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Research Article

Genetic variability in *Astyanax altiparanae* Garutti & Britski, 2000 (Teleostei, Characidae) from the Upper Paraná River basin, Brazil

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Abstract

Allozyme data was used to assess the genetic diversity *Astyanax altiparanae* populations from the floodplain of the Upper Paraná River (PR). Specimens were collected in the southern Brazilian state of Paraná from PR in Porto Rico municipality and Ribeirão Ficha (RF) in Ubiratã municipality. The authors used 15% (w/v) corn starch gel electrophoresis to identify 21 putative *loci* for 13 enzymatic systems: Aspartate aminotransferase, 2.6.1.1 (AAT), Acid phosphatase, 3.1.3.2 (ACP), Esterase, 3.1.1.1 (EST), Glycerol-3-phosphate dehydrogenase, 1.1.1.8 (G3PDH), Glucose-6-phosphate dehydrogenase, 1.1.1.49 (G6PDH), Glucose-6-phosphate isomerase, 5.3.1.9 (GPI), Iditol dehydrogenase, 1.1.1.14 (IDDH), Isocitrate dehydrogenase - $NADP^+$, 1.1.1.42 (IDH), L-Lactate dehydrogenase, 1.1.1.27 (LDH), Malate dehydrogenase, 1.1.1.37 (MDH), Malate dehydrogenase - $NADP^+$, 1.1.1.40 (MDHP), Phosphoglucomutase, 5.4.2.2 (PGM), and Superoxide dismutase, 1.15.1.1 (SOD). The proportion of polymorphic *loci* were estimated as 52.38% in the PR population and 38.10% in the RF population. Expected estimated heterozygosities were 0.1518 ± 0.0493 for the PR population and 0.0905 ± 0.0464 for the RF population. The *A. altiparanae* heterozygosity data were similar to previous estimates for other PR basin characid species. Allele frequencies were significantly different between the PR and RF populations in respect to some loci (*Acp-1*, *G3pdh-1*, *Gpi-A*, *Iddh-1*, *Mdhp-1* and *Mdhp-2*). Wright's statistics for all loci were estimated as F_{is} = 0.3919, F_{it} = 0.4804 and F_{st} = 0.1455. Our results show that the *A. altiparanae* populations studied are genetically different and have a high degree of genetic variability.

Key words: allozymes, *Astyanax*, fishes, genetic variability, Paraná River floodplain.

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Introduction

In the Upper Paraná River (PR) there was an extensive floodplain stretching 480 km between the municipalities of Três Lagoas (20°48' S, 51°43'W) in the Brazilian state of Mato Grosso do Sul and Guaíra (24°04' S, 54°15' W) in the Brazilian state of Paraná. When the Porto Primavera hydroelectric dam (22°30' S, 52°57' W) was inaugurated in 1998 the upper half of the floodplain was submerged by the reservoir and the floodplain reduced to 230 km, this being the last stretch of the Paraná River inside Brazilian territory with a floodplain. The flooding regime of this floodplain is in part controlled by upstream dams (Agostinho *et al*., 2001), with the Paraná River becoming up to 20 km wide at the western side when flooding occurs during the rainy season.

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For almost twenty years, workers at the Center for limnological studies (Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura - NUPELIA), Maringá State University, Paraná, Brazil, have been studying the ecology of fish, plants, benthos, phytoplankton and zooplankton in the PR floodplain. This research program has revealed that the PR floodplain is home to more than 170 fish species (Agostinho *et al*., 2001), 33 of which were introduced from the Middle Paraná River (MPR) basin, after the inauguration of the Itaipu hydroelectric dam in 1982 (Julio Jr *et al*., 2003). While intensive ecological studies have been made on 64 floodplain species (Vazzoler *et al*., 1997, Agostinho *et al*., 2001) only a few species have been investigated in terms of their population genetics. Regarding piscine studies, allozyme data was used by Revaldaves *et al*., (1997) to investigate *Prochilodus lineatus*, by Peres *et al*. (2002) to study *Hoplias malabaricus* and by Zawadzki *et al*., (2005) to study 15 *Hypostomus* species, while DNA markers were used by Oliveira *et al*. (2002) to investigate

Steindachnerina insculpta and *Steindachnerina brevipinna*, Oliveira (2004) to study *Cichla monoculus*, and by Sekine *et al*. (2002) to study *Pseudoplatystoma corruscans*.

According to Vida (1994) *The future of species diversity is in the genetic diversities of the species. In general*, *the higher the maintained genetic diversity*, *the higher the adaptability and*, *consequently*, *the survival probability of species in a changing world* which means that information on genetic variability of the species to be conserved is essential to conservation programs.

Workers NUPELIA have used allozyme electrophoresis to study the genetic variability of ten of the most abundant PR floodplain fish species, including *Astyanax altiparanae* (Garutti & Britski, 2000) which is one of the most abundant fish in the PR basin. It has been reported that in the Paraná River *A. altiparanae* feeds mainly on insects and microcrustaceans (Luz and Okada, 1999) while in streams it feeds mainly on plants (Abes, 1998). In this species, spawning occurs in several batches from September to March (spring through summer in the Southern Hemisphere), the first sexual maturation length being about 6.9 cm and at this length about 50% of *A. altiparanae* are reproducing (Vazzoler *et al.*, 1997). In the PR basin, *A. altiparanae* is recognized as one of the most important forage and food resources for many piscivorous fish species that are commercially exploited. Because of its ecological importance, basic knowledge of the genetic variability of *A. altiparanae* populations is fundamental to in helping ecologists construct future conservation guidelines for the PR basin.

We chose to investigate allozymes because their role in metabolism and adaptation strategies is currently better understood than that of DNA markers. A further advantage of using allozyme electrophoresis is that this technique can detect higher heterozygosity per locus than can be detected using dominant RAPD markers because with these the estimated heterozygosity cannot be higher than 0.50.

The aim of the work described in this paper was to use allozyme data to estimate the genetic variability of two *A. Altiparanae* populations, one from the Upper Paraná River and the other from the small Ribeirão Ficha (both in Paraná state). We also used our data to shed more light on the factors that maintain genetic variability in natural populations.

Material and Methods

From March to August (autumn through winter) 2002, 31 adult *Astyanax altiparanae* (Garutti & Britski, 2000) (Figure 1) were caught from a site (22°45'60" S, 53°15'22" W; Figure 2) on the Upper Paraná River (PR) in Porto Rico municipality where the Paraná River is about 4 km wide and the mean water temperature oscillate between 18 °C in winter and 30 °C in summer (Thomaz *et al*., 1997).

Figure 1 - *Astyanax altiparanae* (lambari-de-rabo-amarelo). Standard lenght = 8 cm.

From April to November (autumn through spring) 2003, 33 adult *A. altiparanae* were caught from a site (24°26'22" S, 53°06'36" W) on the Ribeirão Ficha (RF) in Ubiratã municipality. The Ribeirão Ficha is a narrow creek about 2 m wide that flows into the Piquiri River, a tributary of the Paraná River. Both sides of the creek are covered by a riparian vegetation of about 20 m wide which shades its surface. Water mean temperature oscillates between 15 °C in winter and 27 °C in summer (Estado do Paraná, 1987).

Immediately after capture, white skeletal muscle and liver, gill, stomach, gonad, eye, kidney and heart tissue were removed from each specimen and frozen in liquid nitrogen. Tissues were homogenized with plastic sticks in 1.5 mL microcentrifuge tubes in the presence of Tris/HCl 0.02 M, pH 7.5 buffer (1:1 v:w). Carbon tetrachloride (CCl_4) was added to homogenized liver samples (1:2 v:v) because of the large amounts of fat present in this tissue (Pasteur *et al.*, 1988). Homogenized samples were centrifuged at 45,114 x g for 30 min, at temperatures between 1° and 5 °C. The supernatant fractions were submitted to horizontal electrophoresis in 15% (w/v) corn starch gel (Val *et al*., 1981)

A total of 13 enzymatic systems were evaluated, which are shown in Table 1 along with their abbreviations. Enzyme nomenclature followed the proposals of Murphy *et*

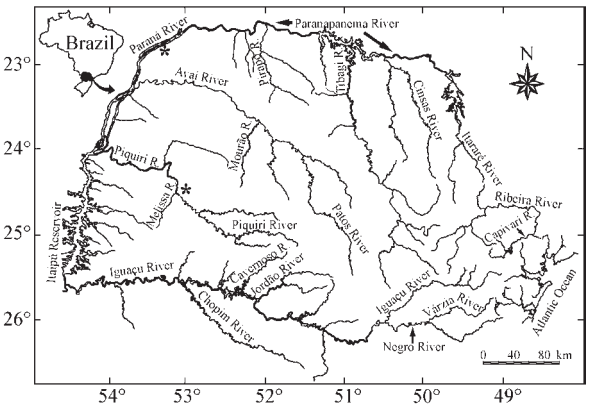

Figure 2 - Sample localities in Upper Paraná River and Ribeirão Ficha, Paraná, Brazil.

Table 1 - Allele frequencies at 21 *loci* of *Astyanax altiparanae* from the Upper Paraná River (PR) and Ribeirão Ficha (RF).

Enzyme*	Tissue	Locus	Allele	PR (n = 31)	RF (n = 33)
Aspartate aminotransferase (AAT)	Liver	*Aat-1*	*a*	1.000	1.000
Acid phosphatase (ACP)	Liver, stomach	*Acp-1*	*a*		0.160
			b		0.240
			c	1.000	0.440
			d		0.160
Esterase (EST)	Liver	*Est-1*	*a*	0.065	0.015
			b	0.903	0.970
			c	0.032	0.015
		Est-2	*a*	0.984	0.985
			b	0.016	0.015
Glucose-3-phosphate dehydrogenase (G-3-PDH)	Liver	*G3pdh-1*	*a*	0.919	1.000
			b	0.081	
		G3pdh-2	*a*	1.000	1.000
glucose-6-phosphate dehydrogenase (G-6-PDH)	Heart	*G6pdh-1*	*a*	0.134	0.020
			b	0.775	0.900
			c	0.091	0.080
glucose phosphate isomerase (GPI)	Heart	*Gpi-A*	*a*	0.113	0.131
			b	0.339	0.109
			c	0.161	0.152
			d	0.258	0.304
			e	0.097	0.130
			f	0.032	0.174
	Heart	*Gpi-B*	*a*	0.968	0.985
			b	0.032	0.015
Iditol dehydrogenase (IDDH)	Liver	*Iddh-1*	*a*	0.581	1.000
			b	0.339	
			c	0.080	
Isocitrate dehydrogenase (IDHP)	Liver	*Idhp-1*	*a*	0.032	0.058
			b	0.968	0.923
			c		0.019
	Heart	*Idhp-2*	*a*	1.000	1.000
Lactate dehydrogenase (LDH)	Gill, heart	*Ldh-A*	*a*	1.000	1.000
		Ldh-B	*a*	1.000	1.000
Malate dehydrogenase (MDH)	Heart	*mMdh-1*	*a*	0.984	1.000
			b	0.016	
		sMdh-A	*a*	1.000	1.000
		sMdh-B	*a*	1.000	0.985
			b		0.015
Malate dehydrogenase $NADP^+$ (MDHP)	Heart, muscle	*Mdhp-1*	*a*	0.800	1.000
			b	0.200	
		Mdhp-2	*a*	0.484	1.000
			b	0.516	
Phosphoglucomutase (PGM)	Liver	*Pgm-1*	*a*	1.000	1.000
Superoxide dismutase (SOD)	Liver	*Sod-1*	*a*	1.000	1.000
Frequency of polymorphic loci				52.38	38.10

al. (1996). Electrophoreses conditions were according to the following authors: Boyer *et al*., (1963) for SOD; Ruvolo-Takasusuki *et al*., (2002) for ACP and EST; Shaw and Prasad (1970) for AAT, G3PDH, G6PDH, GPI, IDDH, IDHP, LDH, MDH, MDHP and PGM. Standard histochemical staining procedures were used to visualize specific enzymes according to Aebersold *et al.* (1987). Genetic interpretation of the gels was based on the quaternary structure of the enzymes (Ward *et al*., 1992).

Data were analyzed using the POPGENE software version 1.31 (Yeh *et al.*, 1999). Genetic variability was estimated using Nei's unbiased heterozygosity (He) or gene diversity (Nei, 1978). The observed (*Ho*) and expected (*He*) heterozygosities for each putative loci and the overall loci means were also calculated. Genotypic frequencies were tested for Hardy-Weinberg equilibrium using the chi-squared (χ^2) test. Wright's (1978) F statistics were tested for significance by the χ^2 test (Workman and Niswander,

1970). Differences in *He* between the two localities were verified using the t-test (Nei, 1987). Nei's unbiased measure of genetic identity and genetic distance were also calculated (Nei, 1987).

Results

The electrophoretic patterns obtained in this study are shown in Figure 3 and described below.

Aspartate aminotransferase (AAT) activity was higher in liver than in other tissue but only one electrophoretic band was observed in all analyzed specimens. This phenotype was interpreted as one monomorphic *locus*.

Acid phosphatase (ACP) activity was restricted to liver tissue and showed the electrophoretic pattern of a dimeric enzyme encoded by a single *locus*.

Esterase (EST) activity was highly expressed in all tissues, except heart and muscle tissue where its expression was weak. This enzyme was better resolved in liver by using á-naphthyl propionate as substrate. Although three regions of expression were detected only two were interpretable so the bands were considered to be an expression of two polymorphic *loci*, *Est-1* with three alleles and *Est-2* with two alleles.

Glucose-3-phosphate dehydrogenase (G-3-PDH) is a dimeric enzyme which showed high expression only in liver tissue, where it showed one polymorphic (*G3pdh-1*) and one monomorphic (*G3pdh-2*) locus.

Glucose-6-phosphate dehydrogenase (G-6-PDH) was weakly expressed in heart and muscle tissues but highly expressed in liver tissue with the presence of only one band for each specimen. This phenotype was considered as resulting from only one polymorphic *locus* with three alleles.

Glucose phosphate isomerase (GPI) is a dimeric enzyme encoded by two polymorphic loci and was highly expressed in liver, heart, muscle, gonad, kidney and stomach tissues where it formed heterodimers. The least anodic bands are encoded by the *Gpi-b* locus and the most anodic bands by the *Gpi-A* locus. The A_2 isozyme was predominant in eye, gonad, gill, kidney and stomach tissues, while the B_2 was prevalent in heart tissue.

Iditol dehydrogenase (IDDH) was expressed only in liver tissue with the presence of only one band for each specimen, suggesting the existence of only one polymorphic locus.

Isocitrate dehydrogenase (IDHP) showed differential expression in the tissues analyzed, one polymorphic locus (*Idhp-2*) with three alleles being expressed in gill and liver tissue while another monomorphic locus (*Idhp-1*) was detected in eye, heart and white muscle tissue. Both *loci* were weakly expressed in gonad, kidney and stomach tissues.

Lactate dehydrogenase (LDH) is a tetrameric enzyme which showed several bands which we interpreted as resulting from the expression of two homozygous loci (*Ldh-A* and *Ldh-B*) with variable heterotetramer formation (Figure 3). Three bands were observed in heart, presumably representing the B_4, A_1B_3 and A_3B_1 isozymes. Two loci were expressed in eye, gill and stomach tissues but the *Ldh-B locus* was preferentially expressed in gonad, kidney, and stomach tissues.

Malate dehydrogenase (MDH) is a dimeric enzyme and presented four bands in eye, gill, heart and white muscle tissues. Only two anodic bands were detected In liver

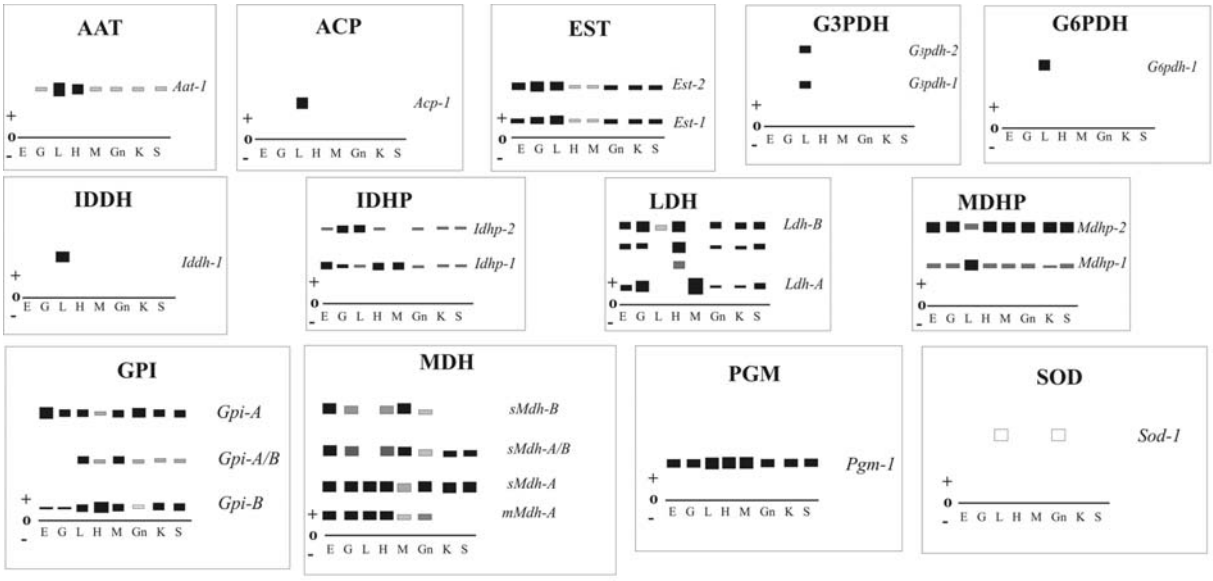

Figure 3 - Electrophoretic pattern and *locus* designation for 13 enzyme systems of *Astyanax altiparanae* from the Upper Paraná River and Ribeirão Ficha, Paraná, Brazil. Type of tissue: E = eye; G = gill; L = liver; H = heart; M = white muscle; Gn = gonad; K = kidney; S = stomach.

tissue, while both kidney and stomach tissue exhibited two intermediary bands. This pattern was interpreted as the expression of three loci ($_{m}$*MDH-1*, $_{S}$*MDH-A* and $_{S}$*MDH-B*) with heterodimer formation between the $_{S}$*MDH-A* and $_{S}$*MDH-B* loci.

Malate dehydrogenase $NADP^{+}$ (MDHP) showed the typical electrophoretic pattern of enzymes encoded by two *loci* (*Mdhp-1 and Mdhp-2*). The *Mdhp-2 locus* was strongly expressed in all tissues except liver, which showed a stronger expression of *Mdhp-1locus.*

Phosphoglucomutase (PGM) exhibited only one band, which is typical of monomeric enzyme encoded by one monomorphic *locus*.

Superoxide dismutase (SOD), expression of this enzyme was restricted to liver and gonad tissues, showing a pattern of only one band encoded by a monomorphic *locus*.

We identified 21 putative enzyme loci in 31 *A. altiparanae* specimens, 11 (52.38%) of the loci being polymorphic in PR fish and 8 (38.10%) in RF fish polymorphic (Table 1). For the PR population *Ho* = 0.0753 ± 0.0404 and *He* = 0.1518 ± 0.0493 while for the RF population *Ho* = 0.0730 ± 0.0399 and *He* = 0.0905 ± 0.0464 (Table 2). The *t*-test revealed that the difference between estimated *He* values for the two populations was not significant (t = 0.9055, 19 degrees of freedom (df)). Nei's unbiased measure of genetic identity was 0.9549 and the genetic distance between populations was 0.0462.

The data revealed that the *G6pdh-1*, *Iddh-1*, *Mdhp-1* and *Mdhp-2* loci in the PR population and the *Acp-1* locus in the RF population were not in Hardy-Weinberg equilibrium. All 31 specimens in the PR population were homozygotes for the putative *Acp-1*(*c*) and *sMdh-B*(*a*) alleles and the 33 specimens in the RF population were homozygotes for the *G6pdh-1*(*a*), *Iddh-1*(*a*), *mMdh-1*(*a*), *Mdhp-1*(*a*) and *Mdhp-2*(*a*) alleles. The Wright's (1978) F statistics showed that there was a significant excess of homozygotes and the F_{st} values showed that the two populations differ significantly at the *Acp-1*, *G3pdh-1*, *Gpi-A*, *Iddh-1*, *Mdhp-1*, and *Mdhp-2* loci (Table 3).

Table 2 - Obtained (*Ho*) and expected (*He*) heterozygosity per *locus* of *Astyanax altiparanae* from the Upper Paraná River (PR) and Ribeirão Ficha (RF).

	PR			RF		
Locus	N	*Ho*	*He*	n	*Ho*	*He*
Aat-1	31	0.0000	0.0000	31	0.0000	0.0000
Acp-1	31	0.0000	0.0000	25	0.5600	0.6976
Est-1	31	0.1290	0.1790	33	0.0606	0.0592
Est-2	31	0.0323	0.0317	32	0.0303	0.0298
G_{3}pdh-1	31	0.1613	0.1483	33	0.0000	0.0000
G_{3}pdh-2	31	0.0000	0.0000	33	0.0000	0.0000
G_{6}pdh-1	22	0.0000	0.3760	25	0.0400	0.1832
Gpi-A	31	0.8387	0.7492	24	0.6667	0.7266
Gpi-B	31	0.1613	0.1483	33	0.0303	0.0298
Iddh-1	31	0.1613	0.5489	23	0.0000	0.0000
Idhp-1	31	0.0000	0.0000	26	0.0000	0.0000
Idhp-2	31	0.0645	0.0624	32	0.1154	0.1442
Ldh-A	31	0.0000	0.0000	33	0.0000	0.0000
Ldh-B	31	0.0000	0.0000	33	0.0000	0.0000
mMdh-1	31	0.0323	0.0317	33	0.0000	0.0000
sMdh-A	31	0.0000	0.0000	33	0.0000	0.0000
sMdh-B	31	0.0000	0.0000	33	0.0303	0.0298
Mdhp-1	31	0.0000	0.4121	32	0.0000	0.0000
Mdhp-2	31	0.0000	0.4995	33	0.0000	0.0000
Pgm-1	31	0.0000	0.0000	33	0.0000	0.0000
Sod-1	31	0.0000	0.0000	33	0.0000	0.0000
Mean		0.0753	0.1518		0.0730	0.0905
Standard error		0.0404	0.0493		0.0399	0.0464

Discussion

Our allozyme starch gel electrophoretic study detected high allozyme variability between the *A. altiparanae* populations from the Upper Paraná River (PR) and the Ribeirão Ficha (RF). Other workers have also used molecular markers to compare the genetic variability of *A. altiparanae* populations from other Brazilian rivers. Moysés and Almeida-Toledo (2002) studied the genetic variability of the mitochondrial DNA (mtDNA) of *Astyanax lacustris* from the São Francisco River basin and *A. altiparanae* from the PR basin using the restriction fragment length polymorphism (RFLP) method and found differences between these two populations. Prioli *et al*. (2002) used random amplified polymorphic DNA (RAPD) and mtDNA markers to demonstrate strong genetic similarities between *A. altiparanae* populations from the Keller and Pirapó rivers in the PR basin and the Iguaçu River. Leuzzi *et al.* (2004) used RAPD to analyze the genetic variability of *A. altiparanae* from four localities in the Capivara dam, one from the Rosana dam (downstream of the Capivara dam) and one from the Jurumirim dam (upstream of the Capivara dam) of the Paranapanema River (a Brazilian tributary of the Paraná River) and reported high genetic variability and high population differentiation.

The *A. altiparanae* phenotypes we obtained for the 13 enzymatic systems studied were similar to those previously demonstrated for other Characiformes from the PR basin (Revaldaves *et al.*, 1997; Renesto *et al.*, 1997, 2001; Chiari and Sodré, 1999; Peres *et al.*, 2002) and for Siluriformes (Almeida and Sodré, 1998; Zawadzki *et al.*, 1999, 2000a, 2000b; Renesto *et al.*, 2000). Our data revealed that *A. altiparanae* carries high genetic variability, 52.38% of polymorphic *loci* in PR and 38.10% in the RF. The expected genetic heterozygosity was estimated as 0.1518 for the PR population and 0.0933 for the RF population, which is higher than the average of 0.051 for 195 piscine species from several world-wide localities reported in the review

Table 3 - Wright's statistics for *Astyanax altiparanae* from the Upper Paraná River (PR) and Ribeirão Ficha (RF).

	PR		RF		Overall		
Locus	n	F_{is}	n	F_{is}	F_{is}	F_{it}	F_{st}
Aat-1	31				0.0000	0.0000	0.0000
Acp-1	31		25	0.1972	0.1972*	0.3838*	0.2324*
Est-1	31	0.2791	33	-0.0233	0.2039	0.2157	0.0148
Est-2	31	-0.0164	32	-0.0154	-0.0159	-0.0159	0.0000
G3pdh-1	31	-0.0877	33		-0.0877	-0.0420*	0.0420*
G3pdh-2	31		33		0.0000	0.0000	0.0000
G6pdh-1	22	1.0000*	25	0.7817*	0.9285*	0.9303	0.0260
Gpi-A	31	-0.1194	24	0.0824	0.1062*	0.1221*	0.0705*
Gpi-B	31	-0.0877	33	-0.0154	-0.0756	-0.0503*	0.0235
Iddh-1	31	0.7062*	23		0.7062*	0.7728*	0.2268*
Idhp-1	31	-0.0333	26	0.2000	0.1295*	0.1358*	0.0072
Idhp-2	31		32		0.0000	0.0000	0.0000
Ldh-A	31		33		0.0000	0.0000	0.0000
Ldh-B	31		33		0.0000	0.0000	0.0000
mMdh-1	31	-0.0164	33		-0.0164*	-0.0081	0.0081
sMdh-A	31		33		0.0000	0.0000	0.0000
sMdh-B	31		33	-0.0154	-0.0154	-0.0076	0.0076
Mdhp-1	31	1.0000*	32		1.0000*	1.0000*	0.1698*
Mdhp-2	31	1.0000*	33		1.0000*	1.0000*	0.3478*
Pgm-1	31		33		0.0000	0.0000	0.0000
Sod-1	31		33		0.0000	0.0000	0.0000
Mean		0.5039		0.1934	0.3919	0.4804	0.1455

* = Statistically significant at p = 0.05.

by Ward *et al.* (1992) but similar to other Brazilian Characiformes. Revaldaves *et al.* (1997) estimated that the expected heterozygosity of *Prochilodus lineatus* from the PR was 0.13, while Chiari and Sodré (1999) analyzed populations of five anostomid species from the Tibagi River (a tributary of the Paranapanema River) and found expected heterozygosity values of 0.142 for *Leporinus elongatus*, 0.132 for *Leporinus frederici*, 0.09 for *Leporinus obtusidens*, 0.092 for *Schizodon nasutus* and 0.072 for *Schizodon intermedius.* The expected heterozygosity of *Hoplias malabaricus* was estimated as 0.14 for both a Paraná River and a lagoon population (Peres *et al.*, 2002). However, lower heterozygosity values have been reported for Iguaçu River (Brazil) fish species, Renesto *et al.*, (1997) having reported that three undescribed *Astyanax* species endemic to the Iguaçu River showed low estimated expected heterozygosity (0.097, 0.082 and 0.061 for *Astyanax* sp. B, C and F). Low estimated expected heterozygosity has also been reported for two other Iguaçu River fish, 0. 063 for *Crenicichla iguassuensis* (Renesto *et al.*, 2001) and 0.024 for *Pimelodus ortmanni* (Renesto *et al*., 2000). These low expected heterozygosity values for Iguaçu River species may be due to regulation of invariable structural genes which guarantee phenotypic plasticity (Chippari-Gomes *et al*., 2003).

In our study, although the expected heterozygosities estimated for the two populations analyzed were not significantly different (t = 0.9054), significant F_{st} values were found for *Acp-1*, *G$_3$pdh-1*, *Gpi-A*, *Iddh-1*, *Mdhp-1* and *Mdhp*-2 (Table 3). Furthermore, a few alleles present in one population were missing in the other. If the *Acp-1*(*c*) and *sMdh-B*(*a*) allele frequencies in the PR population were equal to those of the RF population the probability that all the 31 PR specimens were homozygotes would be 7.835 x 10^{-23} (0.44^{2x31}) for *Acp-1*(*c*) and 0.3918 (0.985^{2x31}) for *sMdh-B*(*a*). On the other hand, if the *G$_3$pdh-1*(*a*), *Iddh-1*(*a*), *mMdh-1*(*a*), *Mdhp-1*(*a*) and *Mdhp-2*(*a*) allele frequencies of the RF population were about the same as for the PR population the probability that all the 33 RF specimens were homozygotes for *G$_3$pdh-1(a)*, *Iddh-1*(*a*), *mMdh-1*(*a*), *Mdhp-1*(*a*) and *Mdhp-2*(*a*) would be 0.0038, 2.7266 x 10^{-16}, 0.3449, 4 x 10^{-7}, 1.5841 x 10^{-21} respectively. Considering the number of fish analyzed, the probability that the *G$_3$pdh-1(a)*, *Iddh-1*(*a*), *mMdh-1*(*a*), *Mdhp-1*(*a*) and *Mdhp-2*(*a*) alleles were not detected in the PR population is extremely low. This may be due to lack of gene flow between the two populations because they are separated by

about 440 km of river which may have allowed the accumulation of different mutations. Our data indicated that the PR and RF populations are genetically differentiated.

The expected heterozygosity for each *locus* ranged from zero to 0.7492 in the *A. altiparane* population from the PR floodplain and from zero to 0.7266 for the RF population, with the *Gpi-A* locus being the most polymorphic (Table 2). Johnson (1974) suggested that glucose phosphate isomerase (GPI) polymorphism could be considered as a metabolic alternative, since this enzyme can have a regulatory function and that GPI polymorphism enhances fitness by providing metabolic compensation in fluctuating environments.

We found a significant excess of homozygotes for the *G6pdh-1*, *Iddh-1*, *Mdhp-1* and *Mdhp-2* loci in the PR population and at the *G6pdh-1* locus in the RF population (Table 3). Such high homozygosity should not be attributable to either inbreeding or genetic drift because *A. altiparanae* is highly abundant in the rivers studied, so it may be that selection for homozygotes is occurring at these loci.

The high genetic variability of *A. altiparanae* detected by us may be explained by natural selection for heterozygote advantage at polymorphic loci. Because of the large populations the high genetic variability of *A. altiparanae* may also be due to the accumulation of neutral or quasi-neutral mutations (Kimura and Ohta, 1971). Cytogenetic studies have suggested that *A. altiparanae* is a complex of cryptic species, several authors having reported the same diploid number (2n = 50) but different karyotype formulae and a different number of NOR-bearing chromosomes in this fish (Vale and Martins-Santos, 1998; Daniel-Silva and Almeida-Toledo, 2001; Pacheco *et al.*, 2001; Porto and Martins-Santos, 2002; Fernandes and Martins-Santos, 2005).

Because of its important ecological role, the genetic variability of *A. altiparanae* should be taken into account in conservation policies to assure the evolutionary future of this species.

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