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Supernumerary chromosome inheritance in the curimbatá *(Prochilodus lineatus)* of the Mogi-Guaçu River

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Prochilodus lineatus is widely used in pisciculture projects, mainly in the south of Brazil. It shows a basic karyotype composed of $2n = 54$ chromosomes, in addition to as many as seven supernumerary chromosomes, also known as B-chromosomes. These additional small chromosomes are frequently heterochromatic, vary in number and morphology, and generally have no homology with the A complement. Intensive studies have investigated the function, origin and inheritance of these supernumerary chromosomes. The present study aimed to determine the inheritance pattern of the B-chromosomes resulting from directed cross-fertilization of *P. lineatus*, Prochilodontidae isolated from the Mogi-Guaçu River, Pirassununga, SP. These cross-fertilization experiments were performed at CEPTA/ICMBio in Pirassununga. The transmission patterns of these micro-chromosomes agreed ($K_B = 0.48$) with the expectation of regular meiotic behavior following a Mendelian transmission model ($K_B = 0.5$). A non-accumulation process was observed for these B-chromosomes in filial generations. Together, our results indicate that the supernumerary chromosomes in *P. lineatus* samples from the Mogi-Guaçu River exist in a neutral state and follow a Mendelian inheritance pattern.

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The eukaryotic genome is not always restricted to genes found in the A chromosome set. It frequently contains a set of supernumerary elements that do not seem to follow the rules of Mendelian inheritance (Camacho et al. 2000). B chromosomes are dispensable DNA fragments that carry some functional genes and exhibit an irregular transmission model. In many cases, the presence of B-chromosomes lowers the fitness of their carriers (Bakkali et al. 2002).

B-chromosomes are found in some individuals of a same population of plants or animals (Jones and Rees 1982; Jones and Puertas 1993; Jones 1995) and in several fungi (Mills and McCluskey 1990; Miao et al. 1991a, 1991b; Tzeng et al. 1992; Gêiser et al. 1996; Leclair et al. 1996). A primary characteristic of these chromosomes is their lack of recombination with complementary chromosomes. These supernumerary DNA elements tend to propagate through a separate evolutionary path (Camacho et al. 2000). These structural elements have been identified in diverse karyotypes, but little is known about their structure, function or behavior.

The inheritance pattern of B-chromosomes has been intensively studied. Almost all of these studies have shown that these elements usually exhibit an accumulation mechanism, explaining their parasitic nature (Jones 1991). However, some cases have been identified in which a frequency differentiation exists between B-chromosomes among several populations (Chiavarino et al. 1995). The variation in the transmission pattern of B-chromosomes is an ordinary aspect of inheritance. The manner in which they are lost in some progenies and increased in number in others is always compared to the Mendelian expectation (Chiavarino et al. 1998).

Genetic control of these transmission patterns has been demonstrated in some animals, including the grasshopper *Myrmeleotettix maculatus* (Shaw and Hewitt 1985; Shaw et al. 1985), mealy bug *Pseudococus affinis* (Nur and Brett 1987, 1988), the grasshopper *Eyprepocnemis plorans* (Herrera et al. 1996) and in some plants including *Hypochoeris maculata* (Parker et al. 1982), *Aegilops speltoides* (Cebriá et al. 1994), and *Allium schoenoprassum* (Bougourd and Plowman 1996).

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Two equilibrium models have been proposed, according to the presence of accumulation mechanisms and their possible effects. The heterotic model suggests a balance between the positive effects of B-chromosomes on host forms that are present when the B-chromosomes occur at low numbers and the negative effects that are a result of high numbers of B-chromosomes. However, this model does not account for an accumulation mechanism (White 1973). The parasitic model (Östergren 1945; Nur 1966a, 1966b, 1977), or selfish model (Jones 1985; Shaw and Hewitt 1990), assumes that B-chromosomes are maintained in the population by accumulation mechanisms, which counterbalance their deleterious effects on the host genome.

Zurita et al. (1998) proposed a non-equilibrium model of long term B-chromosome evolution. According to this model, a parasitic B-chromosome that has lost its accumulation mechanism is destined to disappear from the population. This disappearance is predicted to occur by a fast, slow or very slow mechanism, in a long, random extinction process that ultimately reaches a mitotic and/or meiotic stabilization stage known as neutralization. As a result of this stabilization process, a new B-chromosome variation could arise and reinitiate the cycle (Herrera et al. 1996).

B-chromosomes are expected to be transmitted with a frequency of 0.5 because the transmission of a certain characteristic involves the contribution of one half of the genetic information from each progenitor. Because these chromosomes are mitotically and meiotically unstable, their probability of transmission is expected to be low (Camacho et al. 2000). However, many B-chromosomes display a transmission rate that is clearly higher than 0.5. This results in an accumulation which is the most important property of parasitic B-chromosomes.

The first evidence of B-chromosomes in neotropical fishes was identified in *Prochilodus lineatus* (Pauls and Bertollo 1983). However, studies concerning supernumerary chromosome inheritance in fishes remain scarce (Oliveira et al. 1997). The process of supernumerary chromosome inheritance has been identified in *Poecilia formosa* as a mechanism of paternal inheritance (Schartl et al. 1995). Oliveira et al. (1997) carried out an initial analysis of the inheritance pattern of B-chromosomes in *P. lineatus* from the Mogi Guaçu River by direct crosses. The transmission pattern observed in their study was $K_B = 0.511$, consistent with the Mendelian expectation ($K_B = 0.5$).

Based on the preliminary analyses of the inheritance pattern of supernumerary chromosomes in fishes, especially in the *P. lineatus* species, this study aimed to provide a deep analysis of the transmission pattern of B-elements in *P. lineatus* through directed cross experiments. This study identifies the maintenance relationships of these micro-chromosomes among natural populations and pisciculture elements.

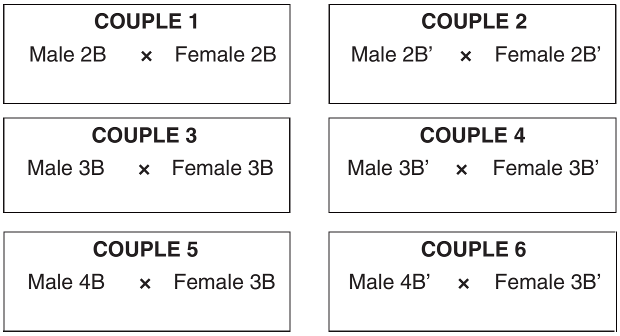

Fig. 1. Crosses involving two females and six males distributed according to their respective supernumerary chromosome numbers. These crosses formed a total of six couples.

MATERIAL AND METHODS

Two groups of *Prochilodus lineatus* samples were cytogenetically analyzed. The first group was composed of six couples formed by gamete combination involving six males and two females. The first 2B carrier female was crossed with a 2B carrier male, as well as with another male that possessed 2B in its karyotypic set. The second 3B carrier female was crossed with two 3B carrier males, as well as with two other males displaying 4B in their karyotypic sets (Fig. 1). Individuals utilized as parents were captured from the Mogi-Guaçu river natural population in Pirassununga, São Paulo, Brazil. Induced reproduction was performed at the Instituto Chico Mendes de Conservação da Biodiversidade, Centro Nacional de Pesquisa e Conservação de Peixes Continentais (CEPTA/ICMBio) in the municipality of Pirassununga, SP.

The second group was composed of 63 individuals obtained by direct cross-fertilization, representing a filial generation. For the analysis, individuals in the filial generation resulting from cross-fertilizations where parents showed equal B-chromosome numbers were grouped, resulting in three possibilities from the three directed crosses (Fig. 2).

Parental chromosome preparations were obtained by lymphocyte culture using the method described by Fenocchio and Bertollo (1988) with some adjustments for this species. Chromosome preparations for the filial generation were obtained using fragments from the anterior kidney tissues following the method of Foresti et al. (1981). All parents were maintained in a fish pond at CEPTA/ICMBio for further studies.

Chromosome morphology was determined as proposed by Levan et al. (1964), and chromosomes were classified

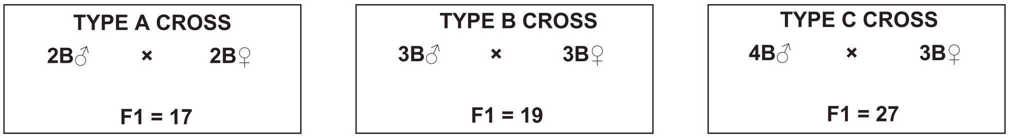

Fig. 2. Formation of three kinds of *Prochilodus lineatus* couples through grouping of parentals by equal B chromosome numbers with their respective filial generations (F_1). The ♂ Symbol indicates male individuals and the ♀ symbol indicates female individuals.

as metacentric (m), submetacentric (sm), subtelocentric (st) or acrocentric (a).

For determination of B-chromosome modal numbers, 30 metaphase cells were analyzed per individual. B-chromosome transmission patterns (K_B) were investigated using the Z test, according to the method of López-Léon et al. (1992).

RESULTS AND DISCUSSION

Eight exemplars of the parental generation, obtained from the Mogi-Guaçu River natural population, Pirassununga, SP, and 63 individuals of the filial generation, obtained by direct crosses, were cytogenetically analyzed. As shown in Fig. 3, in the first crosses where both parents possessed two B-chromosomes, the proportions of B-chromosome manifestation in the filial generation were: 35.8% of individuals carrying one B-chromosome, 25% of individuals showing two B, 25% of individuals showing three B-chromosomes and 14.2% with four B-chromosomes in their genomic conjunct (Type A Cross). In the second cross, where both parents possessed three B-chromosomes, the proportions manifested in the filial generation were: 11% of individuals carrying one B-chromosome, 21% showing two B-chromosomes, 42% carrying three B-chromosomes, 16% carrying four B-chromosomes, and 10% showing five B-chromosomes (Type B Cross). In the final, where males carried four B-chromosomes and females carried three B-chromosomes, the proportions manifested in the filial generation were: 4% showing one B-chromosome, 22% carrying two B-chromosomes, 51% showing three B-chromosomes, 12% carrying four B-chromosomes and 11% showing five B-chromosomes (Type C Cross). These data represent the proportion of supernumerary chromosomes transmitted to the filial generation. Based on these crosses, we determined that the B-chromosome number present in the filial generation never exceeded the sum of the number of B-chromosomes in the respective parents.

In one the few studies on the transmission pattern of supernumerary chromosomes in fishes, Oliveira et al. (1997) described the supernumerary chromosome inheritance in *P. lineatus.* They found that transmission of these micro-chromosomes was consistent with a Mendelian model ($K_B = 0.511$).

Our data shows that the transmission pattern of these genomic elements agrees with the Mendelian expectation ($K_B = 0.48$) (Table 1). These results support the conclusions of Oliveira et al. (1997).

The K_B values obtained for B-chromosome frequency in individuals from different crosses indicate that B-chromosome transmission ($K_B = 0.48$) in *P. lineatus* follows a Mendelian inheritance pattern ($K_B = 0.5$). The possibility of accumulation of B-chromosomes in one sex and their elimination in the other cannot account for the observed B-chromosome inheritance pattern. Specifically, all crosses performed have involved both male and female B-chromosome carriers. This assertion is corroborated by the Oliveira et al. (1997) study.

The Mendelian inheritance pattern for B-chromosomes in *P. lineatus* that we observed following induced reproduction at the CEPTA/ICMBio pisciculture is representative of the B-chromosome maintenance pattern in the Mogi-Guaçu River natural population. According to Cavallaro et al. (2000), the supernumerary chromosomes of the Mogi-Guaçu River natural population have undergone a neutralization process.

Accumulation, neutralization and extinction of these supernumerary chromosomes are related to their method of transmission among carrier generations. An increase in meiotic instability causes an accumulation of these supernumerary elements in the environment. Accumulation of

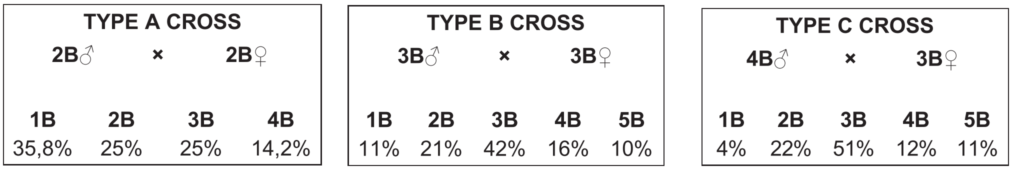

Fig. 3. Proportions of B chromosomes present in the F_1 generation resulting from induced crosses of the respective parental generation. The ♂ symbol indicates male individuals and the ♀ symbol indicates female individuals.

Table 1. *B chromosome transmission pattern in controlled crosses of the Prochilodus lineatus species.*

Types of crosses		B-chromosome number In F_1 generation									B	B transmission index		
♂	♀	0	1	2	3	4	5	6	7	Total	average	KB	Z	P
2	2		7	4	4	2				17	2.05	0.51	0.12	NS
3	3		2	4	8	3	2			19	2.94	0.49	–0.07	NS
4	3		1	6	14	3	3			27	3.03	0.43	–0.68	NS
	Total	0	10	14	26	8	5	0	0	63	2.68	0.48		

K_B B chromosome transmission pattern; Z values higher than +1.96 indicate B chromosome accumulation and Z values lower than –1.96 indicate B chromosome elimination; NS not significant.

these chromosomes is due to transmission frequency above the Mendelian expectation. However, if B-chromosomes reach meiotic stability, the transmission pattern becomes coherent with the Mendelian expectation, reflecting the neutralization stage observed in this work. Finally, a decrease in the B-element frequency of a given chromosome in a generation results in a transmission frequency below the Mendelian expectation, promoting the extinction of the elements from the carrier population.

BAKKALI et al. (2002) analyzed the supernumerary chromosome inheritance pattern in populations of grasshopper, *E. plorans*, based on the Mendelian transmission expectation ($K_B = 0.5$). Their study explored the B_1-chromosome transmission pattern (originating from the Iberian population) in females of this species from three different Morocco populations: Mechra (near Mechra-bel-Ksiri), SO.DE.A (near Ksar-el-Kebir) and Simir (between Ceuta and Tetouan). Following directed crosses, the authors showed that the transmission pattern of the B_1-chromosome in the Mechra population showed an accumulation process ($K_B = 0.575$), as compared to the transmission patterns of the SO.DE.A ($K_B = 0.512$) and Simir ($K_B = 0.463$) populations. These data confirmed the presence of different evolutionary stages for the same B_1-chromosome in different populations. Under the long term evolutionary model, the Mechra population showed a younger B_1 polymorphism, resulting in an accumulation of this supernumerary chromosome through the generations. In the two other grasshopper populations, the B_1-chromosome transmission process exhibited a neutralizing behavior and followed a Mendelian inheritance pattern.

The pattern of supernumerary chromosome transmission in *P. lineatus* is consistent with the Mendelian expectation, supporting the view that this polymorphism is stabilized in the wild population from the Mogi-Guaçu River. This data supports the conclusions of the OLIVEIRA et al. (1997) and CAVALLARO et al. (2000) studies.

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