Allozyme comparison of two populations of *Rineloricaria* (Siluriformes, Loricariidae) from the Ivaí River, upper Paraná River basin, Brazil

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Abstract

Two allopatric morphotypes of the genus *Rineloricaria* were compared through the allozyme electrophoresis technique: one morphotype, *R. pentamaculata*, from the Keller River in the middle stretch of the Ivaí River basin and the other, *R. aff. pentamaculata*, from the São João River in the upper portion of the Ivaí River basin. The morphotype from the São João River was collected upstream from the São João waterfall, which is about 80 m deep. Twelve enzymatic systems (AAT, ADH, EST, GCDH, G3PDH, GPI, IDH, LDH, MDH, ME, PGM and SOD) were analyzed, which allowed to score 22 loci. Only loci Aat-2, Est-3 and Mdh-C showed polymorphism. The two samples differed in allele frequencies at the three polymorphic loci. The average expected heterozygosity for all loci was $0.0806 \pm 0.0447$ in the Keller River sample. For the São João River morphotype, this value was $0.0489 \pm 0.0350$. Nei’s genetic identity and distance between the two populations were respectively 0.9789 and 0.0213. Wright’s $F_{ST}$, $F_{IT}$ and $F_{ST}$ over all loci were estimated as 0.3121, 0.4021 and 1.309, respectively. We consider that the two morphotypes represent species in *status nascendi*.

Key words: allozymes, fish genetics, genetic distance, polymorphism, Loricariidae, Pisces.

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the same species and between 0.35 and 0.85 between two species of the same genus. Therefore, if the genetic identity between two taxonomic units is inferior to 0.85, it is improbable that they are conspecific. If it is inferior to 0.35, they should be considered species of a different genus.

Studies on isozyme electrophoresis have supplied important information about the genetic variability of natural populations, estimation of gene flow, elucidation of interspecific limits and the establishment of evolutionary relationships among different taxa (Solférini and Selivon, 2001). When the morphological criteria do not supply clear evidence for the validity of a genus, estimates of genetic distance can generate objective data (Thorpe, 1982; Nei, 1987).

*Rineloricaria pentamaculata* was described by Langenani and Araújo (1994), based on nine specimens collected from several locations in the upper Paraná River basin. Collections from the Ivaí River basin showed the presence of two morphologically different populations of *R. pentamaculata*. The main characteristic of *R. pentamaculata* is the presence of five dark dorsal stripes over the dorsum. It differs from congeners by having a naked snout tip, without platelets or odontodes, and an upper caudal-fin ray with an extension usually equal in length to the orbital diameter.

Considering that a waterfall can represent a geographical barrier to gene flow and that the two populations present morphological differences as regards the form of the snout, form of the lip, disposition of the dermal plates and form of the caudal fin, this work was done to verify whether or not these two populations belong to the same species.

Two samples of *Rineloricaria* from the Ivaí River basin (Figure 1) were collected between March and July 2006. Thirteen specimens (Figure 2) were collected in the Keller River, Marialva, Paraná State (23°38′30.59″ S; 51°51′32.47″ W) and 22 in the São João River, Prudentópolis, Paraná State (25°4′29.95″ S; 50°59′57.84″ W). The São João River specimens were collected upstream from the São João waterfall (80 m high).

The Keller River sample was identified as *Rineloricaria pentamaculata* and the São João sample was identified as *R. aff. pentamaculata* because it shows morphological differences in relation to specimens from the Keller River. The main morphological differences between *R. pentamaculata* from the São João and Keller rivers are: I) a rounded vs. a pointed, more triangular snout tip; II) a lower lip distal border that is smooth or weakly serrate, vs. strongly serrate; III) each abdominal platelet being surrounded by skin, vs. abdominal platelets that are attached to each other and not surrounded by skin and; IV) an upper caudal-fin spine that usually does not extend beyond the branched ones, vs. an upper caudal-fin spine that is prolonged beyond the branched ones (the extension being similar in length to the eye diameter).

Muscle, liver, eye, stomach, heart, kidney and gill tissue samples were homogenized with a plastic stick in propylene tubes (1.5 mL) containing 100 µL of Tris-HCl buffer 0.02 M, pH 7.5 and centrifuged at 44,720 g at 4 °C for 30 min. Due to the presence of a great amount of fat in the liver, 100 µL of carbon tetrachloride (CCl₄) was added to the tubes (Pasteur et al., 1988).

Gels were prepared with 15% corn starch (Val et al., 1981). Three buffer solutions were used: Tris 0.135 M/Citric acid 0.043 M pH 7.0 (Shaw and Prasad, 1970), Tris 0.18 M/Boric acid 0.1 M/EDTA 0.004 M pH 8.6 (Boyer et al., 1963) and Tris 0.1 M/Maleic acid 0.1 M/EDTA 0.01 M pH 7.4 (Murphy et al., 1996). The enzyme extract was ap-
showed allele variation, with four, three and two alleles, re-

The genetic variability was estimated according to the
average heterozygosity (He and Ho) of Nei (1978). The
homogeneity of the allele frequencies between populations
was verified through a contingency chi-squared test. The
unbiased genetic identity (I) and genetic distance (D) were
calculated following Nei (1978). All of the estimates were
calculated using the software POP GENE 1.31 (Yeh 
et al.
(1996).

Voucher specimens were deposited in the ichthyological
collection of the Núcleo de Pesquisas em Limnologia,
Ictiologia e Aquicultura, Universidade Estadual de Marin-
gá (NUP): Rineloricaria pentamaculata: NUP 2599, from
the Keller River and R. aff. pentamaculata: NUP 4292,
from the São João River. Twelve enzymatic systems (AAT,
ADH, EST, GCDH, G3PDH, GPI, IDH, LDH, MDH, ME,
PGM and SOD) were analyzed, which allowed to score 22
loci. Table 1 shows the analyzed enzymatic systems and the
respective allele frequencies. In the sample from the Keller
River, only Aat-2, Est-3 and Mdh-C (13.64% of all loci)
showed allele variation, with four, three and two alleles,
respectively. In the sample from the São João River, only
Aat-2 and Est-3 (9.09% of all loci) presented variation, with
three alleles each.

Table 2 presents the values of observed and expected heterozygosities, and the effective number of alleles per loc-
us for both samples. The average observed heterozygosity
for all loci was 0.0475 ± 0.031 in the sample from the Keller
River and 0.0411 ± 0.03 in the sample from the São João
River, values that are not statistically different (t = 0.1787;
21 d.f.; p > 0.05). The average expected heterozygosity for
the three polymorphic loci was estimated to be
0.0806 ± 0.0447 for the Keller River sample and
0.0489 ± 0.0350 for the São João River sample, values
that are not statistically different (t = 1.095; 21 d.f.; p > 0.05).
The sample from the Keller River was not in Hardy-
Weinberg equilibrium for Est-3 (χ² = 14.0979; p = 0.003),
while the R. pentamaculata sample from the São João
River was in Hardy-Weinberg equilibrium for the three polymor-
phic loci. The two populations differed in the allele fre-
cuencies of Est-3 (χ² = 9.3778; p = 0.0092), Aat-2
(χ² = 15.56; p = 0.0014) and Mdh-C (χ² = 29.46; p = 0).

Zawadzki et al. (2004) found all of the polymorphic
loci of four populations of Hypostomus from the Keller River
to be in Hardy-Weinberg equilibrium. Paiva et al. (2005)
also detected Hardy-Weinberg equilibrium for three spe-

Table 1 - Allele frequencies at 22 loci of Rineloricaria pentamaculata
from Keller River and Rineloricaria aff. pentamaculata from São João
River, Ivaí River basin.

| Locus   | Keller (n = 13) | São João (n = 22) | Tissue | Buffer |
|---------|----------------|------------------|--------|--------|
| Aat-1   | a              | 1.0000           | L      | TEM    |
| Aat-2   | a              | 0.4615           | 0.7619 | TEM    |
|         | b              | 0.0769           | 0.1667 |        |
|         | c              | 0.2692           | 0.0714 |        |
|         | d              | 0.1923           |        |        |
| Acp-1   | a              | 1.0000           | L      | TC     |
| Acp-2   | a              | 1.0000           | L      | TC     |
| Adh     | a              | 1.0000           | L      | TBE    |
| Est-1   | a              | 1.0000           | L      | TBE    |
| Est-2   | a              | 1.0000           | L      | TBE    |
| Est-3   | a              | 0.4615           | 0.3571 | TBE    |
|         | b              | 0.0385           | 0.3571 |        |
|         | c              | 0.5000           | 0.2857 |        |
| Gdh     | a              | 1.0000           | L      | TEM    |
| G3pdh-1 | a              | 1.0000           | M      | TC     |
| G3pdh-2 | a              | 1.0000           | M      | TC     |
| Idh     | a              | 1.0000           | M      | TC     |
| Ldh-A   | a              | 1.0000           | M      | TC     |
| Ldh-B   | a              | 1.0000           | M      | TC     |
| Mdh-A   | a              | 1.0000           | M      | TC     |
| Mdh-B   | a              | 1.0000           | M      | TC     |
| Mdh-C   | a              | 0.4583           | 1.0000 | M      |
|         | b              | 0.5417           |        |        |
| Me-1    | a              | 1.0000           | M      | TC     |
| Me-2    | a              | 1.0000           | M      | TC     |
| Pgm     | a              | 1.0000           | M      | TC     |
| Gpi-1   | a              | 1.0000           | M      | TC     |
| Gpi-2   | a              | 1.0000           | M      | TC     |

L = liver; M = muscle; n = number of specimens; TEM = Tris/EDTA/
Maleate; TC = Tris/Citrate; TBE = Tris/Borate/EDTA.

Table 2 - Observed (Ho) and expected heterozygosity (He) per polymor-
phic locus and the average over 22 loci, and effective number of alleles
(Ae) for Rineloricaria pentamaculata of the Keller River and
Rineloricaria aff. pentamaculata of the São João River, Ivaí River basin.

| Locus   | Keller (n = 13) | São João (n = 22) | Ho   | He   | Ae   |
|---------|----------------|------------------|------|------|------|
| Aat-2   | 0.3846         | 0.6985           | 3.0450 | 0.3333 | 0.3961 | 1.6303 |
| Est-3   | 0.0769         | 0.5569           | 2.1529 | 0.5714 | 0.6794 | 2.9697 |
| Mdh-C   | 0.5383         | 0.5181           | 1.9862 | 0.0000 | 0.0000 | 1.0000 |
| Average | 0.0475         | 0.0806           | 1.1902 | 0.0411 | 0.0489 | 1.1182 |
| SE      | 0.031          | 0.0447           | 0.2538 | 0.030  | 0.0350 | 0.2384 |

SE = Standard error.
cies of *Hypostomus* of the Maringá Stream. A great number of factors can deviate a population from Hardy-Weinberg equilibrium for a given locus, e.g. inbreeding, assortative mating, natural selection and gene flow (Nei, 1987), in addition to small sample size. Similar values of allele frequencies at the same locus turn the effective number of alleles (Ae) similar to the obtained number (Table 2). The value of Ae could have contributed to an increase in He, even though the number of polymorphic loci in our sample is small.

The value of He for other species of freshwater fish is reported as 0.051 (Ward et al., 1992), when these were analyzed with the same 12 enzymatic systems as in our study. The average of He was 0.0806 in the sample from the Keller River, which is larger than the average for species of fish in general. However, in the population from the São João River, the value of He (0.0489) is smaller than the average estimated by Ward et al. (1992). The high heterozygosity estimated for the population of the Keller River results from the fact that this population has three polymorphic loci, while the population of the São João River presented only two. The high heterozygosity of Keller River population seems paradoxical, because that population was polymorphic at only 13.64% of the loci. Paiva et al. (2005), found 20% polymorphic loci for the population and *Hypostomus strigaticeps* of the Maringá Stream; however, the expected heterozygosity was only 0.028. A similar fact occurred with a *Hypostomus* sp. 3 population collected in the Itapu Reservoir analyzed by Zawadzki et al. (2005), where these Loricariidae showed 24% of polymorphic loci, despite an expected heterozygosity was 0.048. The relatively high heterozygosity estimated in our work, when compared to the above studies, can be explained by the similarity among the allele frequencies in the analyzed polymorphic loci. The value of expected heterozygosity is maximal when the allele frequencies are similar and indeed, at *Mdh-C* we observed similar frequencies for the alleles *Mdh-C* (a) and *Mdh-C* (b) (0.4583 and 0.5417, respectively). In Paiva et al. (2005) and Zawadzki et al. (2005), the allele frequencies at the polymorphic loci were quite divergent, which explains the lower He value.

The He values estimated for species of Loricariidae have high variation, as found in several *Hypostomus* populations. The currently available results show that the family Loricariidae contains species with larger genetic variability than others, i.e. 0.011 and 0.017 for two populations of the Iguacu River basin (Zawadzki et al., 1999), 0.000 and 0.107 for two species of the Paraná River basin (Paiva et al., 2005 and Zawadzki et al., 2005, respectively), and 0.000 and 0.172 for *H. albopunctatus* and *H. hermani*, respectively (Paiva S, MSc Dissertation, Universidade Estadual de Maringá, 2006).

Wright’s *F*<sub>IS</sub>, *F*<sub>IT</sub> and *F*<sub>ST</sub> per polymorphic locus were statistically different from zero, which indicates a significant excess of homozygotes. The averages over all loci were estimated as *F*<sub>IS</sub> = 0.3121, *F*<sub>IT</sub> = 0.4021 and *F*<sub>ST</sub> = 0.1309, respectively. Nei’s genetic identity and genetic distance were estimated as 0.9789 and 0.0213, respectively.

The *F*<sub>IS</sub> value represents the level of gene fixation within subpopulations. *F*<sub>IT</sub> represents the level of gene fixation present in the total population (ignoring the subdivision). The *F*<sub>ST</sub> value indicates the genetic differentiation between two populations (Wright, 1978). Except for *Mdh-C* in the population of the Keller River, *F*<sub>IS</sub> and *F*<sub>IT</sub> values indicate a significant excess of homozygotes, while *F*<sub>ST</sub> values show that 13.09% of the total heterozygosity is due to the differentiation between the two *Rineloricaria* populations analyzed. The excess of homozygotes is possibly a result of inbreeding, since *R. pentamaculata* is a sedentary species.

Values of *F*<sub>ST</sub> above 0.25 can be considered indicative of a great genetic differentiation, whereas values between 0.15 and 0.05 indicate a moderate and below 0.05 a small differentiation between populations (Wright, 1978). Following Wright’s criterion, *R. pentamaculata* of the Keller River and *R. aff* *pentamaculata* of the São João River show a moderate genetic differentiation. The values of *F*<sub>ST</sub> are statistically different from zero for the three polymorphic loci (χ<sup>2</sup> = 15.52, χ<sup>2</sup> = 8.43, χ<sup>2</sup> = 25.26; for Aat-2; Est-3 and *Mdh-C*, respectively). These values show that the two populations are genetically different at these three loci; however, the average for the 22 loci turns the differentiation between the two populations moderate.

According to Thorpe and Solé-Cava (1994), significant variation at any locus represents a barrier to the gene flow for sympatric populations and may result in, at least partial reproductive isolation. In organisms with sexual reproduction, this variation indicates that two populations should be considered different species.

*Rineloricaria pentamaculata* of the Keller River and *R. aff* *pentamaculata* of the São João River of the Ivaí River basin are isolated geographically. As they differ morphologically, as well as in allele frequencies at the polymorphic loci, it is probable that they represent species in *status nascendi* (Dobzhansky, 1970), i.e. in the process of genetic divergence to become two distinct species.

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