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Frequency increase and mitotic stabilization of a B chromosome in the fish *Prochilodus lineatus*

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Abstract

Six populations of the fish *Prochilodus lineatus* were analysed for B chromosome frequency. A study of spermatogenesis revealed the absence of B accumulation during the stages analysed. In one of the populations, from the Mogi-Guaçu river where samples have been analysed over a ten-year period, B chromosome frequency doubled between 1979–80 and 1987–89, whereas no additional changes were noticed in samples collected in 1991–92. The analysis of B chromosome mitotic instability, manifested by intraindividual variation in B chromosome number, indicated a very significant decrease during this time period. This suggests that, in the 1980s, this population was in the final stage of B chromosome invasion, and that there was a possible causal relationship between B mitotic instability and the accumulation mechanism that caused its frequency increase. Mitotic stabilization might thus be a way by which a mitotically unstable B chromosome may become neutralized.

Introduction

Supernumerary or B chromosomes are additional chromosomes carried by some individuals of many animal and plant species. The most widely accepted theory considers them as genome parasites that are maintained in natural populations as a result of accumulation mechanisms which drive up their frequency despite possible harmful effects on the host genome (Östergren 1945, Nur 1966, 1977, Jones 1985, Werren *et al.* 1987). As with other parasites, B chromosomes are subjected to a coevolutionary arms race with the host. The parasitic theory typically states that a B may reach frequency equilibrium as its drive gain is counteracted by loss through harmful effects on host fitness, although it has recently been shown that a B chromosome system may enter into a non-equilibrium state made up of several successive stages of drive, neutralization and polymorphism regeneration (Camacho *et al.* 1997). These different B manifestations emerge as a consequence of the arms race with the host genome. Any extra chromosome possessing an accumulation mechanism would be able to evolve into a B chromosome. Such a parasitic B would rapidly increase in frequency to become a burden to the host genome, whose

response should be to suppress B drive. Once neutralized, the B is condemned to extinction unless a new B variant occurs to subsequently prolong the polymorphism (for review, see Camacho *et al.* 2000).

There is a wide variety of accumulation mechanisms with which B chromosomes can assure their maintenance in natural populations (Jones 1991). One of these is the mitotic instability of B chromosomes coupled with preferential destiny of cells with higher B number towards the germ line. This mechanism was proposed by Nur (1969) for B chromosomes in several grasshopper species, and has been analysed in detail in the locust *Locusta migratoria* (Nur 1969, Kayano 1971, Viseras *et al.* 1990, Pardo *et al.* 1995).

In the present paper, we provide the first evidence for possible neutralization of a mitotically unstable B chromosome, a fact previously predicted by Oliveira *et al.* (1997). The coincidence of very significant B mitotic stabilization with an arrest in frequency increase in a population of the fish *Prochilodus lineatus* (formerly *P. scrofa*), suggests a mechanism (mitotic stabilization) through which a mitotically unstable B chromosome may lose accumulation and thus be neutralized.

Materials and methods

Prochilodus lineatus (*P. scrofa*) (Teleostei, Characiformes, Prochilodontidae) is one of the most abundant fish species in the high basin of the Paraná river, especially in the rivers Grande, Pardo and Mogi-Guaçu (Godoy 1975), and is extensively used in aquaculture programmes in Brazil. Capture–mark–recapture studies have shown a remarkable migratory behaviour in this fish, with maximum migration distances reaching 600–700 km (Godoy 1975, Toledo Filho *et al.* 1986). After spawning, the eggs are usually carried by the stream towards marginal small lakes that, in the dry season, are isolated from the river and present a very appropriate environment for juvenile growth.

A total of 185 individuals (99 males and 86 females) were collected at three natural populations, the Mogi-Guaçu river (MG; Pirassununga) and two marginal small lakes, Lagoa Infernão (LI) and Lagoa Nova (LN), and three piscicultures sited at Paraibuna (PAR), Pindamonhangaba (PIN) and the Universidade Federal de São Carlos (UFSCar), all of which are located in the São Paulo State, Brazil (Table 1). Mitotic and meiotic chromosomes were analysed from kidney and testis cells, respectively. Chromosome preparations were made following the procedure described by Bertollo *et al.* (1978) and C-banding was performed according to Sumner (1972). Spermatogenesis was analysed in 12 males.

Since B chromosomes in *P. lineatus* are mitotically unstable, a same individual shows a distribution of different B numbers in its cells. For this reason, to summarise B number in each individual we have used the median of the distribution of B numbers in a sample of cells, because it probably represents the number of B chromosomes in the zygote stage, as justified by Pardo *et al.* (1995) in the case of *Locusta migratoria*. In addition, these authors provided an appropriate index to quantify the mitotic instability of B chromosomes, *MI*, which is calculated as the sum of the absolute values of every deviation in

Table 1. Materials analysed, collection date and B chromosome frequency.

Population	Type	Year	Mean of median B number	SE	*n*
Mogi-Guaçu (MG)	Natural	1987–89	2.766	0.139	77
Lagoa Infernão (LI)	Natural	1986–89	2.379	0.207	29
Lagoa Nova (LN)	Natural	1986–89	2.920	0.282	25
Paraibuna (PAR)	Pisciculture	1989	1.893	0.181	28
Pindamonhangaba (PIN)	Pisciculture	1989	2.067	0.330	15
UFSCar	Pisciculture	1990	0.273	0.141	11
Total			2.389	1.323	185

B number with respect to the median (M), and normalized by dividing by the median and the number of cells analysed (N) so that the index is independent of the number of Bs and the sample size:

$$MI = \frac{\Sigma(|M - n_i|f_i)}{M.N}$$

where n_i is the number of B chromosomes in the different types of cell that do not coincide with M, and f_i is the number of cells of each particular type.

To estimate the median B number and the MI index, between 5 and 170 cells (mean 13.36 ± 1.02) were analysed per individual. Between-population comparisons of B frequency (median B number) and mitotic instability (MI) were performed by means of one-way ANOVA. Comparisons between germ cells at different developmental stages were done by means of Student's t-test for dependent samples. Condition was defined as the standardized residuals of a regression of body weight on standard length. This parameter was compared between populations and B classes by means of an ANCOVA.

Results

All populations analysed were polymorphic for the presence of B chromosomes. Although some variation was detected in B chromosome size (but always being the smallest elements in the chromosome complement) and morphology (acrocentric, submetacentric or metacentric), all B chromosomes were heterochromatic and most of them were metacentric (Figure 1).

B chromosomes are mitotically unstable as manifested by the intra-individual between-cell variation in B number. The median B frequency was significantly different between the six populations analysed ($F = 11.01$; $df = 5$, 179; $p < 0.000001$), but there was no difference between the three natural samples analysed (MG, LI and LN) ($F = 1.47$; $df = 2$, 128; $p = 0.233$). The lowest B frequency was observed in the UFSCar sample and was significantly lower than those in the two other pisciculture populations (PAR and PIN; ($F = 12.82$; $df = 2$, 51; $p = 0.00003$) (Figure 2).

The mitotic instability index (MI) differed significantly between the six populations analysed ($F = 7.23$; $df = 5$, 165; $p = 0.000004$), and also between the three natural ones ($F = 12.22$; $df = 2$, 126; $p = 0.000014$) (Figure 3).

In the MG population, an analysis of B frequency change and mitotic instability through time was possible by comparing the present sample (collected in 1987–89; see Cavallaro 1992) with those collected in 1979–80 (Pauls & Bertollo 1983) and in 1991–92 (Oliveira *et al.* 1997). Compared with the 1979–80 sample, the 1987–89 sample showed a significantly higher median B number and a lower MI index (Table 2). In contrast, the sample collected in 1991–92 did not differ from the 1987–89 sample for the mean number of Bs nor the mitotic instability measured by the proportion of cells with a non-modal B number (comparison performed by Oliveira *et al.* 1997). Therefore, it is clear that the critical period for the B chromosome polymorphism was the 1980s, during which time B frequency doubled and the MI index decreased more than 100 times (see Table 2).

The possibility of meiotic drive was analysed in males by directly observing B frequency in spermatogonia and metaphase II spermatocytes. For this comparison, B frequency at metaphase II was doubled to account for meiotic reduction in the first division. Student's t analysis revealed no significant difference in B frequency between both stages ($t = 1.897$; $df = 11$; $p = 0.084$). Likewise, a comparison of the MI index failed to show significant differences ($t = 1.44$; $df = 11$; $p = 0.179$). Therefore, our data do not indicate B chromosome meiotic drive in males, at least until the metaphase II stage.

Finally, a regression analysis of body weight (dependent variable) on standard length (independent variable) showed a very significant relationship between these two body measures ($r = 0.903$; $t = 27.8$; $df = 174$; $p < 0.000001$). This permitted the definition of body condition as the standardized residual of the former regression. An ANCOVA with condition as dependent variable, population as random factor and median B number as a covariate, revealed no significant B effect ($F = 3.66$; $df = 1$, 169; $p = 0.057$) but significant differences between populations ($F = 4.39$, $df = 5$, 169; $p = 0.00088$). Similar ANCOVAs, with body weight and standard length as dependent

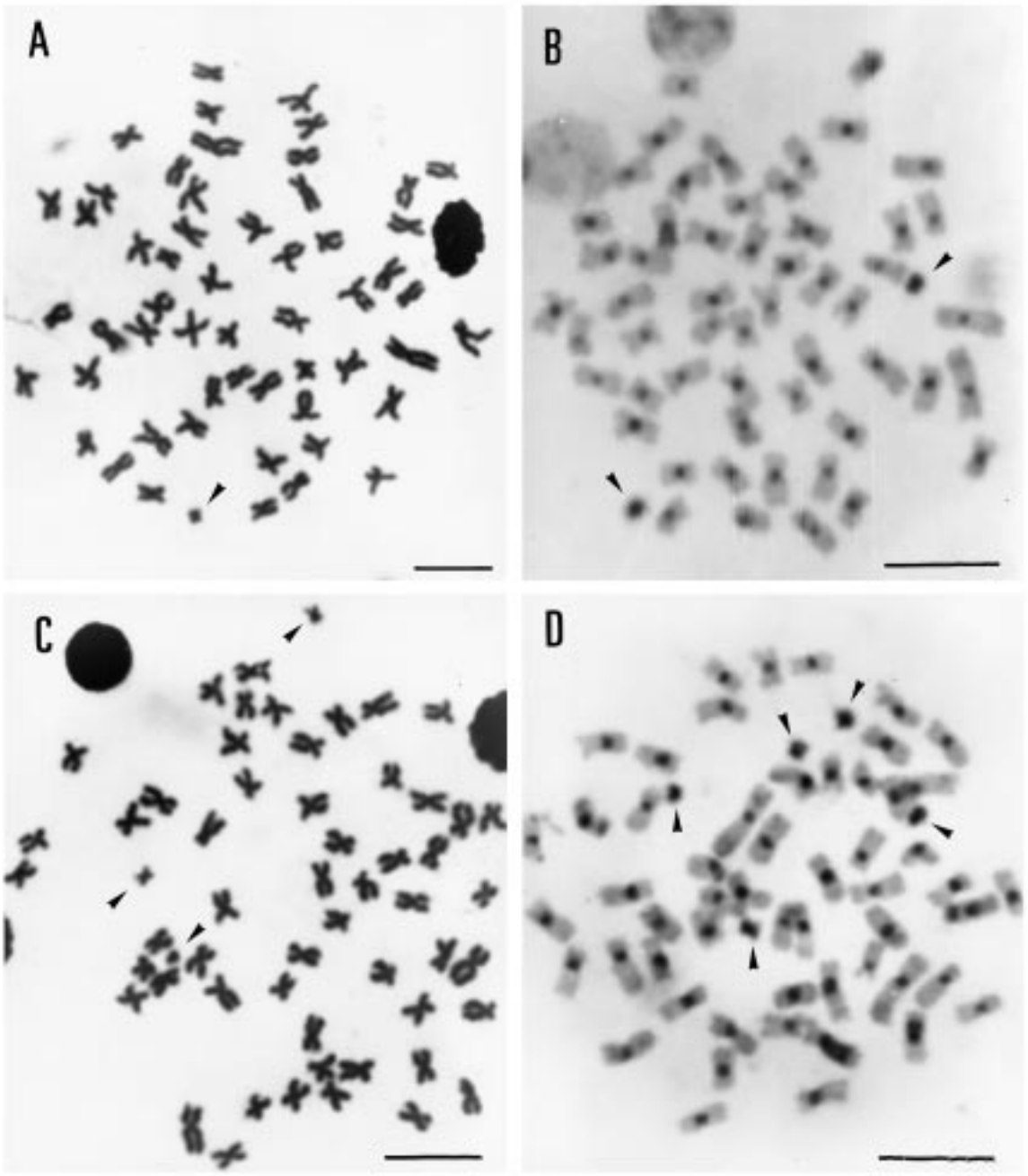

Figure 1. Mitotic metaphase cells showing different B chromosome numbers in *Prochilodus lineatus* (arrowheads). (**A** & **B**) Conventional Giemsa staining. (**C** & **D**) C-banded metaphases showing the heterochromatic B chromosomes. Bar = 5 μm.

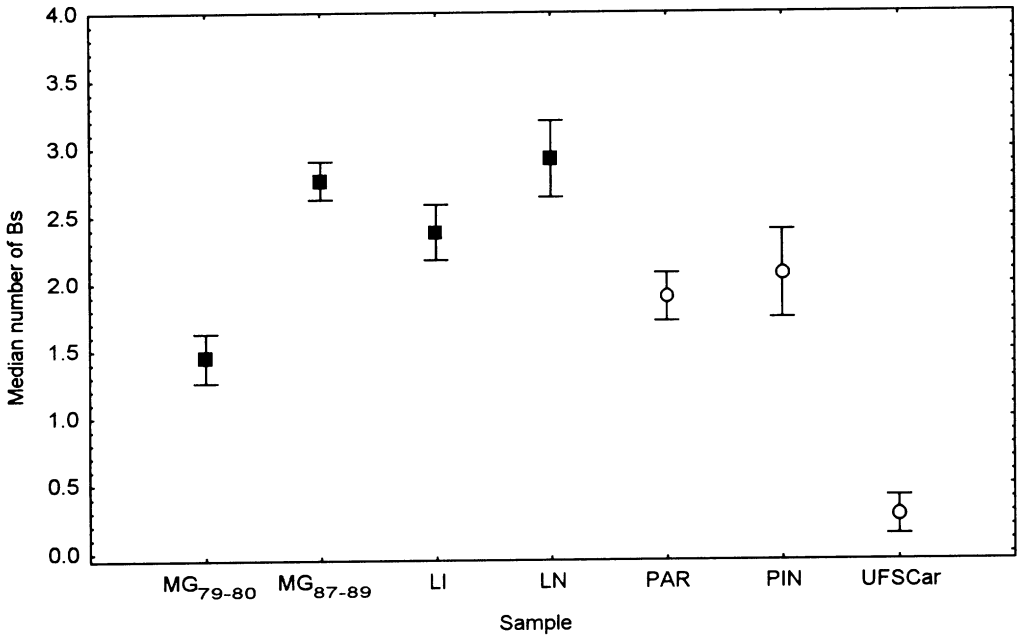

Figure 2. Mean (±SE) of median B numbers in the six population samples analysed, plus the MG79–80 sample (Pauls & Bertollo 1983). Solid squares represent natural populations and white circles piscicultures. See population codes in Table 1.

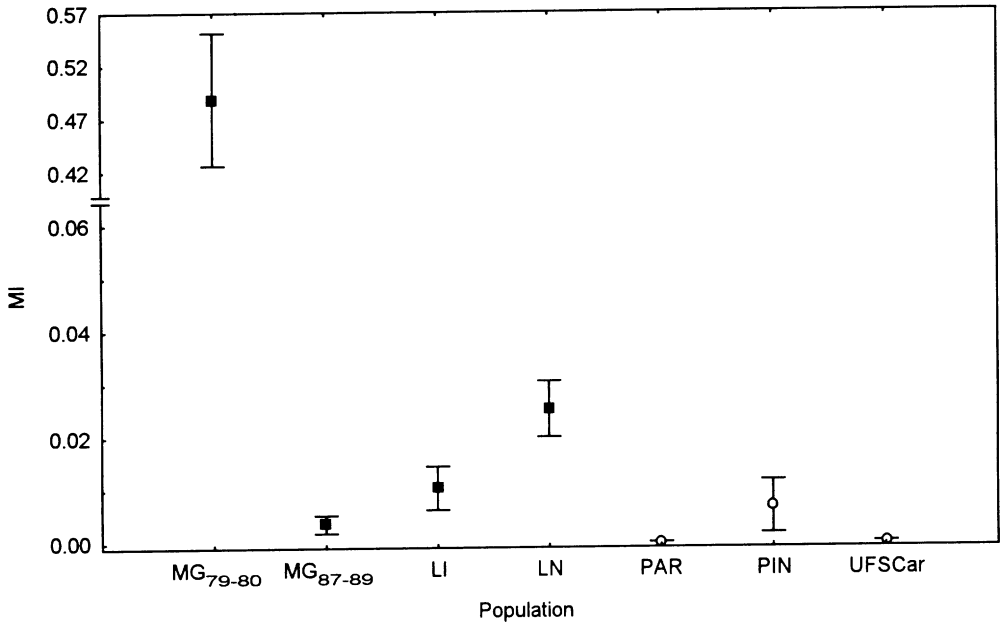

Figure 3. Mean (±SE) of mitotic instability indexes (MI) in the six population samples analysed, plus the MG79–80 sample (Pauls & Bertollo 1983). Solid squares represent natural populations and white circles piscicultures. See population codes in Table 1.

Table 2. B frequency increase and mitotic instability decrease in the Mogi-Guaçu population between 1979–80 and 1987–89.

	Mean		*t*	*df*	*p*	Variance		*F-ratio*	*p*
	1979–80	1987–89				1979–80	1987–89		
Median B number	1.443	2.766	5.742	119	<0.001	1.467	1.498	1.020	0.961
MI	0.486	0.004	12.952	107	<0.001	0.106	0.00023	488.556	<0.001

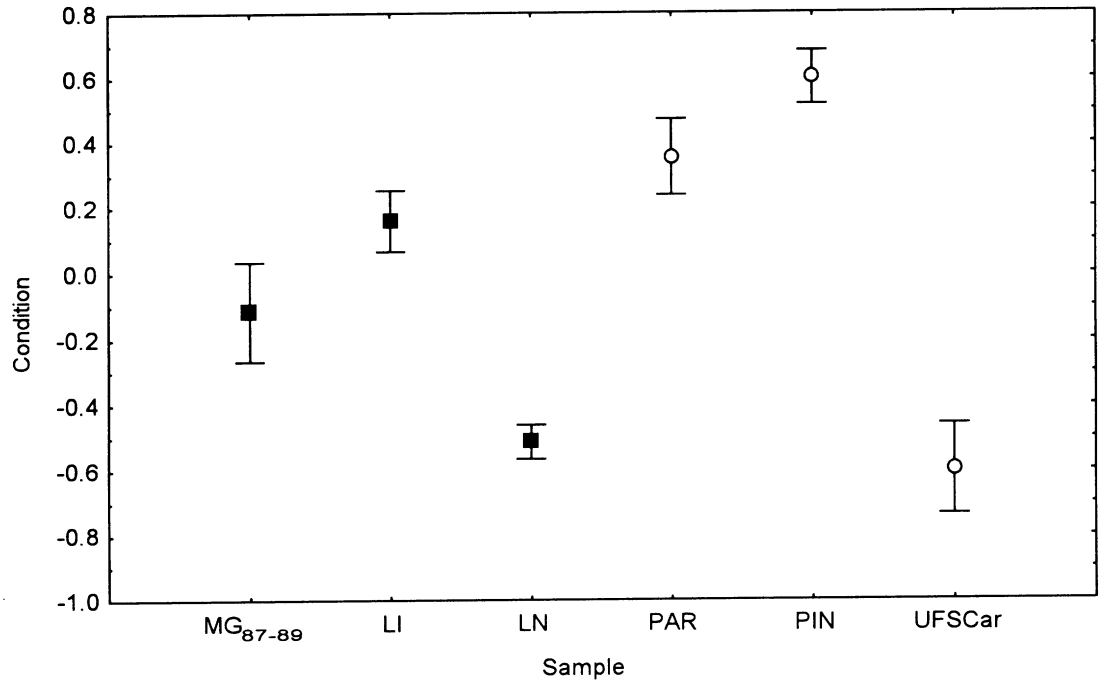

Figure 4. Mean (±SE) of body condition in the six population samples analysed. Solid squares represent natural populations and white circles pisciculture. See population codes in Table 1.

variables, gave similar results, with significant differences between populations but no B effect. The best condition was shown by the individuals from the PAR and PIN pisciculture, the worse being shown by individuals from the UFSCar pisciculture and the natural population at LN. The remaining two natural populations, MG and LI, showed intermediate values (Figure 4).

Discussion

The present data indicate that the B chromosome polymorphism in *P. lineatus* has recently experienced a remarkable increase in frequency in natural populations of the Mogi-Guaçu River, with its frequency doubling in less than ten years. In order to explain this frequency increase, two possible phenomena might have operated during this time, namely the existence of an accumulation mechanism or, alternatively, a beneficial effect of these Bs on carrier fitness. B chromosome effects on carrier fitness have not previously been studied, and our analysis of body condition shows no relationship between Bs and this fitness indicator. While this provides no support for positive B effects on carrier fitness, it will be necessary to analyse other fitness components before reaching a reliable conclusion on this aspect. Alternatively, accumulation mechanisms have

been analysed by Oliveria *et al.* (1997) and us (present paper), and, in both cases, no drive for these B chromosomes has been demonstrated. However, both studies were performed when B frequency had apparently reached its maximum in the MG population (1991–92 and 1987–89, respectively), and thus it is possible that the B drive, if it ever existed, could have been suppressed or very reduced when these samples were taken.

This possibility is compatible with the model for long-term B chromosome evolution proposed by Camacho *et al.* (1997) by which the B polymorphism passes through successive parasitic–neutral–parasitic stages in a non-equilibrium dynamic fashion (see Introduction). In the case of *P. lineatus*, the 1980s seemed to provide a scenario for the final part of a rapid invasion since it took less than ten years to double B frequency from 1.443 to 2.766, with no measurable increase three years later (sampled by Oliveira *et al.* 1997). The absence of accumulation when the invasion had finished (see data in Oliveira *et al.* 1997) suggests the possibility that, at that time, B drive, if it existed previously, had been suppressed.

An interesting fact is the similarity in B frequency between the three natural samples analysed in the present report, i.e. MG, LN and LI. Since LN and LI are two small lakes marginal to the MG river, B frequency similarity could indicate that B invasion occurred almost simultaneously in the three populations, and/or that significant gene flow is taking place between the three localities. Both possibilities are logical bearing in mind the migratory behaviour of this fish. The differences in the *MI* index between the three samples, albeit significant, are many orders of magnitude lower than the change recorded in MG from 1979–80 to 1987–89. Given the rate with which B frequency seems to change during the invasion process (e.g. in the MG population), it is reasonable that slight differences in B colonization time could have led to the differing evolutionary timing (i.e. stabilization) of their respective B systems. Furthermore, B stabilization could have faced different genetic or environmental backgrounds in the different localities.

The apparent mitotic stabilization of the B chromosome during the 1980s suggests the existence of a possible B accumulation suppression mechanism. Mitotically unstable B chromosomes usually show premeiotic accumulation based on their mitotic instability (Nur 1969). For example, B frequency is significantly higher in germ over somatic cells of the individual male (Kayano 1971) but not female locusts *Locusta migratoria* (Viseras *et al.* 1990). B chromosome accumulation in the male germ line is thus probably a result of (1) the generation of cells with different numbers of Bs during early embryogenesis; and (2) the preferential destiny of cells with higher numbers of Bs toward the germ line (Nur 1969, Pardo *et al.* 1995). In theory, mechanisms which suppress B chromosome accumulation could function by impeding either of these two steps. Logically, mitotic stabilization of an unstable B would preclude the variation in B number which provides the raw material for step two. If B chromosome frequency increase in *P. lineatus* was achieved by an accumulation mechanism similar to that in *L. migratoria*, the mitotic stabilization of the B would explain the absence of a significant frequency increase between 1987–89 and 1991–92 (see Oliveria *et al.* 1997), which could thus be interpreted as a symptom of suppression of B accumulation. Likewise, B chromosome variation in size and morphology indicates that Bs are changing, thus providing new variants that could enhance the likelihood of B polymorphism regeneration (Zurita *et al.* 1998). Alternatively, this B frequency stabilization could be the result of a drive–selection balance in which the increase in B frequency due to drive is counteracted by selective elimination of individuals with high B numbers. If this were the case, however, B accumulation should have been apparent in the sample analysed by Oliveira *et al.* (1997). Furthermore, our present results on condition are not suggestive of fitness effects on B carriers and thus the hypothesis of B drive–suppression through mitotic stabilization is more parsimonious than that of drive–selection balance.

To test the drive–suppression hypothesis, two experiments could be done: (1) the introduction of the B chromosome in non-B populations to recreate the invasion period with the presumable accumulation mechanism, and (2) the comparison of B frequency in somatic and germ cells from the same individuals. Testing the drive–selection balance or the beneficial hypotheses would require the analysis of B effects on a large number of

fitness traits, and this would provide insights into the role played by selection on the maintenance of this polymorphism.

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