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Transmission Behavior of B Chromosomes in *Prochilodus lineatus* (Characiformes, Prochilodontidae)

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Key Words

Cytogenetic · Inheritance · Mendelian rate · Supernumerary chromosomes

Abstract

The population of *Prochilodus lineatus* found in the Mogi-Guaçu River is karyotypically polymorphic, carrying acrocentric, metacentric, and submetacentric B chromosomes. The analysis of each B chromosome frequency in this species revealed a variation in the distribution pattern, with the metacentric type having the highest frequency (73.30%), followed by submetacentric (25.22%) and acrocentric B chromosomes (1.48%). The transmission pattern of the supernumerary chromosomes was identified by controlled crosses, and it was shown that the acro- and submetacentric B chromosomes have a transmission pattern below the Mendelian rate (k_B = 0.333 and k_B = 0.385, respectively), but the metacentric variant has a cumulative transmission pattern (k_B = 0.587). These results indicate that the acro- and submetacentric B chromosomes are undergoing an extinction process, while the metacentric B chromosomes appear to be accumulating in frequency with each generation.

B chromosomes have aroused great interest in the scientific community, because they appear to be dispensable yet still persist in populations without being eliminated by natural selection [Carvalho et al., 2008].

Because of various studies on supernumeraries in fish, their remarkable variation in size and morphology can be highlighted, e.g. the genus *Astyanax* has macro-, micro-, and medium-sized supernumeraries, showing meta-, submeta-, and acrocentric morphologies. In addition to the variation in size and morphological types, B chromosomes can also vary in number. Erdtmann et al. [1990] described the occurrence of up to 16 B microchromosomes in *Callichthys callichthys*. However, in *Astyanax* the most common pattern described is the occurrence of only 1 supernumerary chromosome [Daniel et al., 2012].

The Prochilodontidae fish family is included among the Characiformes, whose geographical distribution is restricted to South America [Mago-Leccia, 1972]. According to the Ministério da Pesca e Aquicultura [2011], representatives of the Prochilodontidae family are the highest producers for the continental extractive fishery with a yield of 28.643 t in 2010 and thus have an important role in the fishing industry of Brazil.

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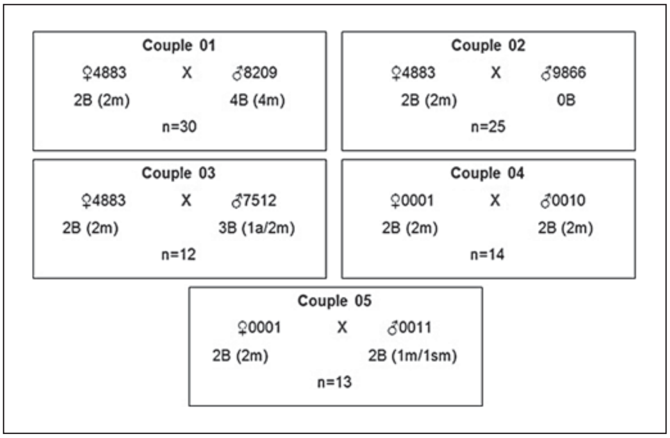

Fig. 1. Controlled crosses of *P. lineatus* conducted at CEPTA/ICMBio. a = Acrocentric, m = metacentric, and sm = submetacentric B chromosome variants, n = number of juveniles obtained from each crossing. The number of magnetic tag in specimens used in the crossings is also indicated.

The occurrence of B chromosomes in the Prochilodontidae family was first described in *Prochilodus lineatus* by Pauls and Bertollo [1983]. These B chromosomes were easily identified as heterochromatic microchromosomes with a variable frequency of 0–9 elements [Voltolin et al., 2011]. Furthermore, Artoni et al. [2006] reported polymorphisms among the supernumerary chromosomes in the population of *P. lineatus* from the Mogi-Guaçu River, representing 3 morphotypes identified as meta-, submeta-, and acrocentric B chromosomes.

Studies about the inheritance of supernumerary chromosomes in neotropical fish are still quite scarce in the literature, and *P. lineatus* is the most representative species studied in relation to the inheritance of B chromosomes in neotropical fish. Oliveira et al. [1997] and Voltolin et al. [2010a] conducted studies about the transmission rate of B chromosomes in *P. lineatus* and found that it was consistent with the expectation of regular meiotic behavior of B chromosomes, according to a Mendelian rate (k = 0.5). Due to the ease of fish capture and handling as well as the high frequency and polymorphisms of supernumerary chromosomes, several studies have used *P. lineatus* as a neotropical fish model investigating the origin, frequency, and evolution of supernumerary chromosomes. However, very little is known about the mechanisms of accumulation, transmission patterns, and meiotic behavior of these extra elements. Hence, the purpose of the present study was to investigate the frequency of B chromosome variants in a natural population of *P. lineatus* to better understand their transmission pattern through controlled breeding. Furthermore, possible events that are responsible for the maintenance of B chromosomes in the population were examined.

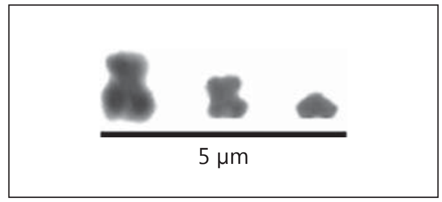

Fig. 2. Morphological types of supernumerary chromosomes found in *P. lineatus*.

Materials and Methods

A cytogenetic analysis of 136 specimens of *P. lineatus* captured from the Mogi-Guaçu River, Cachoeira de Emas, Pirassununga, SP, Brazil was performed. These specimens formed the breeding stock maintained in the breeding tanks of CEPTA/ICMBio, Pirassununga, SP, Brazil. The broodstock were cytogenetically analyzed and cataloged according to the number and morphology of supernumerary chromosome variants present in their somatic cells. Breeders were marked with magnetic tags for later identification during the reproductive period following the protocol proposed by Porto-Foresti et al. [2001]. During the reproductive period of this species (occurring between November and December 2012), a number of matrices were selected and used to induce spawning, which resulted in a filial generation of 94 juveniles from 5 successful crosses (fig. 1).

Cytogenetic analysis of the breeding stock was performed using the lymphocyte culture technique described by Fenocchio and Bertollo [1988] with some adjustments. Analysis of the filial generation was performed using the traditional in vivo method for obtaining metaphase chromosomes [Foresti et al., 1981]. The transmission rate of B chromosomes (k_B) was calculated from the mean number of B chromosomes in the progeny divided by the total number of B chromosomes in the parents according to the method of López-León et al. [1992a]. The analyzes of the transmission of B chromosomes in controlled crosses were performed by the Z-test according to López-Léon et al. [1992b] and χ^2 test to perform frequency comparisons with previous samples in the same population found in the literature by means of contingency tables. Data archiving was not relevant to the results presented in the article.

Results

Cytogenetic analyses of 136 specimens of *P. lineatus* revealed a consistent diploid number of 54 meta- and submetacentric chromosomes in addition to the presence of up to 7 B chromosomes in somatic cells, as has been

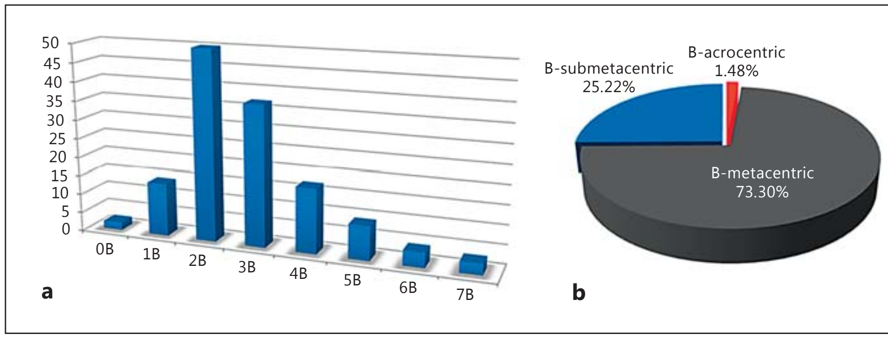

Fig. 3. a Frequency of B chromosomes in each individual of *P. lineatus* from the natural populations of the Mogi-Guaçu River. **b** Frequency of supernumerary variants in *P. lineatus* from the Mogi-Guaçu River obtained from the analysis of 136 specimens between 2011 and 2012.

Table 1. Historical frequency of B chromosomes in the population of *P. lineatus* from the Mogi-Guaçu River

Period	B chromosome frequency								Total	$\bar{x}_B$	Reference
	0B	1B	2B	3B	4B	5B	6B	7B			
1979–1980	18	5	13	6	0	2	0	0	44	1.341	Pauls and Bertollo [1983]
1987–1989	1	11	22	21	15	7	0	0	77	2.766	Cavallaro et al. [2000]
1991–1992	0	0	6	4	5	1	0	0	16	3.063	Oliveira et al. [1997]
2003–2007	8	26	101	84	39	11	5	1	275	2.614	Voltolin et al. [2010b]
2011–2012	2	14	50	37	17	9	4	3	136	2.816	this study

$\bar{x}_B$ = Average number of B chromosomes.

previously described [Pauls and Bertollo 1983, 1990; Penitente et al., 2013].

Among the supernumerary elements, we found 3 different types of B microchromosomes (acrocentric, metacentric, and submetacentric; fig. 2), as was previously described by Artoni et al. [2006]. An interesting feature of B chromosomes in this species is that each type of supernumerary is present in a different frequency in the natural population, with the metacentric type having the highest prevalence at 73.30%, followed by the submetacentric type at 25.22% and the acrocentric type with a rare occurrence in the population at 1.48% (fig. 3b).

The overall frequency of B chromosomes in each individual shows that B non-carrier specimens of *P. lineatus* are rare in the natural population of the Mogi-Guaçu River. Among 136 specimens captured and analyzed, only 2 animals without supernumerary elements were reported. Our analysis also detected 14 individuals carrying 1 B chromosome, 50 with 2, 37 with 3, 17 with 4, 9 with 5, 4 with 6 and 3 with 7 B chromosomes (fig. 3a). The modal numbers of supernumerary elements identified in this sample population were 2 or 3 B chromosomes ($\bar{x}$M = 2.816. These data are consistent with those described by Cavallaro et al. [2000], Oliveira et al. [1997], and Voltolin et al. [2009], who studied B chromosomes in *P. lineatus* from the Mogi-Guaçu River and suggested a stable frequency (table 1). In contrast, intraindividual variation was not found in our study, as was previously proposed by Oliveira et al. [1997] and Cavallaro et al. [2000] and confirmed by Voltolin et al. [2010b]. Nevertheless, the stabilization of B chromosome frequencies in *P. lineatus* does not apply to all the variant forms. We found a variation in frequency of the different morphotypes by observing the k_B obtained from the cytogenetic analysis of the offspring derived from crosses made in 2012. We analyzed 5 controlled crosses involving the 3 morphotypes of B chromosomes (table 2), where the metacentric type showed most of k_B above the Mendelian rate ($k_B > 0.5$) while other types had values below ($k_B < 0.5$). However, when we performed the Z-test, there were no significant differences (Z > 1.96 in absolute values).

The metacentric chromosomes have a mean value of k_B = 0.587, while the acrocentric and submetacentric variants have a $k_B = 0.333$ and $k_B = 0.385$, respectively (table 3). Previous analyses of the 3 variant frequencies between 2004 and 2007 showed that the metacentric type (53.18%) was more prominent than the submetacentric (40.48%) and acrocentric (6.34%) chromosomes in the same population

Table 2. Transmission rates of B chromosomes in controlled crosses of *P. lineatus*

Cross		Variant	Number of offspring with B chromosomes							$\bar{x}_B$	B transmission	
♀	♂		0B	1B	2B	3B	4B	5B	total		k_B	Z
2B(2m)	4B(4m)	Bm	–	1	2	12	13	2	30	3.433	0.572	0.791
2B(2m)	0B	Bm	1	16	8	–	–	–	25	1.280	0.640	1.400
2B(2m)	3B(1a/2m)	Bm	–	–	5	5	2	–	12	2.750	0.688	1.305
		Ba	8	4	–	–	–	–	12	0.333	0.333	–1.159
2B(2m)	2B(2m)	Bm	–	4	7	2	1	–	14	2.000	0.500	0
2B(2m)	2B(1m/1sm)	Bm	–	6	6	1	–	–	13	1.615	0.538	0.275
		Bsm	8	5	–	–	–	–	13	0.385	0.385	–0.833

$\bar{x}_B$ = Average number of B chromosomes. Z indicates significant differences if >1.96 in absolute value. p was not significant in all crosses.

Table 3. Transmission rate of B chromosomes

B chromosome morphotype	$\bar{x}k_B$
Acrocentric	0.333
Metacentric	0.587
Submetacentric	0.385

[Penitente et al., 2013]. The comparison of these data sets with those from our current study using χ^2 test by means of contingency tests reveals a significant change in the variant frequencies in less than 10 years (χ^2 = 21.844, p = 0.00001; table 4) with an increased frequency of the metacentric B chromosome and, consequently, a reduction in frequency of the other chromosome types.

Discussion

The maintenance of supernumerary chromosomes in natural populations has been discussed in relation to 2 models. In the heterotic model proposed by White [1973], it is assumed that in the absence of drive the equilibrium frequency of B chromosomes could be maintained by the benefit that they confer to the carriers with low numbers. Conversely, in the parasitic model discussed by several authors [Östergren, 1945; Nur 1966a, b, 1977; Jones, 1985; Shaw and Hewitt, 1990; Camacho et al., 2000] it is considered that B chromosomes remain in populations due to their own means of accumulation. In the latter case, these chromosomes would be seen as parasitic elements, because their presence would not bring any advantage to the carriers.

The evolutionary dynamics of parasitic B chromosomes seems to be characterized by a rapid invasion followed by neutralization through the evolution of suppressor genes on the chromosomes, which eliminate the accumulation effects of the supernumerary elements [Camacho et al., 1997]. In this model, the accumulation of B chromosomes may increase in a given population over only a few generations.

The observation of the historical frequency of B chromosomes in the natural population of *P. lineatus* of the Mogi-Guaçu River (table 1) allows for the characterization of the invasion of the supernumerary elements from the 1970s on due to the large number of non-carrier individuals and the low average frequency of B chromosomes compared to subsequent years. From the 1980s, a rapid stabilization in frequency is observed, with a modal number of 2 and 3 mitotically stable B chromosomes per individual, as well as the low occurrence of non-carrier individuals, which has remained constant until the present time. In this context, it is possible to characterize the B chromosomes in *P. lineatus* as probable parasitic elements. After the rapid invasion of these supernumerary elements in the population, their mechanisms of accumulation were probably deleted by mitotic stabilization from the 1980s and eventually reached a stage of neutralization [Cavallaro et al., 2000].

The invasion of B chromosomes has also been reported in other species. In the grasshopper *Eyprepocnemis plorans*, the parasitic B_{24} variant invaded the population of Torrox (Malaga, Spain), replacing the neutralized variant B_2 [Zurita et al., 1998]. In addition, the B_1 variant invaded the population of Pollensa (Mallorca, Spain), greatly increasing its frequency in just 10 years [Riera et al., 2004]. Likewise, in the wasp *Trypoxylon albitarse*, the fre-

Table 4. Comparative frequency of B chromosome variants of *P. lineatus* from the Mogi-Guaçu River between 2004–2007 and 2011–2012

Period	Frequency of B chromosome variants			Total	Reference
	acrocentric	metacentric	submetacentric		
2004–2007	9	75	57	141	Penitente et al. [2013]
2011–2012	5	247	85	337	this study
Total	14	322	142	478	

$\chi^2 = 21.844$, p = 0.00001, d.f. = 2.

quency of B chromosomes increased from 0.133 to 0.962 in the population of Nova Ilha (Porto Firme, Brazil) between 1997 and 1999 [Araújo et al. 2002].

Furthermore, between 2004 and 2007, Penitente et al. [2013] analyzed the transmission and inheritance pattern of the B chromosome variants *in P. lineatus* through controlled crosses and found a lower k_B for the acrocentric type chromosome ($k_B = 0.388$), which is lower than the Mendelian rate ($k_B = 0.500$), but the metacentric and submetacentric types have a neutral transmission pattern ($k_B = 0.507$ and $k_B = 0.526$, respectively). These authors proposed that the acrocentric B chromosomes are undergoing an extinction process due to the low transmission pattern and could disappear unless new mutations occur that ensure their permanence in the population. Nevertheless, if previously obtained k_B values are compared with those from our present work, it can be noted that aside from the acrocentric type, the submetacentric type chromosome is also entering an extinction phase, because its transmission pattern is below the Mendelian rate ($k_B = 0.385$). Conversely, the metacentric B variant has an increase in k_B from 0.507 to 0.587 in a few generations. However, despite our results showing different k_B values from the Mendelian rate, when we applied the Z-test there were no significant differences, which may be related to the low number of progeny analyzed in each cross. Apart from the k_B values, the frequency in the natural population is another variable that indicates a change, with the acrocentric and submetacentric variants experiencing a reduction in recent years, whereas the metacentric chromosomes are increasing in the last years (table 4). The χ^2 test by contingency tables between the years 2004–2007 [Penitente et al., 2013] and 2011–2012 (this study) showed a significant difference regarding the frequency of morphotypes in the natural population ($\chi^2 = 21.844$, p = 0.00001). Therefore, the variation in the frequency of different types of B chromosomes in the natural population is our most reliable variable complementing the k_B values obtained in the crossings.

The current scenario shows a remarkable dynamic in the transmission pattern of B chromosomes in *P. lineatus*. In fact, while the acrocentric and submetacentric morphotypes are possibly in the process of extinction, the metacentric morphotype is accumulating in this species due to its active transmission rate demonstrated by the variation in frequency over the years.

According to Zurita et al. [1998] and Camacho et al. [2000], the life cycle of parasitic B chromosomes comprises 3 phases: invasion, neutralization, and extinction. In the neutral phase, supernumerary chromosomes require the presence of new mutations in their DNA sequences so they can reassume their cycle within the population; otherwise they would disappear from the population. The presence of B chromosome variants is an indication that one or more of these different forms can reacquire an accumulation strategy and thus ensure their permanence in the population.

The classic example of polymorphism found in the grasshopper *Eyprepocnemis plorans* shows that different types of supernumerary elements can occur [Henriques-Gil et al., 1984; Henriques-Gil and Arana, 1990]. The frequent generation of new variants in this species indicates a dynamic polymorphism in the carrier population [Lopez-Leon et al., 1994], in which each variant accumulates and increases its average frequency per individual [Camacho et al., 1997].

Different types of strategies are likely related to the mechanism of B chromosome accumulation. Araújo et al. [2002] found that in the wasp *T. albitarse*, one metacentric B morphotype prevails over an acrocentric one, suggesting a higher transmission efficiency of this chromosomal form. Fishman and Saunders [2008] also considered that the accumulation mechanism might be related to the centromere, as observed in the plant species *Mimulus guttatus* during female meiosis. In turn, Endo et al. [2008] showed that non-disjunction of B chromosomes in wheat is controlled by a trans-acting element located in the distal region of the long arm of these chromosomes.

Regardless of the possible processes acting in the transmission of B chromosomes in *P. lineatus*, the results hitherto available provide evidence that these chromosomes are undergoing a dynamic cycle of invasion and neutralization within the population. Some forms of B chromosomes seem to be directed towards a probable extinction, while others, despite their parasitic nature, are being maintained in the population at the expense of accumulation strategies.

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Statement of Ethics

All institutional and national guidelines for the care and use of laboratory animals were followed.

Disclosure Statement

The authors have no conflicts of interest to declare.

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