Extremely high genetic differentiation between two populations of the river goby, *Glossogobius callidus* (Smith, 1937)

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Abstract

Two populations, an estuarine and freshwater population, of *Glossogobius callidus* were studied using protein gel electrophoresis to compare the extent of genetic variation and differentiation within and between them. Thirty protein coding loci were resolved of which eleven loci were polymorphic. The Nhlabane Estuary population displayed higher expected heterozygosity ($H_E = 0.066$) than the freshwater population ($H_E = 0.025$). Rogers' and Nei's genetic distance values as well as genetic heterogeneity estimates (F_{ST} values) indicate a high degree of genetic differentiation between the two populations. The possible reasons for the low variability and the extensive differentiation are discussed.

Introduction

In Southern Africa, the Gobiidae represents a speciose family of 51 genera comprising 107 species (Smith and Heemstra, 1986). The family is found primarily in inshore marine habitats although some species are commonly found in estuaries, coastal lakes and freshwater habitats (Skelton, 1994).

The river goby, *G. callidus* (Smith, 1937), exhibits extensive salinity tolerance as it may be found in estuarine as well as freshwater habitats. This species may also penetrate considerably far inland, especially at the northern limits of its distributional range. Thus, it is evident that estuarine and freshwater populations of *G. callidus* are exposed to widely different environments and therefore selection pressures which, in turn, might favour localised genetic adaptation and ultimately genetic divergence. Furthermore, it has been suggested that specific activities of allozymes may also be affected by salinity (Koehn and Siebenaller, 1981). These are only some of the factors that may influence the genetic structure of estuarine and freshwater populations of the river goby.

The long-term adaptability of populations, and therefore species, is dependent upon a base of genetic variation by means of which to respond to environmental or biotic occurrences (Meffe, 1990). Any management programme involving the river goby will therefore benefit from an understanding of the extent of genetic variation of this species and the preservation of this variation for future adaptation. The need for knowledge regarding the genetic structure of the river goby becomes even more evident when one considers its potential as an ornamental aquarium fish as well as its importance in the fishing industry as a source of protein for subsistence fishermen and/or as bait for the larger game fish species. In light of the above, the genetic structure of an estuarine and a freshwater population of the river goby was examined as part of a broader study on the genetic structure of Southern African gobies.

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Received 20 February 1998; accepted in revised form 17 June 1998.

Materials and methods

A seine net was used to sample a total of 57 and 49 individuals from the Phalaborwa Barrage (24°03' S, 31°08' E) in the Olifants River system and the Nhlabane Estuary (28°38' S, 32°16' E) in KwaZulu-Natal respectively (Fig. 1). Voucher specimens of both populations were deposited in the JLB Smith Institute for Ichthyology. Catalogue numbers are as follows: Phalaborwa Barrage (RUSI 57805) and Nhlabane Estuary (RUSI 57806). After capture, the specimens were transported alive to the laboratory where they were frozen in liquid nitrogen (-196°C) and stored at -40°C to await electrophoresis. Immediately prior to electrophoresis, the samples were thawed and the head, tail, fins, scales and all internal organs were removed from each specimen. Approximately 0.5 g of the remaining tissue was mixed with 1 mℓ distilled water and homogenised using a glass rod. Extracts were absorbed directly onto Whatman Nr. 3 filter paper wicks.

Horizontal starch gel electrophoresis (13% gels), employing three buffer systems, was used to separate the proteins. The following buffer systems were used:

- **A** - a continuous Tris, citric acid (pH 6.9) buffer system (Whitt, 1970);
- **B** - a continuous Tris, boric acid, EDTA buffer (pH 8.6) as described by Markert and Faulhaber (1965);
- **C** - a discontinuous Tris, citric acid (gel pH 8.7), lithium hydroxide, boric acid (electrode pH 8.0) buffer system (Ridgway et al. 1970).

Electrophoresis was carried out at a constant current of 50 mA for 4 to 5 h. Following electrophoresis, enzymes were visualised using the histochemical methods of Harris and Hopkinson (1976) and Hillis and Moritz (1990). Interpretation of gel-banding patterns was as described by Grant (1989). The locus nomenclature described by Shaklee et al. (1990) was used.

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), average observed (H_O) and expected (H_E) heterozygosity per locus. Both H_O and H_E estimates were determined to facilitate

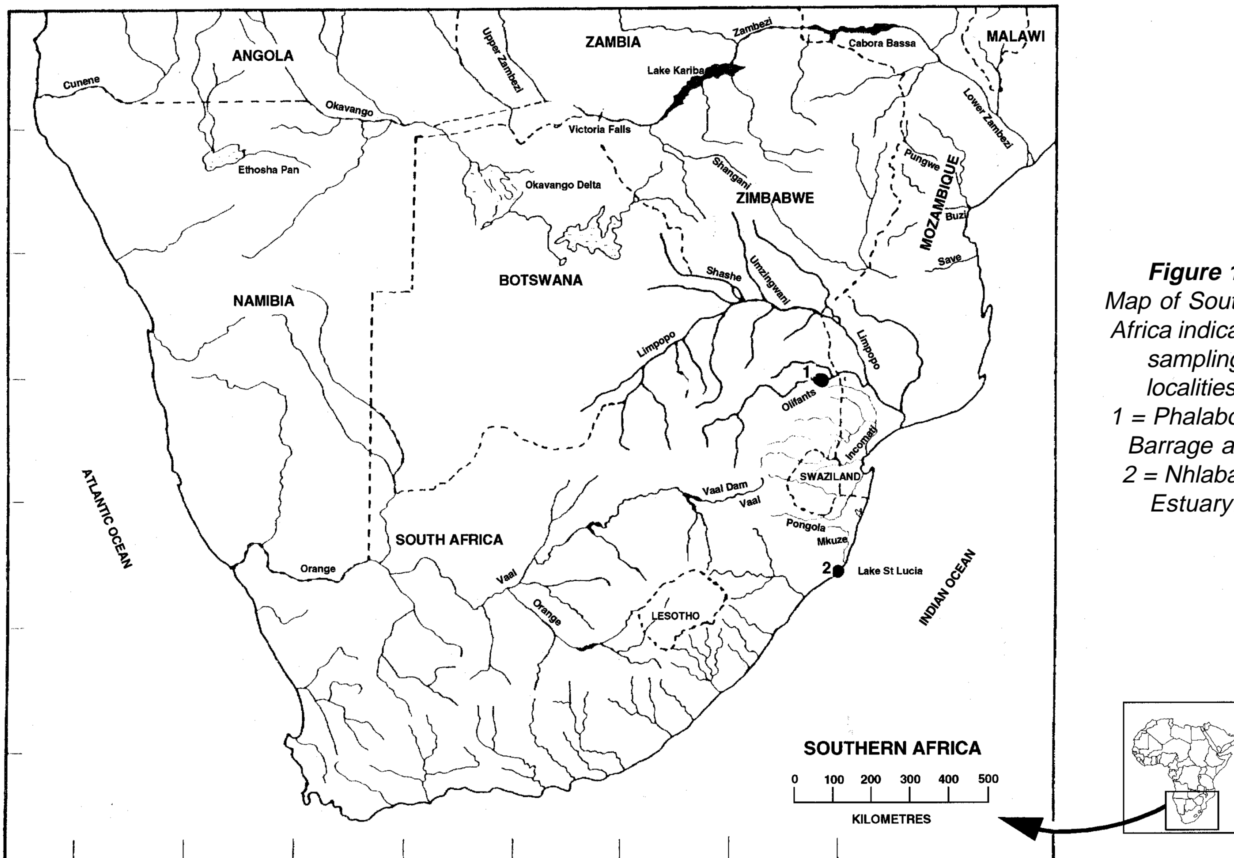

Figure 1
Map of Southern Africa indicating sampling localities.
1 = Phalaborwa Barrage and 2 = Nhlabane Estuary.

TABLE 1
LOCUS ABBREVIATIONS, ENZYME COMMISSION (EC) NUMBERS AND BUFFERS GIVING THE BEST RESULTS FOR EACH PROTEIN ANALYSED. SEE MATERIALS AND METHODS FOR ABBREVIATIONS OF BUFFERS USED

Protein	Locus	EC No	Buffer
Aspartate aminotransferase	AAT-1	2.6.1.1.	C
Creatine kinase	CK-1, 2	2.7.3.2	A
Esterase	EST-1*, 2, 3	3.1.1.-	C
Fumarate hydratase	FH-1	4.2.1.2	B
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH-1*	1.2.1.12	B
Guanine deaminase	GDA-1*	3.5.4.3.	C
Glucose dehydrogenase	GDH-1	1.1.1.47	A
Glycerol-3-phosphate dehydrogenase	G3PDH-1*,2	1.1.1.8	B
Glucose-6-phosphate isomerase	GPI-1*, 2	5.3.1.9	A
Hexokinase	HK-1	2.7.1.1.	B
L-Iditol dehydrogenase	IDDH-1	1.1.1.14	B
Isocitrate dehydrogenase	IDHP-1	1.1.1.42	A
L-Lactate dehydrogenase	LDH-1	1.1.1.27	B
Malic enzyme	ME-1	1.1.1.40	A
Mannose-6-phosphate isomerase	MPI-1*, 2	5.3.1.8	B
Phosphogluconate dehydrogenase	PGDH-1*	1.1.1.44	B
Phosphoglucomutase	PGM-1, 2*	5.4.2.2	B
Peptidase:		3.4.-.-	
Substrate: Phenylalanyl-proline	PEP-D1*		B
Leucyl-tyrosine	PEP-LT1,2*		B
General protein	PROT-1*, 2		B
Superoxide dismutase	SOD-1	1.15.1.1	C
* Polymorphic loci			

comparison with other studies. All polymorphic loci were tested by chi-square (X^2) for goodness-of-fit to Hardy-Weinberg proportions. Heterogeneity amongst populations were determined using contingency chi-square analysis (*p*). Nei's (1972) and Rogers' (1972) genetic distance coefficients and fixation indices (F_{ST}) were calculated to determine the extent of genetic differentiation amongst the two samples.

Results

The names of the proteins examined, locus abbreviations, enzyme commission (EC) numbers, and buffer systems giving the best results are presented in Table 1. Allele frequencies for polymorphic loci, X^2 values of loci which deviate significantly from expected Hardy-Weinberg proportions, $P_{0.95}$, H_O and H_E estimates for the two populations are presented in Table 2.

TABLE 2
ALLELE FREQUENCIES FOR POLYMORPHIC LOCI, X^2 VALUES FOR LOCI WHICH DEVIATE SIGNIFICANTLY FROM EXPECTED HARDY-WEINBERG PROPORTIONS (P > 0.05), PERCENTAGE OF POLYMORPHIC LOCI USING 0.95 CRITERION ($P_{0.95}$) AND AVERAGE OBSERVED (H_O) AND EXPECTED (H_E) HETEROZYGOSITIES FOR TWO POPULATIONS OF *G. CALLIDUS*

Locus	Allele	Population	
		Phalaborwa	Nhlabane
EST-1	105	0.071	
	100	0.929	0.318
	95		0.682
GAPDH-1	100	1.000	0.818
	95		0.182
X^2			10.610
GDA-1	105	0.026	
	100	0.974	1.000
G3PDH-1	105	0.018	
	100	0.982	1.000
GPI-1	105	0.170	0.041
	100	0.830	0.959
X^2		26.129	
MPI-1	100	1.000	0.109
	95		0.022
	90		0.869
X^2			23.920
PGDH-1	105	0.036	0.909
	100	0.964	0.091
X^2		28.000	22.000
PGM-2	100	0.911	0.841
	95	0.089	0.159
X^2			5.290
PEP-D1	100		1.000
	95	1.000	
PEP-LT2	100	1.000	0.271
	95		0.729
X^2			19.203
PROT-1	100	1.000	0.042
	95		0.958
X^2			24.000
$P_{0.95}$		10.000	20.000
H_O		0.014	0.025
H_E		0.025	0.066

The products of 30 protein coding loci were analysed in the present study. Using the 0.95 criterion, the percentages of polymorphic loci were 10% in the Phalaborwa Barrage population and 20% in the Nhlabane Estuary. All heterozygotes observed, confirmed the known quaternary subunit structure of the enzymes. The H_E estimates were lower in the Phalaborwa Barrage (0.025) population compared with that of the Nhlabane Estuary (0.066).

Allele frequencies at seven loci, **GPI-1**, **PGDH-1**, **GAPDH-1**, **LT-2**, **MPI-1**, **PGM-2** and **PROT-1**, deviated significantly from expected Hardy-Weinberg proportions of genotypes. After pooling, **MPI-1** was the only locus where allele frequencies deviated from expected proportions. Significant differences ($p < 0.01$) between allelic frequencies of the two populations were encountered at all polymorphic loci except at the **GDA-1** ($p < 0.25660$), **G3PDH-1** ($p < 0.35222$) and **PGM-2** ($p < 0.28629$). Zymograms of **PEP-D1** and **PGDH-1** showing allele mobility differences for these enzymes between the two populations are presented in Fig. 2.

The genetic distance (Nei, 1972; Rogers, 1972) values obtained between the two populations were $D_N = 0.169$ and $D_R = 0.184$ respectively. Estimates of genetic heterogeneity ($F_{ST} = 0.625$) showed a high level of genetic differentiation between the populations studied.

Discussion

The present study provides the first account of the extent of genetic variation in the river goby. Comparative studies of allozymes using gel electrophoresis provide a direct approach to the study of genetic variability and have been used successfully, amongst others, to solve taxonomic problems, identify cryptic species and in the study of genetic variability within and among natural populations (e.g. Grant et al., 1988; Oosthuizen et al., 1993; Richardson et al., 1986).

Deviations from expected Hardy-Weinberg proportions occurred at seven loci (Table 1) and heterozygote deficiencies were detected at eight loci. This suggest that random mating may not be operating in these populations. However, conformance to Hardy-Weinberg equilibrium is an important but not an absolute criterion for inferring the genetic nature of electrophoretic banding patterns (Grant, 1985). Ideal Hardy-Weinberg populations do not actually exist in nature since several factors can shift the genetic equilibrium, giving rise to change in the genetic structure of a population (Althukov, 1981). Significant departures may occur as a result of natural selection, inbreeding, the Wahlund (1928) effect when samples include individuals from two or more populations, sampling error, population bottlenecks and random genetic drift. It is evident that any of the above factors may be responsible for the Hardy-Weinberg discrepancies attained in this study.

The percentage of polymorphic loci ($P = 20\%$) and expected heterozygosity ($H_E = 0.066$) in the Nhlabane Estuary population, compares favourably with the average value of $P = 20\%$ in fish (Kirpichnikov, 1992) and $H_E = 0.05$ to 0.1 reported by Nevo et al. (1984) for 183 fish species. These values indicate that this population possess sufficient variation to permit adaption to challenging environmental conditions present in estuarine ecosystems. However, the estimates of polymorphism ($P = 10\%$) and heterozygosity ($H_E = 0.025$) obtained for the Phalaborwa Barrage population suggest genetic erosion in this population. The value

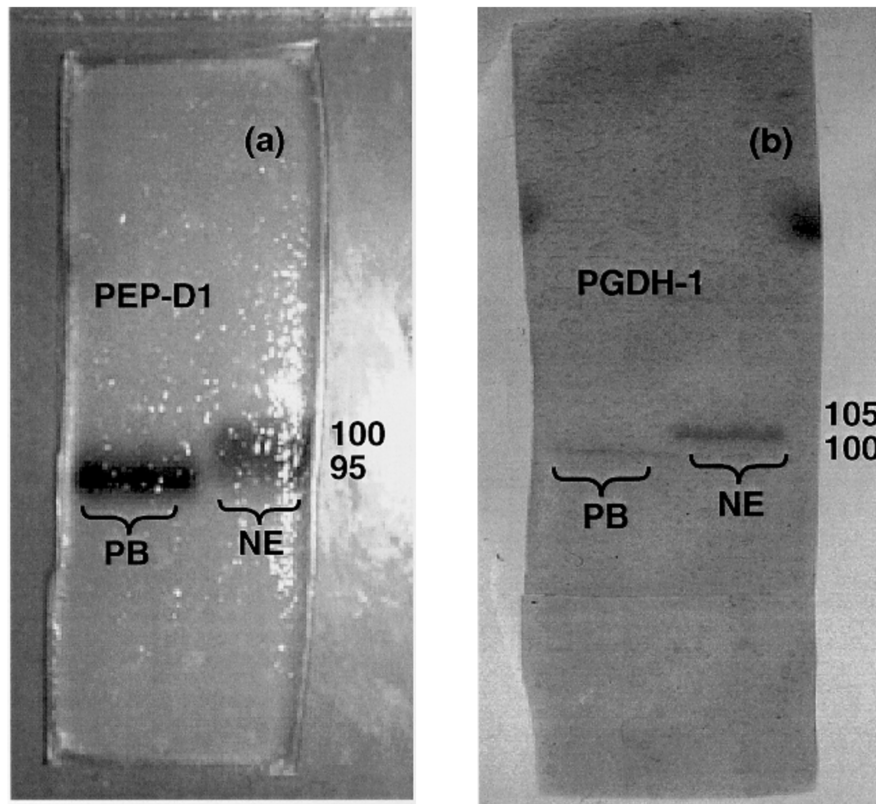

Figure 2
Zymograms showing allele mobility differences between two populations of G. callidus. *a)* ***PEP-D1*** *and b)* ***PGDH-1****. PB = Phalaborwa Barrage and NE = Nhlabane Estuary.*

of P falls in the range of $P = 8$ to 10% that is generally considered as the lower margin of polymorphism in fish (Kirpichnikov, 1992). Furthermore, the estimate of H_E for this population is strikingly lower than the value obtained by Nevo et al. (1984). The values are also lower than $H_O = 0.058$ reported by Wallis and Beardmore (1984a) and $H_E = 0.066$ obtained by Miller et al. (1994) for European and Italian gobies respectively. The estimates obtained for the Phalaborwa Barrage population fall in the lower range of heterozygosity estimates (2-3%) for fishes and other vertebrates (Kirpichnikov, 1992). However, it should be noted that similar low levels of genetic variation have been reported for other Southern African freshwater fish species (Engelbrecht et al., 1997; Van der Bank et al., 1989).

The low H_E estimates obtained for the Phalaborwa Barrage population, may reflect sequential founder events in this populations' phylogenetic history. Genetic variability may be reduced either because of a founder effect, compounded by bottlenecks in small initial population sizes and subsequent loss of rare alleles or because of accelerated selection for particular alleles during the transition from an essentially estuarine to a totally freshwater environment (Nei, 1975). Thus, it is conceivable that frequent bottlenecks could have resulted in considerable genetic drift between the two populations analysed. Moreover, Wallis and Beardmore (1984a) found a correlation between genetic and environmental heterogeneity. This could also account for the differences in H_E obtained between the two populations in the present study. The Phalaborwa Barrage population inhabits a fairly uniform and stable environment and is not subject to the major variables of salinity and temperature operating in coastal and estuarine conditions.

Most animal species show a certain degree of genetic differentiation into local populations which are isolated and locally adapted to varying degrees. If localised populations inhabit similar environments or if gene flow and migration are possible between populations, one would expect them to display largely homogenous arrays of phenotypic or genetic traits. If, however, the populations experience widely different environmental conditions, strong selection pressures and/or genetic drift, noticeable genetic differentiation may arise (Carvalho, 1993). The spatial heterogeneity within a species may also be influenced by factors such as the levels of genetic variability in ecological-significant traits, population size and the number of original founders (Carvalho and Hauser, 1994). Regardless of whether the genetic differences observed in this study are adaptive or not, it is clear that there is substantial genetic differentiation between the two populations analysed.

Wright's (1978) fixation index indicates extensive genetic differentiation between the two populations. The mean F_{ST} value (0.625) indicates that approximately 62% of the total variance of the allelic frequency between both populations is due to genetic differences and 38% of the total genetic diversity is due to intrapopulational variation. It is possible that physiological adaptation to a completely freshwater habitat and possible phylogenetic bottlenecks could have resulted in this situation. However, the low heterozygosity estimates for the Phalaborwa Barrage population may accentuate the interpopulational level of genetic differentiation between the two populations.

The values of genetic distance ($D_N = 0.169$, $D_R = 0.184$) obtained further supports the genetic differentiation between the two populations studied. Shaklee et al. (1982), Thorpe (1982) and Thorpe and Sòl-Cava (1994) showed that genetic distance values (Nei, 1972) for conspecific populations average 0.05 (range: 0.002 to 0.07), for congeneric species it averaged 0.30 (range: 0.03 to 0.61), and for confamilial genera it ranged from 0.58 to 1.21. The distance values obtained in the present study fall within the range of congenerics. Furthermore, these estimates corre-

spond with distance value estimates obtained by Wallis and Beardmore (1984b), McKay (1993) and Miller et al. (1994) for congeneric gobies. Fixed allelic differences at the **PEP-D-1** locus as well as allele frequency differences at four other loci (**PEP-LT-2**, **MPI-1**, **PGDH-1**, **PROT-1**), also support the specific status of the two populations. The presence of fixed allelic differences between populations is a significant evolutionary event as it indicates the absence of gene flow between them - one of the prerequisites for speciation. These large genetic differences are not totally unexpected as the two populations inhabit widely different environments and have also been exposed to different selection processes. One explanation for the extensive genetic divergence might be that the evolution of the Phalaborwa Barrage population has involved extremes of physiological adaptation required for the colonisation of freshwater or a hyposaline environment from an essentially hypersaline environment. Furthermore, anthropogenic influences such as channelisation and the building of dams, overfishing, as well as historical and physical factors such as barriers, droughts, salinity, siltation, pH, temperature, food supply, predators and disease, are all factors that may fragment gene pools and/or be of adaptive significance, both of which may enhance the possibility of population subdivision (Carvalho, 1993).

In conclusion, the genetic structure of populations of *G. callidus* in the area between the two localities sampled in the present investigation is unknown and, therefore, we cannot say for certain whether the extensive genetic differentiation observed represents:

- a gradual cline in gene frequencies indicating that the two populations analysed are geographic races or subspecies; or
- two reproductively isolated and genetically distinct species.

The extent of microgeographic differentiation within and between different river systems and between estuaries should be addressed more closely in order to obtain a better understanding of the genetic structure of populations of the river goby.

Acknowledgements

I wish to acknowledge the co-operation and assistance of Dr Victor Wepener and colleagues at the Department of Zoology of the University of Zululand for sampling the Nhlabane Estuary population and Mr Piet Grobler of the Phalaborwa Water Board for his cooperation with sampling the Phalaborwa Barrage population. I also express my gratitude to the late Beverley du Toit for her efficient and enthusiastic assistance in the laboratory during the analysis.

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