Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v39i1.31793

Genetic variability and evidence of founder effect in *Hemiodus orthonops* (Characiformes: Hemiodontidae) from the upper Paraná River basin, Brazil

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ABSTRACT. *Hemiodus orthonops* is a small fish of the Hemiodontidae family, order Characiformes, with a maximum of 25 cm standard length. Until recently, *H. orthonops* was an endemic species from the Paraná-Paraguay basin and it was absent from the upper Paraná River basin. Since 2008, it has started to be collected in the upper Paraná River, representing up to 10% of catches. Two population samples of *H. orthonops* from two localities of the upper Parana River basin (Porto Camargo and Porto Figueira) were analyzed using the allozymes electrophoresis technique. Twenty-one enzymatic *loci* were detected. The population sample from Porto Camargo displayed a genetic variability (He = 0.1061) higher than that from Porto Figueira (He = 0.0580) and homozygote excess in both of them. The F_{ST} value (0.2081) indicated genetic structure. The excess of homozygotes in both samples was probably due to founder effect in the population.

Keywords: Allozymes, fish, genetic distance, heterozygosity, protein polymorphism.

Variabilidade genética e evidência do efeito do fundador em *Hemiodus orthonops* (Characiformes: Hemiodontidae) da bacia do alto rio Paraná, Brasil

RESUMO. *Hemiodus orthonops* é um pequeno peixe da família Hemiodontidade da Ordem Characiformes com um comprimento padrão máximo de 25 cm. Até recentemente, *H. orthonops* estava ausente da bacia do alto rio Paraná. Desde 2008 ele passou a ser coletado na bacia do alto rio Paraná, representando até 10% das coletas. Duas amostras populacionais de *H. orthonops* provenientes de duas localidades da bacia do alto rio Paraná (Porto Camargo e Porto Figueira) foram analisadas pela técnica de eletroforese de aloenzimas. Vinte um *loci* enzimáticos foram detectados. A amostra proveniente de Porto Camargo revelou uma variabilidade genética (He = 0,1017) superior à amostra de Porto Figueira (He = 0,0558) e excesso de homozigotos em ambas as amostras. O valor de F_{ST} entre elas (0,2081) indica que há estruturação genética. O excesso de homozigotos nas duas amostras é provavelmente devido ao efeito do fundador.

Palavras-chave: Aloenzimas, peixes, distância genética, heterozigosidade, polimorfismo proteico.

Introduction

Habitat alterations caused by water flow control induced by dams, for example, disrupt the structure of the aquatic biota and facilitate the establishment of invasive species (Havel, Lee, & Zanden, 2005). The installation of fish passages in dams, with the aim to preserve migratory species, may pose an additional threat to the biota from upstream stretches by increasing the dispersion of non-native species. This may occur when the dam separates distinct native faunas along the watershed, as observed in the Paraná River (Agostinho et al., 2015). In the case of Paraná River, the Itaipu Reservoir, filled in 1982, covered the Sete Quedas Falls, which separated distinct ichthyofauna provinces. As a consequence, 33 species of native fishes in the lower Paraná basin successfully colonized the upper Paraná River (Julio Junior, Dei Tós, Agostinho, & Pavanelli 2009). In 2002, a channel for fish migration named "Canal de Piracema" was opened, allowing 28 of the 68 fish species registered downstream of the dam to rise towards the Itaipu Reservoir (Agostinho, Gomes, Fernandez, & Suzuki, 2002).

Hemiodus orthonops is a small fish of the Hemiodontidae family, order Characiformes, with a maximum of 25 cm standard length (Froese & Pauly, 2015), described by Eigenmann & Kennedy (1903) from specimens collected in the Paraguay River, near the town of Asuncion.

Until recently, *H. orthonops* was an endemic species from the Paraná-Paraguay basin and it was absent from the upper Paraná River basin (Agostinho et al., 2015). Since 2008, it has started to be collected in the upper Paraná River, representing up to 10% of catches (Agostinho et al., 2015).

In the Paraná River population studied by Agostinho et al. (2015), the maximum standard length observed for *H. orthonops* was 26 cm for males and 29 cm for females; individuals become sexually mature at one year of age when they reach 15.2 cm of length for males and 19.4 cm for females, releasing a maximum amount of 79,653 oocytes with 0.79 mm diameter each and feeding primarily on detritus and algae.

According to Agostinho et al. (2015), the success of *H. orthonops* at colonizing the upper part of Paraná River was due to dispersal ability, favorable environmental conditions, the ability to exploit highly available food resources, early maturation and high body growth rate.

Invasive species are predicted to suffer from reductions in genetic diversity when only a few individuals from the source population colonize new habitats, thus undergoing the founder effect and reducing adaptive potential (Mayr, 1963; Allendorf & Luikart, 2007).

The founder effect on genetic variability of experimental populations of mosquito fish were measured by the allozymes electrophoretic technique by Leberg (1992), who estimated the values of proportion of polymorphic *loci*, number of alleles per *locus* and heterozygosity. Compared to source populations, Leberg found that 23 populations had a smaller He and 15 had an equal or greater He. On average, all three indexes of genetic variability decreased as the effective size of the founding population decreased.

Roman and Darling (2007) reviewed data of 43 introduced aquatic species and found that only 16 (37%) showed clear evidence of significant loss of genetic diversity relative to native populations, while in 27 species there was no alteration.

Dlugosch and Parker (2008) analyzed data from 80 species in the literature covering 18 plants, 2 fungi, and 60 animals (including 7 birds, 6 reptiles, 8 fish, 3 amphibians, 8 mammals, 13 insects, 4 crustaceans, 6 mollusks, 3 annelids, 1 cnidarian, and 1 tunicate) and showed that, in introduced populations, there was an average reduction of 15.5% in allelic richness and a decrease of 18.7% in average heterozygosity compared to the original population.

However, other studies have shown that many invasive species did not lose the variability and in some of them it was even higher. Bossdorf et al., (2005) revealed that 69% of invasive plants had the same or greater genetic diversity than native populations. A similar study of 29 terrestrial and aquatic animal species showed that introduced species have preserved 80% of the genetic variability existent in native populations (Wares, Hughes, & Grosberg, 2005).

Thus, in this study we aimed to estimate the genetic variability of *Hemiodus orthonops* using the allozymes electrophoresis technique and compare it to other species from Paraná River basin, which was also studied with allozymes electrophoretic technique. Furthermore, we verified the presence of a founder effect in the invasive population of this migratory species in the Paraná River.

Material and methods

Hemiodus orthonops individuals were collected in the upper Parana River basin, area of Porto Camargo (23°21'27.87"S, 53°44'54.14"W), municipality of Icaraíma and in Porto Figueira (23°23'43.93"S, 53°48'47.79"W), municipality of Alto Paraíso (Figure 1) since March, 2012 to April, 2013.

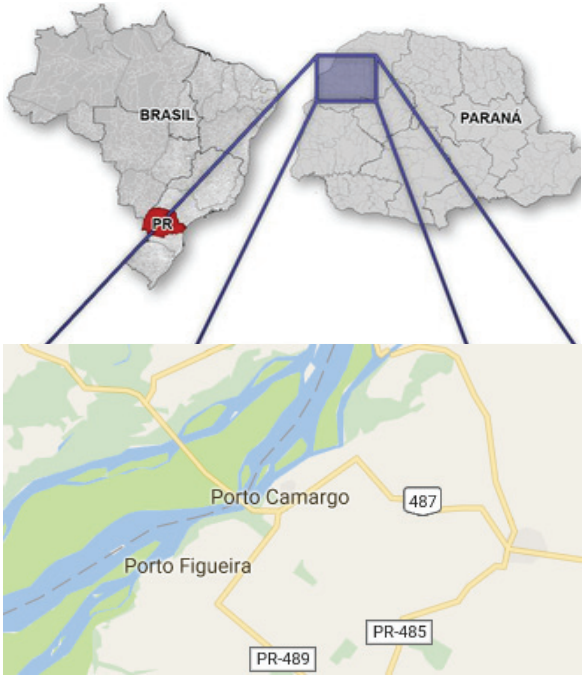

Figure 1. Map showing the places of capture of *Hemiodus orthonops*: Porto Camargo (23°21'27.87"S, 53°44'54.14"W) and Porto Figueira (23°23'43.93"S, 53°48'47.79"W), State of Paraná, Brazil.

Fifteen specimens were captured in the first mentioned municipality, and 13 in the second one. Voucher specimens were deposited in the collection of Nupelia (*Núcleo de Pesquisas em Limnologia,*

Ictiologia e Aquicultura) of the *Universidade Estadual de Maringá* NUP 10610. All specimens were frozen and stored at low temperature (-20° C) until the extraction of enzymes. The enzymes were extracted from samples of muscle, liver and heart tissue, using buffer Tris-HCl, 0.02 M, pH 7.5. The samples were centrifuged in a refrigerated centrifuge at 4° C and 16,434.6 g. The protein extract was applied on corn starch gel (Val, Schwantes, Schwantes, & De Luca, 1981) Penetrose 50 at a concentration of 17%, with small filter paper strips (4 mm x 8 mm) Whatman 3 MM® soaked with the samples. Subsequently, the gel was submitted to a continuous horizontal electrophoresis under refrigeration. Three buffer solutions were used: 0.135 M Tris/Citric Acid 0.043 M, pH 5.9 (TC), diluted 15 times in the preparation of the gel; 0.1 M Tris/Maleic Acid 0.1 M/EDTA 0.004M/Magnesium chloride 0.01M, pH 7.4 (TEM) in tank and diluted 10 times in the gel, and Tris 0.18M/Boric acid 0.1M/EDTA 0.004M, pH 8.6 (TBE), diluted four times in the gel preparation. The polyacrylamide gel, applied for the revelation of Esterase enzyme, was prepared in two glass plates, consisting of two systems of gel with different porosities and pH. The concentration of the separating gel was of 10% of acrylamide-Bisacrylamide and the stacking gel was of 5%.

Muscle samples were prepared as described in the starch gel protocol, however, the buffer employed to macerate was 1.5 M Tris-HCl, pH 8.8 with 10% glycerol, and the samples were centrifuged for 20 minutes at 4°C to 16,434.6 g. Ten enzyme systems in starch gel were analyzed: Aspartate amino Transferase (AAT), Alcohol Dehydrogenase (ADH), Glucose-3-phosphate dehydrogenase (G3PD), Glucose-6-phosphate dehydrogenase (G6PD), Glucose phosphate Isomerase (GPI), Isocitrate Dehydrogenase (IDH), Lactate dehydrogenase (LDH), Malate dehydrogenase (MDH) Malic Enzyme (ME), Phosphoglucomutase (PGM), and Esterase (EST) in the polyacrylamide gel. The nomenclature of enzymes used was previously proposed by Murphy, Sites, Buth, and Haufler (1996). The genetic interpretation of zymograms was based on the quaternary structure of enzymes according to Ward, Skibinski, and Woodwark (1992).

Data were analyzed in the Popgene software 3.1 (Yeh, Yang, Boyle, Ye, & Mao, 1997). The genetic variability was estimated by the calculation of the observed and expected heterozygosity (He and Ho) according to Nei (1978). Considering the values of allele frequencies, the identity (I) and the genetic distance (D) from Nei (1978) were evaluated. To compare the genetic variability of *H. orthonops* with other species of the Paraná River basin, the authors calculated the mean of 74 species distributed in 12 families (49 Loricariidae, three Callichthyidae, five Pimelodidae, one Cynodontidae, one Erythrinidae, six Anostomidae, one Prochilodontidae, one Auchenipeteridae, three Gymnotidae, one Serrasalmidae, three Characidae and one Cichlidae).

Results

In the Porto Camargo sample (PC), 27 alleles distributed in 21 *loci* were detected in *H. orthonops,* six *loci* of which were polymorphic (*Adh, G6pdh, Ldh-1, Est-1, Est-2, Est-3*); in Porto Figueira sample (PF), 25 alleles were detected in 21 *loci,* of which three were polymorphic (*Adh, Est-1, Est-3*).

Table 1 illustrates the allele frequencies of each *locus* of each sample. The Aspartate enzyme Amino Transferase (AAT) expressed two *loci*: *Aat-1*, which codifies cathodic enzymes and *Aat-2* that codifies anodyne enzymes. The *loci Aat-1* and *Aat-2* were monomorphic for the two population samples.

Table 1. Allele frequencies for six polymorphic loci in two samples of *Hemiodus orthonops* from Paraná River at Porto Camargo (PC) and Porto Figueira (PF).

Locus	Allele	PC	PF
Adh	*a*	0.5385	0.5000
	b	0.4615	0.5000
G6pdh	*a*	0.3750	1.0000
	b	0.6250	
Ldh-1	*a*	0.9615	1.0000
	b	0.0385	
Est-1	*a*	0.1538	0.0769
	b	0.8462	0.9231
Est-2	*a*	0.4000	1.0000
	b	0.6000	
Est-3	*a*	0.2308	0.0385
	b	0.7692	0.5385
	c		0.4231

The ADH enzyme expressed one *locus* with two alleles for the two samples in Hardy-Weinberg equilibrium. The G3PDH enzymes were encoded by two *loci,* being both *loci G3pdh-1* and *G3pdh-2* monomorphic. The G6PDH enzyme presented one polymorphic *locus* for *H. orthonops* in the PC region, being in Hardy-Weinberg equilibrium and monomorphic for PF region. Two *loci* for the GPI enzyme were detected, both *Gpi-1* and *Gpi-2* monomorphic for both regions. The IDH enzyme showed two *loci Idh-1* and *Idh-2*, which were both monomorphic. Two *loci* were seen for LDH: *Ldh-1 and Ldh-2*. *Ldh-1* was monomorphic for the PF sample and polymorphic with two alleles in Hardy-Weinberg equilibrium for the PC sample. The MDH enzyme expressed three *loci*, all monomorphic in both samples. Two *loci* for the ME enzyme were detected, named *Me-1* and *Me-2*, which showed no variation. For the PGM enzyme, a monomorphic *locus Pgm-1* was found for both samples.

Table 2 exhibits a summary of the genetic variability results for both population samples. Through analysis of this data, it was possible to verify that the *H. orthonops* sample from the PC region had a polymorphic *loci* proportion of 26.09%, of which six *loci* were polymorphic and presented a ratio of 13.04% for the PF sample with three polymorphic *loci*. The mean number of allele per *locus* was 1.3 and 1.2 for PC and PF, respectively.

Table 2. Measures of genetic variability in *Hemiodus orthonops* samples from upper Paraná River basin at Porto Camargo (PC) and Porto Figueira (PF) in Paraná State. He: = expected heterozygosity; Ho = observed heterozygosity; P% = proportion of polymorphic loci; K = mean number of alleles by *locus*; SD = standard deviation.

Sample	He ± SD	Ho ± SD	P%	K ± SD
PC	0.1017 ± 0.1843	0.0668 ± 0.1683	26.09	1.286 ± 0.4629
PF	0.0558 ± 0.1558	0.0330 ± 0.1206	13.04	1.191 ± 0.5118

In the present study, the average Ho and He were of 0.0668 and 0.1017 for samples from PC, and 0.033 and 0.058 for samples from PF. The fixation index (F) was 0.34 for PC and 0.41 for PF, that is, there was an excess of 34% of homozygotes in the PC sample and 41% in the PF sample.

In order to verify the inbreeding level and the population structure, Wright's F statistics (1965), F_{IS}, F_{IT} and F_{ST} were calculated for the two *H. orthonops* population samples (Table 3). F_{IS} measures the mean excess or deficiency of heterozygotes in the two populations and showed that are an excess of 35.8% of homozygotes. The F_{IT} (0.4917) coefficient showed that if the two populations were united in one there would be an excess of 49.17% of homozygotes. The F_{ST} value (0.2081) represents a differentiation measure of the two populations. In other words, 20.81% of the expected heterozygosity was due to the separation between the populations. All F ratios were statistically significant (Table 3). Bringing the two samples together in a single population, we obtained the values of H_O = 0.0497, H_T = 0.0979 and H_S = 0.0791. The identity and the genetic distance between them were estimated according to Nei (1978) in I = 0.9558 and D = 0.0452.

Table 3. F Statistics summary for each *locus* of *Hemiodus orthonops* samples from Paraná River at Porto Camargo and of Porto Ferreira in the Paraná State. (N = sample size; χ^2 = significant test; * P<0.05; **P<0.01)

Locus	**N**	F_{IS}	χ^2	F_{IT}	χ^2	F_{ST}	χ^2
Adh	26	-0.1573	4.09*	-0.1556	8.09**	0.0015	0.08
G6pdh	25	0.4667	11.67**	0.7091	35.45**	0.4545	22.72**
Ldh-1	26	-0.0400	1.04	-0.0196	1.02	0.0196	1.02
Est-1	26	1.0000	26.00**	1.0000	52.00**	0.0145	0.08
Est-2	23	1.0000	23.00**	1.0000	52.00**	0.4286	19.72**
Est-3	26	0.3043	7.91**	0.392	20.38**	0.1321	13.74**
Mean	26	0.3581	9.31**	0.4917	25.57**	0.2081	10.82**

Discussion

The data obtained in this work showed that *H. orthonops* has little mean number of alleles by *locus* (1.29 for PC sample and 1.19 for PF sample) and revealed that there is an excess of homozygotes in the two samples. Based on data of literature, the authors calculated the genetic variability for 74 species from Paraná River that were also studied with the allozymes electrophoresis technique. The result was a mean of 21.5 *loci*, 24.4% of which were polymorphic, 1.3 allele per *locus* and values of Ho and He of 0.037 and 0.076, respectively. In this work, we found mean values of Ho and He of 0.05 and 0.10 respectively (Table 2), 26.09% of polymorphic *loci* and 1.3 of alleles per *locus*. Therefore *H. orthonops* has greater genetic variability than the mean of 74 species from Paraná River basin and greater than the mean of 195 fish species estimated (He = 0.051) by Ward et al. (1992).

F_{ST} values show that, despite the short distance separating the two sampled populations (7 Km), they are genetically structured. According to Wright (1978), F_{ST} values ranging from 0.0 to 0.05 may indicate slight genetic differentiation; from 0.05 to 0.15 indicates moderate differentiation; from 0.15 to 0.25 indicates large differentiation and over 0.25 indicates a very large differentiation. Therefore, it can be assumed that the value obtained (0.2081; χ^2 = 10.82; P<0.01)) demonstrates a large differentiation among the two populations.

The F_{IS} coefficient measures the heterozygosity reduction due to non-random mating within a subpopulation. Observing Table 3, a significant F_{IS} value (0.3581; χ^2 = 9.31; P<0.01) can be noticed, indicating an excess of homozygotes. Moreover, the average F_{IT} value (0.4917; χ^2 = 25.57; P<0.01) denotes an excess of homozygotes if the two populations were grouped in only one. Such high excess of homozygotes may be owing to a considerable level of consanguinity or to a founder effect due to the recent colonization of the sampled environment. The results obtained by Agostinho et al., (2015) points that the number of captured individuals in the samplings increased from 8 in 2008 to 753 in 2013, which means that this species multiplied 94 times in five years. Therefore, the population dwelling in the Paraná River should be numerous, avoiding consanguinity. In accordance with such data, it is likely that the excess of homozygotes is due to founder effect of the population that had multiplied quickly.

We believe that the high variability is an intrinsic feature of the species, more related to high fertility and population size. To support such hypothesis,

there are the results obtained by Ribeiro, Morán, and Caballero (2008) in Atlantic salmon (*Salmo salar*). The mentioned authors found that reducing the salmon population size from the Eo River, Spain, did not reduce the level of genetic diversity.

Allelic richness is generally predicted to be more sensitive to founder effects than heterozygosity is (Nei, Maruyama, & Chakraborty, 1975; Allendorf 1986; Leberg 1992). For studies that reported both metrics, a paired comparison showed that proportional losses of allelic richness were on average 5.1% more severe (more negative) than losses of heterozygosity in *Hypericum canariense* (Dlugosch & Parker, 2008). This difference is expected because allelic richness will reflect the loss of rare alleles that contributed little to heterozygosity (Dlugosch & Parker, 2008). In this study, we had not analyzed samples of *H. orthonops* from localities downstream Itaipu Reservoir to verify if the values of He, proportion of polymorphic loci and mean number of alleles were reduced or not. The fact of these values in the sample of PF is lower than that of PC is suggestive that the colonization of the upper Parana River basin by *H. orthonops* had reduced those values.

Founder effect was detected in natural populations of various species (Dlugosch & Parker, 2008) and in experimental populations of the mosquito fish *Gambusia Holbrooki* (Leberg, 1992). It was not yet evaluated if the species that migrated through Canal de Piracema passed through a bottleneck due to founder effect. This artificial corridor may contribute to founder effects in other fish species that are capable to transpose these barriers and colonize new environments upstream as did *H. orthonops*.

To support or refute these findings, it would be interesting to conduct genetic analysis with DNA markers in both populations here analyzed and in the Paraguay River populations, where the species is native.

Conclusion

We conclude that the population of Hemiodus orthonops dwelling the upper Paraná River basin is genetically structured and they show homozygote excess probably due to founder effect.

Acknowledgements

The Authors would like to thank Henrique Bueno Ruiz and Rafael Vieira for helping with field work; to Rafaela Granzotti for helping with the language; to Corn Products Brazil for providing Penetrose 50.

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Received on April 30, 2016.
Accepted on October 11, 2016.