Genetics and Molecular Biology, 31, 1 (suppl), 270-274 (2008)

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Short Communication

Chromosomal evidence of population subdivision in the freshwater fish *Leporinus elongatus* in the Upper Paraná River basin

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Abstract

Cytogenetic analyses performed in populations of the Neotropical freshwater fish *Leporinus elongatus* of the Upper Paraná River basin showed a chromosome polymorphism involving heterochromatic segments of the nucleolar organizing pair in this species. The NOR-bearing chromosome pair was characterized by two phenotypes identified by the absence (C^1) or presence (C^2) of a heterochromatic segment located in an interstitial position of the long arm in this pair. The meiotic segregation of these variations results in three distinct cytotypes, C^1C^1, C^1C^2 and C^2C^2. Both populations were in Hardy-Weinberg equilibrium. Sex-related cytotype divergence was identified and multivariate analyses revealed that the C^2C^2 individuals presented morphometric differentiation relative to body height in relation to the other cytotypes. A chromosome differentiation and a cytotype proportion difference observed between both populations suggest the occurrence of population subdivision within this species along the Upper Paraná River basin. These findings might contribute to the knowledge of the population biology of this fish and for its conservation.

Key words: Characiformes, Anostomidae, heterochromatin, chromosome polymorphism.

Received: September 1, 2006; Accepted: May 14, 2007.

The ever-growing and disordered anthropic activities in the different Brazilian hydrographic systems have been causing grave concern among researchers with respect to the possibility of extinction of large sets of fish species in many localities. Destruction, alteration and fragmentation of natural environments can cause an enormous loss of biodiversity, including at the genetic level (Avise, 1996). It is often claimed that genetic diversity is fundamental for the adaptation and speciation of a taxon (Hutchinson *et al.*, 2003) and populations that have suffered severe size reductions or bottlenecks are more likely to become extinct (Frankham, 1996).

Moreover, because both genetically or phenotypically divergent populations should be considered independent evolutionary taxa (Mesquita *et al.*, 2005), the geographic delimitation of a particular species and the amount of genetic variability within and between its populations are essential in the determination of appropriate geographical scales for conservation and in the determination of the management strategies to be adopted (Palumbi, 2003). This is particularly important in freshwater fish which display conspicuous migratory behavior and can be believed to comprise large and single population in a hydrographic system (*e.g.*, Revaldaves *et al.*, 1997).

Little is known about the genetic structure of Brazilian freshwater fishes. Recently, molecular markers have been used for genetic structure analyses in different migratory fish species (*e.g.*, Martins *et al.*, 2003; Hatanaka *et al.*, 2006) but the results are still controversial. The occurrence of single and large population was claimed to explain the results obtained for *Prochilodus lineatus* from Upper Paraná River (Revaldaves *et al.*, 1997) and *Pseudoplatystoma fasciatum* from the Bolivian basin (Coronel *et al.*, 2004) for instance, while population subdivisions along a single hydrographic system were reported in *Prochilodus argenteus* from the São Francisco River (Hatanaka *et al.*, 2006) and *Pseudoplatystoma corruscans* from the Paraná River (Sekine *et al.*, 2002). All these studies employed highly polymorphic molecular marker systems. In contrast, most of cytogenetic studies focus on general pattern descriptions, including chromosome banding of a particular species or on an evolutionary approach of related species. Chromosome polymorphism have been reported in fish (*e.g.*, Giuliano-Caetano and Bertollo, 1988; Jankun and Ráb, 1997;

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Jorge and Moreira-Filho, 2000) but rarely studied in the population context (Turner *et al.*, 1985).

In this work the occurrence of a heterochromatin polymorphism in the Neotropical freshwater species *Leporinus elongatus* inhabiting a single major hydrographic system was able to document the occurrence of population subdivision within this species. In addition, the chromosome polymorphism was related to a set of morphological features.

Cytogenetic analysis. Twenty-eight *L. elongatus* specimens (17 males and 11 females) from the Mogi-Guaçú River sampled in the municipality of Pirassununga (SP) and 53 (43 males and 10 females) from the Paranapanema River collected near the municipality of Ourinhos (SP), both rivers belonging to the Upper Paraná basin (Southeastern Brazil), were analyzed. The chromosome preparations were obtained from anterior kidney tissue by direct tissue dissociation (Bertollo *et al.*, 1978) or by short term culture (Fenocchio *et al.*, 1991). The mitotic chromosomes were submitted to C-banding (Sumner, 1972) using Giemsa staining or the 4'-6-diamidine-2-phenylindole fluorochrome (C/DAPI banding). Ag-NOR sites were stained according to Howell and Black (1980). Replication bands were obtained through the *in vitro* incorporation of the 5-Brdu base analogue (Giles *et al.*, 1988).

The populational chromosome differentiation was investigated based on the Wright fixation index *Fst* (Weir and Cockerham, 1984) implemented in the computer program FSTAT 2.9.3.2 (Goudet, 2001). The index was used to quantify the proportion of genetic variation that lies between subpopulations within the total population. Chi-square test performed with the software BIOESTAT 3.0 (Ayres *et al.*, 2003) was also used to compare cytotype frequency difference between populations, as well as between sexes. Hardy-Weinberg equilibrium was calculated manually.

Morphometric analysis. Body measurements of 23 morphological features were performed on 81 specimens of *L. elongatus* (Table 1) using the truss net method (Strauss and Bookstein, 1982). Size-free canonical discriminant analysis (Reis *et al.*, 1990) was performed using the SAS-PC statistical software (SAS Institute Inc., 1988) to estimate differences within and between stocks. Through the projection of the individual scores on the canonical axes of a bidimensional graph, this analysis allows to view the morphological similarity patterns between the individuals of these groups. It is also possible to obtain the discriminant features of each group.

The karyotype of *L. elongatus* of both the Mogi-Guaçú and the Paranapanema populations had 2n = 54 chromosomes for both sexes, with ZW sex chromosomes characterizing the female karyotype, as already reported by Galetti *et al.* (1981). Most chromosomes are slightly heterochromatic in their telomeric regions, with some pairs presenting centromeric and/or pericentromeric blocks (Figure 1).

Table 1 - Characters used in the canonical variate analysis of *Leporinus elongatus* from the Mogi-Guaçú and Paranapanema Rivers. Weight of the morphometric variables in the first (CV1) and second canonical (CV2) axes of the combined samples.

Measured character	CV1	p	CV2	p
snout to pectoral fin	-0.25	0.0289	0.23	0.0411
length of head	-0.24	0.0326	0.03	0.8106
pectoral fin to head	-0.01	0.9178	0.14	0.2232
dorsal pectoral distance	0.22	0.0534	0.16	0.1598
pectoral pelvic distance	-0.33	0.0037	0.10	0.4018
head to dorsal fin origin	-0.06	0.6302	0.34	0.0022
head to pelvic fin	0.08	0.4825	0.11	0.3264
dorsal origin to pelvic fin	0.36	0.0013	-0.03	0.8088
dorsal base length	0.09	0.4472	-0.08	0.5059
dorsal origin to anal origin	0.42	0.0001	-0.36	0.0015
pelvic fin to last dorsal fin ray	0.37	0.0008	0.11	0.3582
pelvic fin to anal origin	0.30	0.0072	0.13	0.2683
anal origin to the last dorsal fin ray	0.49	0.0001	-0.28	0.0123
last dorsal ray to adipose fin	0.19	0.1033	-0.11	0.3431
last dorsal ray to the last anal ray	0.49	0.0001	-0.11	0.3337
anal fin origin to adipose fin	-0.22	0.0588	-0.07	0.5520
anal fin base length	-0.11	0.3581	0.02	0.8447
adipose fin to last anal ray	0.05	0.6522	-0.007	0.9488
adipose fin to superior caudal base	-0.25	0.0291	0.08	0.4913
adipose fin to inferior caudal base	-0.33	0.0034	-0.17	0.1426
last anal ray to superior caudal base	-0.13	0.2699	-0.10	0.4066
last anal ray to inferior caudal base	-0.18	0.1148	-0.03	0.8148
least depth caudal peduncle	-0.19	0.0917	-0.29	0.0093

The nucleolar organizing region was detected in the telomeric portion of the long arm of a large submetacentric pair (# 5) (Figure 1). The NOR-bearing chromosomes were observed in two variant forms denominated hereafter as C^1 and C^2. The C^1 variant had positive C-bands in the telomeric region of its short and long arm, in the latter coinciding with a secondary constriction. Besides the characteristics present in the C^1 phenotype, the larger C^2 variant also presented a large C-banded and late-replicating heterochromatic block observed in the interstitial portion of the long arm (Figure 1). This heterochromatin-related polymorphism found here was already previously described in *L. elongatus* (Köehler *et al.*, 1997; Molina *et al.*, 1998), and enables the identification of two phenotypes of the NOR-bearing chromosomes differing in the absence (C^1) or presence (C^2) of a conspicuous heterochromatic segment interstitially positioned in the long arm of this chromosome. The comparison of these phenotypes with the nucleolar chromosome of *L. friderici*, considered the basic type for the group (Galetti *et al.*, 1984), shows an apparently higher

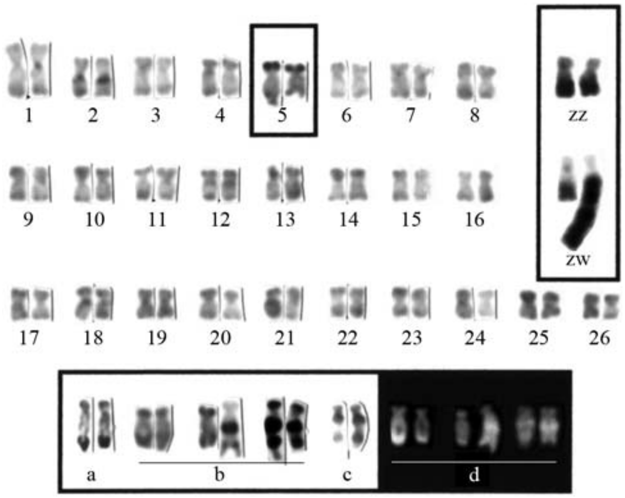

Figure 1 - C-banding patterns in a somatic metaphase of *Leporinus elongatus*. In the lower frame the three variants of the nucleolar organizing pair (5th) found in this species, (a), Ag-NOR site in the C^1C^1 variant, (b), C-banded C^1C^1, C^1C^2 and C^2C^2 cytotypes, (c), replication bands in the C^2C^2, and (d) C/DAPI-banded C^1C^1, C^1C^2 and C^2C^2 cytotypes.

Table 2 - Chromosome variants (C^1 and C^2) in both sexes (F, females and M, males) detected in *Leporinus elongatus* in both studied populations. N = number of individuals; *f* = frequency

Population	Sex	N			$f(C^1)$	$f(C^2)$
		C^1C^1	C^1C^2	C^2C^2		
Mogi-Guaçú	F	2	7	2	0.50	0.50
	M	8	6	3	0.65	0.35
	sum	10	13	5	0.59	0.41
Paranapanema	F	5	5	0	0.75	0.25
	M	28	11	4	0.77	0.23
	sum	33	16	4	0.77	0.23

similarity of *L. friderici* with the C^1C^1 cytotype than with the C^2C^2 and it is suggested that the C^2 is a derived phenotype.

The three possible combinations of these variants, C^1C^1, C^1C^2 and C^2C^2 (Figure 1b), were found in both sexes (Table 2). C^1C^1 and C^1C^2 were the most frequent cytotypes in both populations. The presence of a large heterochromatic block inside the euchromatic segment on the long arm of the chromosome of C^2 phenotype could promote negative position effects or changes in the gene regulatory processes which could lead to physiological and morphological changes in C^2C^2 individuals. However, both populations were in Hardy-Weinberg equilibrium (Mogi-Guaçú, $\chi^2 = 0.47$, DF = 1; $0.95 > p > 0.80$; Paranapanema, $\chi^2 = 1.01$, DF = 1; $0.30 > p > 0.20$) suggesting the occurrence of neutral effects of these cytotypes. Although C^1 was observed relatively more frequent in both populations, its frequency only was evidently higher in the Paranapanema population. There was no significant cytotype frequency difference between sexes within each population, but it was significant when both populations were pooled ($\chi^2 = 5.754$, DF = 2, p = 0.05). The biological significance of this sex-related divergence is still an open question, although a sampling bias comprising three times more males than females could not be discarded.

In contrast, the morphometric multivariate analysis revealed that the first canonical axis retains 61.6% of the total variance of the original data matrix, allowing the discrimination of the C^2C^2 individuals from the remaining ones (Figure 2). The particular morphometric characters for this cytotype refer to the distance from the dorsal-fin origin to anal-fin origin, the distance from the pelvic fin to last dorsal-fin ray, the distance from the anal-fin origin to the last dorsal-fin ray, as well as the distance from the last dorsal-fin ray to the last anal-fin ray. These variables are related to the fish body height and indicate a body divergence of the C^2C^2 individuals comparing to the other cytotypes.

The cytotype frequencies between populations were very close to being significantly different when using the Chi-square test ($\chi^2 = 5.535$, DF = 2, p = 0.06) and they differed significantly when *Fst* was used (*Fst* = 0.0648, p = 0.05), suggesting a population subdivision within this species. These results were concordant with a previous study in this species which detected mtDNA haplotype divergence between representatives of different populations (Martins *et al.*, 2003). Due to the great hydroelectric potential of the Upper Paraná River basin, the physical continuity between the Mogi-Guaçú and Paranapanema Rivers is partially blocked due to several dams constructed in the past 50 years between the two collection sites. A genetic drift

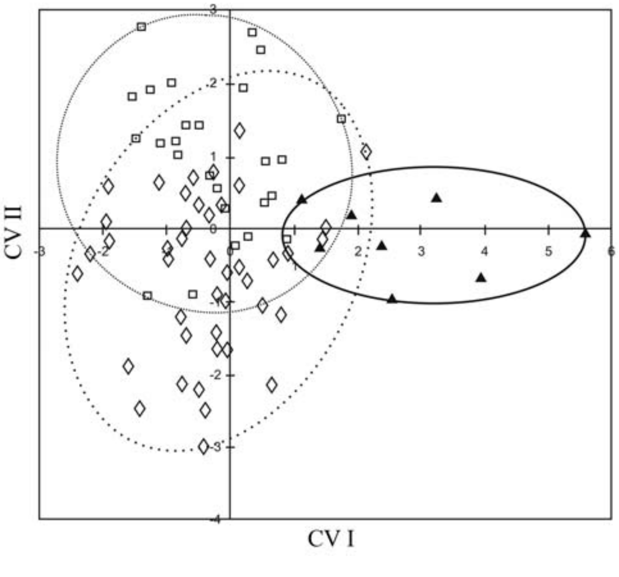

Figure 2 - Projection of the individual scores obtained in a size-free canonical discriminant analysis of 23 morphological body features of the combined samples of *Leporinus elongatus* of the Mogi-Guaçú and Paranapanema populations. The diamonds (◊) correspond to cytotype C^1C^1, squares (❒) to cytotype C^1C^2 and the dark triangles (▲), to C^2C^2.

caused by the dam constructions and a reduced or absent gene flow between these currently isolated populations could be promoting the observed chromosome divergence. However, because their potentially high effective population size and considering that no more than fifty generations have passed since the dam constructions, the detection of genetic drift effects under such conditions would be unlikely. Thus, a chromosome divergence that had already occurred before the dam constructions can not be rejected.

The detection of a differential occurrence of three cytotypes between two recently isolated populations indicating a population subdivision in *L. elongatus* in the Upper Paraná River basin might constitute important information to better understand the population biology of this fish and for its conservation.

Acknowledgments

The authors are grateful to João Henrique Pinheiro Dias for the facilities during fish collection in the Paranapanema River and Reinaldo O Alves de Brito for significant suggestions. This work was supported by the Coordenação de Aperfeiçoamento de Pessoal Docente do Ensino Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Editor: Iracilda Sampaio