Baseline Biochemical and Genetic Profiles of Four Fishes from the River Nile

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Abstract

Six enzyme systems coding for 10 loci and 6 proteins were examined in the blood of *Polypterus senegalus, Clarias lazera, Tilapia nilotica* and *Protopterus annectens*, using electrophoresis. Six loci were polymorphic in all the four species, three polymorphic in three species and one polymorphic in *T. nilotica*. Four protein loci were monomorphic in all the four species with variants in *P. senegalus* and *T. nilotica*. Haemoglobin can be used as a species-specific marker. Polymorphism was 53-56 per cent and average heterozygosity was 0.1-0.15.

Introduction

The river Nile supports up to two hundred fish species. A few comparative biochemical or genetic studies have been conducted on these Nile fish (Babiker and El-Hakeem 1979). Morphological characteristics are the only parameters that have been studied in some detail for taxonomic purposes between and within different genera (Bishai and Abu-Gideiri 1967). To understand the genetic variation within a species, a preliminary study was carried out on some biochemical and genetic characteristics of the four Nile fish species that are being commercially exploited along the Nile waters in the Sudan: (i) *Polypterus senegalus* (Cuvier), a chondrostians derivative; (ii) *Clarias lazera* (Cuvier and Valenciennes), a teleost catfish (Silurid); (iii) *Tilapia nilotica* (Linnaeus), an advanced spiny teleost with a complete aquatic breathing adaptation; and (iv) *Protopterus annectens* (Owen), the lungfish from Western Sudan. Polymorphism of haemoglobin (HB), erythrocyte glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49, D-glucose: NADP oxidoreductase); malate dehydrogenase (MDH; E.C. 1.1.1.37, L-malate: NAD oxidoreductase); phosphoglucomutase (PGM; E.C. 2.7.5.1, D-glucose-1-phosphate phosphotransferase); catalase (CAT; E.C. 1.11.1.6, H_2O:H_2O oxidoreductase); glucose phosphate isomerase (GPI; E.C. 5.3.1.9, D-glucose-6-phosphate ketol isomerase); lactate dehydrogenase (LDH; E.C. 1.1.1.27, L-lactate: NAD oxidoreductase); serum esterase (EST; E.C. 3.1.1.1, aryl-esterase or pseudocholine esterase); haptoglobin (HP); transferrin (TF); albumin (ALB) and caeruloplasmin (CP) were investigated, whenever possible, in all the four species. The aim of the present study was to examine inter- and intraspecies biochemical/genetic variation to obtain baseline information on the genetic structure of these fish, which could be used in further taxonomic and population genetic studies.

Materials and Methods

Fifty specimens of *P. senegalus*, one hundred of *C. lazera* and fifty of *T. nilotica* were obtained from the Jebel Aulia area, 45 km south of Khartoum, on the White Nile. Sixty specimens of aestivated *P. annectens* were collected from the dry bed of the Khor Abu-Habil stream in Kordofan Province, Western Sudan.

Blood samples were obtained by severing the caudal peduncle, placed in centrifuge tubes, allowed to clot and centrifuged at 5000 *g* for 10 minutes. Serum was then pipetted and kept for one week at -20°C until used for electrophoretic analysis. Erythrocytes were separated from freshly drawn heparinized blood. The cells were then washed three times with isotonic saline (0.9 NaCl) and the buffy coat was sucked. The washed packed cells were lysed three times by freezing and thawing.

Haemoglobin, erythrocyte enzymes, serum esterase and serum proteins were separated on horizontal starch-gel electrophoresis according to Menezes and Taniguchi (1990). Typing and nomenclature of gene locus designations followed that of Shaklee *et al.* (1990). When adequate numbers of fish were typed for any protein, allelic and phenotypic frequencies were calculated and deviation from the Hardy-Weinberg equilibrium was tested according to Emery (1976). The degree of polymorphism and average heterozygosity was calculated by including both monomorphic and polymorphic loci in each species according to Ayala (1983).

Results and Discussion

Figure 1 Shows a diagramatic representation that summerizes the allelic products of eight loci revealed in *P. senegalus, C. lazera. T. nilotica* and *P. annectens*. Whenever polymorphism was observed, the type and number of bands were recorded. The allele frequencies and chi-square values for the differences between observed and expected phenotype frequencies of each polymorphic enzyme are given in Table 1. Genotypes for species conformed to Hardy-Weinberg proportions at all loci with the exception of G6PD* in *P. annectens* ($p<0.05$) which was deficient in heterozygotes. The reason for this result is unknown. Haemoglobin was monomorphic in all four species, but was species-specific. It appeared as one anodal band in *C. lazera* and *P. annectens*, two anodal bands in *P. senegalus* and three bands (two anodal and one cathodal) in *T. nilotica*. Six loci: PGM*; MDH-1*; LDH*A; LDH*B; HP* and TF* were polymorphic in all four species. G6PD* was monomorphic in C. lazera while CAT* was monomorphic in *T. nilotica*. Serum esterase (EST-2*) was not examined in *T. nilotica*, but it was polymorphic in the remaining species, while GPI* was polymorphic in *T. nilotica*. Albumin (ALB*) and CP* were monomorphic in all species. The degree of polymorphism ranged between 53-56, and average heterozygosity ranged between 0.10-0.15 in the four species.

The phenotypic distribution of the polymorphic systems (Table 1) was at the Hardy-Weinberg equilibrium. In *P. senegalus* (χ^2 =18.86 *d.f.* 17) and in *T. nilotica* (χ^2 = 20.56 *d.f.* 17). There was an extreme degree of deviation in *C. lazera* (χ^2 = 82.53 *d.f.*16) and *P. annectens* (χ^2 = 25.39 *d.f.*17) with an excess of heterozygotes.

The data represented in this study provide baseline biochemical genetic

Table 1. Allele frequency at five polymorphic loci in Polypterus senegalus, larias lazera, Tilapia nilotica and Protopterus annectens.

Polymorphic systems	Genotypes	*P. senegalus* (n = 50)	C. lazera (n =100)	*T. nilotica* (n = 100)	*P. annectens* (n = 60)
G6PD*	*G6PD*1* *G6PD*2*	0.79 0.21	0.00 1.00	0.57 0.43	--- ---
PGM*	*PGM*1* *PGM*2* *PGM*3*	0.41 0.59 0.00	0.37 0.63 0.00	0.17 0.83 0.00	0.82 0.08 0.10
MDH*	*MDH*1* *MDH*2*	0.75 0.25	0.34 0.66	0.45 0.55	0.42 0.58
CAT*	*CAT*1* *CAT*2*	0.43 0.57	0.39 0.61	--- ---	0.46 0.54
HP	*HPI*1* *HPI*2* *HPI*3*	0.75 0.19 0.06	0.56 0.40 0.04	0.33 0.60 0.07	0.59 0.39 0.02

Table 2. The distribution of allelic products of protein loci, degree of polymorphism and average heterozygosity in *P. senegalus, C. lazera, T. nilotica* and *P. annectens*.

Locus	*P. senegalus*	*C. lazera*	*T. nilotica*	*P. annectens*
*G6PD**	Poly 1 2-1 2	Mono	Poly 1 2-1 2	Poly 1 - 2
*PGM**	Poly 1 2-1 2 -	Poly 1 2-1 2 -	Poly 1 2-1 2 -	Poly 1 2-1 3-1 3-2
*MDH-1**	Poly A AB B	Poly A AB B	Poly A AB B	Poly A AB B
*CAT**	Poly 1 2-1 2	Poly 1 2-1 2	Mono	Poly 1 2-1 2
*GPI**	*	*	Poly 1 2-1 2	*
*EST-1**	Poly 1 2	Poly 1 2 3	*	Poly 1 2 -
*HP**	Poly 1 2-1 - 3-1	Poly 1 2-1 2 3-1	Poly 1 2-1 2 3-1	Poly 1 2-1 2 3-1
*HB**	1 band**	1 band	3 bands**	1 band
*LDH**	***(32)	***(46)	***(46)	***(50)
*TF**	***(52)	***(72)	***(42)	***(57)
*ALB**	1 band (52)	1 band (72)	1 band (42)	1 band (57)
*CP**	1 band (52)	1 band (72)	1 band (42)	1 band (57)
P	0.53	0.56	0.53	0.53
H_a: *Obs.* *Exp*	0.15 0.12	0.13 0.10	0.10 0.09	0.15 0.10

Poly Polymorphic
Mono Monomorphic
* Broad diffuse band – could not be typed
** Two loci
*** Highly polymorphic – could not be typed
P Proportion of polymorphic loci
H_a Average heterozygosity

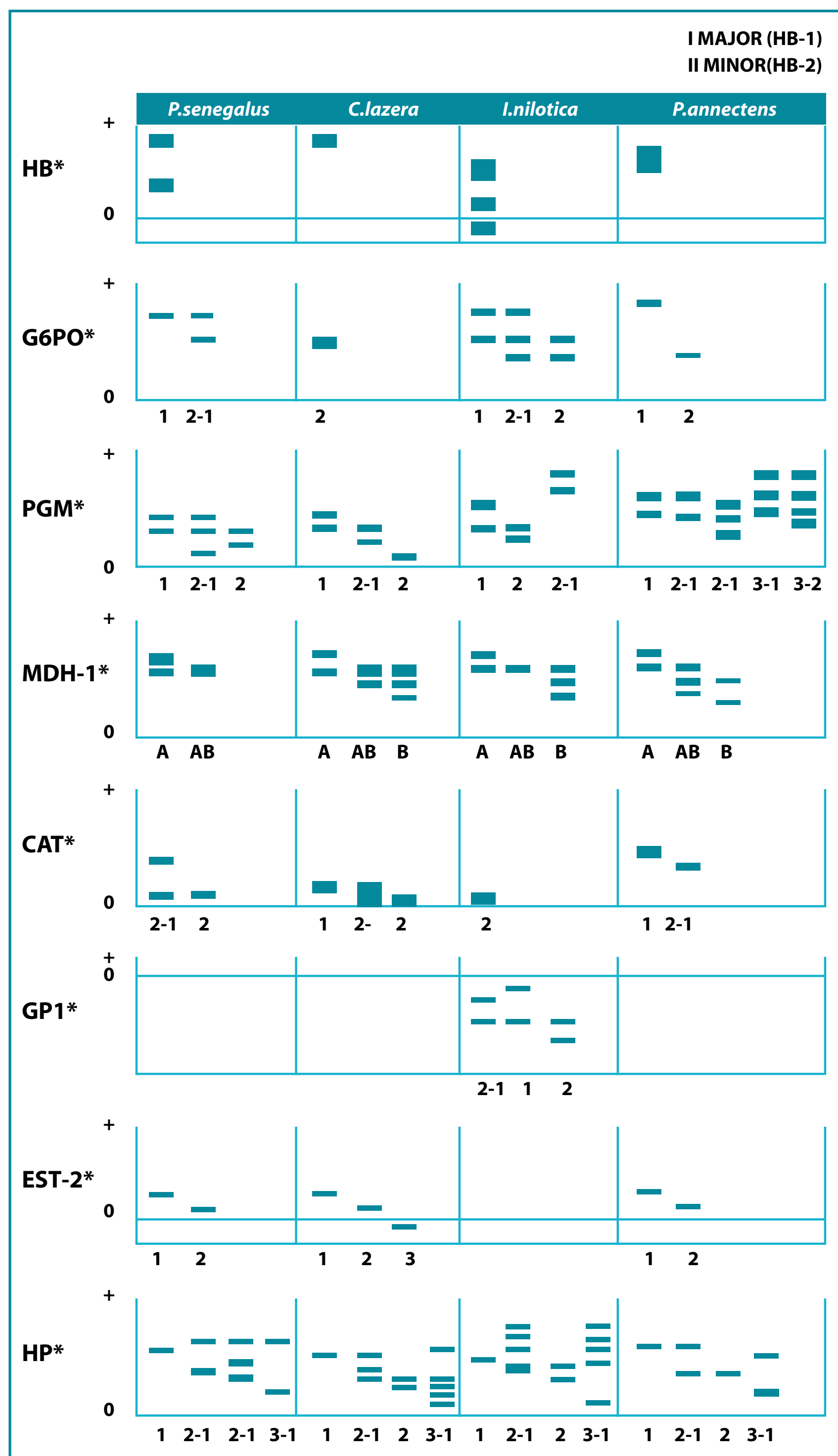

Figure 1. Diagramatic representation of allelic products of enzymes and proteins examined in *P. senegalus, C. lazera, T. nilotica* and *P. annectens*.

Note: O = Origin of application. Mod = Modified variant.

profiles that were distinctly different in the species examined. The high degree of polymorphism (Table 2) in the four species probably reflected a high adaptive response to a wide range of environmental conditions. The average heterozygosity was very high in all species except *T. nilotica*. It is reasonable to assume that the amount of isozyme variations detected reflects the relative amount of genetic variation found at other loci in the genome (Scott and Harrington, 1990; Coppes, 1993; Mckay, 1993, Monteiro. et *al*, 1993). High levels of heterozygosity and polymorphism have also been reported in other species of fish (Cook, et *al*., 1992; McCracren, et al, 1993; Menezes 1993; Menezes et al. 1993; Dobrovolov, 1994; Ohtani et al. 1997). According to Nevo (1978); Kirpichnichov (1981); Van-Der- Bank, et al. (1992); Van-Der- Walt, et al. (1993); and Strussmann, et al. (1997), high heterozygosity values are found in tropical vertebrates that are ecologically generalists (widespread, common, vagile, broad-niched species).

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