Allozyme variation in the river sardine, *Mesobola brevianalis* (Pisces, Cyprinidae)

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Abstract

A population of *Mesobola brevianalis* was examined for genetic variation using horizontal starch gel electrophoresis. Gene products of 27 protein coding loci were consistently resolved and revealed polymorphism at five loci. Observed genotype frequencies at three loci, **CK-1**, **HK-1** and **PGDH-1**, deviated significantly from Hardy-Weinberg expectations. Genetic variability estimates were congruent with those obtained for fish in general. Possible reasons for the low variability values obtained are discussed in relation to natural and anthropogenic influences in the river system. The results are of value for conservation authorities as well as for aquarists concerned with commercial production of the species.

Introduction

Members of the genus *Mesobola*, commonly referred to as neobolins, are relatively small, shoaling species which prefer the upper stratum of open water in rivers and dams. The genus, which comprises four species, is endemic to Africa. A single species, the river sardine (*M. brevianalis*), occurs in Southern Africa (Bell-Cross and Minshull, 1988; Skelton, 1993).

In terms of commercial potential, the river sardine must be one of the most underestimated fish species in Southern Africa. According to Bell-Cross and Minshull (1988), it is invaluable in dam management where it is introduced as fodder for large game fish and other predatory fish such as tigerfish (*Hydrocynus vittatus*), bass (*Micropterus* spp.), nembwe (*Serranochromis robustus*) and silver catfish (*Schilbe intermedius*). This species is also harvested to some extent by subsistence fishermen and is used as bait for predatory fish by anglers. Furthermore, the river sardine is also popular amongst ornamental fish aquarists. Despite these attributes, the river sardine seldom features in large-scale commercial aquacultural ventures.

Information regarding the genetic variation occurring in the river sardine is a basic prerequisite for further fundamental and applied research designed to develop a basis for successful management of this species. Such an understanding of detectable genetic variation will also be important for the establishment of proposed aquaculture ventures involving the species. The present study aims to provide objective information regarding the genetic structure of the potentially commercially important river sardine, using allozyme electrophoresis.

Materials and methods

Sixty individuals were collected with a seine net and by electronarcosis from the Phalaborwa Barrage (24°03' S, 31°08' E) in the Olifants River, Limpopo River system. 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 buffer systems used, electrophoretic procedures, histochemical staining techniques and interpretation of gel-banding patterns are as described in Engelbrecht et al. (1997).

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 using the 95% criterion ($P_{0.95}$) and average observed (H_O) and expected (H_E) heterozygosity per locus. Both H_O and H_E estimates were determined to facilitate comparison with other studies. All polymorphic loci were tested by chi-square (X^2) analysis for goodness-of-fit to Hardy-Weinberg proportions.

Results

The products of 27 protein coding loci were detected and provided interpretable results for population analysis. The names of the proteins examined, locus abbreviations, enzyme commission (E.C.) numbers, and buffer systems giving the best results are presented in Table 1. Twenty-two of the 27 loci (81%) were monomorphic. All polymorphic loci displayed allozyme banding patterns consistent with that expected from the known quaternary structure of the proteins (Ward, 1977). Allele frequencies for polymorphic loci, X^2 values of loci which deviated significantly from expected Hardy-Weinberg proportions, $P_{0.95}$, H_O and H_E estimates for the two populations are presented in Table 2.

Observed genotype frequencies deviated significantly from expected Hardy-Weinberg proportions at three loci, **CK-1**, **HK-1** and **PGDH-1**. These deviations were associated with a deficit of heterozygotes at all three the above-mentioned loci (Table 2). The percentage of polymorphic loci ($P_{0.95}$) was calculated at 14.8%. Estimates of observed and expected heterozygosity were H_O = 0.011 and H_E = 0.025 respectively.

Discussion

Successful management of a species depends on data regarding the genetic structure of populations. The data obtained may be used in management plans by conservation authorities as well as by

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Received 10 June 1998; accepted in revised form 2 February 1999.

TABLE 1
LOCUS ABBREVIATIONS, ENZYME COMMISSION (E.C.) NUMBERS AND BUFFERS GIVING THE BEST RESULTS FOR EACH PROTEIN ANALYSED

Protein	Locus	E.C. No.	Buffer
Aspartate aminotransferase	**AAT-1**	2.6.1.1.	B
Creatine kinase	**CK-1***	2.7.3.2	A
Esterase	**EST-1, 2**	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.	B
Glucose dehydrogenase	**GDH-1**	1.1.1.47	B
Glycerol-3-phosphate dehydrogenase	**G3PDH-1***	1.1.1.8	B
Glucose-6-phosphate isomerase	**GPI-1**	5.3.1.9	B
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	A
Malate dehydrogenase	**MDH-1,2**	1.1.1.37	B
Malic enzyme	**ME-1,2**	1.1.1.40	C
Phosphogluconate dehydrogenase	**PGDH-1***	1.1.1.44	B
Phosphoglucomutase	**PGM-1**	5.4.2.2	B
Peptidase:		3.4.-.-	
Substrate: Phenylalanyl-proline	**PEP-D1,2**		A
Leucyl-tyrosine	**PEP-LT1,2**		C
General protein	**PROT-1, 2**		B
Superoxide dismutase	**SOD-1**	1.15.1.1	C

A - a continuous Tris, citric acid (pH 6.9) buffer system (Whitt, 1970)
B - a continuous Tris, boric acid, EDTA buffer system (pH 8.6) (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)
* - Polymorphic loci ($P_{0.95}$)

aquaculturists concerned with artificial production of the species. This study provided the first account of the genetic structure of a population of the river sardine.

Deviations of observed genotype frequencies from expected Hardy-Weinberg proportions at the **CK-1**, **HK-1** and **PGDH-1** coding loci, were associated with a deficit of heterozygotes in each instance. Conformance to Hardy-Weinberg proportions should be an important, but not absolute criterion for inferring the genetic nature of electrophoretic banding patterns (Grant, 1985). Several factors may contribute to Hardy-Weinberg disequilibrium in populations. These include, amongst others, natural selection, small effective population sizes, sampling error, self-sorting crossings, founder effects, the Wahlund effect and even anthropogenic influences. However, knowledge regarding the general biology of the river sardine is too limited to ascribe unequivocally one or more of the above-mentioned factors as being responsible for the present Hardy-Weinberg discrepancies.

Apart from deviations from expected Hardy-Weinberg proportions, estimates of *P* and *H* are also frequently used to assess the genetic structure of populations. The estimate of $P_{0.95}$ = 14.8% compares favourably with estimates obtained by Nevo et al. (1984) for polymorphism in fish in general (P = 15.2%). However, the H_E = 0.025 obtained in the present study falls in the range of values (H = 0.02 to 0.03) which are generally considered as the lower margins of genetic variability for fishes (Nevo et al., 1984; Kirpichnikov, 1992). The low heterozygosity estimates obtained in the present study merit further discussion.

A sufficient amount of genetic variation is essential for the long-term survival and adaptability of populations and species, because this enables these species to respond to adverse environmental and biotic occurences (Leberg, 1990). A reduction in genetic variability may place constraints on the future adaptive potential and hence survival of populations or species. However, the accuracy of genetic variability estimates may be influenced by, amongst others, the number and type of loci studied, sample size, effective population size, degree of migrations between different populations, population bottlenecks and founder events, phyletic age and the variability of the environment (Valentine, 1976;

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 WITH STANDARD ERROR (SE) FOR *M. BREVIANALIS*

Locus	Allele	Frequency	X^2
CK-1	105	0.050	25.282
	100	0.950	
G3PDH-1	105	0.069	
	100	0.931	
GPI-1	105	0.019	
	100	0.981	
HK-1	105	0.053	57.000
	100	0.947	
PGDH-1	105	0.183	26.617
	100	0.817	
$P_{0.95}$		14.8	
H_O		0.011	SE (0.006)
H_E		0.025	SE (0.012)

Kirpichnikov et al., 1990). Graur (1985) recommended that at least 20 loci should be analysed in population genetic studies and a minimum sample size of 50 individuals is required for reliable inferences from electrophoretic data. Thus, the estimates of *P* and *H* obtained may be considered accurate when the above limitations and suggestions are taken into account.

Several natural and anthropogenic factors may also cause a reduction in variability. Grant and Leslie (1993) found that population extinction and recolonisation, i.e. bottlenecks and founder events, through cycles of drought and rainfall, appear to play a larger role in Southern African metapopulation dynamics and genetic variability of vertebrates, than is the situation in vertebrates in the Northern Hemisphere. Periodic bottlenecks and/or founder events as a result of adverse climatic conditions such as droughts, may increase the probability of mating between relatives. This in turn, increases the frequency of homozygous individuals and decreases the frequency of heterozygous individuals relative to that which may be expected from random mating, leading to a reduction in genetic variability (Avise, 1977). It is thus conceivable that inbreeding, which usually results in a deficit of heterozygotes, may have been a contributing factor to the Hardy-Weinberg discrepancies and the low heterozygosity estimates obtained in the present study. It should be noted that similar low estimates of genetic variability have been reported for several Southern African fish species from the same river system (Van Vuuren, 1989; Engelbrecht et al., 1997; Engelbrecht and Mulder, 1999).

In conclusion, despite the low genetic variability observed in the population analysed, the data will nevertheless be of value to conservation authorities as well as aquaculturists who may consider commercial production of the species, whether for human consumption, the aquarium trade or as a fodder species for predatory fishes, i.e. dam management. We recommend that more populations from across the species' distributional range should be analysed to obtain a better understanding of the extent of genetic diversity in the species.

Acknowledgements

We wish to acknowledge Mr Piet Grobler of the Phalaborwa Water Board for his co-operation with the sampling.

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