Allozyme variation in a freshwater mussel population (*Coelatura kunenensis* Mousson, 1887) from Southern Africa

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Abstract

Gene products of 35 protein coding loci in *Coelatura kunenensis* (Mollusca, Lamellibranchiata) were examined by horizontal starch gel electrophoresis. Electrophoretic analysis of enzymatic proteins revealed genetic variation at 12 (34.3%) of the loci studied. Values of 28.57 (0.95 criterion), 1.43 (±0.12) and 0.075 (±0.025) were obtained for the percentage of polymorphic loci, the mean number of alleles per locus and average heterozygosity respectively. Genetic variation compares favourably with values obtained for other species in general, but it is less than previous estimates based on fewer loci for intertidal mollusc and freshwater bivalve species.

Introduction

Coelatura is the valid name for the genus of unionid bivalves previously amended to *Caelatura* (Rosenberg et al., 1990), and although many Southern African species of the family Unionidae have been described, only four are recognised (Appleton, 1979). These are *Unio caffer, C. framesi, C. kunenensis* and *C. mossambicensis*. *C. kunenensis* is a western species confined to the Kunene, Okavango and Upper Zambezi River systems, and found in lentic and often seasonally inundated areas. Appleton (1979) was able to identify functional females (with marsupia and embryos), suggesting a dioecious mode of reproduction.

Despite the above-mentioned reports, no information is available on genetic variation or other aspects regarding the biology of this species. Mussels are, however, suitable for monitoring heavy metal pollution (Balogh, 1988; Pynnoenen, 1990), trace element pollution (Baudo and Galanti, 1988), the effect of pH on electrolyte balance in hemolymph in hard and soft water (Pynnoenen, 1991), tolerance against insecticides (Varanka, 1987), the relationship between fluctuations in environmental factors and meat to shell ratio (Blay, 1990), and DNA-adduct measurements as biomarkers in the assessment of both the biologically relevant exposure to carcinogens and the pathological consequences of such exposure (Kurelec et al., 1989).

Some freshwater mussel species are threatened with extinction due to indiscriminate use of insecticides, pollution and/or deterioration in the quality of mussel habitats by eutrophication through agricultural runoff (Hochwald and Bauer, 1988). Mussels are known for their nutritional value and the feasibility of application of protein enrichment by electropolarity treatment in mussel culture had a positive effect on protein content (Mani et al., 1988). Not only are mussels an excellent source of protein for human consumption and suitable as indicator species for pollution monitoring, but other species also rely on freshwater mussels to survive. For example, Nystroem and Pehrsson (1988) found that small individuals may be selected against since mussel-feeding diving ducks prefer them to larger mussels. The European bitterling, *Rhodeus amarus*, also practices rather unusual methods of spawning by using the favourable conditions in the gills of living freshwater mussels for the embryonic development of its fry (Heschl, 1989). This dependency of some species on mussels may also explain why a single emphemeropteran nymph and small leeches were found in the pallial cavities of 16.3% of the *C. kunenensis* (Appleton, 1979). In addition, the dissection of catfish showed the presence of freshwater mussels in their alimentary tracts (personal observations). As a result of the above-mentioned findings and applications for freshwater bivalves, there is great potential for their artificial culture. Mussel culture would also be beneficial for conservation stocking purposes. Successful rearing of juvenile parasitic freshwater mussels (Unionidae) is possible (Hudson and Isom, 1984) and *in vitro* culture and rearing of mussels involve only nominal cost (Isom, 1987).

The success of conservation efforts can be enhanced by knowing the genetic structure of the species in question and since the artificial culture of freshwater mussels is possible, the apparent importance of such fundamental and applied data (e.g. to be used in genetic selection programmes) has become evident. This study therefore aims to provide information on the allozyme diversity of wild *C. kunenensis*. Variability in nuclear DNA, as studied with allozyme electrophoresis, was chosen as a suitable biochemical technique based on recommendations by Grant and Leslie (1993). These authors suggested that it be used in preference to the analysis of uniparentally inherited organellar DNA (such as mitochondrial DNA) because species from Southern Africa showed little or no variation when the latter method was used compared to high levels of variability with allozyme electrophoresis.

Material and methods

Fifty *C. kunenensis* were obtained from the Upper Zambezi River system near Katima Mulilo, Namibia (24°25'S, 17°29'E). Samples were stored in liquid nitrogen and transported to the laboratory. Total body extracts were prepared and analysed by starch gel electrophoresis (12% gels), using the electrophoretic procedures, buffers, method of interpretation of gel banding patterns and locus nomenclature referred to by Van der Bank et al. (1992). Loci were numbered beginning at the anodal end of the gel, and cathodally migrating allozymes were designated by a minus sign. Statistical analysis of allozyme data was done using **BIOSYS-1** (Swofford and Selander, 1981).

It is important to note that distilled water or buffer solutions should not be added to samples prior to homogenation since

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TABLE 1
LOCUS ABBREVIATIONS, ENZYME COMMISSION NUMBERS (E.C. NO.), TISSUES AND BUFFERS GIVING THE BEST RESULTS ARE LISTED AFTER EACH PROTEIN

Protein	Locus	E.C. No.	Buffer
Adenylate kinase	(**AK-1, -2**)	2.7.4.3	TC
Creatine kinase	(**CK-1, -3**)	2.7.3.2	RW
	*(**CK-2**)		MF
Esterase	(**EST-1, -2, -3, -5**)	3.1.1.-	RW
	*(**EST-4**)		RW
Guanine deaminase	(**GDA**)	3.5.4.3	MF
Glycerol-3-phosphate dehydrogenase	(**GPD-1, -2**)	1.1.1.8	TC
Glucose-6-phosphate isomerase	*(**GPI-1**)	3.5.1.9	RW
	(**GPI-2**)		RW
Hexokinase	*(**HK**)	2.7.1.1	MF
Isocitrate dehydrogenase	(**IDH**)	1.1.1.42	TC
Malate dehydrogenase	*(**MDH-1**)	1.1.1.37	RW
	(**MDH-2**)		RW
Malic enzyme	(**ME-1, -2**)	1.1.1.38	RW
Mannose-6-phosphate isomerase	(**MPI**)	5.3.1.8	MF
Peptidase:		3.4.-.-	
Substrate: Leucyl-tyrosine	*(**PEP-C,1,**		MF
	-C,2, -C,3)		MF
	(**PEP-C,4**)		MF
6-Phosphogluconate dehydrogenase	*(**PGD**)	1.1.1.44	TC
Phosphoglucomutase	(**PGM**)	5.4.2.2	RW
Purine-nucleoside	(**NP-1**)	2.4.2.1	MF
phosphorylase	*(**NP-2**)		MF
General protein	(**PROT-1, -3**)		RW
	*(**PROT-2**)		RW
Superoxide dismutase	*(**SOD-1**)	1.15.1.1	MF
	(**SOD-2**)		MF

* = Polymorphic loci
MF = a continuous tris, boric acid, EDTA buffer (pH 8.6) described by Markert and Faulhaber (1965).
RW = a discontinuous tris, citric acid, (gel pH 8.7), lithium hydroxide, boric acid (electrode pH 8.0) buffer system (Ridgway et al., 1970).
TC = a continuous tris, citric acid (pH 6.9) buffer system (Whitt, 1970).

mussels retain a lot of fluid. In some instances it was necessary to freeze-dry samples to concentrate enzymes.

Results

Thirty-five protein coding loci provided interpretable results, of which 34.3% displayed polymorphism. Locus abbreviations, enzyme commission numbers, tissues and buffers giving the best results are listed in Table 1. Twenty-three of the 35 loci displayed monomorphic gel banding patterns (Table 1). Products of the **AK-2** and **EST-5** protein coding loci migrated cathodally. In addition to these loci, I stained for alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, **PEP-A** (using glycyl-L-leucine as substrate), L-iditol dehydrogenase, L-lactate dehydrogenase, **PEP-B** (using L-leucylglycylglycine as substrate) and **PEP-D** (using phenylalanine-proline as substrate), which, together with **MDH-3** and **ME-3**, did not show sufficient activity or resolution to score it satisfactorily. Relative allele frequencies for polymorphic loci, sample size, observed and expected numbers of heterozygotes, coefficients for heterozygote deficiency or excess, X^2 values, and individual heterozygosity values per locus are presented in Table 2. Allozyme phenotypes of putative heterozygotes at loci were congruent with those expected on the basis of the quaternary structure of the enzyme (Ward, 1977). Thus heterozygotes were four-banded for the trimeric enzyme **NP** and triple-banded, as expected for dimeric enzymes at **CK, GPI, MDH, PGD** and **SOD**. Heterozygotes at the monomeric enzymes **EST, HK, PEP-C** and **PROT** were double-banded. No heterozygotes were detected at the **CK-2** locus (only alternate homozygotes). The number of alleles for the **MDH-1** locus was four and three for **PROT-2** whereas two alleles were present at all of the other polymorphic protein coding loci studied.

Genotypic frequencies at three loci (**GPI-1, PEP-C,3** and **PGD**) closely approximated Hardy-Weinberg expectations (Table 2). A deficiency of heterozygotes occurred at the other polymorphic loci. Polymorphic loci had an average of 1.43 (±0.12)

TABLE 2
SAMPLE SIZE, RELATIVE MOBILITY (RM) OF ALLELES, RELATIVE ALLELE FREQUENCIES OF POLYMORPHIC LOCI, OBSERVED (OBS) AND EXPECTED (EXP) NUMBER OF HETEROZYGOTES, COEFFICIENTS FOR HETEROZYGOSITY DEFICIENT (D) OR EXCESS, X^2 VALUES WITH DEGREES OF FREEDOM (DF) AND INDIVIDUAL HETEROZYGOSITIES (H)

Locus	2N	RM	Frequency	OBS	EXP	D	X^2	DF	h
CK-2	100	100	0.920	0	3.68	-1.00	25.00*	1	0.147
		110	0.080						
EST-4	100	0	0.410	3	24.19	-0.88	38.37*	1	0.484
		100	0.590						
GPI-1	100	100	0.450	25	24.75	0.01	0.01	1	0.495
		110	0.550						
HK	50	100	0.890	1	9.79	-0.90	40.31*	1	0.196
		110	0.110						
MDH-1	100	80	0.030	4	6.66	-0.40	100.01*	6	0.133
		90	0.930						
		100	0.030						
		110	0.010						
PEP-C1	100	90	0.050	3	4.75	-0.37	6.79*	1	0.095
		100	0.950						
PEP-C2	100	90	0.851	3	9.37	-0.68	17.09*	1	0.253
		100	0.149						
PEP-C3	20	90	0.020	2	1.96	0.02	0.02	1	0.039
		100	0.980						
PGD	70	0	0.010	1	0.99	0.01	0.01	1	0.020
		100	0.990						
NP-2	78	90	0.064	1	4.68	-0.79	24.11*	1	0.120
		100	0.936						
PROT-2	88	80	0.023	12	22.41	-0.47	12.77*	3	0.509
		90	0.409						
		100	0.568						
SOD-1	100	80	0.080	2	7.36	-0.73	26.52*	1	0.147
		100	0.920						

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

alleles per locus, whereas individual heterozygosities ranged from 0.020 (**PGD**) to 0.509 (**PROT-2**), and averaged 0.075 over all 35 loci, with a standard error of 0.025. The percentage of polymorphic loci (0.95 criterion) was 28.57.

Discussion

It may be possible to select for *C. kunenensis* individuals with increased growth rate since genetic variation was obtained at the **GPI** protein coding locus (Table 2), which proved to be an excellent biochemical genetic marker associated with rapid growth in the catfish *Clarias gariepinus* (Van der Walt et al., 1993). *C. kunenensis* is a moderately large (62.5 x 31.9 mm) species (Appleton, 1979) and therefore a suitable size for aquacultural purposes. Some mussel species showed a high fecundity (>30 000 eggs per female) and can reproduce with peak densities in excess of 200 individuals m^{-2} (Hebert et al., 1989). Furthermore, the results obtained by Garton and Haag (1991) indicated that mussel length was significantly correlated with multiple locus heterozygosity, since heterozygotes grew faster than homozygotes. In addition, Baudo and Galanti (1988) found that larger (older) molluscs contained higher concentrations of metal and Buhse (1989) reported that the tolerance of freshwater mussels to salts manifests itself according to the age and the species of individuals. Peculiarities among populations were also observed concerning variability in body size and shape of shells and also the number of released glochdia and differences in the preference of host fish (Engel and Waechtler, 1989). These results all support the view that genetic selection might be possible in *C. kunenensis* and should be attempted to increase the size of individual freshwater bivalves, in culture.

Genotypic frequencies deviated from expected Hardy-Weinberg proportions at the **CK-2, EST-4, HK, MDH-1, PEP-C,1, -2, NP-2, PROT-2** and **SOD-1** loci (Table 2). High levels of significance at some variable loci were due to an excess of homozygotes and a deficiency of heterozygotes. It is evident that scarce and rare alleles are present at these loci. Various factors can, however, shift the equilibrium and disrupt the stability of a population, giving rise to change in the genetic structure, as ideal Hardy-Weinberg populations do not actually occur in nature (Altukhov, 1981). For instance, small sample sizes and recurrent bottlenecks involving founder populations with little variability could account for deviations at loci. The latter hypothesis is supported by the incidence of recurrent severe droughts over the past 22 years in Southern Africa, which undoubtedly produced numerous population bottlenecks. It is also true that certain factors (e.g. natural selection) can result in the production of a larger number of progeny by some genotypes than by others (Altukhov, 1981; Kirpichnikov, 1981). Other factors include non-random mating and selfsorting crossings and linking (Allendorf and Utter, 1979). The effects that such factors could have had on *C. kunenensis* are unknown since very little is known about their biology.

Grant et al. (1984) studied gene products of 19 protein coding loci in intertidal mussels over four locations and obtained an average heterozygosity (***H***) of 0.27. However, these authors noted that this estimate may be high because variable substrate enzymes such as the peptidases were included in their study, which tend to be more polymorphic than glucose-metabolising or Krebs cycle enzymes (Johnson, 1974). Hebert et al. (1989) obtained polymorphism at 73.9% of 23 loci examined in a freshwater bivalve, *Dreissena polymorpha*. Individual heterozygosity averaged 31.6%. This high level of genotypic variation was attributed to the population being founded from a substantial number of individuals which did not undergo a bottleneck subsequent to founding. Garton and Haag (1991) obtained an ***H*** value of 0.307 and an average of 3.43 alleles per locus in a recently established freshwater bivalve species. Allozyme variation at 16 enzyme coding loci in three species of freshwater snails was studied by Chung (1984), who obtained allelic variation at seven of these loci, with ***H*** ranging from 6 to 19%, and Ahmad et al. (1977) obtained an ***H*** value of 9.5% in a natural population of *Mytilus*. The latter authors examined 29 protein coding loci. From the above information, it is evident that lower estimates of **H** were achieved when natural populations (compared to recently established populations founded from a substantial number of individuals) and a large number of loci were studied. The relatively low value of heterozygosity (7.5%) obtained for *C. kunenensis* in the present study may also be explained because different species are compared, using different sample sizes, and the choice of loci (which may have differences in heterozygosities) also varied. Lewontin (1974) reviewed the effects of such factors on genetic variation. The estimate of ***H*** obtained in the present study is also congruent with those found for other species (Powell, 1975; Nevo, 1979; Avise and Aquadro, 1982; Mork et al., 1982; Van der Bank et al., 1992; Grant and Leslie, 1993; Ward et al., 1994).

As far as could be ascertained, this is the first account of electrophoretic variants of this economically potentially important species. Furthermore, no information was available on freshwater mussel species from the Southern Hemisphere and comparisons were made to species from the Northern Hemisphere. The results of the present study indicate that the species examined possesses less genetic variation than most other bivalves, but still has sufficient variation to allow it to adapt to environmental changes. The isozymes described in this study provide a good basis for estimating the amount and pattern or distribution of genetic variation within this species; they describe the biological characteristics of the species (i.e. to assess the impact of recurrent bottlenecks, natural selection and possibly non-random mating on current genetic variability), and the enzymatic polymorphism also revealed interesting data for both fundamental and applied research (especially when the artificial production of mussels for aquaculture is considered). Even though little is known regarding the biology of this species, it is conceivable that this is a slow-growing and long-lived species. They may, however, breed when approximately one year old, compared to 3 to 4 years for larger freshwater mussel species (e.g. from the genus *Aspatharia*). The above inferences were made from reports for the congeneric species, *C. mossambicensis* (Appleton et al., 1987).

The data obtained in the present study offer the hitherto unavailable opportunity to improve freshwater bivalves genetically and to breed them selectively. The potential use of these organisms to relieve the ever-increasing demand for protein for human consumption world-wide, the fact that other organisms such as catfish use it as a source of nutrition (personal observations), and the vast range of other applications (e.g. conservation stocking purposes, pollution monitoring, etc., as mentioned in the introduction) indicate the importance of the results obtained in the present study. In addition, these results will undoubtedly aid in improving the systematics and resolving the phylogeny of freshwater mussels in general, especially when genetic differentiation among populations and species is compared.

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