



AUSTRALIAN INSTITUTE  
OF MARINE SCIENCE

**Methods for allozyme  
electrophoresis of the top  
snails *Trochus* and *Tectus*  
(Prosobranchia: Trochidae)**

**Philippe Borsa and John A.H. Benzie**

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## INTRODUCTION

Allozyme electrophoresis provides data (simultaneous phenotype for multiple enzymes in large numbers of individuals), that can directly be interpreted in genetic terms. These data have proven useful in studies of population genetics, phylogeny and systematics in a wide range of organisms.

Details concerning preliminary screening for enzyme activity, selection of enzymes, choice of buffers, and description of zymograms no longer appear in papers of population genetics using allozyme electrophoresis. This information is however highly desirable because it varies from one group to another: it saves time and resources in studies of a species for which the electrophoretic techniques have already been refined. Such data help to assess the quality of the genetic interpretation of allozyme patterns, which is the basis of subsequent genetic data processing. It is also useful, in the event further developments are attempted, to know the methods tried previously which did not work and which are rarely reported.

The information presented in this report is a record of the development of genetic markers for comparative studies on populations and species of the top snails *Trochus* and *Tectus* (Prosobranchia, Trochidae).



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## MATERIALS AND METHODS

### Sample collection and processing

Samples of *Trochus niloticus*, *T. maculatus*, *T. conus* and *Tectus pyramis* (plus, among these, two species subsequently revealed by electrophoresis and referred to as *Trochus (conus) sp.*, and *Tectus (pyramis) sp.*) were collected by SCUBA diving on various reefs of the Northern, Central, and Southern sections of the Great Barrier Reef. The reef crest's turf zone was the preferred habitat of *T. niloticus* and *Tectus sp.* The other species were more frequent in less exposed parts of the reef.

Catch per unit of effort of the abundant *Trochus niloticus* varied between 1.00 individual per minute per diver (in some pristine Southern reefs) and 0.03 ind. min<sup>-1</sup>.diver<sup>-1</sup> (e.g. in heavily harvested reefs, e.g. Bowden Reef).

Animals were kept alive on board ship in running seawater, until they were dissected. The shell was crushed with a hammer in such a way that columellar muscle and digestive gland and gonad were preserved intact. Shells were first hit sharply on the side, to expose the animal. Soft parts could also be removed by drilling a hole near the apex of the conch and flushing the tissues out with water. This allowed the shell to be preserved for later morphological observations.

Cubes of approximately 0.1 cm<sup>3</sup> were dissected from the tender part of the columellar muscle and from the digestive gland, put into small polypropylene tubes, and immediately frozen in liquid nitrogen. They were then stored at -80°C until analysis.

Before each electrophoretic run, tissues were homogenized in a 0.04% solution of β-mercaptoethanol, slightly coloured with Bromophenol Blue. Muscle tissue was homogenized with an equal volume of grinding solution in a ceramic dish using a stainless steel pestle and crushed glass. When a paste of even colour and texture was obtained, this was centrifuged at 7000 g for 5 minutes and the supernatant used for electrophoresis. Digestive gland tissue was homogenized with an equal volume of grinding solution by squeezing it into a hole in a perspex plate.



## Electrophoresis

Electrophoresis was performed according to standard horizontal starch gel methods as described in Pasteur *et al.* (1988), and cellulose acetate gel methods as described in Richardson *et al.* (1986). Starch gels were made of 13% Sigma starch in buffer (12% when buffer TEB was employed). Cellulose acetate gels (Cellogel™) were purchased from Chemetron, Milan, Italy. Recipes of the different electrode and gel buffers used for starch and cellogel are given in Table 1. Those for starch were adapted from Boyer, Fainer & Watson-Williams (1963), Shaw & Prasad (1970) and Soltis *et al.* (1983). Those for cellogel were from Richardson *et al.* (1986).

## Stain recipes

Staining followed Richardson *et al.* (1986) for all enzymes except AAT and EST-D for which the stain recipes were those of Pasteur *et al.* (1988).

**Table 1.** Details of buffer systems used with starch gels (12% Sigma starch) or cellulose acetate gels (Cellogel™, Chemetron, Milan) in a preliminary screening for enzyme activity in *Trochus niloticus*.

Buffer	pH	Composition (electrodes)	(gel)
<b>Starch</b>			
TC	8.0	172 mM Tris 39 mM citric acid	1/6 <sup>(*)</sup> electrode concentration
TEB	8.4	150 mM Tris 3 mM EDTA 117 mM boric acid	1/3 electrode concentration
TEC	7.9	135 mM Tris 4 mM EDTA 32 mM citric acid	1/6 electrode concentration
TM	7.4	100 mM Tris 100 mM maleic acid 10 mM EDTA 1 mM MgCl <sub>2</sub> 12.5 mM NaOH	1/10 electrode concentration
<b>Cellogel</b>			
CP	6.4	10 mM Na <sub>2</sub> HPO <sub>4</sub> 2.5 mM citric acid	-
Phos	7.0	11.6 mM Na <sub>2</sub> HPO <sub>4</sub> 8.4 mM NaH <sub>2</sub> PO <sub>4</sub>	-
TM	7.8	50 mM Tris 20 mM maleic acid	-

(\*) or 1/12 for TC 8.0 [1/2]

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## RESULTS

### Activity

Thirty-eight enzymes were tested for activity in *Trochus niloticus*. These included: (1) all enzymes that have been reported active in other allozyme surveys in molluscs and (2) some enzymes commonly reported active in other groups.

Both muscle and digestive gland tissues from the same 4-10 individuals were tested for each enzyme, together on the same gels. Up to 4 buffer systems in starch and 3 buffer systems in cellogel (Table 1) were tested for each enzyme.

Results of this preliminary step are reported in Table 2. Details of activity according to tissue and buffer for enzymes that showed some activity in this preliminary step are reported in Table 3.

### Polymorphism

Seventeen enzymes were selected on the basis of their activity and resolution for the next step of the study: screening for polymorphism. This was done using the combinations of tissue and buffer that had given the best results in earlier tests. An average number of 60 individuals were tested, chosen in similar proportions from the Northern, Central, and Southern sections of the Great Barrier Reef (Northern: Evening Reef and Low Islets; Central: Davies Reef; Southern: Hunt Reef and Little Chauvel Reef). Results are reported in Table 4.

#### 4 Results

**Table 2.** Enzymes tested for activity in *Trochus niloticus* from the Great Barrier Reef, with abbreviation (Abbr.) and Enzyme Commission number (E.C.#). o Buffer tested. Tissues assayed: M muscle; DG digestive gland. Details of the buffers used are given in Table 1.

Enzyme	Abbr.	E.C.#	Buffer (starch)				Buffer (cellogel)			Activity(*) in:		
			TC8	TEB	TM	TEC	CP	Phos	TM	M	DG	
Aspartate aminotransferase	AAT	2.6.1.1	o	o	o			o	o	o	-	-
Acid phosphatase	ACP	3.1.3.2	o	o	o						+	+
Adenosine deaminase	ADA	3.5.4.4						o	o	o	-	-
Alcohol dehydrogenase	ADH	1.1.1.1	o	o	o						-	-
Alkaline phosphatase	ALP	3.1.3.1	o	o	o						-	-
Aldehyde dehydrogenase	ALDH	1.2.1.5	o	o	o						-	-
Diaphorase	DIA	1.6.-.-	o	o	o	o					-	+
Enolase	ENOL	4.2.1.11	o	o	o	o		o	o	o	+	-
Esterase (4MU)	EST-D	3.1.1.1	o	o	o	o		o	o	o	+	-
Esterase (Naphthyl acetate)	EST-N	3.1.1.1	o	o	o	o		o	o	o	-	+
Glucose dehydrogenase	GDH	1.1.1.47	o	o	o	o					w	-
Glutamate dehydrogenase	GLDH	1.4.3.1	o	o	o						-	-
Glyoxalase	GLO	4.4.1.5	o	o	o						w	-
Glyceraldehyde-3-phosphate dehydrogenase	G3PDH	1.2.1.2	o	o	o	o		o	o	o	+	-
$\alpha$ Glycerol-3-phosphate dehydrogenase	$\alpha$ GPD	1.1.1.8	o	o	o						w	w
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	o	o	o			o	o	o	w	w
Glucose-phosphate isomerase	GPI	5.3.1.9	o	o	o			o	o	o	+	+
Glutathione reductase	GSR	1.6.4.2	o	o	o						-	-
Hydroxybutyrate dehydrogenase	HBDH	1.1.1.30						o	o	o	-	-
Hexokinase	HK	2.7.1.1	o	o	o			o	o	o	w	-
Isocitrate dehydrogenase	IDH	1.1.1.42	o	o	o	o		o	o	o	+	+
Leucine aminopeptidase	LAP	3.4.11.1	o	o	o			o	o	o	-	+
Lactate dehydrogenase	LDH	1.1.1.27	o	o	o						-	-
Malate dehydrogenase	MDH	1.1.1.37	o	o	o			o	o	o	+	w
Malic enzyme	ME	1.1.1.40	o	o	o			o	o	o	+	w
Mannose-phosphate isomerase	MPI	5.3.1.8	o	o	o	o		o	o	o	+	-
Nucleoside phosphorylase	NP	2.4.2.1	o	o	o			o	o	o	w	+
Octopine dehydrogenase	OPDH	1.5.1.11	o	o	o						-	-
Peptidase (Ala Pro)	PEP(AP)	3.4.11.-					o				-	w
Peptidase (Leu Ala)	PEP(LA)	3.4.11.-					o				+	+
Peptidase (Leu Gly Gly)	PEP(LGG)	3.4.11.-	o	o	o	o		o	o	o	+	+
Peptidase (Leu Pro)	PEP(LP)	3.4.13.9					o				-	+
Peptidase (Leu Tyr)	PEP(LT)	3.4.11.-	o	o	o	o		o	o	o	+	+
Peptidase (Val Leu)	PEP(VL)	3.4.11.-	o	o	o	o		o	o	o	+	+
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44	o	o	o						w	w
Phosphoglucomutase	PGM	2.7.5.1	o	o	o			o	o	o	+	+
Superoxide dismutase	SOD	1.15.1.1	o	o	o	o		o	o	o	w	+
Xanthine dehydrogenase	XDH	1.2.1.37	o	o	o						-	-

(\*) - no activity  
w weak  
+ activity sufficient to warrant further investigation

Table 3. Details of buffers showing acceptable results (activity and resolution) for enzyme systems selected from preliminary screening for activity (Table 2). Capitals: starch; small letters: cellogel. Activity and resolution: bold: good; standard: acceptable.

Enzyme	Muscle		Digestive Gland	
	Activity	Resolution	Activity	Resolution
ACP	TEB	TEB	ALL	<i>poor</i>
DIA	TEB TEC TM	<i>poor</i>	TEB TEC TM	TEB TEC
ENOL	phos tm cp	phos tm	-	-
EST-D	TEB TM all	TEB TM TEC tm	-	-
EST-N	-	-	ALL all	TEB TEC TM tm
GDH	<i>poor</i>	TEC	-	-
GLO	<i>poor</i>	TEB	-	-
G3PDH	ALL all	TC tm cp	-	-
$\alpha$ GPD	<i>poor</i>	<i>poor</i>	<i>poor</i>	<i>poor</i>
G6PD	<i>poor</i>	<i>poor</i>	<i>poor</i>	<i>poor</i>
GPI	ALL all	<i>poor</i>	ALL all	TC phos cp
HK	TC all	TC phos tm	-	-
IDH	ALL all	TC TEC phos	ALL all	TC
LAP	-	-	<i>poor</i>	<i>poor</i>
MDH	ALL tm cp phos	TM cp tm phos	<i>poor</i>	<i>poor</i>
ME	ALL all	TM phos	-	-
MPI	ALL tm	<i>poor</i>	-	-
NP	TM tm	<i>poor</i>	TM tm	<i>poor</i>
PEP(LGG)	TEB TEC TC	TEB TEC	ALL	<i>poor</i>
PEP(LT)	TEC TC TEC	TEC TEC	ALL	<i>poor</i>
PEP(VL)	TC TEB TEC	TEB TEC	ALL	<i>poor</i>
6PGD	<i>poor</i>	TC	<i>poor</i>	<i>poor</i>
PGM	ALL all	TC cp TEB TM	ALL all	TC cp phos TEB TM
SOD	TEB TEC	TEB TEC	TEB TEC tm	TEB TEC tm

## 6 Results

Table 4. Results of preliminary screening for variability in samples of *Trochus niloticus* from various regions of the Great Barrier Reef, for 17 enzymes selected from Tables 2 and 3. Choice of buffers was based on the results presented in Table 3. M muscle; DG digestive gland. N Northern; C Central; S Southern Great Barrier Reef; + Enzyme variable; - no variability in the sample.

Enzyme	Buffer used (medium)	Tissue	Number of activity zones	Preliminary screening				Variability	
				N	C	S	Total		
DIA	TEB 8.4 (starch)	DG	2	(DIA-1)	20	22	20	62	-
				(DIA-2)	20	22	20	62	-
ENOL	Phos 7.0 (cellogel)	M	1		5	20	20	45	-
EST-D	TM 7.4 (starch)	M	1		20	19	19	58	-
EST-N	TM 7.4 (starch)	DG	1		20	19	19	58	-
GDH	TEC 7.9 (starch)	M	1			12		12	+
G3PDH	TC 8.0 (starch)	M	1		25	20	20	65	-
GPI	TC 8.0 (starch)	DG	1		25	19	19	63	+
HK	TC 8.0 (starch)	M	1		20	14	20	54	-
IDH	TC 8.0 (starch)	M	2	(IDH-1)	20	19	19	58	-
				(IDH-2)	20	19	19	58	-
MDH	TM 7.4 (starch)	M	2	(MDH-1)	20	19	19	58	-
				(MDH-2)	20	19	19	58	-
ME	TM 7.4 (starch)	M	1		20	19	19	58	-
MPI	TC 8.0 (starch)	M	1		20	19	24	63	+(1)
PEP(LT)	TEB 8.4 (starch)	M	3	(PEP-2)	20	20	20	60	+(2)
6PGD	TC 8.0 (starch)	M	1		25	20	20	65	-
PGM	TC 8.0 (starch)	M	1		25	19	19	63	-
SOD	TEB 8.4 (starch)	DG	2	(SOD-1)	20	27	20	67	-

(1)

MPI patterns were variable among individuals, but resolution too poor to allow reliable scoring.

(2)

Only PEP-2, intense and polymorphic, was considered at that stage. PEP-1 and PEP-3 zones of activity were eventually considered too weak to be scored.

## Effect of freezing / defreezing

During the phase of screening for polymorphism, a subset of 3-10 individuals of those to be run were ground the day before each run, and their enzyme extracts stored overnight at -80°C. These extracts were then assayed on the same gels as fresh extracts from the same individuals, on the day of the run.

Overnight freezing of the enzyme extract affected enzyme activity in all cases except GDH, for which a clear test was not made. This was of no importance for enzymes showing sharp and intense bands of activity such as GPI, MDH-2, IDH-1 and EST-D, because residual activity was still sufficient for clear interpretation. For the other enzymes, it was preferable to avoid overnight freezing, so grinding was made just before each run. This was in the morning when DG tissue extracts were to be run during the same day, or in the afternoon, prior to overnight runs with muscle tissue, because of the time required to thoroughly grind this tissue (2-3 minutes per individual).

## Routine screening

Considerations of maximum return for minimum cost and effort lead us to adopt the schedule given in Table 5.

Table 5. Combinations of buffer and tissue extract for routine screening of 11 enzymes in *Trochus niloticus*. M muscle; DG digestive gland.

Buffer	Tissue extract	
	M	DG
TC 8.0 [1/2]	-	GPI PGM
TEB 8.4	-	SOD DIA EST-N
TEC 7.9	GDH IDH PEP (LT)	-
TM 7.4	EST-D MDH ME	-

### Detail of 12 enzyme systems

A detailed description of the zymograms obtained for enzymes routinely scored in *Trochus niloticus*, and other species, follows. Except where indicated, relative mobilities of electromorphs were verified by running individuals of different phenotypes on the same gels. Where more than one zone of activity was observed, these were numbered consecutively from the slower migrating to the faster. Intense enzyme activity was indicated by black spots on the figures. Weaker enzyme activity was shown by dotted spots. Differences in resolution were expressed as differences in spot sizes.

#### Diaphorase

Two zones of NADH-dependent diaphorase activity were observed in all 6 species studied (Fig. 1). The only individual of *Trochus conus* analysed showed three bands of activity. The two slower ones were close to one the other, and similar in appearance, whereas the faster one was more intense. The quaternary structure of this enzyme is monomeric in man (Harris & Hopkinson, 1976). We assumed that the two zones of activity therefore corresponded to two distinct loci, with the individual of *Trochus conus* being presumptively heterozygous at the locus encoding the slower enzyme. However additional data (e.g. scores from a larger sample of *Trochus conus*) are required before a clear genetic interpretation of DIA zymograms can be proposed.

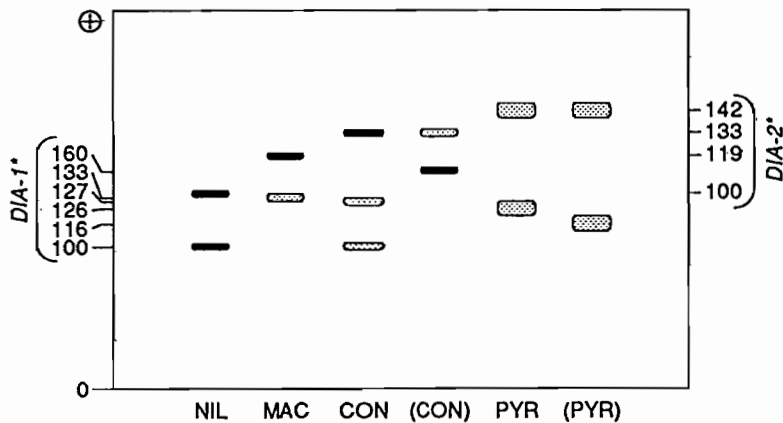


Figure 1. Enzyme variants observed for DIA. DIA-2\* is represented by the fastest migrating band in each lane.

*Esterase-D*

This enzyme specifically used radical 4-methylumbelliferyl acetate (4MU) as substrate. It did not show any activity with another substrate ( $\alpha$  naphthyl acetate) which is hydrolysed by non-specific esterases. Also, its presumed quaternary structure (dimeric, inferred from the number of bands displayed by presumed heterozygotes) is different from that of the latter (monomeric). It therefore can be considered as an analog of the vertebrate EST-D (Hopkinson *et al.*, 1973; Ward & Beardmore, 1977). A 4MU-specific, presumptively dimeric esterase is also present in bivalves (Skibinski, Ahmad & Beardmore, 1978; Borsa & Thiriot-Quievreux 1990).

Staining of this enzyme is inexpensive and simple. It gave very clear and intense bands of activity in all *Trochus* and *Tectus* species studied so far (Fig. 2). It is likely to be useless as a genetic marker in *Trochus niloticus* because it was almost completely

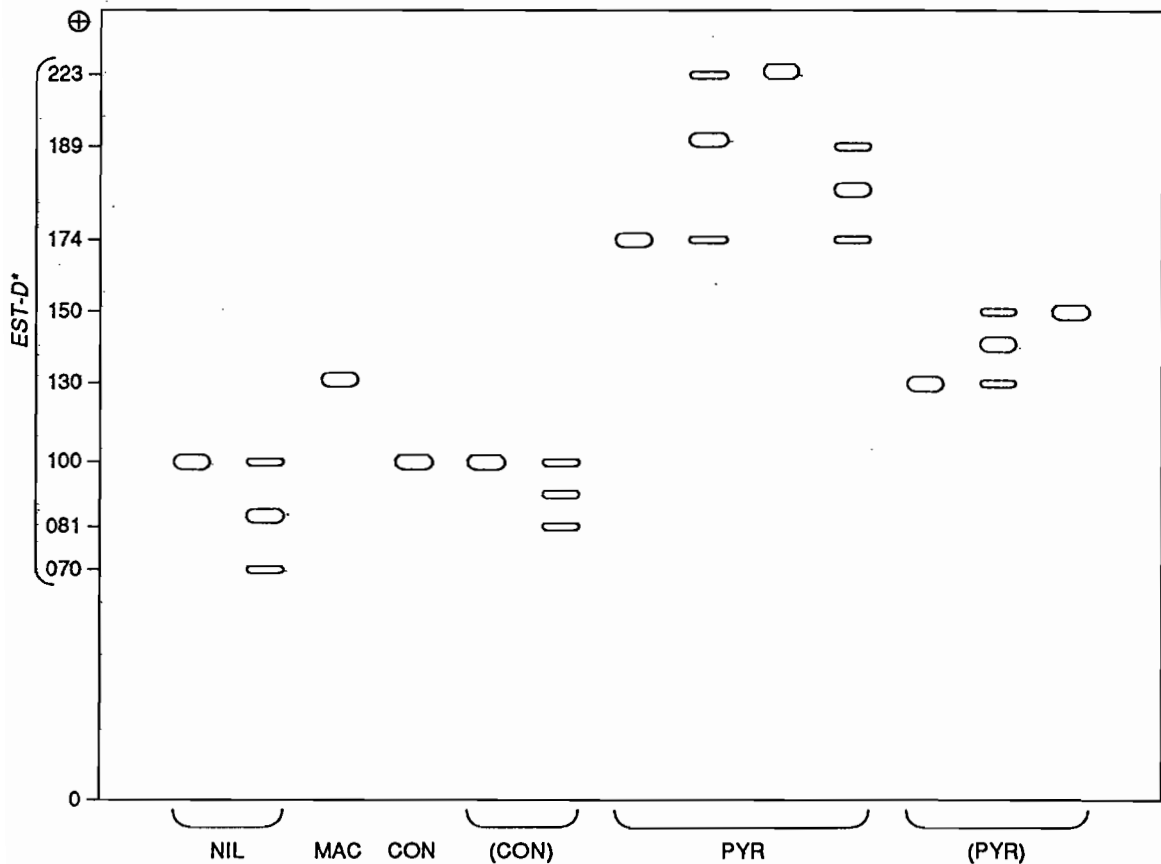


Figure 2. Enzyme variants observed for EST-D. PYR and (PYR) heterozygotes were not symmetrical and the patterns observed on the gels are reproduced exactly in the illustration.



monomorphic in our samples of that species (one single variant over 839 individuals analysed). A higher level of variation was present in *Trochus sp.*, *Tectus pyramis* and *Tectus sp.* Best results were achieved using muscle tissue extract as the source of enzyme. EST-D was also present in digestive gland tissue extracts. When these were run, additional faster bands of non-specific EST-N activity also appeared.

#### *Esterase-N*

In presence of radical  $\alpha$ -naphthyl acetate and a soluble diazo dye (Fast Garnet GBC, Fast Black K), several zones of activity appeared in digestive gland extracts of *Trochus niloticus*, one of which (the fastest one) was sharp and intense. The same result could be obtained with 4MU as substrate, except that bands of EST-D additionally appeared. This fast and sharp band was assumed to correspond to a locus (*EST-N\**) totally monomorphic in *T. niloticus*. Bands with habitus and mobilities similar to those of *T. niloticus* were present with DG extracts of other *Trochus* and *Tectus* species (Fig. 3, produced from a run on cellogel TM). The patterns shown in Fig. 3 are typical of a polymorphic locus with co-dominant alleles encoding a monomeric enzyme. The fine resolution of cellogel TM was not always achieved with starch gels.

#### *Glucose dehydrogenase*

Preliminary tests using NAD as cofactor showed no GDH activity. New attempts were made later in the study, using NADP as cofactor. These demonstrated weak GDH activity. When present, this enzyme showed two anodal zones of activity in *T. niloticus*. The weaker, slower one was interpreted as the expression of a monomorphic locus (not scored at all), the faster one as that of a polymorphic locus here called *GDH.\** *GDH\** was scored in only those few gels exhibiting clear banding patterns (Fig. 4) for all individuals. TEB and TEC were the only buffers tested for NADP-dependent GDH activity: similar results were obtained with both these buffers.

#### *Glucose-phosphate isomerase*

This enzyme exhibited some variation in *Trochus niloticus* (4 allelomorphs in a sample of 839 individuals). It was found to be very variable in *Tectus pyramis*, with 7 allelomorphs in 58 individuals (Fig. 5). Half the concentration of gel buffer (TC8 [1/2]) was preferred because it increased the speed of migration, hence the separation between electromorphs, otherwise low in *T. niloticus*. This did not affect the intensity or the sharpness of the bands of activity which were in any case very good. Good results were also obtained with TEC [1/2]).

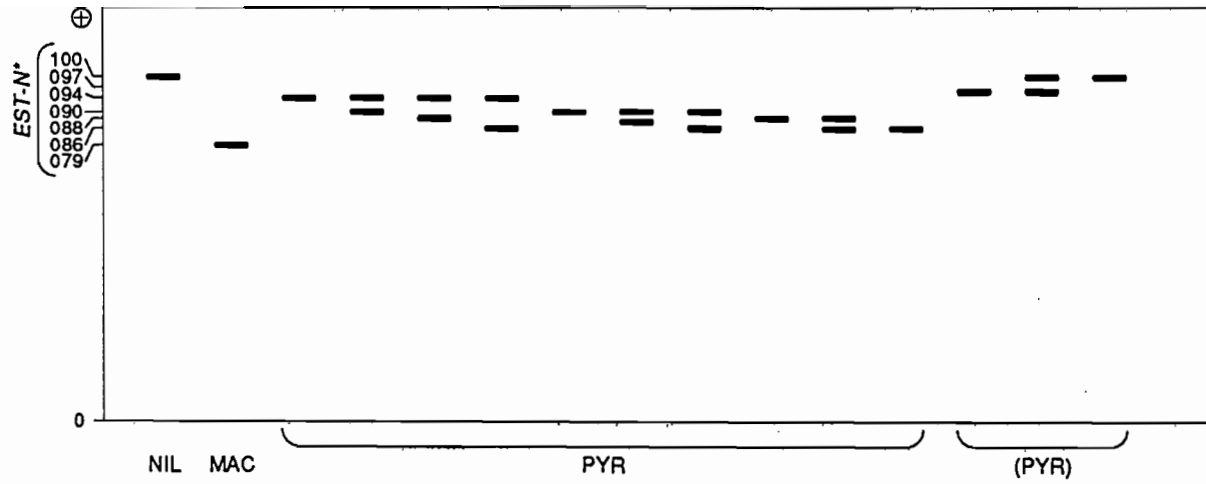


Figure 3. Enzyme variants observed for EST-N. No activity was observed for CON and (CON).

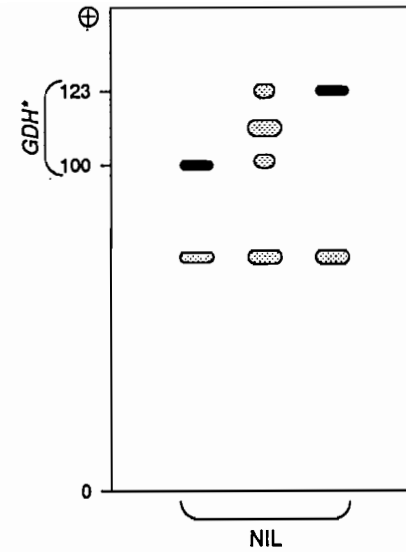


Figure 4. Enzyme variants observed for GDH.

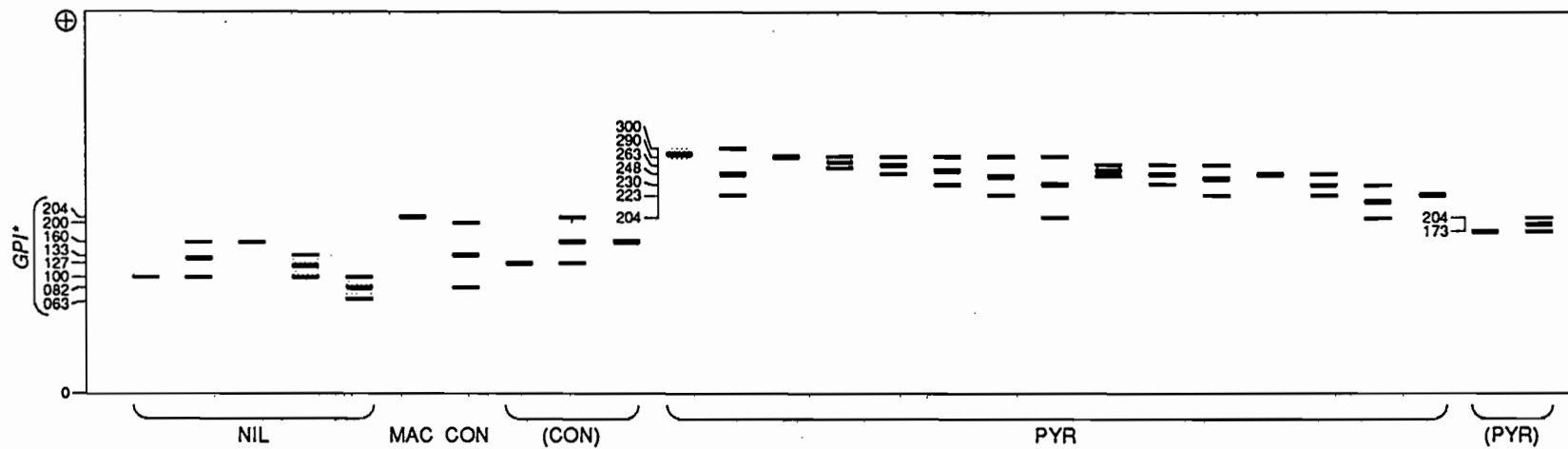


Figure 5. Enzyme variants observed for GPI.

### Isocitrate dehydrogenase

Two isocitrate dehydrogenase zones of activity were usually present. The patterns shown in Fig. 6 suggest that *IDH-1\** and *IDH-2\** were encoded by two different loci. *IDH-1\** of *Trochus niloticus* exhibited sub-bands whose presence did not interfere with the clarity of the IDH zymograms. Whether electromorph \*030 of *Trochus conus* was different from the rare electromorph \*021 of *T. niloticus* could not be verified by running both samples side-by-side.

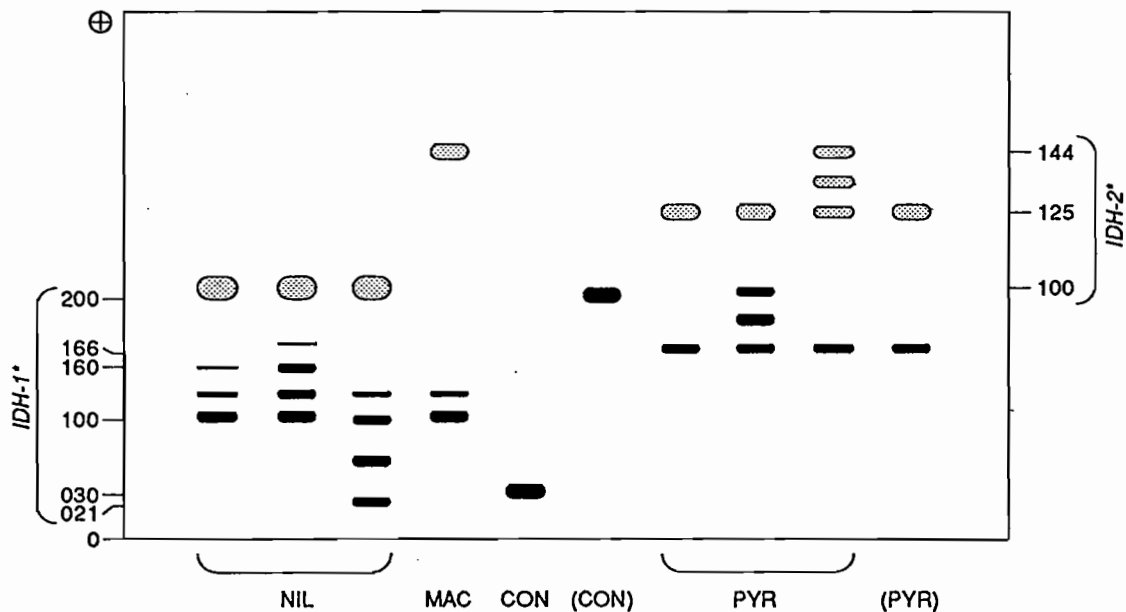


Figure 6. Enzyme variants observed for IDH.

### Malate dehydrogenase

Two malate dehydrogenase zones of activity were present in all 6 species except *Tectus pyramis* (Fig. 7). These were clearly distinct, and could be ascribed to two different loci, thanks to the occurrence of distinct heterozygous patterns in *Trochus niloticus*. Because of intense sub-banding, heterozygous phenotypes usually were not of the classical three-banded type as would have been expected for that presumptively dimeric enzyme, although three-banded heterozygous individuals were observed on some gels for *Tectus pyramis*. *MDH-2\** was highly polymorphic in *Tectus pyramis*, but was monomorphic in *Tectus sp.*, *Trochus maculatus* and almost monomorphic in *T. niloticus*.

Electromorph \*128 of *Trochus sp.* might be identical to the rare electromorph \*125 of *T. niloticus* but this could not be verified by side-by-side sample running.

Despite potentially confusing sub-banding, assessing the presence of heterozygotes at one or the other *MDH\** loci in *T. niloticus* was not problematical.

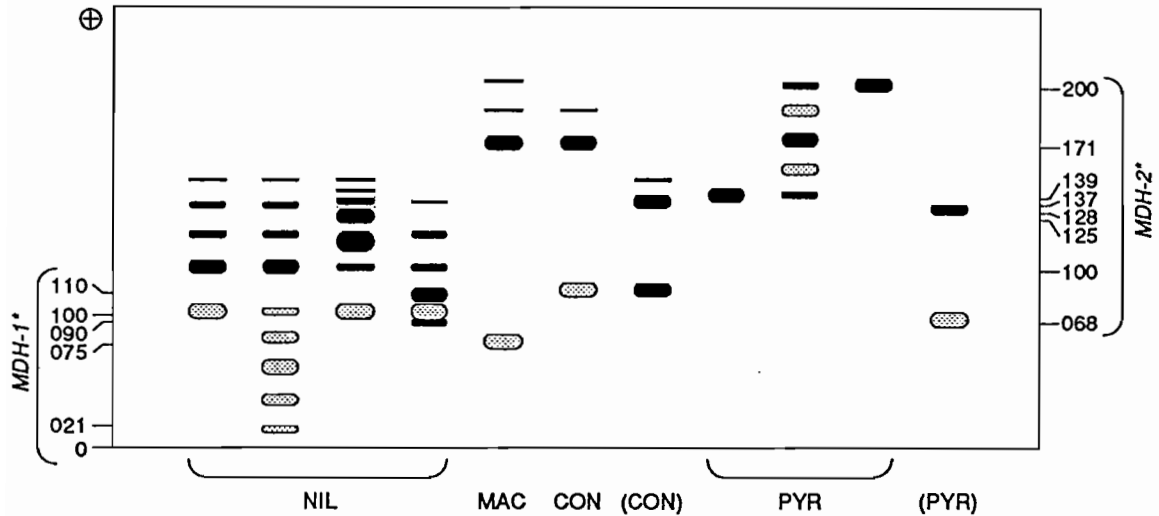


Figure 7. Enzyme variants observed for MDH

### Malic enzyme

Staining for this dehydrogenase required the same substrate as MDH (L- malic acid) and NADP as cofactor. Its quaternary structure is tetrameric in vertebrates (Nevaldine, Bassel & Hsu 1974; Hopkinson *et al.*, 1976). Heterozygotes are expected to have a five-banded phenotype in the best case, or a roughly beer barrel-shaped smear if resolution of the gel is not perfect. The latter patterns were observed in *Trochus* and *Tectus* (Fig. 8). The presence of both homozygotes and heterozygotes for the two presumptive alleles in *Tectus pyramis* reinforced the reliability of our genetic interpretation.

A slower migrating faint band (designated "*ME-1\**" in Fig. 8) was often observed on gels of *T. niloticus* stained for ME. In *Trochus niloticus*, homozygotes for the rare \*087 presumptive allele were not observed. It is therefore possible that the latter was identical to allele \*092 of *T. maculatus*.

### Non-specific dehydrogenase

Weak but clear-cut bands were sometimes found to appear after 24 hrs of incubation on gels stained for both GDH and IDH, thus identified as NADP-dependent non-specific dehydrogenase (NDH) zones of activity. This enzyme showed electrophoretic

variation between species, but not within species (Fig.9), although numbers of individuals scored for *NDH\** in each of them were low (see Appendix). In *Trochus niloticus* and *Tectus sp.*, *NDH\** bands overlapped those of *IDH-1.\**

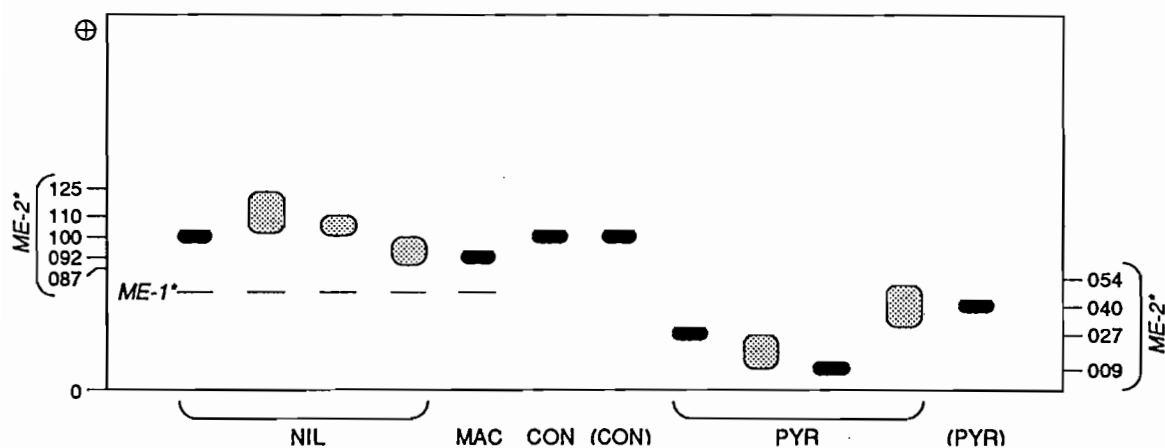


Figure 8. Enzyme variants observed for ME

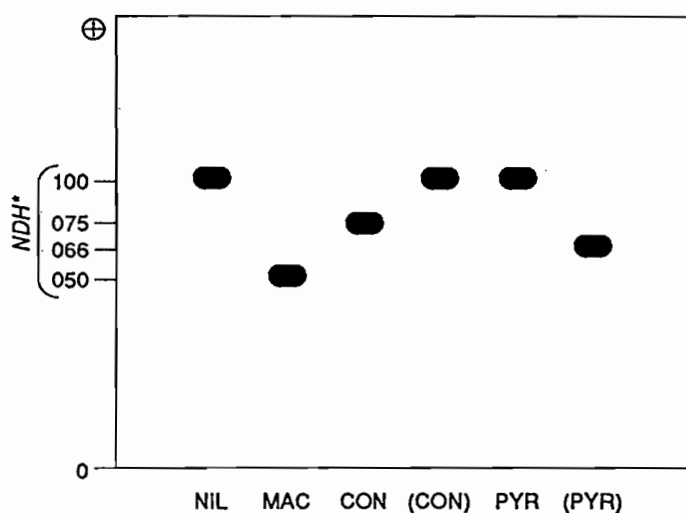


Figure 9. Enzyme variants observed for NDH

### Peptidase (LeuTyr)

In *Trochus niloticus*, a comparative run involving the muscle tissue extracts of 10 individuals and different combinations of buffer (TEB 8.4, TEC 7.9) and substrate (Leu-Gly-Gly, Leu-Tyr, Val-Leu) was made in addition to the preliminary screening described above. Results for intensity, resolution and variability are summarized in Table 6.

Table 6. Intensity (I), resolution (R) and enzyme polymorphism (P) in the three peptidase zones of activity observed in 6 combinations of buffer and enzyme substrate. See text for details.

Buffer	Substrate		
	LeuGlyGly	LeuTyr	ValLeu
<b>TEB 8.4</b>			
<i>PEP-1*</i>	I: weak R: poor P: present (slight)	I: intense R: not sufficient P: present (same as LGG)	I: weak R: ok P: absent
<i>PEP-2*</i>	I: good R: poor P: present	I: good R: borderline P: present (same as LGG)	I: good R: ok P: absent
<i>PEP-3*</i>	I: good R: good P: absent	I: good R: good P: absent	I: very weak R: ok P: absent
<b>TEC 7.9</b>			
<i>PEP-1*</i>	I: good R: good P: present (same as above)	I: sufficient R: poor P: present (same as above)	I: very weak R: poor P: ?
<i>PEP-2*</i>	I: good R: borderline P: present (same as above)	I: intense R: good P: present (same as above)	I: very weak R: poor P: ?
<i>PEP-3*</i>	I: weak R: poor P: ?	I: weak R: poor P: absent	I: very weak R: poor P: ?

Three zones of peptidase activity were present with buffers TEB 8.4 and TEC 7.9 when gels were stained using either Leu-Gly-Gly, Leu-Tyr or Val-Leu as substrates. The relative mobilities of each zone were identical when compared on slices of the same gel, stained using these different substrates. However, the relative intensities, sharpness, and electromorph mobilities within each zone were variable. Resolution was better with TEC than with TEB for LGG and LT. Intensity of PEP (VL) was greater with TEB.

*PEP-1\** and *PEP-2\** each showed allozyme variability with LGG and LT, but not with VL. *PEP-1* (VL) had the same mobility as the most common electromorph of *PEP-1\** (LGG or LT). *PEP-2\**(VL) had the same mobility as electromorph \*100 of *PEP-2\** (LGG or LT). *PEP-3\** was monomorphic in the 10 individuals for all

combinations tested. It was intense in TEB, but weak enough in TEC to prevent being confused with any possible fast electromorph of PEP-2.

Only *PEP-2\** (LT) was therefore considered for further analysis, and it was routinely scored on TEC 7.9 (Fig. 10).

*PEP-2\** (LT) was also the only peptidase scored in the five other *Trochus* and *Tectus* species. Because of sub-banding, and resolution not sufficient to separate overlapping electromorphs with suspected different mobilities, assessment of *PEP-2\** phenotypes was more difficult than with *T. niloticus*. We feel that refining procedures similar to those developed for *T. niloticus* (Table 6) might result in increased resolution in these other species.

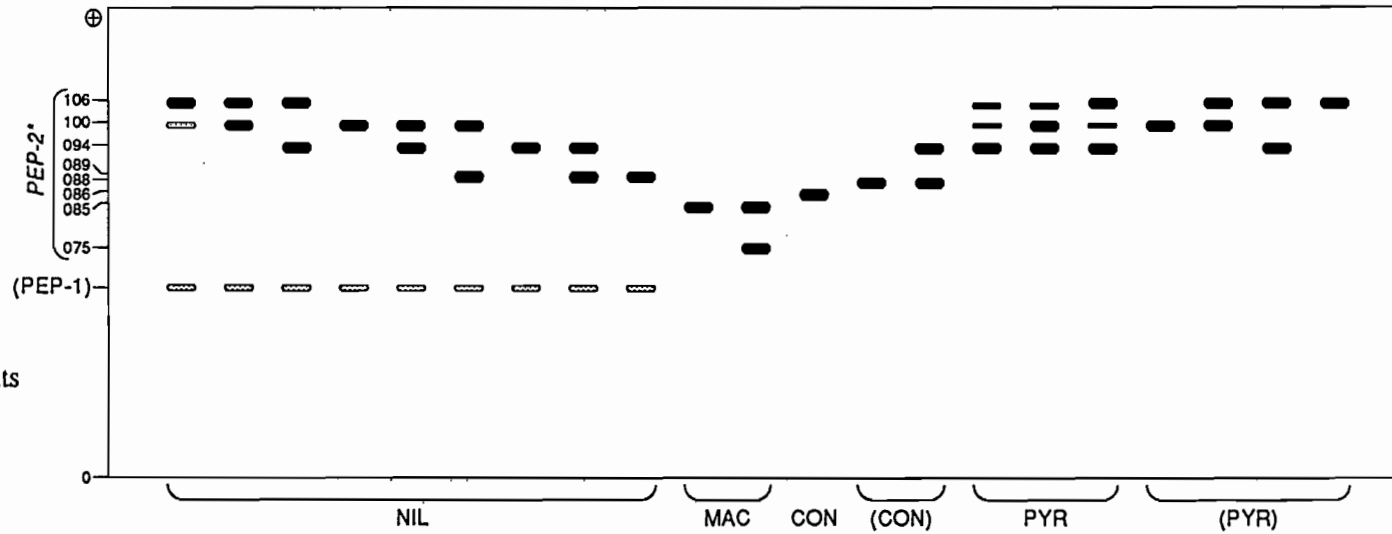
As stressed by Richardson *et al.* (1986), peptidases are usually ambiguous markers: their electrophoretic patterns may be determined by an unknown number of loci and the isozyme products of separate loci often overlap. However, gels obtained for *T. niloticus* usually exhibited clear *PEP-2\** (LT) one-banded or two-banded patterns, in accordance with expectations for a monomeric enzyme encoded by several co-dominant alleles.

### *Phosphoglucomutase*

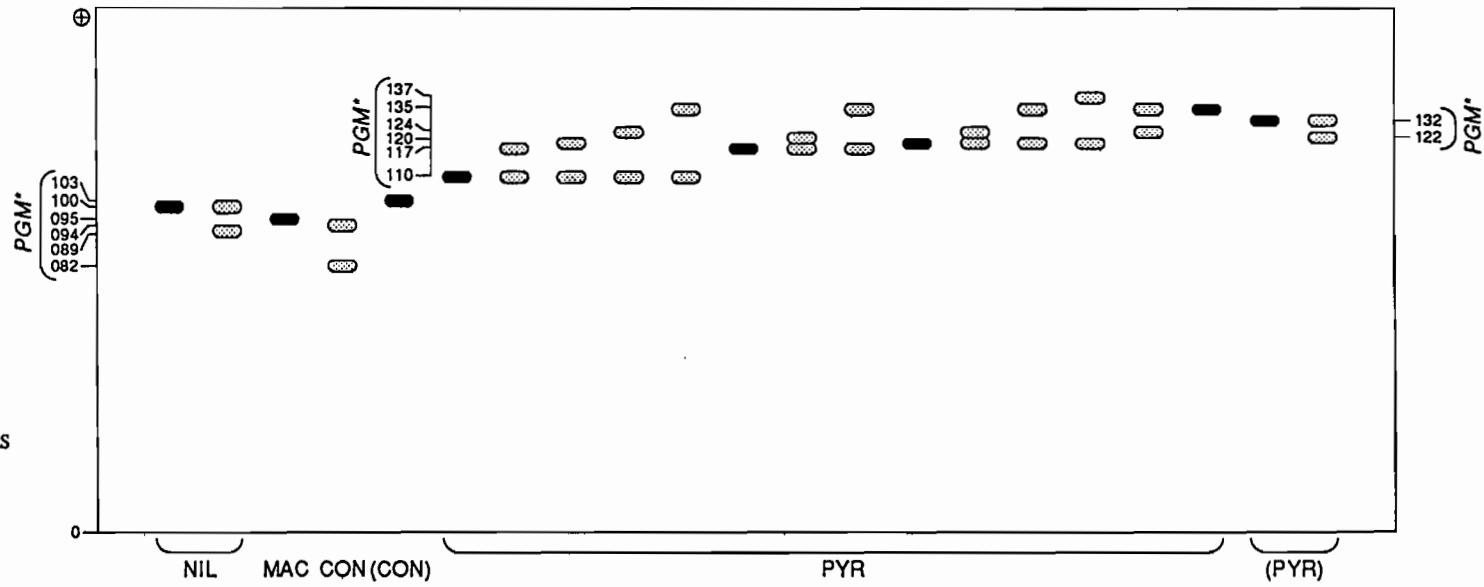
Interpretation of zymograms of this presumptively monomeric enzyme was straightforward (Fig. 11). Caution should be taken, however, in assessing the mobilities of the different electromorphs of PGM in the highly polymorphic *Tectus pyramis*. Ideally, one would use a large proportion of the different available phenotypes as reference markers on each gel, but this was not always possible (see Appendix).

In *T. niloticus*, only one intense zone of activity was present in digestive gland tissue extracts. Two zones appeared in muscle. One had the same mobility as that of digestive gland and was therefore assumed to correspond to the same locus, although only monomorphic samples of *T. niloticus* could be compared between these tissues. The other slower zone was considered to correspond to another locus, whose existence in other molluscs (bivalves) has been inferred from zymograms in which variation was displayed at both loci (Worms & Pasteur, 1982; Borsa & Thiriot-Quievreux, 1990).

**Figure 10.**  
Enzyme variants  
observed for  
PEP-2 (LT).



**Figure 11.**  
Enzyme variants  
observed for  
PGM.





### Superoxide dismutase

Two zones of SOD activity, best visualized with digestive gland tissue extract, were observed. The faster one was intense but not sharp enough for reliable interpretation. The weaker, slower one (*SOD-1\**) was clear enough for comparisons between species, and for assessing polymorphism in *Tectus sp.* (Fig. 12). In *Trochus niloticus*, faster sub-bands were often present. These may have masked possible rare heterozygotes.

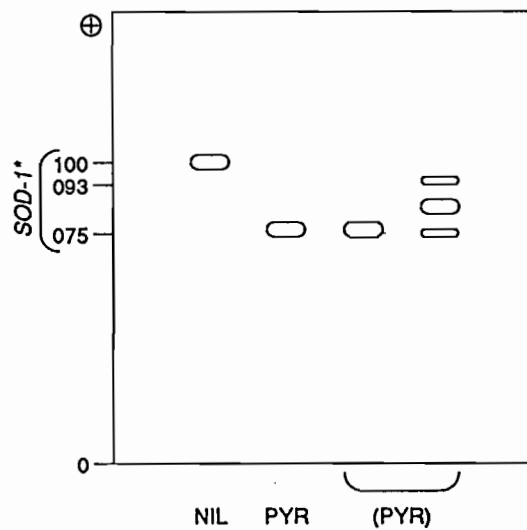


Figure 12. Enzyme variants observed for SOD. No activity was observed for MAC, CON or (CON).

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## DISCUSSION

The methods presented here were originally selected to screen for variation in populations of *Trochus niloticus* from the Great Barrier Reef. They were extended later to other species belonging to the genera *Trochus* and *Tectus* (both Trochidae). In several instances, this comparative approach was useful in reinforcing the reliability of our genetic interpretation of zymograms for *T. niloticus*: (1) Some enzyme loci monomorphic in *T. niloticus* (e.g. *EST-N\** and *IDH-2\**) and some others (*EST-D\**, *ME\** and *PGM\**) slightly polymorphic in *T. niloticus* and lacking the phenotype expected for the rare homozygote, displayed polymorphism in other trochids, which was in accordance with the expectations derived from a simple genetic model and the known enzyme's quaternary structure. (2) An enzyme (*NDH\**) revealed using IDH staining, and appearing to have the same mobility as *IDH-1\** in *T. niloticus* was clearly distinct from *IDH-1\** in another species (*Tectus sp.*), thus confirming its separate status.

No breeding experiments have been attempted yet to demonstrate that the observed allozyme banding patterns in *Trochus* and *Tectus* have a simple genetic basis. In most cases of polymorphism, the zymograms conformed to typical patterns of codominance. Breeding experiments performed in another prosobranch species, *Littorina saxatilis*, have demonstrated that the patterns observed for enzymes *GPI\**, *IDH-1\**, *IDH-2\**, *PEP-2\** and *PGM\** are inherited in a simple Mendelian fashion (Ward, Warwick & Knight, 1991). Similar results have been obtained in a few other molluscan (bivalve) species (Beaumont, Beveridge & Budd, 1983; Hvilsom & Theisen, 1984; Foltz, 1986), adding *EST-D\** and one *MDH\** to the above set of enzymes.

A variety of factors other than genetic polymorphism can result in banding variation: environmental effects (Oxford, 1975), protein degradation (Harris & Hopkinson, 1976), uncontrolled variation in gel quality (Borsa, Jousselin & Delay, 1991, reinterpreting Monti *et al.*, 1986) or other stain artifacts (Harris & Hopkinson, 1976). However, the two following criteria suggested by Selander *et al.* (1970) that are usually considered acceptable among population geneticists (Burton, 1983), were met in *Trochus* and *Tectus* with polymorphic enzymes for which no breeding data are available: (1) Consistency of banding patterns with known quaternary structure and (2) presence of all phenotypes expected to be in non-marginal proportions according to a Mendelian model.

Nevertheless, definitive assessment of the genetic control of banding variation requires formal genetic analyses. In *Trochus* and *Tectus*, this requires that pairs of individuals be induced to spawn and their offspring cultivated, prior to comparing the allozyme phenotypes of offspring and parents. Heslinga & Hillmann (1981) have shown that experimental spawning of *Trochus niloticus* and subsequent rearing of juveniles could easily be achieved in standard mariculture conditions. Sexing might be possible prior to cross-breeding by carefully drilling a hole in the apical region of the shell, through which it is then possible to visualize the gonad's colour and texture (green, granulated for females; creamy for males). Since no harmless way of assessing each parent's phenotype seems to be available, the pairs to be cross-bred can only be randomly chosen. A few such random crossings would be expected to give valuable results with sufficiently variable enzymes like *PEP-2\** in *Trochus niloticus*, *EST-N\**, *GPI\**, *MDH\** and *PGM\** in *Tectus pyramis* and *EST-D\**, *EST-N\** and *PEP-2\** in *Tectus sp.*

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## APPENDIX

**Table A1** Key to allelomorphs in Table A2

Locus	Allelomorphs
<i>DIA-1*</i>	A=100, B=116, C=126, D=127, E=132
<i>DIA-2*</i>	A=100, B=119, C=133, D=142
<i>EST-D*</i>	A=070, B=081, C=100, D=130, E=150, F=174, G=189, H=223
<i>EST-N*</i>	A=079, B=086, C=088, D=090, E=094, F=097, G=100
<i>GDH*</i>	A=100, B=123
<i>GPI*</i>	A=082, B=100, C=127, D=160, E=173, F=200, G=204, H=223, I=230, J=236, K=248, L=263, M=290, N=300
<i>IDH-1*</i>	A=030, B=100, C=120, D=127, E=160, F=166, G=200
<i>IDH-2*</i>	A=100, B=125, C=144
<i>MDH-1*</i>	A=075, B=090, C=100, D=110
<i>MDH-2*</i>	A=100, B=128, C=137, D=139, E=171, F=200
<i>NDH*</i>	A=050, B=066, C=075, D=100
<i>ME-2*</i>	A=009, B=027, C=040, D=092, E=100, F=054
<i>PEP-2*</i>	A=075, B=085, C=086, D=088, E=089, F=094, G=100, H=106
<i>PGM*</i>	A=082, B=094, C=095, D=100, E=103, F=110, G=117, H=120, I=122, J=124, K=132, L=135, M=137
<i>SOD-1*</i>	A=075, B=093, C=100

**Table A2** Individual multilocus genotypes in four species of genus *Trochus*, *T. niloticus* (L. 1758), *T. maculatus* (L. 1758), *T. conus* (Gmelin 1791) and *Trochus* sp., and two species of genus *Tectus*, *T. pyramis* (Lmk. 1822) and *Tectus* sp. from the Great Barrier Reef.

#	DIA-1*	DIA-2*	MDH-1*	MDH-2*	IDH-1*	IDH-2*	NDH*	PGM*	GPI*	EST-N*	EST-D*	ME-2*	PEP-2*	SOD-1*	GDH*
<i>Trochus niloticus</i> , Escape Reef															
01	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GF	CC	
02	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	EE	CC	
03	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	FE	CC	
04	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GF	CC	
05	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
06	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	FF	CC	
07	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
08	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GF	CC	
09	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
10	AA	AA	CC	AA	EB	AA		DD	BD	GG	CA	EE	FF	CC	
11	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
12	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
13	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
14	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
15	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	FF	CC	
16	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
17	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GF	CC	
18	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	FE	CC	
19	AA	AA	CC	AA	BB	AA		DD	BB	GG	CC	EE	GG	CC	
20	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GG	CC	
21	AA	AA	CC	AA	BB	AA		DD	BD	GG	CC	EE	GF	CC	
22	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	
23	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	
24	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	
25	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GG	CC	



Table A2 (continued)

#	DIA-1*	DIA-2*	MDH-1*	MDH-2*	IDH-1*	IDH-2*	NDH*	PGM*	GPI*	EST-N*	EST-D*	ME-2*	PEP-2*	SOD-1*	GDH*
26	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	FE	CC	AA
27	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	BB
28	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	GE	CC	AA
29	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	AA
30	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GG	CC	AA
31	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GG	CC	AA
32	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GE	CC	AA
33	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	BB
34	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	GG	CC	AA
35	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	GG	CC	AA
36	AA	AA	CC	AA	BB	AA	DD	DD	BB	GG	CC	EE	GF	CC	AA
37	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	GF	CC	AA
38	AA	AA	CC	AA	BB	AA	DD	DD	BD	GG	CC	EE	FF	CC	AA

*Trochus maculatus*, Davies Reef (\*Square Reef)

01	EE	BB	AA	EE	BB	CC	AA	CC	GG	AA	DD	DD	DD		
02	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD		
03	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD		
04	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DA		
05	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD		
06	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
07	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
08	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
09	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
10	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		
11	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD		

**Table A2 (continued)**

#	DIA-1*	DIA-2*	MDH-1*	MDH-2*	IDH-1*	IDH-2*	NDH*	PGM*	GPI*	EST-N*	EST-D*	ME-2*	PEP-2*	SOD-1*
12	EE	BB	AA	EE	BB	CC		CC	GG	AA	DD	DD	DD	
14	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD	
15	EE	BB	AA	EE	BB	CC		CC	GG		DD	DD	DD	
16	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
17	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
18	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
V3*	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
V4	EE	BB	AA	EE	BB	CC	AA	CC	GG		DD	DD	DD	
<i>Trochus conus</i> , Davies Reef														
V5	DA	CC	DD	EE	AA		CC	BA	FA		CC	EE	CC	
<i>Trochus (conus) sp.</i> , Davies Reef (*Escape Reef)														
01	GG	CC	DD	CC	GG		DD	EE	CC		CB	EE	FD	
V6	FF	CC	DD	CC	AA		DD	EE	JD		CC	EE	DD	
V7*	GG	CC	DD	CC	GG		DD	EE	GC		CC	EE	DD	
<i>Tectus pyramis</i> , Davies Reef														
01	CC	DD		FD	FF	BB		FF	ML	BB	FF	BB	FF	AA
02	CC	DD		DD	FF	BB		HF	MH	BB	FF	BB	FF	AA
03	CC	DD		DD	FF	BB		FF	KK	EE	FF	BB	FF	AA
04	CC	DD		DD	FF	BB		GF	NH	EB	FF	BB	FF	AA
05	CC	DD		FD	FF	BB		JH	MM	DD	FF	BB	FF	AA
06	CC	DD		DD	FF	BB		FF	MH	EE	FF	BB	FF	AA
07	CC	DD		FF	FF	BB		FF	KH	EC	FF	BB	FF	AA
08	CC	DD		DD	FF	BB		HG	MK	EB	FF	BB	FF	AA

Table A2 (continued)

#	DIA-1*	DIA-2*	MDH-1*	MDH-2*	IDH-1*	IDH-2*	NDH*	PGM*	GPI*	EST-N*	EST-D*	ME-2*	PEP-2*	SOD-1*
09	CC	DD		FD	FF	BB		FF	MH	BB	FF	BB	FF	AA
10	CC	DD		FD	FF	BB		GF	MM	DD	FF	BB	FF	AA
11	CC	DD		FD	FF	BB		GG	MK	ED	FF	BB	FF	AA
12	CC	DD		DD	FF	BB		HH	MH		FF	BB	FF	AA
13	CC	DD		DD	FF	BB			LK	EC	FF	BB	FF	
14	CC	DD		FD	FF	BB			ML	EE	FF	BB	FF	
15	CC	DD		DD	FF	BB			LK	DB	FF	BB	FF	
16	CC	DD		FD	FF	BB			MM	EB	FF	BB	FF	
17	CC	DD		DD	FF	BB			LI	EE	FF	BB	FF	
18	CC	DD		DD	FF	BB			HH	ED	FF	BB	FF	
19	CC	DD		DD	FF	BB			MM	DD	FF	BB	FF	
20	CC	DD		FD	FF	BB			MH	EC	FF	BB	FF	
21	CC	DD		DD	FF	BB			MK	EE	FF	BB	FF	
22	CC	DD		DD	FF	BB			MH	ED	FF	BB	FF	
23	CC	DD		FD	FF	BB			MG	DD	FF	BB	FF	
24	CC	DD		DD	FF	BB			MK	DD	FF	BB	FF	
25	CC	DD		DD	FF	BB			MK	EC	GF	BB	FF	
26	CC	DD		DD	FF	BB			MM	EE	FF	BB	FF	
V1	CC	DD		FD	FF	BB			LH	CC		BB	FF	
<i>Tectus pyramis</i> , Square Reef														
01	CC	DD		DD	FF	BB		LG	KK	EB	FF	BB	FF	
02	CC	DD		FD	FF	BB		HH		EC	FF	BB	GF	
03	CC	DD		DD	FF	BB		HH	MK	CC	FF	BB	FF	
04	CC	DD		FF	FF	BB		LF	MK	EE	HH	BB	FF	
05	CC	DD		DD	FF	BB		GF	IG	EB	HF	BB	FF	
06	CC	DD		FD	FF	BB		FF	MM	EC	FF	BB	FF	
07	CC	DD		FD	FF	BB		LG	MI	DD	FF	BB	FF	

Table A2 (continued)

#	DIA-1*	DIA-2*	MDH-1*	MDH-2*	IDH-1*	IDH-2*	NDH*	PGM*	GPI*	EST-N*	EST-D*	ME-2*	PEP-2*	SOD-1*
08	CC	DD		DD	FF	BB		GF	KI	DB	HF	BB	FF	
09	CC	DD		FD	FF	BB		FF	MM	BB	FF	BB	FF	
10	CC	DD		DD	FF	BB		HF	MK	ED	FF	BB	FF	
11	CC	DD		FD	FF	BB			MM	DC	FF	BB	FF	
12	CC	DD		DD	FF	BB			KK	DD	FF	BB	FF	
13	CC	DD		DD	FF	BB				CC	FF	BA	FF	
14	CC	DD		DD	FF	BB				EB	FF	BB	FF	
15	CC	DD		DD	FF	BB			MK	EE	FF	BA	FF	
16	CC	DD		DD	GF	BB			KH	DB	FF	BB	FF	
17	CC	DD		FF	FF	BB				DD	FF	BA	FF	
18	CC	DD		DD	FF	BB				BB	GF	BB	FF	
19	CC	DD		FD	FF	BB				CB	FF	BB	FF	
20	CC	DD		FD	FF	CB			NM	EB	FF	AA	FF	
21	CC	DD		FD	FF	BB			MK	EC	FF	BB	FF	
22	CC	DD		DD	FF	BB			MK	DC	FF	BB	FF	
23	CC	DD		DD	FF	BB			MK	DD	FF	BB	FF	
24	CC	DD		DD	FF	BB			KK	DB	FF	BB	FF	
25	CC	DD		FD	FF	BB			KH	DB	FF	BB	FF	
26	CC	DD		FD	FF	BB			MK	CC	FF	BB	FF	
27	CC	DD		DD	FF	BB			MH	DC	FF	BB	FF	
28	CC	DD		FD	FF	BB			MM	BB	FF	BB	HF	
29	CC	DD		DD	FF	BB			LK		HF	BB	FF	
30	CC	DD		DD	FF	BB			LH	DD	FF	BB	FF	
<i>Tectus pyramis</i> , Escape Reef														
02	CC	DD		DD	FF	BB	DD	GF	KH	DD	FF	FB	FF	AA
06	CC	DD		FF	FF	BB	DD	GF	MK		FF	BB	FF	AA

Table A2 (continued)

#	DIA-1*	DIA-2*	MDH-1*	MDH-2*	IDH-1*	IDH-2*	NDH*	PGM*	GPI*	EST-N*	EST-D*	ME-2*	PEP-2*	SOD-1*
<i>Tectus (pyramis) sp.</i> , Escape Reef														
01	BB	DD	BB	BB	FF	BB	BB	KK	EE	FF	ED	CC	GG	AA
03	BB	DD		BB	FF	BB	BB	KK	EE		DD	CC	HG	AA
04	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	AA
05	BB	DD		BB	FF		BB	KK	EE		EE	CC	GG	AA
07	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	AA
08	BB	DD		BB	FF		BB	KK	EE		ED	CC	GG	AA
09	BB	DD		BB	FF		BB	KK	EE		ED	CC	HH	AA
10	BB	DD		BB	FF		BB	KF	EE	FF	EE	CC	HG	AA
11	BB	DD		BB	FD		BB	KK	EE		DD	CC	HF	BA
12	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	BA
13	BB	DD		BB	FF		BB	KK	EE		DD	CC	HH	AA
14	BB	DD		BB	FF		BB	KK	EE		EE	CC	HF	BA
15	BB	DD		BB	FF		BB	KK	EE		ED	CC	HG	AA
16	BB	DD		BB	FF		BB	KK	EE		EE	CC	HF	AA
17	BB	DD		BB	FF		BB	KK	EE		EE	CC	HF	AA
18	BB	DD		BB	FF		BB	KK	EE		DD	CC	GG	AA
19	BB	DD		BB	FF		BB	KK	EE		EE	CC	GG	AA
20	BB	DD		BB	FF		BB	KK	EE		ED	CC	GG	AA
21	BB	DD		BB	FF		BB	KK	GE	GG	EE	CC	GG	AA
22	BB	DD		BB	FF		BB	KK	EE	GG	EE	CC	HG	AA
23	BB	DD		BB	FF		BB	KK	EE	GF	EE	CC	HH	BA
24	BB	DD		BB	FF		BB	KK	EE	GG	DD	CC	HH	BA
25	BB	DD		BB	FF		BB	KK	EE	FF	EE	CC	HH	AA
26	BB	DD		BB	FF		BB	KK	EE	GF	EE	CC	HG	AA
27	BB	DD		BB	FC		BB	KK	EE	GF	ED	CC	HG	BA
28	BB	DD		BB	FF		BB	KF	EE	FF	ED	CC	HH	BA
29	BB	DD		BB	FF		BB	KK	EE	GG	ED	CC	HG	AA

**Table A2** (continued)

#	DIA-1*	DIA-2*	MDH-1*	MDH-2*	IDH-1*	IDH-2*	NDH*	PGM*	GPI*	EST-N*	EST-D*	ME-2*	PEP-2*	SOD-1*
30	BB	DD		BB	FF		BB	KF	EE	FF	ED	CC	HG	AA
31	BB	DD		BB	FF		BB	KK	GE		EE	CC	HH	
32	BB	DD		BB	FF		BB	KK	GE		EE	CC	HH	
33	BB	DD		BB	FF		BB	KK	EE		ED	CC	HH	
34	BB	DD		BB	FF		BB	KK	GE		ED	CC	HF	
35	BB	DD		BB	FF		BB	KK	EE		EE	CC	HG	
36	BB	DD		BB	FF		BB	KK	EE		ED	CC	HG	
37	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	
38	BB	DD		BB	FF		BB	KK	EE		EE	CC	HH	
V2	BB	DD	BB	BB	FF	BB	BB	KK	EE		EE	CC	GG	

**Voucher specimens**

The shells of individuals # V1 to V7 were sent as voucher specimens to the Australian Museum, Sydney, with registration numbers C.168458, C.168454, C.168457, C.168459, C.168463, C.168462 and C.168455, respectively. The shells of individuals *Trochus maculatus* # 01 and 15 were registered as C.168460 and C.168461, respectively.



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