A preliminary biochemical comparison of two species of the genus *Barbus* from the Vaal River system

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Abstract

A preliminary electrophoretic analysis was performed on two populations of *Barbus aeneus* and *Barbus kimberleyensis* using seven enzyme systems and two electrophoretic techniques. Differences in allelic frequencies and allele mobility were observed in three enzyme systems. This technique indicated a distinct possibility of *B. aeneus* and *B. kimberleyensis* being conspecific, lending for the first time significantly more credibility to the opinion of hybridisation between the two species, shared by a number of authors.

Introduction

The taxonomy of the genus *Barbus* has been the subject of considerable controversy and many species and subspecies have been named (Groenewald, 1958). Even more complex is the situation in the case of hybrids (Gaigher, 1976).

Both *B. kimberleyensis* (Gilchrist and Thompson, 1913) and *B. aeneus* (Burchell, 1822) are endemic to the Orange-Vaal system. The two species are closely related and it is sometimes difficult to distinguish between them by using traditional morphological characteristics only. To complicate matters, the possibility of hybridisation between the two species has been mentioned several times in the literature (Gaigher, 1976; Eccles, 1986).

Taking the effect of hybridisation on the genetic structure of a species into consideration, it is essential to know the genetic diversity of each species in order to identify and to conserve pure, natural and panmictic populations containing the total genetic variation of the species.

A preliminary study of blood and muscle proteins/enzymes using electrophoresis, was initiated to determine the genetic status of each population. This method can also provide useful genetic information about the populations by calculating the degree of in or outbreeding.

Materials and methods

B. kimberleyensis were collected from the Vaal Dam using gill nets and *B. aeneus* from the Barrage using casting nets. Fifty-two specimens of *B. aeneus* and thirty-three specimens of *B. kimberleyensis* were collected over a period of eight months.

Blood (0,6 mℓ) was drawn from the caudal artery of the live fish and diluted with 0,4 mℓ 0,9% saline. After coagulation, the serum was collected. Skeletal muscle was taken from each individual and all samples were stored at -20°C for up to six months until laboratory analysis could be conducted.

Polyacrylamide gel electrophoresis was conducted as described by Avtalion and Wojdani (1971). Serum transferrin and serum esterase phenotypes were determined by using a continuous vertical tris-citrate buffer (Table 1) and 6% gels. Horizontal starch gel electrophoresis was used to detect five muscle proteins (Table 1). The gels consisted of a 13% hydrolysed potato starch (Sigma S-4501).

The following buffer systems were used to achieve maximum resolution of the proteins in the gels:

RW) Gel, (pH 8,5):	tris 0,03 M; citric acid 0,005 M
Tray, (pH 8,1):	lithium hydroxide 0,06 M; boric acid 0,3 M (Ridgeway *et al.*, 1970).
TC) Gel:	1:20 dilution of tray solution.
Tray, (pH 6,1):	citric acid 0,04 M adjusted to pH 6,1 with N (3-aminopropyl) - morpholine (Clayton and Tretiak, 1972).
MF) Gel:	1:4 dilution of tray solution.
Tray, (pH 8,7):	tris 0,18 M; boric acid 0,1 M; EDTA 0,004 M (as described by Markert and Faulhaber, 1965).

Allelic nomenclature as described by Allendorf and Utter (1979) was applied. Multiple loci coding for functionally similar proteins were designated numerically from the cathodic end of the gel. Alleles at each locus were distinguished by their mobilities relative to the mobility of the most common allele.

Statistical procedures

Statistical procedures were conducted as described by Grant *et al.* (1980), Grant *et al.* (1983) and Grant and Leslie (1983). Deviations from the Castle-Hardy-Weinberg proportions at each locus were detected using the log likelihood-ratio test for goodness of fit (Sokal and Rohlf, 1969) with the degrees of freedom equal to the number of phenotypes minus the alleles.

Estimates of average population heterozygosity, H, were computed by averaging h over all loci, including monomorphic loci.

Results and discussion

Electrophoretic variation

The products of ten protein-coding loci were examined. The enzymes, with locus abbreviations and buffer systems producing best results, are presented in Table 1. Only two loci were polymorphic, the others were monomorphic.

Adenylate kinase

A single, monomorphic locus was observed (Table 2). All samples

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TABLE 1
A SUMMARY OF PROTEINS SCREENED IN *B. AENEUS* AND *B. KIMBERLEYENSIS*. IN EACH CASE THE BUFFER SYSTEM WHICH GAVE BEST RESOLUTION AND THE PROBABLE NUMBER OF LOCI CODING FOR EACH PROTEIN ARE GIVEN

Protein	Abbreviation	E.C. No.	Buffer	Number of loci
Adenylate kinase	AK	2.7.4.3	TC	1
Esterase	EST	3.1.1.1	Tris/glycine	1
Lactate dehydrogenase	LDH	1.1.1.27	TC	2
Malic enzyme	ME	1.1.1.40	MF	2
Purine nucleoside phosphorylase	NP	2.4.2.1	RW	1
Superoxide dismutase	SOD	1.15.1.1	MF	1
Transferrin	TF	-	Tris/glycine	1

were homozygous and no difference in migration rate was observed between the two *Barbus* species. This enzyme can therefore not be used to distinguish between them.

Serum esterase

A total of four bands (A, B, C and D) were observed on a single locus for both populations. *B. aeneus*, however, did not show the B-allele, while the C-allele was absent from the population of *B. kimberleyensis*. Heterozygotes of both populations showed different combinations of two bands. It is known that esterase may have a monomeric enzyme structure and these banding patterns indicate such a structure in both *B. aeneus* and *B. kimberleyensis*.

This locus showed a high degree of polymorphism; a shortage of heterozygotes was still observed. The largest shortage was found in the population of *B. kimberleyensis*.

TABLE 2
ALLELIC FREQUENCIES OF ELECTROPHORETIC VARIANTS OF *B. AENEUS* AND *B. KIMBERLEYENSIS*. ALLELES ARE DESIGNATED BY THEIR MOBILITIES RELATIVE TO THE COMMON ALLELE

Locus	Allele	Allelic frequency *B. aeneus* (N=52)	Allelic frequency *B. kimberleyensis* (N=33)
AK	100	1,00	1,00
EST	88	0,212	0,442
	95	0,442	-
	96	-	0,212
	100	0,346	0,346
LDH-1	100	1,00	1,00
LDH-2	100	1,00	1,00
ME-1	100	0,5	0,5
	189	0,5	0,5
ME-2	100	0,5	0,5
	139	0,5	0,5
NP-1	100	1,00	1,00
NP-2	100	1,00	1,00
SOD	92	-	1,00
	100	1,00	-
TF	90	-	0,365
	100	0,394	0,635
	105	0,154	-
	113	0,144	-
	120	0,154	-
	128	0,154	-

Lactate dehydrogenase

Two monomorphic LDH loci were scored in both species. Three interlocus bands between these two loci are very common (Grant *et al.* 1983; Frankel, 1983; McAndrew and Majumdar, 1983) and consist of the subunits of both LDH-A and LDH-B. As described by Kaplan (1964), the three bands represent the $A_1 B_3$, $A_2 B_1$ and $A_3 B_1$ combination of subunits.

Malic enzyme

Two monomorphic loci were scored. ME-1 showed five alleles for all samples; this indicates heterozygotes for this tetrameric enzyme. A natural population consisting of only heterozygotes is very rare and this situation was found only once for malic enzyme in *Salmo trutta* (Allendorf *et al.*, 1977). This species is, however, tetraploid and the explanation for the high number of heterozygotes in the *Barbus* populations may also be found in the tetraploidy, especially since the genus *Barbus* belongs to evolutionary tetraploids (Rab, 1980; Triantaphyllidis *et al.*, 1981; Stratil *et al.*, 1983). The second, more anodal locus had the same configuration and serves to strengthen the above argument.

Purine nucleoside phosphorylase

Two monomorphic loci appeared on the gel after staining. This was unexpected as NP usually has only one locus, although Harris and Hopkinson (1976) mentioned two loci observed in humans. Both species also displayed the same allele and it can therefore not be used as a marker.

Two interlocus products were observed. These zones represented the $A_1 B_2$ and $A_2 B_1$ isozymes.

Superoxide dismutase

A single, monomorphic locus was observed for both *Barbus* populations. All samples were homozygous for the SOD locus, but the alleles of *B. aeneus* had a higher migration rate than those of *B. kimberleyensis*. It appears that SOD can be used as a genetic marker to distinguish between *B. aeneus* and *B. kimberleyensis*.

It is, however, possible that the second homozygote of, for instance, *B. aeneus* has the same migration rate as that of the observed homozygote for *B. kimberleyensis*, and *vice versa*. The migration rate for SOD homozygotes in both species would then be identical and the enzyme would have no use as a genetic marker. It is therefore necessary to study more populations of each species in an attempt to find both homozygotes.

Transferrin

A total of six different alleles were visible on a single locus. *B. aeneus* showed a total of five alleles in different combinations of two. *B. kimberleyensis* had only the E and F bands (Table 2).

The large number of alleles in the population of *B. aeneus* can be attributed to the fact that this is a river population with an accordingly larger genetic variation.

The average heterozygosity (H) appeared to reflect the geographic heterogeneity of the sampling site. The mean H of the river population of *B. aeneus* was 0,266 and that of *B. kimberleyensis*, obtained from a dam, was 0,233 (Table 3). Although it seems that the *B. aeneus* population may have a larger gene pool than the *B. kimberleyensis* population, no definite conclusions may be drawn from this, particularly if the relatively large standard deviations on the average heterozygosity values are taken into consideration.

The higher degree of gene mixing in the river population of *B. aeneus* may, however, give rise to a larger effective gene pool. This would retard the loss of genetic variation due to inbreeding.

Genetic distance

Only serum transferrin, esterase and superoxide dismutase revealed differences between *B. aeneus* and *B. kimberleyensis*. All other enzyme systems examined were monomorphic. This high degree of similarity emphasises the fact that these two species are relatively closely related. A graph drawn up by Thorpe (1982) provides a means of determining qualitative relationships between populations, species, genera and higher taxa. These genetic distances have been determined by using groups of fishes that were originally classified by using traditional morphological methods, and a certain degree of overlap does occur between groups. The genetic distance of 1,027 as determined between *B. aeneus* and *B. kimberleyensis* fall inside such an overlap zone, positioned in such a way as to indicate a small chance of the two populations being congeneric.

TABLE 3
AVERAGE POPULATION HETEROZYGOSITY AND STANDARD DEVIATION OF *B. AENEUS* AND *B. KIMBERLEYENSIS*

Species	Population heterozygosity	Standard dev.
B. aeneus	0,266	±0,108
B. kimberleyensis	0,233	±0,094

Conclusion

This preliminary study thus indicates that enzyme analysis by electrophoresis may provide valuable information on certain aspects of the genetic status of a species, and may provide a means of distinguishing between species and species hybrids. The genetic distance of 1,027 is a definite indication of conspecific status. It is emphasised by te fact that a number of authors have mentioned the possibility of hybridisation between *B. aeneus* and *B. kimberleyensis* (Eccles, 1986; Gaigher, 1976). A more thorough investigation should establish the taxonomic status and genetic diversity of the populations beyond doubt.

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