Increased B chromosome frequency and absence of drive in the fish *Prochilodus lineatus*

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The neotropical freshwater fish species *Prochilodus lineatus* (Pisces, Prochilodontidae) shows $2n = 54$ chromosomes plus supernumerary microchromosomes ranging in number from zero to seven among different animals. The transmission rates of B chromosomes were studied by the analysis of the parental and F_1 generations in 10 controlled crosses performed with specimens from a natural population. The mean transmission rate observed for B chromosomes ($k_B = 0.511$) was consistent with that expected from a regular meiotic behaviour of Bs in both sexes and with the theoretical value under a Mendelian mode of transmission (0.5). Possible explanations for the dramatic increase in B frequency observed in this population during the last 10 years are discussed, bearing in mind the current absence of drive.

Keywords: B chromosome evolution, B chromosomes inheritance, fish cytogenetics, supernumerary chromosomes.

Introduction

Supernumerary or B chromosomes are found in about 15 per cent of all species of both plants and animals (Jones & Rees, 1982; Beukeboom, 1994). However, the structure, function and behaviour of these supernumerary chromosomes are quite particular in different groups, making it difficult to reach a general conclusion about their importance for the species.

Despite the fact that only a few studies have been undertaken concerning the inheritance of B chromosomes, almost all the results have demonstrated that they usually show a mechanism of accumulation (drive), which explains their selfish nature (Jones, 1991). However, at least two species, *Allium schoenoprasum* (Bougourd & Parker, 1979; Bougourd & Plowman, 1996) and *Eyprepocnemis plorans* (López-León *et al.*, 1992), do not show such accumulation mechanisms and represent two cases in which the selfish model is now being submitted to critical test. Whereas Bs in *A. schoenoprasum* seem to be essentially heterotic because of the advantage of B-carrying plants during seed to seedling development (Holmes & Bougourd, 1989), B chromosomes in *E. plorans* were originally selfish but have been neutralized, thus losing drive, by the presence of

drive-suppressor genes in the A genome (Herrera *et al.*, 1996; Camacho *et al.*, 1997; J. P. M. Camacho *et al.*, unpublished observations).

B chromosomes apparently are not widespread among fishes. Thus, among about 2800 karyotyped species (Klinkhardt *et al.*, 1995), only 40 species with B chromosomes have been described (1.5 per cent). Such a reduced frequency may represent a real situation or, alternatively, may reflect the fact that many of the early cytogenetic studies on fishes were performed on a very small number of specimens and usually employed low-resolution techniques. The first occurrence of B chromosomes among neotropical freshwater fishes was reported by Pauls & Bertollo (1983) in *Prochilodus lineatus* (= *P. scrofa*), and this species was recently restudied by Cavallaro (1992). Considering that the species *P. lineatus* has been bred successfully in captivity and has been used extensively in aquaculture programmes in Brazil, the objective of the present study was to analyse the mode of inheritance of B chromosomes in this fish species. No previous studies involving B chromosome inheritance have been performed in fishes, apart from the demonstration of paternal inheritance in the gynogenetic fish *Poecilia formosa* (Schartl *et al.*, 1995). The results showed an absence of drive for *P. lineatus* Bs, which may provide another useful test for the selfish theory of B chromosome evolution.

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Materials and methods

Two groups of *P. lineatus* specimens were analysed: the first consisted of 10 couples formed by gamete combination involving six females and 10 males (two females were crossed with two males each and a third female was crossed with three males), captured from a natural population in the Mogi-Guaçu river (Pirassununga, State of São Paulo, Brazil) in 1991/1992; these represented the parental generation. The second group consisted of 131 specimens obtained from the parental generation in controlled crosses and represented the F₁ generation.

Chromosome preparations from kidney tissue were obtained using the method of Foresti *et al.* (1993). For the establishment of the modal number of Bs, 30 cells with $2n = 54$ A chromosomes were analysed in each individual. The transmission rate of B chromosomes (k_B) was investigated using the Z-test (López-León *et al.*, 1992), and karyotypic frequencies were compared by the nonparametric Kruskal–Wallis and Mann–Whitney tests.

Results

The present study confirms the diploid number of $2n = 54$ chromosomes in *P. lineatus* as observed previously by Pauls & Bertollo (1983) and Cavallaro (1992). The chromosomes of this species are metacentric or submetacentric ranging in size from 2.5 to 5.0 μm .

The analysis of 16 specimens collected in the Mogi-Guaçu river in 1991/1992 and their F₁ offspring (131 specimens) showed the presence of zero to seven supernumerary chromosomes of very small size (about 0.7 μm) and very similar morphology (metacentric). As shown in Table 1, previous studies of B chromosome frequency performed on this population by Pauls & Bertollo (1983) and Cavallaro (1992) on specimens collected in 1979/1980 and 1987/89, respectively, showed a dramatic increase in mean B frequency from 1.341 to 2.766 during this period (Mann–Whitney test: $z = 5.18$, $P < 0.0001$), whereas the mean number of

Bs was only slightly and nonsignificantly increased between 1987/89 and the present study (Mann–Whitney test: $z = 0.90$, $P = 0.37$).

All of the 10 controlled crosses showed an absence of significant drive for the B chromosomes, with a high consistency among crosses (Table 2). The mean rate of B chromosome transmission ($k_B = 0.511$) observed in the 10 controlled crosses was consistent with that expected from regular meiotic behaviour of Bs in both sexes and also closely similar to the 0.5 Mendelian theoretical value.

Therefore, no net accumulation or elimination of B chromosomes seems to exist in *P. lineatus*, which is in accordance with the fact that the mean number of Bs in the 131 F₁ progeny analysed (3.212) did not differ significantly from that observed in the 16 adult individuals (3.063) used as the parental generation (Mann–Whitney test: $z = 0.17$, $P = 0.86$).

However, the possibility that Bs are accumulated in one sex and eliminated in the other cannot be ruled out on the basis of the present results, because all our crosses involved males and females carrying Bs. The high frequency reached by the B chromosomes, however, virtually prevents the detection of zero B individuals in the natural population and, hence, it was not possible to analyse uniparental B transmission.

In the present study, the number of cells with B chromosome numbers differing from the modal number for the individual was very low (3.4 per cent), thus indicating a low degree of mitotic instability for the B chromosomes in the natural population of *P. lineatus* from the Mogi-Guaçu river. The same situation was observed by Cavallaro (1992) in the specimens analysed 2 years before. These observations contrast with the high mitotic instability of Bs in this same population reported by Pauls & Bertollo (1983) in a sample collected about 10 years before, which reached 44.1 per cent. These data suggest either a substitution of the original B chromosome type by a B variant with a higher mitotic stability or a progressive coadaptation

Table 1 Evolution of B chromosome frequency in the population of *Prochilodus lineatus* from the Mogi-Guaçu river

Year	Number of individuals with								Total	Mean no. of Bs	References
	0B	1B	2B	3B	4B	5B	6B	7B			
1979–80	18	5	13	6	0	2	0	0	44	1.341	Pauls & Bertollo (1983)
1987–89	1	11	22	21	15	7	0	0	77	2.766	Cavallaro (1992)
1991–92 (parents)	0	0	6	4	5	1	0	0	16	3.063	This paper
1991–92 (offspring)	1	5	40	33	39	10	2	1	131	3.212	This paper

Table 2 Transmission analysis of B chromosomes in controlled crosses involving couples of wild specimens of *Prochilodus lineatus*

Type of cross		Code	Number of specimens from the F ₁ generation with modal number of Bs								Mean number of Bs	B transmission			
♀	♂		0	1	2	3	4	5	6	7		Total	<i>k</i> _B	<i>Z</i>	<i>P</i>
2	2	C1	1	3	14	4	2				24	2.1250	0.5313	0.3062	NS
2	2	C2		2	4	4	1				11	2.3636	0.5909	0.6030	NS
		Total	1	5	18	8	3				35	2.2000	0.5500	0.5916	NS
3	3	C3			4	6		2			12	3.0000	0.5000	0.0000	NS
3	3	C4			1	2	6	1			10	3.7000	0.6167	0.7379	NS
		Total			5	8	6	3			22	3.3182	0.5530	0.4975	NS
4	4	C5			1	3	12	1			17	3.7647	0.4706	−0.2425	NS
2	3	C6			5	6					11	2.5455	0.5091	0.0603	NS
2	4	C7			5	1	4				10	2.9000	0.4833	−0.1054	NS
5	2	C8			5	3	2	4			14	3.3571	0.4796	−0.1527	NS
5	4	C9				2	4	1	1	1	9	4.4444	0.4938	−0.0370	NS
5	4	C10			1	2	8	1	1		13	3.9231	0.4359	−0.4623	NS
		Total			1	4	12	2	2	1	22	4.1364	0.4596	−0.3790	NS
Totals			1	5	40	33	39	10	2	1	131	3.5442	0.5111		

k_B , transmission rate of B chromosomes. Z indicates significant differences if greater than 1.96 in absolute value. NS, not significant.

between Bs and the host genome, leading to a regularization of the mitotic behaviour of the Bs. In any case, the consequence seems to be the fixation of a mitotically stable B chromosome system in this population.

Discussion

Studies on the temporal stability of B chromosome polymorphisms have shown that some populations reach a stable frequency over periods from 6 to 9 years (Hewitt & John, 1967; Jackson & Cheung, 1967; Kayano *et al.*, 1970). Some populations of mealy bugs (Nur, 1966) and rye (Jones & Rees, 1982) showed an increase in the number of B chromosomes over some generations, similar to that observed in *P. lineatus* from the Mogi-Guaçu river. On the other hand, a decrease in B frequency was observed in a Japanese rye population under strong selective pressure (Moss, 1966). As pointed out by Jones & Rees (1982), these examples, taken together, provide strong evidence for a marked influence of varying kinds of selection on B chromosome frequency.

Two kinds of selection may favour the increase in B frequency: (i) phenotypic selection, in which case Bs should be considered 'heterotic' by providing a

beneficial effect on carrier fitness (White, 1973); and (ii) nonphenotypic selection, caused by the existence of drive (accumulation mechanisms), in which case Bs should be considered 'selfish', because they would be maintained in natural populations despite being harmful to carriers (see Henriques-Gil & Arana, 1990). Ascertaining which of these two kinds of selection process is responsible for B maintenance in natural populations is a major task, demanding extensive investigation in many species. Although most Bs seem to fit the selfish model, there are some cases lacking significant drive, which, at first glance, are good candidates for heterotic Bs. This could be the case for *P. lineatus* Bs in the Mogi-Guaçu river population, if the increase in frequency from 1979/80 to 1987/89 resulted from a beneficial effect on carriers. Although this interpretation is a possibility that needs to be tested by the analysis of the phenotypic effects of Bs in this species, there is a second possibility that is strongly supported by recent findings in the grasshopper, *Eyprepocnemis plorans*, in which a B chromosome lacking drive and phenotypic effects on fitness shows a widespread geographical distribution (Camacho, 1993) that is incompatible with the hypothesis that the B never had drive. This species provides the first case of a B chromosome that was initially selfish but has been

neutralized by the evolution of drive-suppressor genes in the A genome (Herrera *et al.*, 1996; Camacho *et al.*, 1997). In *P. lineatus*, it is possible that the B was selfish in origin, facilitating its initial increase in frequency in the population, that it was still selfish in 1979/80, but that drive had been neutralized by 1991/92. Mitotic instability can be important in B chromosome accumulation (Jones, 1991), so in the present case a reduction in drive may have been accompanied by an increase in mitotic stability.

Further studies analysing (i) B chromosome inheritance in each sex separately, by performing crosses in which one of the parents lacks Bs, (ii) the genetic control of B chromosome transmission, for which purpose interpopulation crosses might be very useful, (iii) the existence of phenotypic fitness effects related to B presence, and (iv) B chromosome frequency in many other populations in order to determine large-scale geographical distribution of these Bs, will provide valuable information on the evolutionary significance of this B chromosome polymorphism in natural populations of *P. lineatus* and may contribute to the genetic improvement of lines in breeding programmes.

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