Allozyme variation in two populations of *Hydrocynus vittatus* (Pisces, Characidae)

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The isozymes described in this study provide the first account of the amount, pattern and distribution of genetic variation within this genus. Twenty-five enzyme coding loci in two populations of *H. vittatus*, from Namibia and South Africa, were analysed by horizontal starch gel-electrophoresis. Electrophoretic analysis of liver, white muscle, heart and testis samples revealed genetic variation at 20% (Upper Zambezi River, Namibia) and 36% (Olifants River, South Africa) of the protein coding loci studied. Average heterozygosity values ranged from 1.9% (Upper Zambezi River) to 4.6% (Olifants River), with a genetic distance value of 0.005 between these populations. The low amount of genetic variability in the former population compared to that of other fish species from the same geographical area, and to that of *H. vittatus* from the Olifants River, can be attributed to restricted gene flow owing to isolation. Although morphological differences exist between the two populations, the extent thereof is not sufficient to regard them as subspecies.

Die iso-ensieme wat in hierdie studie beskryf word, is die eerste weergawe van die aantal, patroon en verspreiding van die genetiese variasie binne die genus. Vyf-en-twintig ensiemkoderende loki in twee *H. vittatus* populasies van Namibië en Suid-Afrika onderskeidelik, is geanaliseer met behulp van stysel-gel-elektroforese. Elektroforetiese analise van lewer-, spier-, hart- en testismonsters het genetiese variasie by 20% (Bo Zambezirivier) en 36% (Olifantsrivier, Suid-Afrika) van die proteïen-koderende loki getoon. Gemiddelde heterosigositeit-waardes was tussen 1.9% (Bo Zambezirivier) en 4.6% (Olifantsrivier), met 'n genetiese afstandswaarde van 0.005 tussen die populasies. Die lae genetiese variasie in eersgenoemde populasie in vergelyking met ander visspesies van dieselfde geografiese area, en in vergelyking met *H. vittatus* van die Olifantsrivier, kan toegeskryf word aan beperkte geenvloei as gevolg van isolasie. Alhoewel morfologiese veskille tussen die twee populasies voorkom, is die mate daarvan nie voldoende om hulle as subspesies te beskou nie.

Keywords: tigerfish, allozyme variation, polymorphism

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Introduction

The scientific name of the tigerfish from southern African freshwaters was, for many years, *Hydrocynus vittatus* (Castelnau). The name means 'striped water dog'. The application of the genus name *Hydrocynus* to the African tigerfish has been a subject of controversy (Weitzman & Fink, 1983; Brewster, 1986). With a revision of the genus of tigerfish species from Africa, Brewster (1986) concluded that the southern African species was the same species as *H. forskahlii* (Cuvier), until then known only from the Nilo-Sudanic region (West and North Africa). More recently, Paugy &

Guégan (1989) re-investigated the taxonomy of certain tigerfish species. Because of major morphological differences and supportive parasitological evidence, the latter authors re-instated *H. vittatus*. They concluded that *H. vittatus* occurs in southern Africa, whereas two other species, *H. forskahlii* and *H. brevis* occur further north in the Nilo-Sudanic region. The ecological differences between the species is further manifested in their morphology and ecology (Skelton, 1990).

Hydrocynus is endemic to Africa and belongs to the Characidae which is one of the largest families of freshwater fishes found in Africa and the neotropics (Kenmuir, 1972; Skelton, 1993). *Hydrocynus vittatus* has an extensive distribution in Africa, from the Nile and Congo Rivers to rivers of West Africa through to the Zambezi, Okavango, Limpopo and the Pongola Rivers in the south (Jubb, 1967; Gaigher, 1970). It is notably absent from the Kunene, Kafue, and the Upper Save-Runde Rivers and Lake Malawi (Skelton, 1993). In most of these open water systems, the tigerfish represents the major piscivorous fish and mainly feeds on cichlid fishes, *Hepsetus odoe* and small characids (Winemiller & Kelso-Winemiller, 1994).

In southern Africa, tigerfish are found in the northern (upper), central (middle) and southern (lower) regions of the Zambezi River and associated flood plains (Winemiller & Kelso-Winemiller, 1994). South from the Zambezi, *H. vittatus* is mainly restricted to certain rivers in the Kruger National Park as well as to the lower reaches of the Pongola River System in northern KwaZulu-Natal (Pienaar, 1978). This species supports important commercial and recreational fisheries in southern Africa, especially in the Zambezi, Okavango and Chobe Rivers and Lake Kariba (Winemiller & Kelso-Winemiller, 1994). It has a well-deserved reputation as the most sought-after sport fish on the African continent. In South Africa increasing numbers of dams, weirs and reduced flow conditions in relevant rivers effectively prevent the annual migration of tigerfish to their spawning areas. This leads to the establishment of isolated populations in such rivers (Van Loggerenberg, 1983).

Tigerfish from the Upper Zambezi River display a unique colouration (Steyn *et al.,* 1996). Spectacular colour differences exists between mature males and females with the males having very prominent yellow ventral and caudal fins. The ventral section of the caudal fin has a bright red colouration. Mature females from the Upper Zambezi River have orange fins, which resembles the colouration of both sexes from the Kruger National Park. With the recent possibility to exploit the commercial potential of *H. vittatus* by artificial spawning (Steyn *et al.*, 1996), the importance of fundamental knowledge of the genetic structure of the species became evident. A literature survey revealed that genetic variability in tigerfish has not been studied previously.

The use of molecular methods to study genetic variation of natural populations has provided considerable insight into population genetics and evolutionary processes (Grant & Leslie, 1993). Research on DNA markers is proceeding in several international laboratories and it is hoped that DNA-based genetic data continue to become available and at the same time more affordable. Isozyme analysis has been the standard molecular technique in fish population genetic research for three reasons, firstly it is inexpensive, secondly the method allows for quick processing times and the third asset is that isozyme data often constitute the largest existing genetic data set for many organisms, both within and between species.

This study therefore aims to provide information on the amount and pattern of genetic variation within and between two morphologically different populations of tigerfish, and to determine the amount of genetic differentiation between these populations by applying enzyme gel-electrophoresis.

Material and methods

Heart, liver, muscle and testis samples were obtained from 35 and 40 sexually mature individuals from the Upper Zambezi River (24°18'S; 17°28'E) and the Olifants River System (31°07'S;

24°27'E) respectively. These populations will be referred to as ZAMBEZI and OLIFANTS henceforth. Samples were stored in liquid nitrogen and transported to the laboratory. Tissues were analysed by horizontal starch gel-electrophoresis using **TC** (Whitt, 1970), **RW** (Ridgway *et al.*, 1970) and **MF** (Markert & Faulhaber, 1965) buffer systems and 13% gels as applied by Van der Bank *et al.* (1992). Samples were analysed for activity at 15 enzymes, comprising 25 loci. Gel banding patterns were interpreted according to the method used by Van der Bank *et al.* (1989) and the locus nomenclature used was as described by Shaklee *et al.* (1990).

Average heterozygosity (*H*) was calculated, using the formula of Nei (1975). The *G*-test for goodness-of-fit was used to test deviations of allele frequencies from expected Hardy-Weinberg proportions for polymorphic loci (Sokal & Rohlf, 1969), and the likelihood-ratio statistic was used to test the equality of allelic frequencies among samples within and between populations (Smouse & Ward, 1978). Genetic distance (*D*) was calculated from allele frequencies using Nei's (1972; 1978) formulae. Different fixation indices were used to analyse genetic differentiation between populations (Wright, 1978), using **BIOSYS-1** (Swofford & Selander, 1981), where F_{IT} and F_{IS} are the fixation indices of individuals compared with the total population and its subpopulations respectively and F_{ST} measures the amount of differentiation among subpopulations compared with the limiting amount under complete fixation.

Results

Twenty-five enzyme-coding loci provided interpretable results. Locus abbreviations, enzyme commission numbers, tissues and buffers giving the best results are listed in Table 1. Products of the following loci migrated cathodally: ***ADH-2***, ***GAPD-2***, ***G3PDH***, ***LDH-3***, ***MDH-3***, ***PGD-2*** and ***SDH***. In addition to these loci, staining was done for ***AK***, ***CK***, ***GPI***, ***HK*** and ***PEP*** but neither of these systems showed sufficient activity or resolution to score it satisfactorily.

The heterozygotes at the ***LDH*** locus were five-banded, the allozyme products for ***IDH***, ***MDH*** and ***PGD*** produced triple banded heterozygotes as expected for dimeric enzymes, and double banded heterozygotes for ***ES*** and ***PGM*** as expected for monomeric enzymes. These allozyme phenotypes were therefore in agreement with the quartenary structure of the corresponding enzymes (Ward 1977). All four ***ES*** loci were best resolved with α-naphthyl acetate as staining substrate using liver tissue. No intraspecific genetic variation was found at the two ***ADH*** loci and because no heterozygotes were observed at these loci, the subunit structure of this enzyme could not be established.

Liver tissue showed two monomorphic zones of ***GAPD*** activity. This is in accordance with that of cichlid fish where two loci were detected in muscle tissue (Cruz *et al.*, 1982). One monomorphic locus each was observed for ***GDA***, ***NP***, ***G3PDH***, ***MPI***, ***SDH*** and ***SOD*** in liver tissue and for ***MEP*** in muscle tissue. According to McAndrew & Majumdar (1983) and Basiao & Taniguchi (1984) two ***G3PDH*** loci occur in cichlids. In this study only one locus for ***G3PDH*** was observed and three ***LDH*** loci were best resolved in heart tissue. McAndrew & Majumdar (1983) found high activities in skeletal muscle, heart and liver of ***LDH-1***, ***-2*** and ***-3*** respectively. Two ***MDH*** loci were observed in muscle and one locus (***MDH-3***) in liver tissue in this study. The three loci for ***MDH*** is in accordance with results by Basiao & Taniguchi (1984) who observed three ***MDH*** loci, one being muscle specific, one being liver specific and a third being heart specific in tilapias. Two ***PGD*** loci were expressed in liver tissue. In cichlids only one locus was expressed for ***PGD*** (Cruz *et al.*, 1982).

Allele frequencies, coefficients for heterozygosity deficiency or excess (***d***), observed number of heterozygotes, Chi-square (χ^2) values and individual heterozygosity values (h) are presented in Table 2. Loci where significant ($p < 0.05$) deviations of allele frequencies from expected Hardy-Weinberg proportions occurred, are also listed. The degree of freedom for polymorphic loci was

Table 1 Locus abbreviations, enzyme commission numbers (E.C. No.), tissues and buffers giving the best results for each isozyme

Enzyme	Locus	E.C.No.	Tissue	Buffer
Alcohol dehydrogenase	*ADH-1	1.1.1.1	M	MF
	*ADH-2		L	MF
Esterase	ES-1 to -4	3.1.1.-	L	RW
Glyceraldehyde-3-phosphate dehydrogenase	*GAPD-1, -2	1.2.1.12	L	RW,TC
Guanine deaminase	*GDA	3.5.4.3	T	MF
Glycerol-3-phosphate dehydrogenase	*G3PDH	1.1.1.8	L	TC
Isocitrate dehydrogenase	IDH	1.1.1.42	L	TC
L-Lactate dehydrogenase	*LDH-1	1.1.1.27	H,L,M,T	MF
	LDH-2		H,M	MF
	LDH-3		H,L	MF
Malate dehydrogenase	MDH-1	1.1.1.37	M	MF
	*MDH-2		M	MF
	*MDH-3		L	MF
Malic enzyme	*MEP	1.1.1.38	M	MF
Mannose-6-phosphate isomerase	*MPI	5.3.1.8	L	MF
6-Phosphogluconate dehydrogenase	PGD-1	1.1.1.44	L	TC
	*PGD-2		L	TC
Phosphoglucomutase	PGM	5.4.2.2	L,M	RW
Purine-nucleoside phosphorylase	*NP	2.4.2.1	T	MF
Superoxide dehydrogenase	*SDH	1.1.1.14	L	TC
Superoxide dismutase	*SOD	1.15.1.1	L	TC

* Monomorphic loci; **H** heart; **L** liver; **M** muscle; **T** testis; **MF** a continuous Tris, boric acid, EDTA buffer (pH 8.6) described by Markert & Faulhaber (1965); **RW** a discontinuous Tris, citric acid (gel pH 8.7), lithium hydroxide, boric acid (electrode pH 8.0) buffer system (Ridgeway *et al.*, 1970); **TC** a continuous Tris, citric acid (pH 6.9) buffer system (Whitt, 1970).

one in all cases.

ZAMBEZI had three heterozygous loci (***ES-1, -3, -4***) of which the allele frequencies approximated Hardy-Weinberg expectations. Deviations of allele frequencies occurred at ***IDH*** and ***LDH-3*** (Table 2) and no heterozygotes were observed at the latter locus. OLIFANTS showed close approximation of allele frequencies to Hardy-Weinberg proportions at four of the nine polymorphic loci (Table 2). Deficiencies of heterozygotes occurred at the ***ES-1, -4, IDH, PGD-1*** and ***PGM*** enzyme coding loci for OLIFANTS, where heterozygotes were observed only at the ***PGM*** locus. The mean number of alleles per locus (*A*) was 1.20 (± 0.08), ***h*** values ranged from 0.028 to 0.202 and the ***H*** value was 1.9% (± 0.009) for ZAMBEZI. OLIFANTS had an *A* of 1.36 (±0.10), ***h*** values ranged from 0.025 to 0.399 and ***H*** was 4.6% (±0.021).

Population differences were examined by calculating fixation indices for each locus as well as the mean value across all loci. The mean $\mathbf{F_{IS}}$ and $\mathbf{F_{IT}}$ values across all loci were 0.646 and 0.673 respectively. Wright's (1978) measure of differentiation, $\mathbf{F_{ST}}$, between the populations studied was 0.076 and the loci that contributed most to population differences were **ES-1** ($\mathbf{F_{ST}}$ = 0.101) and **PGM** ($\mathbf{F_{ST}}$ = 0.143). Nei's (1972; 1978) genetic distances were both 0.005 between the two populations studied.

Table 2 Relative mobility (RM) of alleles, relative frequencies of polymorphic loci observed (OBS) and expected (EXP) number of heterozygotes, coefficients for heterozy gosity deficient (*d*) or excess, χ^2 values and individual heterozygosities (*h*) for two popula tions of *Hydrocynus vittatus*

		Upper Zambezi River population (N = 35)						Olifants River (Kruger National Park) population (N = 40)					
Locus	RM	Frequencies	OBS	EXP	*d*	χ^2	*h*	Frequencies	OBS	EXP	*d*	χ^2	*h*
ES-1	100	0.957	3	2.87	0.045	0.070	0.082	0.725	0	15.95	−1.00	40.000*	0.399
	105	0.043						0.275					
ES-2	90	1.000						0.988	1	0.99	0.013	0.006	0.025
	95							0.012					
ES-3	70	0.971	2	1.94	0.029	0.030	0.056	0.975	2	1.95	0.026	0.026	0.049
	85	0.029						0.025					
ES-4	60	0.986	1	0.99	0.014	0.007	0.028	0.975	0	1.95	−1.000	40.000*	0..049
	55	0.014						0.025					
IDH	100	0.886	2	7.09	−0.718	18.000*	0.202	0.975	0	1.95	−1.000	40.000*	0.049
	95	0.114						0.025					
LDH-2	65	1.000						0.975	2	1.95	0.026	0.026	0.049
	50							0.025					
LDH-3	−100	0.943	0	3.77	−1.000	35.000*	0.108	1.000					
	−95	0.057											
MDH-1	100	1.000						0.988	1	0.99	0.013	0.006	0.025
	105							0.012					
PGD-1	100	1.000						0.925	0	5.55	−1.000	40.000*	0.139
	95							0.075					
PGM	90	1.000							0.250				
		Average heterozygosity = 0.019 (± 0.009)						Average heterozygosity = 0.046 (± 0.021)					

* = Loci where significant ($p < 0.05$) deviations of alleles from expected Hardy-Weinberg proportions occurred.

Discussion

Genetic variation

ZAMBEZI displayed 20% polymorphism (at ***ES-1***, ***-3***, ***-4***, ***IDH*** and ***LDH-3***), whereas OLIFANTS displayed a much higher percentage (36%) at ***ES-1*** to ***-4***, ***IDH***, ***LDH-2***, ***MDH-1***, ***PGD-1*** and ***PGM***. The two populations showed close similarity in genetic variability with *A* values of 1.20 and 1.36 for ZAMBEZI and OLIFANTS respectively. Deviations of allele frequencies from expected Hardy-Weinberg proportions in ZAMBEZI occurred at three of the five polymorphic loci, and for OLIFANTS at five of the nine polymorphic loci (Table 2). Such deviations can be due to non-random mating, gene flow, mutations, genetic drift and selection (Soltis & Soltis, 1988). Non-random mating and gene flow can therefore be excluded since these processes generally affect all loci. Heterozygote deficiencies occurred at all the loci which deviated in allele frequencies from expected Hardy-Weinberg proportions for both populations studied. The observed heterozygote deficiencies (Table 2) may be due to mutations and/or natural selection.

The estimate of ***H*** for ZAMBEZI was 1.9% (±0.009) whereas OLIFANTS had a much higher value (4.6%, ± 0.021). Previous studies reported ***H*** values ranging from 4.7 to 31.6% (Avise & Aquadro, 1982). These authors compared the electrophoretical characters of more than 77 freshwater fish species and estimated ***H*** to average 5.4% for panmictic populations. The ***H*** value obtained for OLIFANTS compares favourably with estimates reported by the above authors. Although it was thought OLIFANTS was more isolated (Van Loggerenberg, 1988), this population has more genetic variation than ZAMBEZI. The ***H*** value for ZAMBEZI is low if compared to ***H*** for other fish species from the same geographical area. Average heterozygosity values ranged from 1.3 to 4.7% in a study of fifteen southern African cichlids, and 8.5% for *Synodontis leopardinus* (Van der Bank *et al.*, 1989; Van der Bank, 1993).

The higher level of genetic variation in OLIFANTS can be attributed to random sampling. During winter tigerfish migrate to Mozambique to escape the relatively colder winter conditions of the Kruger National Park. During spring, before flooding, an upstream migration occurs probably to search for new breeding grounds and to expand their range (Gagiano, 1997). Samples were collected when the sexually active fish congregated below a barrier near the joining of the Letaba and Olifants Rivers in the Kruger National Park. Therefore, it is conceivable that a random sample of the total gene pool of this population was taken to effectively eliminate sample error. On the other hand, the lower level of heterozygosity in ZAMBEZI may be due to inbreeding. Inbreeding can occur as a result of the isolation of populations. Isolated populations can form since the tigerfish is a significant predator at the interface between river backwaters and channel habitats and food resource competition exists owing to a high degree of habitat partitioning between larger size classes of the species (Badenhuizen, 1966; Winemiller & Kelso-Winemiller, 1994). Freshwater fish are often topographically subdivided into smaller breeding populations and small or isolated breeding populations tend to lose variation as a result of selection and inbreeding (Utter *et al.*, 1973; Grant & Stahl, 1988). Isolation of ZAMBEZI also occurred by the Victoria Falls, which forms a major barrier and prevent fish from migrating. Colour variations of the mature male tigerfish from the Upper Zambezi River have not been found in the tigerfish from Lake Kariba. This probably indicates that tigerfish do not survive the drop over the Victoria Falls, giving rise to a distinct gene pool above the falls (Steyn *et al.*, 1996). Adult fish, which display distinct territoriality, were sampled with artificial lures in the separate upper part of the Zambezi River at the Caprivi Strip, Namibia.

Genetic differentiation

The genetic distance was calculated between the two populations studied to determine if genetically different species are involved, since ZAMBEZI differs morphologically from OLIFANTS. The morphological differences are described in the introduction. Genetic distance values between congeneric species range between 0.03 and 0.61 with an average of 0.3 (Shaklee *et al.*, 1982). The ***D*** value (0.005) obtained for tigerfish in this study does not fit in this range. This indicates that conspecific populations (and not congeneric species as hypothesized) are involved. This is also reflected by the **F**-statistics. The population structure, as determined with the hierarchical F-statistics, gives the total inbreeding coefficient estimate ($\mathbf{F_{IT}}$) as 0.67 for the tigerfish studied. Therefore, the inbreeding in the individuals relative to the total populations is quite large and can be due to random genetic drift among the subpopulations studied. Values of $\mathbf{F_{IS}}$ in most natural populations are typically close to zero, which indicates random mating within subpopulations (Nei, 1986). The high $\mathbf{F_{IS}}$ value of 0.63 calculated in the present study is indicative of effective barriers to gene flow between the populations. No gene flow is possible between the two populations due to geographical constraints. The low fixation index ($\mathbf{F_{ST}}$ = 0.076), which is the amount of inbreeding due solely to pop-

ulation subdivision, also shows that there is low genetic differentiation between the two populations.

To conclude, we have shown that ZAMBEZI and OLIFANTS are conspecific populations and in addition sufficient intraspecific genetic variability exists to distinguish the two populations. Individuals from OLIFANTS, with higher heterozygosity, should be used in artificial propagation programmes. Artificial breeding can lead to the establishment of domesticated broodstock which in turn may resolve the problems associated with the artificial production of wild tigerfish (Steyn & Van Vuren, 1991; Steyn, 1993; Steyn *et al.*, 1996). Restocking of tigerfish propagated by an artificial breeding programme is important for conservation and recreational fishing purposes.

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