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CORRESPONDENCE BETWEEN THE BUFFER SYSTEMS SUITABLE FOR ELECTROPHORETIC RESOLUTION OF BIVALVE AND GASTROPOD ISOZYMES

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Abstract—1. A marine bivalve (*Mercenaria mercenaria*) and a freshwater gastropod (*Goniobasis proxima*) were compared by examining the suitability of five buffer systems for electrophoretic resolution of 28 enzymes. Results were scored as 'good', 'poor', or 'no activity'.

2. Bivalve and gastropod results corresponded in 65.9% of the cases, not a strikingly strong relationship but significantly greater than random expectation.

INTRODUCTION

Selection of a suitable buffer system for electrophoretic separation of isozymes is generally a trial-and-error process that must be repeated for every new enzyme and each new species studied. Typically a researcher will make a selection from the scores of buffers that have been developed for these purposes (e.g. Brewer, 1970; Shaw and Prasad, 1970) and then experiment to see whether the enzyme under examination retains its activity and migrates in a clear, discrete band or bands. This process is then repeated with other buffers until satisfactory results are obtained.

A great deal of research has compared populations and species with regard to the number of loci coding for particular enzymes, the structure and function of these enzymes, and the frequencies of the genes coding for any isozymes present. However, I am unaware of any previously published research comparing species by the buffer systems suitable for the resolution of isozyme bands.

Both of the animals selected for comparison here have been the objects of considerable protein electrophoretic study. *Goniobasis proxima* (Say) is a freshwater prosobranch snail widespread in small, upland streams from Virginia to Georgia. A number of unusual biological attributes make *Goniobasis* an excellent subject for population genetic studies (Chambers, 1978, 1980; Dillon and Davis, 1980; Dillon, 1982, 1984). By contrast, genetic research involving the marine hard clam, or quahog, *Mercenaria mercenaria* (L.), has focused on more commercial concerns. Protein electrophoretic studies have been performed by Pesch (1972, 1974), Humphrey (1982), and Adamkewicz *et al.* (1984a,b).

As members of the same phylum, *Mercenaria* and *Goniobasis* would be expected to share broad biological similarities. But clearly a marine clam and a freshwater snail are quite different organisms living in quite different environments. Further, it should be remembered that gastropods and bivalves had already diverged from primitive molluscan stock by the Cambrian Period. Thus it is not clear what sort of similarity is to be expected in *Mercenaria* and *Goniobasis* enzymes or the chemical conditions necessary to analyse them. An investigation into this question is the subject of this report.

METHODS

Goniobasis proxima were collected from Cripple Creek, a tributary of the New River in Wythe County, Virginia (station CRIP of Dillon, 1984). The shell was cracked and the animal frozen whole at −70°C in 0.05 M Tris (hydroxymethyl) aminomethane ('Tris') buffer adjusted to pH 7.4 with H_3PO_4. *Mercenaria mercenaria* were collected from the Folly River near Charleston, South Carolina. Shells were cracked and tissue samples taken from both viscera and foot muscle. These samples were also frozen at −70°C in the Tris tissue buffer described above. Freshly killed animals were analysed as available during the course of the study. In no case was the loss of enzymatic activity in the frozen samples significant.

For electrophoresis, tissue samples were ground with a glass rod, centrifuged, and soaked in tabs of Whatman No. 3 filter paper. These tabs were blotted and applied to a freshly cut edge of the gel. A minimum of four different individual snails and eight different clams were analysed per electrophoretic run. Viscera and foot tissues were compared for at least two clams. Gels were composed of 14% hydrolysed starch, a mixture of three parts Sigma starch to one part Electrostarch. A description of the apparatus employed and detailed electrophoretic methods can be found in Dillon and Davis (1980) and Dillon (1982).

The five buffer systems selected for this investigation were as follows. AP6—The electrode buffer was 0.04 M citric acid (monohydrate) adjusted to pH 6.0 with *N*-(3-aminopropyl)-morpholine. The gel buffer was a 19:1 dilution of the electrode buffer. TC6—The electrode buffer was 0.237 M Tris and 0.085 M citric acid (monohydrate), pH 6.0. The gel buffer was a 3.5% solution of the electrode buffer. TME7—The electrode buffer was 0.10 M Tris, 0.10 M maleic acid, 0.01 M disodium ethylenediaminetetraacetic acid (EDTA), and 0.01 M $MgCl_2 \cdot 6H_2O$, adjusted to pH 7.4 with NaOH. The gel buffer was a 9:1 dilution of the electrode buffer. TEB8—The electrode buffer was 0.50 M Tris, 0.645 M boric acid, and 0.0179 M EDTA, pH 8.0. The gel buffer was 0.05 M Tris, 0.097 M boric acid, and 0.0018 M EDTA, pH 8.0. POUL—Poulik's (1957) discontinuous buffer system, electrode buffer 0.30 M boric acid and 0.05 M NaOH, pH 8.0. The gel buffer was 0.076 M Tris and 0.005 M citric acid (monohydrate), pH 8.8.

Gels were sliced and stained for one of the following enzymes (Enzyme Commission number in parentheses): ADH—alcohol dehydrogenase (1.1.1.1), GLOD—glycerol-3-phosphate dehydrogenase (1.1.1.8), SDH—sorbitol dehydrogenase (1.1.1.14), LDH—lactate dehydrogenase (1.1.1.27), MDH—malate dehydrogenase (1.1.1.37), ME—malic enzyme (1.1.1.40), ICDH—isocitrate dehydrogenase (1.1.1.42), PGD—phosphogluconate dehydrogenase (decarboxylating 1.1.1.44), G6PD—glucose-6-phosphate dehydrogenase (1.1.1.49), GLYD—glyceraldehyde-phosphate dehydrogenase (1.2.1.12), XDH—xanthine dehydrogenase (1.2.1.37), AO—aldehyde oxidase (1.2.3.1), GLUD—L-glutamate dehydrogenase (1.4.1.3), OPDH—octopine dehydrogenase (1.5.1.11), SOD—superoxide dismutase (1.15.1.1), AAT—aspartate aminotransferase (2.6.1.1), HK—hexokinase (2.7.1.1), AK—adenylate kinase (2.7.4.3), PGM—phosphoglucomutase (2.7.5.1), EST—arylesterases (3.1.1.2), ALP—alkaline phosphatases (3.1.3.1), ACP—acid phosphatase (3.1.3.2), LAP—leucine aminopeptidase (3.4.1.1), PEP—peptidases (3.4.1), ALD—fructose-biphosphate aldolase (4.1.2.13), FH—fumarate hydratase (4.2.1.2), MPI—mannose phosphate isomerase (5.3.1.8), GPI—glucose phosphate isomerase (5.3.1.9).

Enzyme staining methods were taken from various sources (Shaw and Prasad, 1970; Bush and Huettel, 1972; Schaal and Anderson, 1974; Harris and Hopkinson, 1976). Many of the recipes were modified, particularly for the use of agar overlay. Details of the staining procedures are given in Dillon and Davis (1980) and Dillon (1982).

Gels were scored using the following criteria: if the enzyme activity was resolved into discrete bands, the buffer system was considered 'good'. If enzymatic activity was present on the gel, but in the form of streaks, irregular patches, etc. the buffer system was considered 'poor'. If no enzymatic activity was apparent on the gel at all, the buffer system was scored 'no activity'. Occasionally cases were found where several loci apparently coded for different classes of isozyme bands, and particular buffers were suitable to resolve only a subset of these bands. Then if results were good for any class of isozyme bands on the gel, the buffer was considered 'good'.

RESULTS

Overall results are presented in Table 1. Notes regarding individual enzyme sytems follow.

ADH and GLOD—Two loci seem to encode ADH in both *Mercenaria* and *Goniobasis*, and identical results were obtained with either ethanol or hexanol as substrate. One of the ADH loci also seems to account for the GLOD activity observed, although the buffers suitable for isozyme resolution differ considerably when glycerol-3-phosphate serves as substrate.

LDH—Pesch (1972) succeeded in resolving *Mercenaria* LDH clearly using polyacrylamide gel electrophoresis with a Tris–HCl pH 8.9 gel buffer and a Tris–glycine pH 8.3 buffer in the electrode vessel. Attempts to reproduce these results using starch gels were unsuccessful.

MDH—Several zones of activity are present in both *Mercenaria* and *Goniobasis*, some of which were poorly resolved in all buffer systems examined and others of which formed clear bands on all gels.

ME—Only one locus apparently codes for this enzyme in *Goniobasis*. A second locus seems to be present in *Mercenaria*, but the second isozyme was found only in viscera, never in foot muscle.

OPDH—No activity for this enzyme was demonstrated in *Mercenaria* tissues, although it has been found in a number of other marine bivalves (Storey and Dando, 1982). But since conversion of pyruvate to octopine is but one of many alternative terminal steps for anaerobic glycolysis, it seems possible that OPDH may be completely absent from *Mercenaria*.

AK—No additional isozyme bands appeared in assays for creatine kinase (EC 2.7.3.2) in either *Mercenaria* or *Goniobasis*.

EST—As would be expected, many bands of activity can be resolved in both *Mercenaria* and *Goniobasis*, undoubtably the products of a number of loci. Buffer suitability was identical regardless of whether α-napthyl acetate or α-napthyl propionate was provided as substrate, although some isozymes seem to show some specificity. EST activity is very low and difficult to resolve in *Mercenaria* foot tissue.

LAP, PEP—As usual, a large number of loci seem to encode peptidases in both *Mercenaria* and *Goniobasis*. The locus coding for isozymes with strong activity when L-leucine β-napthylamide is the available substrate (LAP) has received particular attention in the literature (Koehn *et al.*, 1976; Adamkewicz *et al.*, 1984a,b) and is separated in Table 1. In *Mercenaria*, but not *Goniobasis*, these isozymes are also among the many that show activity when L-leucyl-L-tyrosine or L-leucylglycylglycine is provided as the substrate. Results from the latter two gels are combined in the column labeled 'PEP'. Activity for many of these isozymes was reduced or absent in *Mercenaria* foot tissue.

Table 1. Suitability* of five buffer systems for resolving bands of enzymatic activity in *Mercenaria* and *Goniobasis*

	Enzymes												
Buffer	ADH	GLOD	SDH	LDH	MDH	ME	ICDH	PGD	G6PD	GLYD	XDH	AO	GLUD	OPDH
AP6	na/g	g	na	p	g	na	p	g	p	g	na	g/na	na	na/g
TC6	na/g	p	na	na	g	na	g	p	p	p	na	p/na	na/p	na/g
TME7	g	g	g	p/na	g	g	g	p/g	p	g	g/na	p/na	g/na	na/g
TEB8	g	na	na	p	g/p	na	p/g	p/g	p	na	g	na	g/na	na/g
POUL	g	g/na	na/g	p/g	g/p	g	na	p/na	p/g	g/na	g/na	g	g/p	na/g

Continued -														
	SOD	AAT	HK	AK	PGM	EST	ALP	ACP	LAP	PEP	ALD	FH	MPI	GPI
AP6	g	g	p	p	g	p/g	-	p	g/p	g/na	na	p	g	g
TC6	na	g	p	g	g	p/g	-	p	g/p	g	na	g/p	g	g
TME7	g	g/p	p	g	g	p/g	p	p	p/g	g	na/p	g/p	p/g	g
TEB8	g	g/p	na	p	g/p	p/g	p	-	g	g	na/p	g/p	g	g
POUL	g	na	p	p	g	g	na/g	-	p/g	g	na	p/g	p/g	g

*g = good, p = poor, na = no activity. If results for the mollusk species differed, the *Goniobasis* results follow the *Mercenaria* results and a slash.

ALD—No activity for this enzyme was demonstrated in *Mercenaria* tissues under any conditions, but it seems unlikely that aldolase could be completely absent.

GPI—Although the enzyme activity resolved into discrete bands using TME7 buffer, there was no difference in the mobility of the *Mercenaria* and *Goniobasis* isozymes. Since clear differences were seen in the mobility of the bands in every other buffer system, TME7 would seem to be a poor choice.

DISCUSSION

Since no ALD or OPDH activity was demonstrated in *Mercenaria* regardless of gel conditions, it would seem advisable to exclude the results of these two assays from further discussion. Then of the 126 observations remaining in Table 1, *Mercenaria* and *Goniobasis* showed identical results 83 times, or 65.9%. That is, there are 83 cells in Table 1 where both species are 'good', both are 'poor' or both are 'no activity'. This degree of similarity is not striking, but it is clearly not a random assortment of two variables, each with three character states.

For *Mercenaria* there were 59 'good' results, 44 'poor' results, and 23 gels with no activity apparent. For *Goniobasis*, these figures were 61, 36, and 29, respectively. If these two variables were independent, the probability of a match between them would be:

$$P_M = \frac{N_{GC}N_{GS} + N_{PC}N_{PS} + N_{NC}N_{NS}}{N_T^2},$$

where N_{GC}, N_{PC}, and N_{NC} are the numbers of good gels, poor gels, and 'no activity' gels for *Mercenaria* ('clams') and N_{GS}, N_{PS}, and N_{NS} take the analogous values for *Goniobasis* ('snails'). N_T is the total number of observations, or 126.

Thus if there were no relationship between the suitability of a particular buffer for *Mercenaria* and *Goniobasis*, the probability of a match between them would be only 0.368, a little more than half the observed frequency. The value of chi-square from a goodness-of-fit test to the observed frequency of 0.659 was 45.7, which with one degree of freedom is significant at much better than the 0.001 level.

Pooling the numbers of 'good', 'poor', and 'no activity' observations across both *Goniobasis* and *Mercenaria* to obtain an overall estimate of the probability of the three states has very little effect on this conclusion. Interestingly, the two distributions (59/44/23 and 61/36/29) are not significantly different. Thus the revised value of P_M is still 0.371, much lower than observed. So in summary, although considerable difference exists in the electrophoretic conditions in the electrophoretic conditions suitable to resolve isozyme bands in freshwater snails and marine clams, a relationship is still apparent.

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