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 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 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 $F_{is} = 0.3919$, $F_{rt} = 0.4804$ 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.

For almost twenty years, workers at the Center for limnological studies (Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura - 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). 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 (CCl₄) 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 al., 718 Peres et al. (1981).
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 ($\chi^2$) test. Wright’s (1978) F statistics were tested for significance by the $\chi^2$ test (Workman and Niswander, 2002).

### 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 | 0.240 |
| | | b | 1.000 | 0.440 |
| | | c | 0.160 | 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 | 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 | 0.080 |
| Isocitrate dehydrogenase (IDHP) | Liver | Idhp-1 | a | 0.032 | 0.058 |
| | b | 0.968 | 0.923 |
| | c | 0.080 | 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 | 0.016 |
| | sMdh-A | a | 1.000 | 1.000 |
| | sMdh-B | a | 1.000 | 0.985 |
| | b | 0.015 | 0.015 |
| Malate dehydrogenase NADP⁺ (MDHP) | Heart, muscle | Mdhp-1 | a | 0.800 | 1.000 |
| | b | 0.200 | 0.200 |
| | Mdhp-2 | a | 0.484 | 1.000 |
| | b | 0.516 | 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

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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 tissues 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 α-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 A3 isozyme was predominant in eye, gonad, gill, kidney and stomach tissues, while the B2 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 B4, A1B3 and A3B1 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.
tissue, while both kidney and stomach tissue exhibited two intermediary bands. This pattern was interpreted as the expression of three loci (\(\text{MDH}-1, \text{MDH}-A\) and \(\text{MDH}-B\)) with heterodimer formation between the \(\text{MDH}-A\) and \(\text{MDH}-B\) loci.

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

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 \(A.\) \textit{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 \(H_o = 0.0753 \pm 0.0404\) and \(H_e = 0.1518 \pm 0.0493\) while for the RF population \(H_o = 0.0730 \pm 0.0399\) and \(H_e = 0.0905 \pm 0.0464\) (Table 2). The \(t\)-test revealed that the difference between estimated \(H_e\) values for the two populations was not significant (1 = 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 \(G_6pdh-1, Iddh-1, \text{Mdhp-1}\) and \(\text{Mdhp-2}\) 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 \(G_6pdh-1(a), Iddh-1(a), mMdhp-1(a), \text{Mdhp-1}\) and \(\text{Mdhp-2}\) alleles. The Wright’s (1978) \(F\) statistics showed that there was a significant excess of homozygotes and the \(F_s\) values showed that the two populations differ significantly at the \(Acp-1, G_3pdh-1, \text{Gpi-A, Iddh-1, Mdhp-1,}\) and \(\text{Mdhp-2}\) loci (Table 3).

**Discussion**

Our allozyme starch gel electrophoretic study detected high allozyme variability between the \(A.\) \textit{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 \(A.\) \textit{altiparanae} populations from other Brazilian rivers. Moysés and Almeida-Toledo (2002) studied the genetic variability of the mitochondrial DNA (mtDNA) of \(A.\) \textit{lacustris} from the São Francisco River basin and \(A.\) \textit{altiparanae} from the PR basin using the restriction fragment length polymorphism (RFLP) method and found differences between these two populations. Prioli \textit{et al.} (2002) used random amplified polymorphic DNA (RAPD) and mtDNA markers to demonstrate strong genetic similarities between \(A.\) \textit{altiparanae} populations from the Keller and Pirapó rivers in the PR basin and the Iguacu River. Leuzzi \textit{et al.} (2004) used RAPD to analyze the genetic variability of \(A.\) \textit{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 \(A.\) \textit{altiparanae} phenotypes we obtained for the 13 enzymatic systems studied were similar to those previously demonstrated for other Characiformes from the PR basin (Revaldaves \textit{et al.}, 1997; Renesto \textit{et al.}, 1997, 2001; Chiari and Sodré, 1999; Peres \textit{et al.}, 2002) and for Siluriformes (Almeida and Sodré, 1998; Zawadzki \textit{et al.}, 1999, 2000a, 2000b; Renesto \textit{et al.}, 2000). Our data revealed that \(A.\) \textit{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 2** - Obtained \((H_o)\) and expected \((H_e)\) heterozygosity per locus of \(A.\) \textit{altiparanae} from the Upper Paraná River (PR) and Ribeirão Ficha (RF).

| Locus | PR |  |  | RF |  |  |
|-------|----|---|---|----|---|---|
| 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-gdh-1 | 31 | 0.1613 | 0.1483 | 33 | 0.0000 | 0.0000 |
| G-gdh-2 | 31 | 0.0000 | 0.0000 | 33 | 0.0000 | 0.0000 |
| G-gdh-3 | 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 |
| 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 |
| mMdhp-1 | 31 | 0.0323 | 0.0317 | 33 | 0.0000 | 0.0000 |
| sMdhp-A | 31 | 0.0000 | 0.0000 | 33 | 0.0000 | 0.0000 |
| sMdhp-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 | 0.4893 | 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 |
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 Iguacu River (Brazil) fish species, Renesto et al., (1997) having reported that three undescribed Astyanax species endemic to the Iguacu 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 Iguacu River fish, 0.063 for Crenicichla ignassuensis (Renesto et al., 2001) and 0.024 for Pimelodus ortmanni (Renesto et al., 2000). These low expected heterozygosity values for Iguacu 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 Fst values were found for Acp-1, Gpdh-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} (0.442x31) for Acp-1(c) and 0.3918 (0.985 2x31) for sMdh-B(a). On the other hand, if the G3pdh-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 G3pdh-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 Gpdh-1(a), Iddh-1(a), Mdhd-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

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

| Locus  | PR n | Fis  | RF n | Fis  | Overall n | Fis  |
|--------|------|------|------|------|------------|------|
| Aat-1  | 31   | 0.0000 | 31   | 0.0000 | 31         | 0.0000 |
| Acp-1  | 31   | 0.1972* | 31   | 0.1972* | 31         | 0.1972* |
| Est-1  | 31   | 0.2039 | 31   | 0.2039 | 31         | 0.2039 |
| Est-2  | 31   | 0.0159 | 31   | 0.0159 | 31         | 0.0159 |
| G3pdh-1| 31   | 0.0877 | 31   | 0.0877 | 31         | 0.0877 |
| G3pdh-2| 31   | 0.0000 | 31   | 0.0000 | 31         | 0.0000 |
| G6pdh-1| 22   | 0.7817* | 25   | 0.7817* | 25         | 0.7817* |
| Gpi-A  | 31   | 0.1062* | 24   | 0.1062* | 24         | 0.1062* |
| Gpi-B  | 31   | 0.0000 | 33   | 0.0000 | 33         | 0.0000 |
| Idhp-1 | 31   | 0.7062* | 26   | 0.7062* | 26         | 0.7062* |
| Idhp-2 | 31   | 0.0000 | 32   | 0.0000 | 32         | 0.0000 |
| Ldh-A  | 31   | 0.0000 | 33   | 0.0000 | 33         | 0.0000 |
| Ldh-B  | 31   | 0.0000 | 33   | 0.0000 | 33         | 0.0000 |
| mMdh-1 | 31   | 0.0164* | 33   | 0.0164* | 33         | 0.0164* |
| sMdh-A | 31   | 0.0000 | 33   | 0.0000 | 33         | 0.0000 |
| sMdh-B | 31   | 0.0000 | 33   | 0.0000 | 33         | 0.0000 |
| Mdhp-1 | 31   | 1.0000* | 32   | 1.0000* | 32         | 1.0000* |
| Mdhp-2 | 31   | 1.0000* | 33   | 1.0000* | 33         | 1.0000* |
| Pgm-1  | 31   | 0.0000 | 33   | 0.0000 | 33         | 0.0000 |
| Sod-1  | 31   | 0.0000 | 33   | 0.0000 | 33         | 0.0000 |
| Mean   | 0.0539 | 0.1934 | 0.3919 | 0.4804 | 0.1455     | 0.1455 |

* = Statistically significant at p = 0.05.
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. altiparanae* 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-I, Iddh-I, Mdhp-1* and *Mdh-2* loci in the PR population and at the *G6pdh-I* 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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