Allozyme variation in *Chiloglanis paratus* and *C. pretoriae* (Pisces, Mochokidae) from the Limpopo River system, Southern Africa

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Abstract

The gene products of 32 protein coding loci in two populations of *Chiloglanis paratus* and one population of *C. pretoriae* were examined by horizontal starch-gel electrophoresis. Genetic variability estimates were low in all three populations. The percentage of polymorphic loci using the 95% criterion ranged from 6.3% to 9.4% and observed (H_O) and expected heterozygosity (H_E) values for the three populations ranged from H_O = 0.022 to 0.031 and H_E = 0.025 to 0.036 respectively. Fixed allele mobility differences between the two species were observed at 17 loci and deviations of genotypes from Hardy-Weinberg expectations occurred at six loci. Genetic distance and Wright's fixation index estimates were consistent with values expected for conspecific populations but indicated extensive genetic differentiation between the congeneric species.

Introduction

The genus *Chiloglanis* comprises 34 species of which eight occur in Southern Africa. Members of the genus, commonly known as suckermouths, are small catlets adapted for life in fast flowing rocky streams or rapids and rocky stretches of rivers (Skelton, 1993). They are valuable in river management programmes as they are useful indicator species of pollution (Skelton, 1993). Furthermore, the suckermouths are popular aquarium fish.

Despite their popularity among aquarists and their importance in river conservation studies, there is no information available on the extent of genetic variation in suckermouths. A scientific approach to conservation and management of biotic resources requires a thorough understanding of the genetic structure of the species concerned. The applicability of allozyme electrophoresis for determining the genetic structure of populations is well documented (e.g. Grant et al., 1988; Van der Bank, 1996) and was the method of choice in the present study.

Two sympatric species comprising two populations of *C. paratus* and one population of *C. pretoriae* were analysed. Both species are endemic to Southern Africa and are superficially similar in appearance, distinguished by the presence of a serrated dorsal spine in *C. paratus* and a smooth dorsal spine in *C. pretoriae*. The purpose of this study was to provide information on the extent of genetic diversity and variability in *C. paratus* and *C. pretoriae* and to compare the data obtained with those for other freshwater fish species.

Materials and methods

Specimens were collected by electro-narcosis at two localities in the Limpopo River system (Fig. 1). Population 1, *C. paratus* (N = 50), was sampled at the confluence of the Shashe and Limpopo Rivers (22°14' S, 29°22' E) whereas Population 2, *C. paratus* (N = 48), and Population 3, *C. pretoriae* (N = 46), were collected at the Phalaborwa Barrage (24°03' S, 31°08' E) in the Olifants River. Voucher specimens of each population were deposited in the JLB Smith Institute for Ichthyology. The catalogue numbers are as follows: Population 1 (RUSI 57514), Population 2 (RUSI 57512) and Population 3 (RUSI 57513).

After capture, the specimens were frozen in liquid nitrogen (-196°C) for transportation purposes and then stored at -40°C in the laboratory to await electrophoresis. Prior to electrophoresis, approximately 0.5 g skeletal muscle tissue was mixed with 0.5 mℓ distilled water and homogenised using a glass rod. The samples were analysed by horizontal starch-gel electrophoresis using the experimental procedures, methods of interpretation of gel-banding patterns, locus nomenclature and statistical analysis as described and referred to in Engelbrecht and Mulder (1999). The buffer system of choice for separation of the proteins was a continuous Tris, boric acid, EDTA buffer (pH 8.6) as described by Markert and Faulhaber (1965). All allozyme data were analysed using the BIOSYS-1 programme of Swofford and Selander (1981). Genetic variability was assessed by calculating the percentage of polymorphic loci ($P_{0.95}$) and average observed (H_O) and expected heterozygosity (H_E) per locus. Deviations of allele frequencies from expected Hardy-Weinberg proportions for each locus were tested using the X^2-test for goodness-of-fit. Nei's (1972) genetic distance coefficients and fixation indices (F_{ST}) were calculated to determine the extent of genetic differentiation among the populations (Wright, 1978).

Results

Locus abbreviations, enzyme commission numbers and polymorphic loci are listed in Table 1. Eighteen enzyme systems coded by 32 loci were analysed of which 21 loci (66%) were monomorphic. Allele frequencies for polymorphic loci are presented in Table 2. Fixed allele mobility differences between the two species were observed at **AAT-2**, **CK-1**, **EST-1**, **EST-2**, **GPDH-1**, **GPI-2, IDH-1**, **IDDH-1, LDH-1**, **MPI-1**, **PEP-LT- 1**, **PGDH-1**, **PROT-1, PROT-2, PROT-3, PROT-4** and **SOD-1**. A zymogram of the **GPI** protein coding loci showing allele mobility differences for these enzymes between the three populations are presented in

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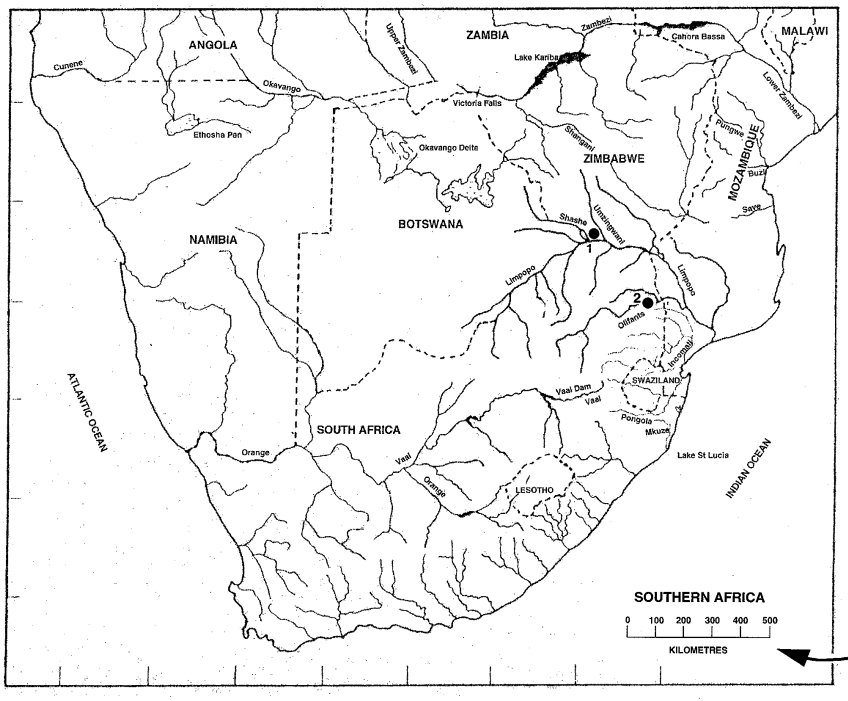

Figure 1
Localities at which C. paratus *(1 and 2) and* C. pretoriae *(2) were sampled during the present study. 1 = Confluence of the Shashe and Limpopo Rivers; 2 = Phalaborwa Barrage.*

TABLE 1
LOCUS ABBREVIATIONS AND ENZYME COMMISSION (E.C.) NUMBERS FOR EACH PROTEIN ANALYSED IN TWO POPULATIONS OF *C. PARATUS* AND ONE POPULATION OF *C. PRETORIAE*

Protein	Locus	E.C. No.
Aspartate aminotransferase	AAT-1, 2	2.6.1.1.
Creatine kinase	CK-1, 2, 3	2.7.3.2
Esterase	EST-1, 2, 3*	3.1.1.-
Fumarate hydratase	FH- 1	4.2.1.2
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH- 1	1.2.1.12
Glycerol-3-phosphate dehydrogenase	G3PDH- 1	1.1.1.8
Glucose-6-phosphate isomerase	GPI- 1*, 2	5.3.1.9
Hexokinase	HK- 1	2.7.1.1.
L-Iditol dehydrogenase	IDDH- 1, 2	1.1.1.14
Isocitrate dehydrogenase	IDHP- 1	1.1.1.42
L-Lactate dehydrogenase	LDH- 1, 2	1.1.1.27
Mannose-6-phosphate isomerase	MPI- 1	5.3.1.8
Peptidase:		
Substrate: Phenylalanyl-proline	PEP-D- 1*	3.4.13.11
Leucyl-tyrosine	PEP-LT- 1, 2	
Phosphogluconate dehydrogenase	PGDH- 1	1.1.1.44
Phosphoglucomutase	PGM- 1, 2*	5.4.2.2
General protein	PROT- 1, 2, 3, 4, 5	
Superoxide dismutase	SOD- 1	1.15.1.1

* Polymorphic loci ($P_{0.95}$)

Fig. 2. Deviations of genotypes from Hardy-Weinberg expectations occurred at six loci, **EST-3, GPI-1**, **PEP-D- 1**, **PEP-LT- 2, PGM-1** and **PGM-2**. Estimates of $P_{0.95}$ (range: 6.3% to 9.4%), H_O (range: 0.022 to 0.031) and H_E (range: 0.025 to 0.036), were low in all three populations (Table 2).

The value of Wright's (1978) fixation index of individuals relative to the subpopulation, F_{IS} is 0.116; for individuals relative to the total population, F_{IT} = 0.916; and F_{ST} = 0.905 for the amount of differentiation among subpopulations relative to the limiting amount under complete fixation. The genetic distance estimate (Nei, 1972) (D_N) between the two *C. paratus* populations was very small (0.017), whereas much larger genetic distance values were obtained between *C. pretoriae* and *C. paratus* from the Limpopo River (1.050) and between *C. pretoriae* and *C. paratus* from the Olifants River (0.987).

Discussion

Knowledge of the genetic structure of natural populations is essential for effective

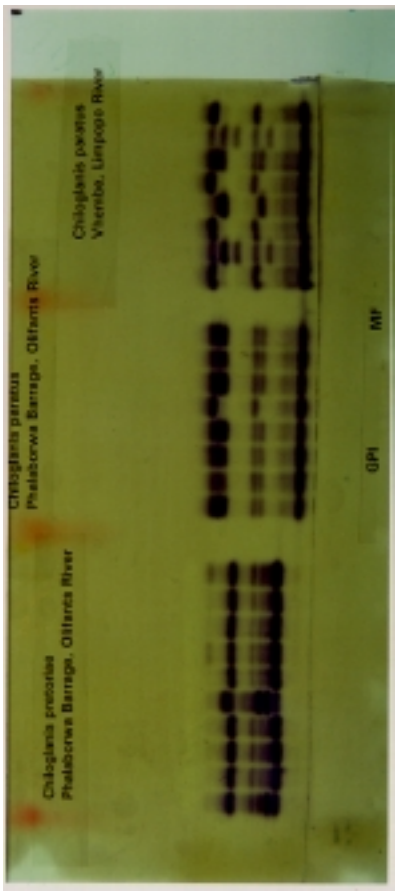

Figure 2
Zymogram showing allele mobility differences for ***GPI*** *between three populations of* C. paratus *and* C. pretoriae.

TABLE 2

ALLELE FREQUENCIES FOR POLYMORPHIC LOCI, O^2 VALUES FOR LOCI WHICH DEVIATE SIGNIFICANTLY FROM EXPECTED HARDY-WEINBERG PROPORTIONS (P > 0.05), PERCENTAGE OF POLYMORPHIC LOCI USING THE 0.95 CRITERION ($P_{0.95}$), AND AVERAGE OBSERVED (H_O) AND EXPECTED (H_E) HETEROZYGOSITIES WITH STANDARD ERRORS (SE) IN PARENTHESES FOR *C. PARATUS* AND *C. PRETORIAE*. POPULATION 1 = *C. PARATUS* FROM THE CONFLUENCE OF THE SHASHE AND LIMPOPO RIVERS; POPULATION 2 = *C. PARATUS* FROM THE PHALABORWA BARRAGE; AND POPULATION 3 = *C. PRETORIAE* FROM THE PHALABORWA BARRAGE

Locus	Allele	Population		
		Population 1	Population 2	Population 3
AAT-2	105			1.000
	100	1.000	1.000	
CK-1	105			1.000
	100	1.000	1.000	
EST-1		1.000	1.000	
				1.000
EST-2	100	1.000	1.000	
	95			1.000
EST-3	100	1.000	1.000	0.024
	95			0.500
	90			0.238
	85			0.214
	80			0.024
X^2				27.602
GPDH-1	105			1.000
	100	1.000	1.000	
GPI-1	105	0.687		
	100		0.562	1.000
	95	0.188		
	90		0.438	
	85	0.125		
X^2			4.840	
GPI-2	105			0.012
	100			0.988
	95	1.000	1.000	
IDH-1	105			1.000
	100	1.000	1.000	
IDDH-1	105			1.000
	100	1.000	1.000	
LDH-1	110			0.012
	105	1.000	1.000	
	100			0.988
MPI-1	105			1.000
	100	0.988	1.000	
	95	0.012		
PEP-D- 1	100	1.000	0.923	0.083
	95		0.077	0.917
X^2			13.000	24.000
PEP-LT- 2	100	0.976	1.000	
	95	0.024		0.955
	90			0.045
X^2		41.000		
PGDH- 1	105			1.000
	100	1.000	0.990	
	95		0.010	
PGM-1	105	0.031	0.010	0.023
	100	0.969	0.990	0.977
X^2		21.095		
PGM-2	100	0.830	0.948	0.957
	95	0.170	0.052	0.043
X^2			6.466	
PROT-1	105			1.000
	100	1.000	1.000	
PROT-2	105			1.000
	100	1.000	1.000	
PROT-3	100	1.000	1.000	
	95			1.000
PROT-4	100	1.000	1.000	
	95			1.000
PROT-5	105			0.036
	100	1.000	1.000	0.964
$P_{0.95}$		6.3	9.4	6.3
H_O		0.022 (0.015)	0.031 (0.027)	0.024 (0.014)
H_E		0.029 (0.018)	0.025 (0.017)	0.036 (0.021)

management in conservation biology (Meffe, 1990; Lavery and Fielder, 1993). Since collection of wild stock as well as artificial propagation of these popular aquarium fish are being carried out, a thorough understanding of the spectrum of genetic diversity of wild populations becomes imperative. This is important from a conservation point of view and for future stock selection and identification. Such fundamental information is important for freshwater fishes because of the opportunities for restricted gene flow and, therefore, heightened levels of population subdivision and possibly unique gene pools (Allendorf and Leary, 1988). Population geneticists commonly use five statistically based methods to assess the genetic structure of populations: conformance to Hardy-Weinberg expectations; the percentage of polymorphic loci; average observed and expected heterozygosity; Wrights' F-statistics; and several genetic distance coefficients.

Significant departures ($P < 0.05$) from Hardy-Weinberg expectations were encountered at six loci (Table 2). Fit to Hardy-Weinberg expectations is an important criterion for inferring the genetic nature of electrophoretic banding variants. However, caution should be exercised when relying excessively on fit to these expectations when inferring the genetic nature of populations, since several factors may contribute to Hardy-Weinberg disequilibrium and ideal Hardy-Weinberg populations actually do not exist in nature (Althukov, 1981).

Estimates of genetic variability, e.g. percentage of polymorphic loci (P) and heterozygosity (H), are two useful parameters to employ when analysing the genetic structure of populations. The long-term adaptability of populations, and hence species, is dependent upon a sufficient amount of genetic variation to enable continued adaptation to environmental and biotic challenges. As the ultimate goal of conservation is to maintain biological lineages over evolutionary time, a thorough understanding of the extent of genetic variation and diversity in populations is of critical importance in any conservation management plan. Moreover, genetic variability data may also be used as a tool by aquaculturists for stock selection. However, the reliability of P and H are closely correlated with the effective population size, the degree of migration, variability of the environment and the number and choice of loci analysed (Simon and Archie, 1985; Kirpichnikov, 1992). The sample size and number of loci analysed in the present study are in accordance with recommendations in the literature (Gorman and Renzi, 1979; Grant et al., 1988).

Values for P and H obtained in the present study (Table 2) fall in the range of $P < 10\%$ and $H = 0.02$ to 0.03, that are generally considered to be the lower range of genetic variability estimates in fish (Nevo et al., 1984; Kirpichnikov, 1992). The low variability estimates are similar to values obtained for other fish species from the same river system. Van Vuuren (1989) obtained $H = 0.0109$ for *Labeo ruddi*; an estimate of $H = 0.007$ was determined for *Schilbe intermedius* (Engelbrecht et al., 1997); $H = 0.025$ for both *Glossogobius callidus* (Engelbrecht and Mulder, 1999) and *Mesobola brevianalis* (Engelbrecht and Mulder, 1999). The low genetic variability observed in the suckermouths could reflect a small effective population size, i.e. a reduced gene pool, caused by repeated bottlenecks, random drift and reduced gene flow between populations. This may be the result of adverse environmental conditions caused by droughts, excessive water extraction and pollution. Siluroids in particular appear to be very sensitive to agrochemicals. Abban and Skibinski (1988) and Agnèse (1991) implicated the indiscriminate use of agrochemicals as being responsible for the low variability estimates obtained in their studies on West African siluroids. The Olifants River catchment area is exposed to the effects of pollution from agriculture, industry

and mining. It is thus conceivable that pollution and agrochemicals may have contributed to bottlenecking and ultimately a reduction in genetic variability in the populations analysed.

The electrophoretic banding patterns observed in this study clearly showed similarities and differences among the populations analysed. As expected, the results obtained separated the congeneric *C. paratus* from *C. pretoriae* and revealed a high degree of similarity between conspecific populations of *C. paratus*. Furthermore, several diagnostic loci were identified that may be used as reliable genetic markers to distinguish the two species. Wright's (1978) fixation index is valuable to describe the degree of differentiation between populations. The high mean $F_{ST} = 0.905$ value is an indication of extensive genetic differentiation between the populations resulting from genetic drift. The low F_{ST} value (0.244) between the two *C. paratus* populations indicates very little genetic differentiation. However, the F_{ST} value obtained between the two conspecific populations of *C. paratus* and *C. pretoriae* (0.909 and 0.911), reflects considerable interspecific genetic differentiation. The F_{ST} values are supported by D_N values (range: 0.987 to 1.050) calculated for *C. paratus* and *C. pretoriae*, which are consistent with the range of values (D_N = 0.58 to 1.21) expected for confamilial genera (Shaklee et al., 1982; Thorpe, 1982; Thorpe and Sòle-Cava, 1994). The low D_N value (0.017) between the two populations of *C. paratus*, is indicative of conspecific populations and suggest marginal gene flow or, at least, a recent separation of the two gene pools. This low D_N value is to be expected from two conspecific populations inhabiting the same river system. The very high D_N values for the congenerics are rather surprising. A possible hypothesis for this large genetic differentiation might be that the southward dispersal of the genus coincided with considerable radiation of populations and species as a result of genetic drift. It would be interesting to extend the present study, by analysing more enzyme systems and different populations and species from across the genus' distributional range in Southern Africa, in order to determine the genetic diversity and phylogenetic relationships of the suckermouth species.

In conclusion, this study provides the first account of the genetic structure of two species of suckermouths in Southern Africa as a basis for future population genetic studies involving these species. The populations analysed revealed that the low levels of genetic variability is comparable with those of other fish species from the same river system. The D_N and F_{ST} values indicate extensive genetic differentiation between the species. Seventeen loci were found to be fixed for presumably different alleles in the two species studied. These differences may have resulted from different selective forces acting on the gene pools of the two species or from stochastic processes. We recommend electrophoretic analysis of more populations and different species of the suckermouths to obtain a better understanding of the amount and pattern of allozyme variation in the genus.

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References

ABBAN EK and SKIBINSKI DOF (1988) Protein variation in *Schilbe mystus* (L.) and *Eutropius niloticus* (Rüppel) (Pisces: Siluriformes) in the Volta Basin of Ghana, West Africa. *Aquacult. Fish. Manage.* **19** 25-37.

AGNÈSE JF (1991) Taxonomic status and genetic differentiation among West African populations of the *Chrysichthys auratus* complex (Pisces; Siluriforme), based on protein electrophoresis. *Aquacult. Fish. Manage.* **22** 229-37.

ALLENDORF FW and LEARY RF (1988) Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Cons. Biol.* **2** 170-84.

ALTUKHOV YP (1981) The stock concept from the viewpoint of population genetics. *J. Fish. Aquat. Sci.* **38** 1523-1538.

ENGELBRECHT GD and MULDER PFS (1999) Extremely high genetic differentiation between two populations of the river goby, *Glossogobius callidus* (Smith, 1937). *Water SA* **25** (1) 85-90.

ENGELBRECHT GD and MULDER PFS (1999) Allozyme variation in the river sardine, *Mesobola brevianalis* (Pisces, Cyprinidae). *Water SA* **25** (3) 293-296.

ENGELBRECHT GD, VAN DER BANK FH and MULDER PFS (1997) Allozyme variation in *Schilbe intermedius* Rüppel, 1832 (Pisces: Siluriformes) from two localities in the Limpopo River system, South Africa. *Comp. Biochem. Physiol.* **117**B (2) 179-84.

GORMAN GC and RENZI J (1979) Genetic distance and heterozygosity estimates in electrophoretic studies: Effects of sample size. *Copeia* **2** 242-49.

GRANT WS, DEMPSTER YL and DA SILVA FM (1988) Use of protein electrophoresis in evolutionary systematics. *Trans. R. Soc. South Afr.* **46** (4) 295-311.

KIRPICHNIKOV VS (1992) Adaptive nature of intrapopulational biochemical polymorphism in fish. *J. Fish Biol.* **40** 1-16.

LAVERY S and FIELDER DR (1993) Low allozyme variation in the coconut crab *Birgus latro. Comp. Biochem. Physiol.* **104**B (2) 353-59.

MARKERT CL and FAULHABER I (1965) Lactate dehydrogenase isozyme patterns of fish. *J. Exp. Zool.* **159** 319-32.

MEFFE GK (1990) Genetic approaches to conservation of rare fishes: Examples from North American desert species. *J. Fish Biol.* **37**A 105-12.

NEI M (1972) Genetic distance between populations. *Am. Naturalist* **106** (549) 283-92.

NEVO E, BEILES A and BEN-SHLOMO R (1984) The evolutionary significance of genetic diversity, ecological, demographic and life history correlates. Evolutionary dynamics of genetic diversity. *Lecture Notes Biomath.* **53** 13-213.

SHAKLEE JB, TAMARU CS and WAPLES RS (1982) Speciation and evolution of marine fishes studied by electrophoretic analysis of proteins. *Pacific Sci.* **36** 141-157.

SIMON C and ARCHIE J (1985) An empirical demonstration of the lability of heterozygosity estimates. *Evol.* **39** 463-67.

SKELTON PH (1993) *A Complete Guide to the Freshwater Fishes of Southern Africa.* Southern Book Publishers, Halfway House.

SWOFFORD DL and SELANDER RB (1981) BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72** 281-283.

THORPE JP (1982) The molecular clock hypothesis: Biochemical evaluation, genetic differentiation and systematics. *Ann. Rev. Ecol. Syst.* **13** 139-68.

THORPE JP and SÒLE-CAVA AM (1994) The use of allozyme electrophoresis in invertebrate systematics. *Zool. Scripta* **23** 3-18.

VAN DER BANK FH (1996) Inter- and intraspecific allozyme comparisons of mormyrids (Pisces, Mormyridae) from South Africa and Namibia, with reference to an undescribed species. *Water SA* **22** (3) 285-90.

VAN VUUREN NG (1989) An Investigation Into the Genetic Differences and Population Genetics of Nine *Labeo* species in South Africa. Ph. D. Thesis, Rand Afrikaans University, Johannesburg. (In Afrikaans).

WRIGHT S (1978) *Evolution and the Genetics of Populations. Vol. 4. Variability Within and Among Natural Populations.* University of Chicago, Chicago.