ISOZYMES IN THE AXOLOTL

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Over the last few years, we have used polyacrylamide gel electrophoresis (PAGE) and isoelectric focussing (IEF) to test for possible isoelectric variants in a number of different enzyme systems. Our objective has been to uncover additional markers that might be of use for genetic mapping or as tissue specific markers for transplantation experiments. Of the enzymes studied, MDH (malate dehydrogenase) and LDH (lactate dehydrogenase) do not appear to show phenotypic variation, whereas two other systems, PGM (phosphoglucomutase) and EST (esterase) show promise as markers since they exhibit phenotypic variation in predictable Mendelian ratios. They have already been used to test the efficiency of our gynogenetic and androgenetic procedures. In addition, the location of the Pgm and Est genes with respect to their centromere(s) has been determined.

MATERIALS AND METHODS

Adult animals used in this study were maintained as described in Hronowski and Armstrong (1977), and spawned using the procedures listed by Gillespie and Armstrong in this newsletter (Number 10, Winter 1981). Gynogenetic larvae were produced by suppression of second polar body release (Gillespie and Armstrong, 1979). Larvae raised specifically for isozyme testing were maintained individually, from hatching, in 50 ml cups and were changed and fed daily. Under these conditions, the animals reached a size sufficient for analysis (about 5 cm in length) within 50-60 days of hatching. Animals less than 40 days of age do not express isozyme patterns clearly enough to be of use. When raised together, animals were generally smaller and had to be raised longer before sampling. In addition, mortality was higher and we wanted to avoid possible distortion of the ratios resulting from our inability to determine the genotypes of animals that died early.

Samples of tail muscle were collected by cutting off the tip of the animal's tail with scissors. To the tissue, 3 volumes of ice-cold Triton solution was added (1:200 v/v Triton, 2.5% glycerol in water) after which it was homogenized, centrifuged, and the supernatant frozen.

PGM was studied by IEF on a slab gel electrophoresis apparatus, according to the procedures of Ames and Nikaido (1976). A 5% polyacrylamide gel was used with a 2%

ampholyte concentration made up of 0.6 ml pH 5/7 and 0.15 ml pH 3/10 solution to set up a pH gradient of 4.5 to 7.5. 20 mM NaOH and 10 mM H_3PO_4 were the electrolytes of the cathode and the anode respectively. After a one hour prerun, 10 ul samples were injected directly onto the gel which was then run at 260 volts for 4.5 hours at 4° C. Gels were stained using the recipe in Shaw and Prasad (1970).

EST isozymes were run on a 12% polyacrylamide gel buffered with Tris borate EDTA, pH 8.9. After a one hour prerun, samples were injected onto the gel and run at 400 volts for 2 hours. Staining was according to Shaw and Prasad (1970).

MDH was tested on 30 randomly chosen animals. Samples were run on PAGE buffered with Tris borate EDTA, at 300 volts for 2.5 hours. Resolution was better with IEF using the same conditions as for PGM. Both types of gels showed two zones of activity for every animal tested. The staining recipe was obtained from Shaw and Prasad (1970).

A normal acrylamide gel buffered as in the EST system, but run for 6 hours at 400 volts, was used to test 35 animals for LDH activity. Further resolution was possible using IEF under the same running conditions as for PGM. Both types of gels were stained using Shaw and Prasad's recipe (1970).

NOMENCLATURE

Isozymes generated by more than one locus were numbered assigning the number 1 to that with the highest velocity of migration toward the anode. For molecular forms generated by multiple alleles (allozymes), that migrating fastest toward the anode was designated a and the following b etc., in order of decreasing anodal mobility, with a "silent" or null allele being designated o.

RESULTS AND DISCUSSION

Phosphoglucomutase (E.C.2.7.5.1.)

Three phenotypes, A, AB, B and were found among animals in the colony (Figure 1). Data from crosses of the various phenotypes are shown in Table 1. From these data we have postulated a single locus model where the 2 Pgm alleles (a and b) are expressed codominantly, each giving rise to one band on the gel. Crosses between two A or two B animals genetically homozygous (ie. either aa or bb) produced progeny phenotypically identical with the parents. Other crosses produced phenotypic ratios which were consistent with the model but all of which segregated fewer B animals than expected. This suggests that the allele b might have a

detrimental effect on survival. At the time of writing, there were only 14 B animals in the colony out of 214 tested. There were, however, 95 heterozygotes and the distribution is not significantly different from that expected for a Hardy-Weinberg equilibrium based on a gene frequency of 0.29 for the b allele.

Table 1: Phosphoglucomutase Normal Spawning Data

Parents	Progeny Observed Ratio (#) aa	ab	bb	Expected ratio	X^2
ab x ab	23	53	17	1:2:1	2.52
ab x bb	0	36	39	0:1:1	0.12
ab x aa	15	22	0	1:1:0	1.32
aa x aa	80	0	0	1:0:0	n.a.
aa x bb	0	82	0	0:1:0	n.a.
bb x bb	0	0	17	0:0:1	n.a.

The above data does not include the spawning results from 84-1 or 84-4.

Note

84's x aa	6	13	0	0:1:0
84's x ab	12	17	12	0:1:1
84's x bb	0	0	17	0:0:1

Two sibling animals, 84-1 and 84-4, presented anomalous results when their progeny were tested for PGM isozymes. Both appeared to be genotype bb but they also carry 2 recessive lethal genes (c and t) and several of the colour mutations. When crossed with aa animals, the model predicts that only AB animals should result. However, 6 out of the 19 larvae produced by such crosses were phenotypically A. The results of crosses between the 84's and AB animals differ in like manner from our expectation. The model predicts that these larvae would be one half AB and one half B, but a significant number of A progeny were also obtained. These unexpected A progeny can be explained if we postulate that the 84's carry a null allele, o, not expressed on the gel, and are thus genotypically bo rather than bb. The "A" progeny are, therefore, ao. Aside from these two animals, we have no evidence of other animals in the colony carrying the proposed null allele.

The results of uniparental spawnings bear out the codominance model. Homozygotes produced progeny of the same

phenotypes as themselves, while heterozygotes produced some progeny of all types. Homozygous gynogenetic progeny of a heterozygote (ab) would result from normal segregation of chromosomes during meiosis while the ab progeny would result from crossing over. Neither 84-1 nor 84-4 were used to produce uniparental larvae.

Soluble Esterases (E.C.3.1.1.)

Esterase gels displayed 4 zones of activity, each of which may be under separate genetic control. However, variation was seen only for the second band from the origin (Est-3), which was either present or absent (Figure 1). We propose that this may be accounted for by the existance of a null allele, such that those with the band present (phenotype A) are genotypically aa or ao and those without (phenotype 0) are oo. Phenotype A animals were spawned and their progeny tested in order to determine the actual genotype of the animal. As shown in Table 2, the results for all spawnings tested for esterase were not statistically significantly different from those predicted by such a model. Crosses between homozygous recessive parents produced only type 0 progeny while only type A progeny were produced by crosses between homozygous dominant parents. Crosses involving heterozygotes resulted in the appropriate proportion of type 0 progeny.

Table 2: Esterase Normal Spawning Data

Parents	Offspring			
	Observed ratio (#)			
	Type A	Type O	Expected Ratio	X^2
aa x aa	47	0	1:0	n.a.
ao x oo	40	40	1:1	0.0
ao x aa	43	0	1:0	n.a.
aa x oo	58	0	1:0	n.a.
oo x oo	0	32	0:1	n.a.
ao x ao	57	21	3:1	0.15

Since the PGM genotype frequencies approximated a Hardy-Weinberg equilibrilum distribution, we can calculate the frequency of the EST null allele o from the frequency of homozygous recessive O animals, if we assume that EST is also at equilibrium. At the time of writing, there were 73 O animals amongst the 200 axolotls which had been checked,

giving a frequency of 0.6 for the allele o.

The results of uniparental spawnings were consistent with this model. Homozygotes produced progeny which were the same phenotype as themselves, while heterozygotes produced both phenotypes. Since the type A larvae include both the aa and ao animals (the latter resulting from crossing over), the numbers of type A progeny are greater than the numbers of type 0.

LDH (E.C.1.1.1.27.)

As seen by conventional electrophoresis in most vertebrates, LDH isozymes occur in five molecular forms. Each form is a tetramer of two structurally distinct subunits, A and B, which assemble in all possible combinations to form five isozymes, identified as LDH-1 to LDH-5. LDH-1 is the most anodal and is found in rapidly respiring tissure, such as heart muscle. LDH-5 on the other hand, is the most cathodal and is found in tissues such as skeletal muscle. When we used a normal acrylamide gel to test for isozyme variation, a rather puzzling 8 banded pattern was obtained. Further bands were resolved by IEF. However, the basic pattern seemed to be one of 5 bands corresponding to LDH-1 to LDH-5 with each of these accompanied by 2 "satellite" bands, all of which were not visible on the normal gels. LDH-5 was the predominant band, as expected for skeletal muscle. The pattern was reminiscent of that obtained by Brahma and van der Saag (1974) who reported single satellite bands for all but LDH-1. Their Figure 1, however, suggests a second satellite for LDH-3 and LDH-5. In total 35 animals were tested but no variation in the pattern was seen.

Mapping

Data from progeny of uniparental spawnings can be used to map the distance between a gene and its kinetochore or centromere. The number of heterozygotes resulting from the uniparental spawning of a heterozygote depends on the distance of the gene from the centromere. In the case of Pgm 26 out of 57 larvae (45%) were heterozygous. Using the mapping function of Armstrong (1983), it is possible to calculate that Pgm is 23.6 map units from its centromere.

For Est, the homozygous dominant larvae produced from a gynogenetic spawning of a heterozygote are indistinguishable electrophoretically from the heterozygotes. However, it is possible to estimate the number of ao' s by assuming that the number of homozygous dominants equal the number of homozygous recessives (Armstrong, 1983). Of the 48 larvae produced gynogenetically from ao parents, 38 were type A while 10 were type 0. Using our assumption, we arrive at

the estimate that 28 of the type A larvae were heterozygous, or 58%. This is not significantly different from the value of 67% heterozygosity that is obtained for a gene distant from its centromere. Under these circumstances a precise map distance cannot be determined. No linkage has yet been found between either of these isozyme markers and any of the other genes known to be found in our colony.

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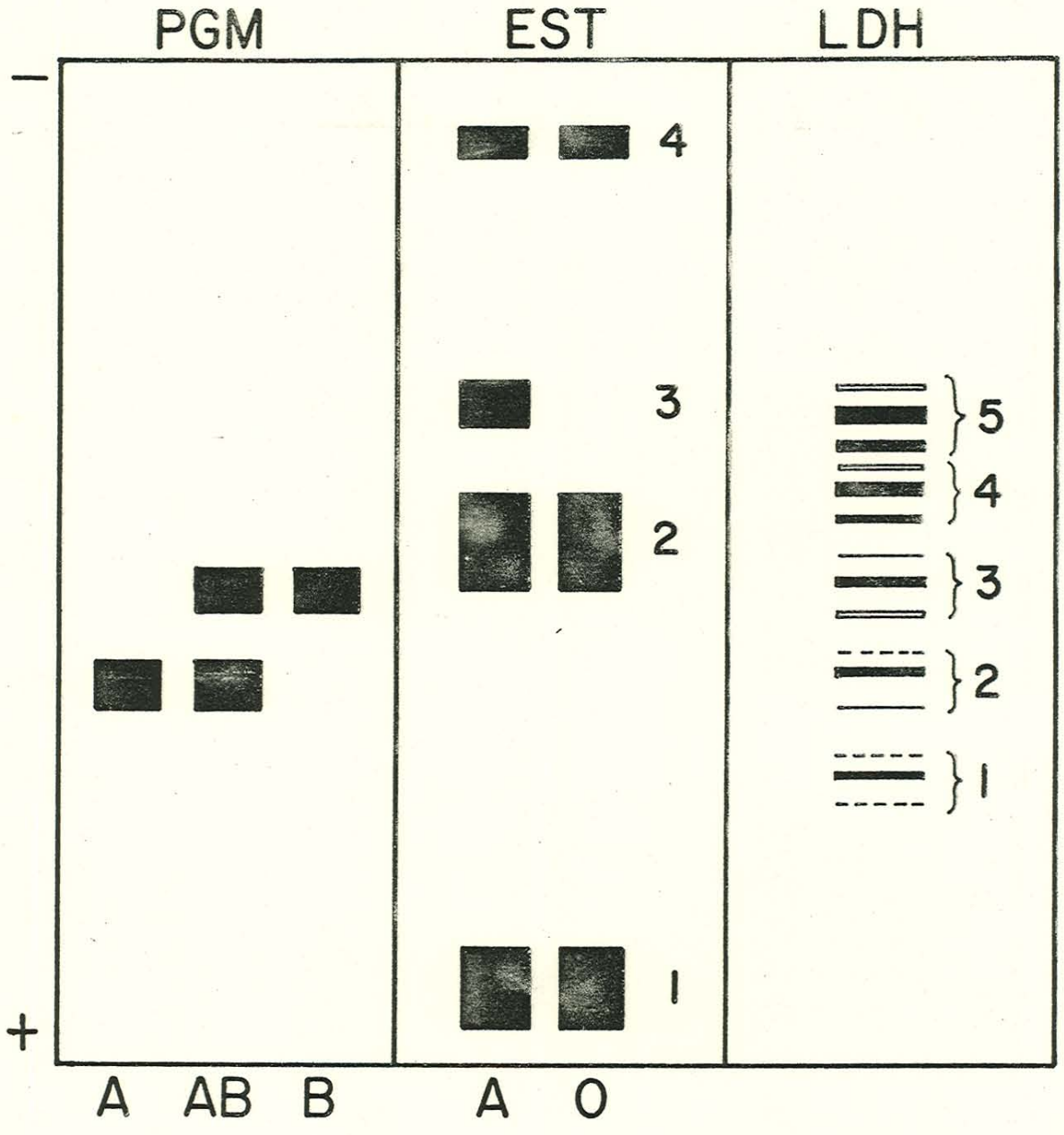

Figure 1