An estimate of the amount of genetic variation in a population of the Bulldog *Marcusenius macrolepidotus* (Mormyridae)

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Abstract

As far as could be ascertained, this is the first account of allozyme variation in mormyrids. Five (3 continuous and 2 discontinuous) buffer systems were used and 29 enzymes and proteins were stained for, of which 26 revealed interpretable results. Gene products of 52 protein coding loci in *M. macrolepidotus* were examined by horizontal starch gel electrophoresis and genetic variation was observed at 21 (40.4%) of these loci. The percentage of polymorphic loci, the mean number of alleles per locus and the average heterozygosity per locus were 30.8% (0.95 criterion), 1.44 (±0.08) and 0.09 (±0.02) respectively. Genetic variation is comparable to values obtained for other freshwater fish species from the Zambezi River.

Introduction

Representatives of the family Mormyridae, of which there are 18 genera and approximately 200 species in Africa (Skelton, 1993), have large brains comparable (relative to body mass) to those of humans. These fishes use their electric sense for location and communication and have been shown to be both electrogenic and electroreceptive (Lissmann, 1958; Kramer, 1990; 1994). *Marcusenius macrolepidotus* (Peters, 1852) is popular with aquarists, is a favourite bait among anglers for catching tigerfish, and some mormyrids have been utilised to monitor changes in water quality (Geller, 1984; Kunze and Wetzstein, 1988; Kunze, 1989). Mormyrids can also be trained by rewarding them with treats for executing the appropriate action when a previously tape-recorded electric organ discharge was sent (Kramer, 1979; Graff and Kramer, 1992). Despite the peculiarities and applications of mormyrids, no information is available on genetic variation in these fishes. We estimated the amount, distribution and pattern of genetic variability in *M. macrolepidotus* from the Zambezi River.

Materials and methods

Muscle and liver samples from 50 *M. macrolepidotus*, collected from the Upper Zambezi River near Katima Mulilo (24°26'S, 17°29'E), were stored in liquid nitrogen and transported to the laboratory. Tissue extracts were prepared and analysed by starch gel electrophoresis (12% gels) following procedures, method of interpretation of gel banding patterns and locus nomenclature of Van der Bank et al. (1992). Statistical analysis of allozyme data was done using **BIOSYS-1** (Swofford and Selander, 1981).

The following buffer systems were used: **HC** - a continuous histidine, citrate buffer, pH 6.5, (Kephart, 1990); **MF** - a continuous Tris, boric acid, EDTA buffer, pH 8.6, (Markert and Faulhaber, 1965); **P** - a discontinuous Tris, citric acid (gel 8.7), NaOH, boric acid (electrode pH 8.2) buffer (Poulik, 1957); **RW** - a discontinuous Tris, citric acid, (gel pH 8.7), lithium hydroxide, boric acid (electrode pH 8.0) buffer (Ridgway et al., 1970); and **TC** - a continuous Tris, citric acid (pH 6.9) buffer system (Whitt, 1970).

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Results

Locus abbreviations, enzyme commission numbers, tissues and buffers giving the best results in *M. macrolepidotus* are listed in Table 1. Thirty-one of the 52 loci (59.6%) were monomorphic (Table 1). Products of the following loci migrated cathodally: **AK-3, ADH, FH-2, GAPDH-3, GPD-2, SORD-2, IDH-2, LDH-3, MDH-2, ME-2, PER-2** and **SKDH-2**. We also stained for aspartate aminotransferase (E.C. 2.6.1.1), dihydrolipoamide dehydrogenase (E.C. 1.8.1.4) and purine-nucleoside phosphorylase (E.C. 2.4.2.1) which, together with **GAPDH-1, HK-2, SORD-1** and **SKDH-1**, did not show sufficient activity or resolution for satisfactory scoring.

Table 2 presents allele frequencies, coefficients for heterozygosity deficiency or excess (D), Chi-square (X^2) values and degrees of freedom for all the polymorphic loci, as well as individual heterozygosity values (h). Loci where significant ($P<0.05$) deviations of alleles from expected Hardy-Weinberg proportions occurred are also listed in Table 2. Allozyme phenotypes of putative heterozygotes were congruent with those expected on the basis of the quaternary structure of the enzyme (Ward, 1977). Thus heterozygotes at **GAPDH** and **LDH** were five-banded, triple banded at **ADH, GPD, GPI, IDH, MDH** and **SOD**, as expected for dimeric enzymes, and heterozygotes at the monomeric enzymes **AK, EST, SORD, PEP, MNR, ME, MPI** and **PGM** were double banded.

Of the 52 protein coding loci which provided interpretable results in *M. macrolepidotus*, 40.4% were polymorphic. Genotypic frequencies at nine loci (**ADH, EST-4, GAPDH-3, GPD-2, SORD-2, LDH-3, MDH-1** and **SOD**) in *M. macrolepidotus* closely approximated Hardy-Weinberg expectations (Table 2). Deficiencies of heterozygotes occurred at all of the other loci, except at **PEP-S3** (Table 2). The number of alleles at the **GPD-1** and **MDH-1** loci were three whereas two alleles were present at all of the other polymorphic protein coding loci studied. The mean number of alleles per locus was 1.44 (±0.08), h values ranged from 0.039 to 0.500, and the average heterozygosity per locus (H) was 9.2% (±0.02).

Discussion

Deviations of allele frequencies from expected Hardy-Weinberg proportions occurred at the **AK-2, CK-2, EST-3, GPD-1,**

TABLE 1
LOCUS ABBREVIATIONS, ENZYME COMMISSION NUMBERS (E.C. NO.), TISSUES AND BUFFERS GIVING THE BEST RESULTS ARE LISTED AFTER EACH PROTEIN
SEE "MATERIAL AND METHODS" FOR ABBREVIATIONS OF BUFFERS USED

Protein	Locus	E.C. No.	Buffer	Tissue
Adenylate kinase	*(AK-1,-3)	2.7.4.3	MF,P,HC	L
	(AK-2)			M,L
Alcohol dehydrogenase	(ADH)	1.1.1.1	MF,P	L
Creatine kinase	*(CK-1)	2.7.3.2	MF,TC	M,L
	(CK-2)			M
Esterase	*(EST-1,-2)	3.1.1.-	MF,HC,P	M,L
	(EST-3)			M,L
	(EST-4)			L
Fumarate hydratase	*(FH-1)	4.2.1.2	MF	M
	*(FH-2)			M,L
General protein	*(PROT-1, to -4)		MF	M,L
Glyceraldehyde-3-phosphate	*(GAPDH-2)	1.2.1.12	MF	M
dehydrogenase	(GAPDH-3)			L
Guanine deaminase	*(GDA)	3.5.4.3	MF	M,L
Glycerol-3-phosphate	(GPD-1)	1.1.1.8	MF,RW	M,L
dehydrogenase	(GPD-2)			L
Glucose-6-phosphate isomerase	(GPI-1,-2)	3.5.1.9	MF	M,L
Hexokinase	*(HK-1)	2.7.1.1	TC	M,L
L-Iditol dehydrogenase	(SORD-2)	1.1.1.14	MF	L
Isocitrate dehydrogenase	*(IDH-1)	1.1.1.42	TC	M,L
	(IDH-2)			L
L-Lactate dehydrogenase	(LDH-1)	1.1.1.27	RW	M,L
	*(LDH-2)			M
	(LDH-3)		MF	L
Malate dehydrogenase	(MDH-1)	1.1.1.37	P	M,L
	*(MDH-2)			L
Malic enzyme	(ME-1)	1.1.1.38	MF	M
	*(ME-2)			M,L
Mannose-6-phosphate isomerase	(MPI-1)	5.3.1.8	MF,TC	M,L
	*(MPI-2)			M
Menadione reductase	(MNR-1)	1.6.99.-	P	M
	*(MNR-2)			L
Peptidase:		3.4.-.-		
Substrate: Glycyl-L-leucine	*(PEP-A1,-A2)	MF	M,L	
Leucylglycylglycine	*(PEP-B1,-B2)	RW	M,L	
Leucyl-tyrosine	*(PEP-S1,-S2)	MF	M,L	
	(PEP-S3)		M,L	
Peroxidase	*(PER-1,-2)	1.11.1.7	MF,P	L
6-Phosphogluconate	*(PGD)	1.1.1.44	MF	M,L
dehydrogenase				
Phosphoglucomutase	*(PGM-1)	5.4.2.2	MF	M,L
	(PGM-2)			L
Shikimate dehydrogenase	*(SKDH-2)	1.1.1.25	TC	L
Superoxide dismutase	(SOD)	1.15.1.1	MF	M,L

* = monomorphic loci; M = muscle; L = liver

TABLE 2
SAMPLE SIZE (N), ALLELE FREQUENCIES, HETEROZYGOSITY DEFICIENCY OR EXCESS (*D*), X^2 VALUES AND DEGREES OF FREEDOM (*DF*), AND INDIVIDUAL HETEROZYGOSITIES (*h*) FOR POLYMORPHIC LOCI

Locus	N	Allele frequency			*D*	X^2	DF	*h*
		A	B	C				
AK-2	50	0.060	0.940		-0.645	20.826*	1	0.113
ADH	50	0.060	0.940		0.064	0.204	1	0.113
CK-2	50	0.980	0.020		-1.000	50.000*	1	0.039
EST-3	45	0.722	0.278		-0.502	11.319*	1	0.401
EST-4	43	0.233	0.767		0.042	0.077	1	0.357
GAPDH-3	45	0.056	0.944		0.059	0.156	1	0.105
GPD-1	47	0.149	0.840	0.011	-0.608	20.768*	3	0.271
GPD-2	50	0.040	0.960		0.042	0.087	1	0.077
GPI-1	50	0.220	0.780		-0.417	8.705*	1	0.343
GPI-2	48	0.042	0.958		-0.478	10.979*	1	0.080
SORD-2	38	0.053	0.947		0.056	0.117	1	0.100
IDH-2	38	0.171	0.829		-0.722	19.787*	1	0.284
LDH-1	47	0.181	0.819		-0.641	19.308*	1	0.296
LDH-3	50	0.050	0.950		0.053	0.139	1	0.095
MDH-1	46	0.011	0.978	0.011	0.017	0.023	3	0.043
ME-1	46	0.500	0.500		-0.565	14.696*	1	0.500
MNR-1	31	0.339	0.661		-0.640	12.696*	1	0.448
MPI-1	41	0.817	0.183		-0.429	7.541*	1	0.299
PEP-S3	45	0.522	0.478		0.381	6.515*	1	0.499
PGM-2	37	0.824	0.176		-0.533	10.528*	1	0.290
SOD	50	0.020	0.980		0.020	0.021	1	0.039

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

GPI-1, -2, IDH-2, LDH-1, ME-1, MNR-1, MPI-1, PEP-S3 and **PGM-2** protein coding loci (Table 2). No heterozygotes were observed at the **CK-2** locus, a slight excess of ***h*** was observed at **PEP-S3**, and a deficit of heterozygotes at other (above-mentioned) loci resulted in deviations of allele frequencies from expected Hardy-Weinberg proportions. Deficiencies of heterozygotes also occurred at all of the other loci where X^2 values exceeded critical values (Table 2). This indicates that rare alleles are present at these loci. Hardy-Weinberg proportions of allele frequencies were obtained at nine loci (Table 2). Various factors (e.g. recurrent bottlenecks involving small sample sizes of founder populations, natural selection, selfsorting crossings and linking, etc.) can shift the equilibrium to disrupt the stability of a population and to give rise to changes in the genetic structure. The extent of the effect that such factors could have had on *M. macrolepidotus* is unknown because very little is known about the biology of these fishes.

Previous studies on freshwater fish, mussels and snails reported H values ranging from 4.7-31.6% (Powell, 1975; Nevo, 1979; Avise and Aquadro, 1982; Hebert et al., 1989; Garton and Haag, 1991). The estimate of H obtained in the present study (8.1%) also is congruent with those found for other species from the same localities (Van der Bank, 1993). These results also indicate that the species examined possess sufficient variation to permit them to adapt to environmental changes or to be used in selection programmes.

The Bulldog is one of nine mormyrid species (from seven different genera) which naturally occurs in rivers in Namibia but as yet, very little is known about their phylogenetic relationships. The extent of genetic variation within *M. macrolepidotus* can provide information which could be used in subsequent studies to delineate phylogenetic relationships among species within the family Mormyridae (see e.g. Buth, 1984; Abban, 1988; Sodsuk and McAndrew, 1991; Davis and Nixon, 1992).

The isozymes described in this study provide a good basis for estimating the amount and pattern of genetic variation within this species. The enzymatic polymorphisms also revealed interesting data for both fundamental and applied research. This information is especially relevant when the artificial production of snoutfish for the aquarium trade is considered. These fishes also serve as one of the major sources of nutrition for catfish (*Clarias gariepinus* and *C. ngamensis*) during pack hunting (Merron, 1993), and they are an important species for human consumption. Thus the Bulldog is a very important species to study and our estimate of genetic variability will be of interest (i.e. to conservation authorities, hobbyists, and aquaculturists).

We recommend that representatives of the other mormyrid genera be analysed electrophoretically and compared, especially the populations which show dimorphism in electric organ discharges (i.e. *M. macrolepidotus* from the Sabi and Zambezi River Systems, *Pollimyrus castelnaui* from the Zambezi and Kwando River Systems, and *Hippopotamyrus ansorgii* from the Zambezi River). It is possible that different races or species are involved (Kramer, 1994, Van der Bank and Kramer, in prep.). Sympatric sibling species of *H. ansorgii* possibly coexist in the Zambezi River system since pulse discharge differences (not sexual dimorphism, as histology has shown) were encountered by Van der Bank and Kramer (in prep.). An electrophoretic analysis of such populations should provide a better understanding of the genetic divergence and biogeography of the snoutfishes.

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