublished data), only the Tauá Stream ones (present study) have shown extra chromosomes. The intrapopulational variation of these chromosomes and the inter-individual frequency in this population ranging from 2.8 to 33.3% (Table 1), coupled with the fact that they are totally heterochromatic (Figure 1d), allowed them to be characterized as B microchromosomes.

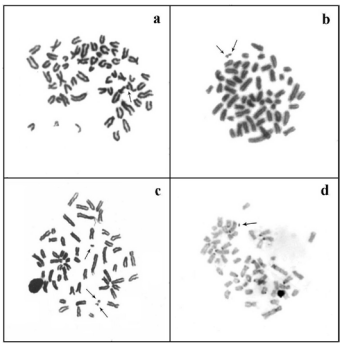

Figure 1. Somatic metaphases from *Rineloricaria pentamaculata*, from the Tauá Stream containing B chromosomes. **a.** One B chromosome. **b.** Two B chromosomes. **c.** Three B chromosomes. **d.** Distribution pattern of the constitutive heterochromatin. Arrows indicate the B chromosomes.

Table 1. Number of diploid cells analyzed in *Rineloricaria pentamaculata* from the Tauá Stream, showing the occurrence of B microchromosomes.

Specimens/sex	Number of B chromosomes/cells				Cells analyzed	Cells with B microchromosomes (%)
	0	1	2	3		
1/-	10	-	-	-	10	0%
2/-	9	2	-	-	11	18.1%
3/♂	29	3	1	-	32	12.5%
4/♂	32	14	1	-	47	31.9%
5/♂	29	2	-	-	31	6.4%
6/♂	36	7	-	-	43	16.3%
7/♂	24	4	-	-	28	14.3%
8/♂	38	4	-	-	42	9.5%
9/♂	55	10	3	1	69	20.3%
10/♂	28	7	4	-	39	2.8%
11/♂	6	2	-	-	8	25.0%
12/-	20	5	-	-	25	20.0%
13/-	6	3	-	-	9	33.3%
Total	323	63	9	1	345	-

Camacho et al. (2000) suggest that cells have mechanisms that promote rapid heterochromatinization of extra elements soon after their formation, avoiding a possible pairing between B and A complement chromosomes, thus leading to a differentiation in B chromosomes. The variation of inter- and intraindividual B chromosomes in *R. pentamaculata* reflects the high instability of the mitotic B chromosomes during cell division, also observed in the majority of B chromosomes found in Neotropical fishes. According to Jones and Rees (1982)

these variations are due to non-Mendelian segregation of chromosomes during cell division.

The analysis of male meiotic metaphases showed 28 bivalent chromosomes in metaphases I and II and small univalent ones in 49.02% of the cells examined (Figure 2b, c, d and Table 2). The majority of the cells displayed only a single univalent chromosome (31.4%). However, cells in meiotic metaphase II with up to 3 univalent ones were observed (Figure 2d). These data reinforce the classification of these elements as B chromosomes, indicating meiotic instability in the transmission to offspring.

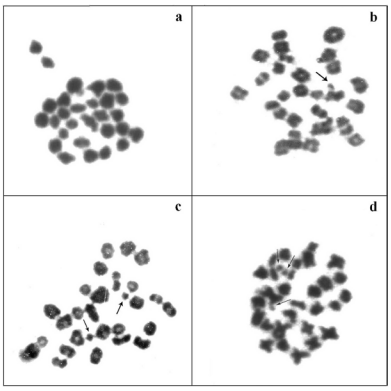

Figure 2. Meiotic metaphases in *Rineloricaria pentamaculata*. Metaphase I: **a.** No B chromosome. **b.** One B chromosome. **c.** Two B chromosomes. Metaphase II: **d.** Three B chromosomes. Arrows indicate the B chromosomes.

Table 2. Number of haploid cells found in males of *Rineloricaria pentamaculata* from the Tauá Stream, indicating the occurrence of B chromosomes in metaphase I.

Specimens	Number of B chromosomes/cells				Cells analyzed
	0	1	2	3	
4	3	2	-	-	5
6	18	11	4	5	38
8	5	3	-	-	8
Total	26	16	4	5	51

In both types of cell division, a greater number of cells were observed with no chromosomes than those with only one B chromosome. According to Camacho et al. (2000), the small number of these chromosomes in diploid cells represents the maximum that a species is able to tolerate in adults.

The cytogenetic study in the *R. pentamaculata* population from Tauá Stream was shown to have two karyomorphs, sympatric and syntopic, with a constant diploid number of 2n = 56 (Porto FE, unpublished data). The standard karyotype in 57.15% of individuals was associated with a karyotype formula of 8m/sm + 48st/a and FN = 64 while 42.85% of the specimens showed a karyotype formula of 9m/sm + 47st/a and FN = 65. This difference was due to the presence of one heteromorphic chromosome pair (submeta/acrocentric). Meiotic non-disjunction and centric fusion rearrangements were suggested as being responsible for the formation of the submetacentric chromosome of the heteromorphic pair (Porto FE, unpub-

lished data). The B chromosomes were present in both karyomorphs: specimens with the standard karyotype and specimens with the heteromorphic pair. Several authors have discussed the origin of B chromosomes, but in general, it has been widely accepted that they derived from the standard complement (Jones and Rees, 1982). Camacho et al. (2000) suggest that these elements are a secondary product of the standard karyotype evolution and also consider that these elements could have derived from A chromosome polysomy, from centric fragments resulting from fusions or from the amplification of the paracentromeric region of the A chromosome. Thus, we suggest that centric fragments from chromosomal rearrangements that formed the heteromorphic pair in *R. pentamaculata* from the Tauá Stream may be responsible for the origin of the B microchromosomes observed in this population (Figure 3).

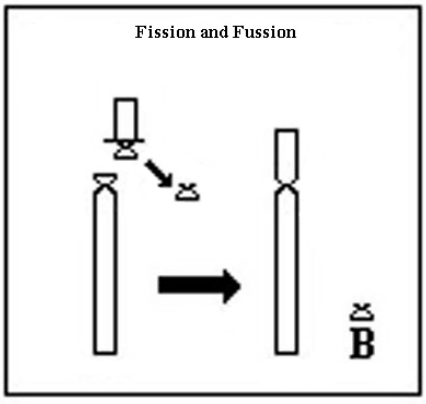

Figure 3. Scheme proposed to explain the origin of B chromosomes in *Rineloricaria pentamaculata* from the Tauá Stream.

In the Loricariinae subfamily, B chromosomes were observed only in *Loricaria* sp and *L. prolixa* (Paraná River) present in 100% of the specimens examined, with high intra- and interindividual variation, with the number ranging from 0 to 5 in both species (Scavone and Júlio-Jr, 1994).

For the family Loricariidae, besides the occurrence of B chromosomes in the Loricariinae already mentioned, there are also reports of these chromosomes in two populations of *Hisonotus leucofrenatus*, cited as *Microlepdogaster leucofrenatus*, the Hypoptopomatinae subfamily (Poço Grande River and Marumbi River; Andreata et al., 1993). In this study, the authors observed a frequency of 1-2 B chromosomes showing high intraindividual mitotic stability.

The characteristics shown by the B chromosomes of *R. pentamaculata* as heterochromatic, mitotic and high meiotic instability, where there is a greater number of cells without B chromosomes, suggest that their origin is recent in the genus and that they play an apparently neutral role in the genome of this species.

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