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INTRASPECIFIC VARIATION OF ISOZYMES IN *ARENICOLA MARINA* (POLYCHAETA:ANNELIDA)

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ABSTRACT: Six enzyme systems, namely acid phosphatase, leucine aminopeptidase, phosphoglucose isomerase, tetrazolium oxidase, esterases and malate dehydrogenase were studied electrophoretically in *Arenicola marina* from various localities in United Kingdom. Out of 13 presumed loci, ten were found monomorphic. The three loci which appeared to be polymorphic are LAP-1, EST-2 and TO-1. Due to small sample size allele frequencies and genetic identity were not calculated. However, results indicate genetic difference among the populations of *A. marina*.

KEY WORDS: *Arenicola marina* - Polychaeta - isozyme - electrophoresis

INTRODUCTION

The lugworm *Arenicola marina* is a sedentary polychaete and inhabits the tidal zone where it burrows in sandy beaches. These sandy beaches may be separated by stretches of unsuitable coast possibly causing local isolation. The lugworm is neither an active swimmer nor an energetic traveller at any stage of its life-history. It lives most of its life in the same burrow (Wells, 1957). It has no pelagic larval phase and therefore the larval dispersal is also limited (Newell, 1948). Genetic differentiation between populations of such species is, therefore, not unexpected.

The present study was initiated to investigate the genetic variation of geographically separated populations of *A. marina*, as shown by isozyme variation. Study of lugworm isozymes by electrophoresis has hitherto not been reported.

MATERIALS AND METHODS

Samples of *A. marina* were obtained from six different localities in the United Kingdom (Fig.1). All samples were collected in the summer of 1980, except those from the Plymouth locality, which was collected in winter, 1980. Live worms were kept in a tank with sand and seawater at 10-15°C. These tanks were provided with circulating artificial seawater. Worms were used for preparing samples within two weeks of collection.

SAMPLE PREPARATION:

Each worm, with the gut cleared, was relaxed by adding magnesium chloride crystals to 7% and then dissected in ice-cold seawater. The body wall was cut open and the coelomic cavity was washed thoroughly with ice-cold seawater to remove any developing gametes. Worms were blotted dry and weighed. Each worm with an

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equal amount of homogenisation mixture (consisting of 40% sucrose in electrode buffer, 0.05 M tris, 0.38 M glycine, pH 8.5) was added. After that, the worm was homogenised in a tissue grinder. The tissue grinder was kept immersed in ice-cold water during homogenisation to avoid heating due to friction. The resulting homogenate was spun in a refrigerated centrifuge at 0-4°C for 90 minutes at 28,000 g. The clear supernatant was removed and aliquots were stored at -20°C until required for electrophoresis.

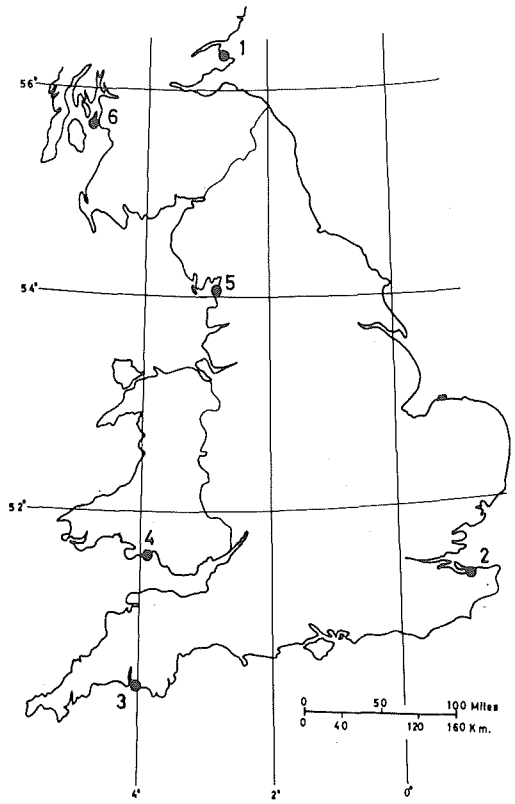

Fig.1. Map showing locations of *A. marina* populations sampled; 1.St. Andrews, 2.Whitstable, 3.Plymouth, 4.Swansea, 5.Lancaster and 6.Millport.

ELECTROPHORESIS:

Electrophoresis was performed at 8-10°C for $3\frac{1}{2}$ and $4\frac{1}{2}$ hours for 7.5% and 15% acrylamide gels, respectively. The 15% acrylamide gel was used for acid phosphatase whereas 7.5% gel was used for other enzymes. Stock and working solutions for setting polyacrylamide gel (small pore) of pH 8.5 were prepared according to the procedure described in the operating instructions of analytical polyacrylamide electrophoresis apparatus, supplied by Shandon Scientific Company Ltd. (London). The Pharmacia vertical slab gel electrophoresis apparatus GE-4 was used in the presenty study. The electrode buffer used was 28.8 g glycine, 6 g tris, made up to 1 litre with water (pH 8.5). Usually 5-10 µl samples were applied to the gel and after the run completed, gels were sliced into three slices by using a gel slicer and a slicing frame. These slices were used for three different enzyme assays.

Two internal standards, horese-heart myoglobin (Type III Sigma) and horse-spleen ferritin (Type I Sigma) were used (Johnson, 1971). The ferritin and myoglobin ratio on 7.5% gel was 5.25±0.18 (S.D.). No measurements were made on gels for which this ratio was outside the range of 5.1 - 5.4. In 15% gel, only myoglobin was run as the standard protein. Gels were stained for acid phosphatase (ACP), leucine aminopeptidase (LAP), phosphoglucose isomerase (PGI), tetrazolium oxidase (TO), esterases (EST) and malate dehydrogenase (MDH). Staining solutions were prepared according to Shaw and Koen (1968) for ACP, LAP, TO and EST and according to Marcus (1977) for MDH and PGI.

RESULTS

The results of the six enzyme assays gave rise to thirteen zones of activity. Presumed loci were assigned an abbreviation for the enzyme name and number, in order of decreasing mobility, if more than one zone (locus) was present. Presumed alleles at a locus were labelled by assigning a value of 100 to the myoglobin (internal standard) and then computing the mobility difference of each appropriate band variant, that is, an allele which migrated 10 mm less than the myoglobin was labelled 90.

Malate dehydrogenase and phosphoglucose isomerase were monomorphic in all the six samples of 33 individuals.

Acid phosphatase was identified as three zones of activity, each exhibiting a single band. The slowest band (ACP-3) stained heavily whereas the two faster bands (ACP-2 and ACP-1) stained lightly. The pattern was assumed to represent monomorphism at the three loci. These loci were designated as ACP-3^{94}, ACP-2^{108}, ACP-1^{112}.

Leucine aminopeptidase (Fig.2) exhibited a complex pattern and could not be analyzed with confidence, especially due to small sample size. The two slow bands LAP-3 and LAP-2 were detected only in one sample of six worms from St. Andrews locality. These two bands presumeably represent monomorphism at the two loci, LAP-3^{105} and LAP-2^{112}. The other five samples of 27 individuals showed no enzyme activity at these two loci. The fast zone (LAP-1) exhibited great variability. The sample from Lancaster (n=4) showed two bands, LAP-1^{117} and LAP-1^{120}, whereas samples from Millport (n=4), Plymouth (n=8) and Whitstable (n=6) showed one band, LAP-1^{120}. The sample from St. Andrews exhibited intrapopulation difference with respect to this zone. Out of six individuals three had LAP-1^{120} band while the other three lacked it. The sample from Swansea of five individuals exhibited an entirely different zymogram consisting of a single band. This band was not sharp and due to diffusion it was not possible to measure the mobility precisely. The average mobility for five worms, was 108±0.7 (S.D.).

Esterase activity was detected at two zones (Fig.3). The slow zone (EST-2) exhibited a single band but with different mobilities. Thes most common band was EST-2^{94} and this was observed in 21 individuals from Lancaster, Swansea, Whitstable and St. Andrews populations, whereas Plymouth and Millport samples of 12 individuals had EST-2^{92}. The fast zone (EST-1) consisted of a single band, stained heavily, in all the samples of 33 individuals. This was presumed as a single monomorphic locus and designated as EST-1^{130}.

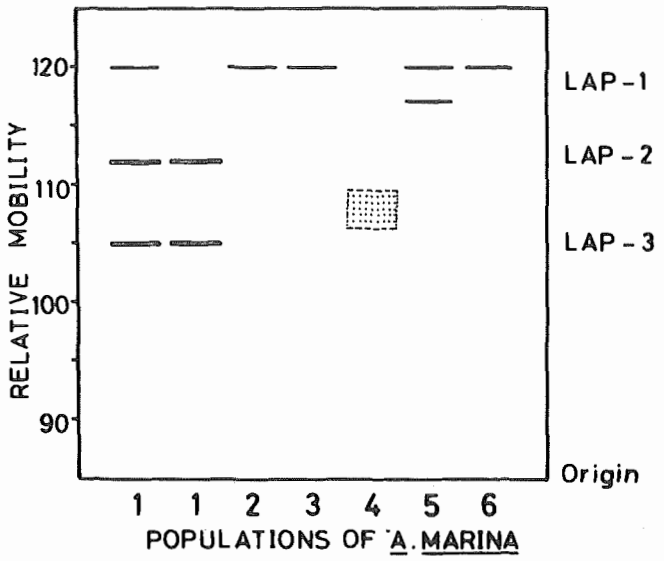

Fig.2. Diagram of leucine aminopeptidase zymogram of different populations of *A. marina*: 1.St.Andrews, 2.Whitstable, 3.Plymouth, 4.Swansea, 5.Lancaster and 6.Millport.

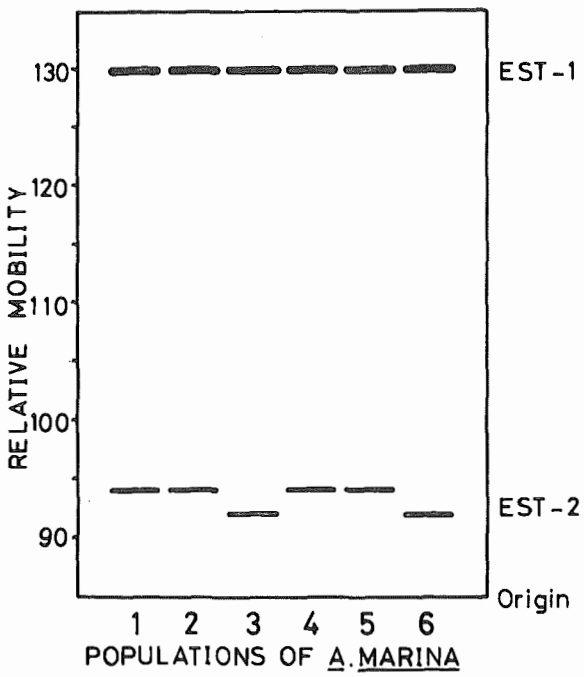

Fig.3. Diagram of esterase zymogram of different populations of *A. marina*: 1.St. Andrews, 2. Whitstable, 3.Plymouth, 4.Swansea, 5.Lancaster and 6.Millport.

Tetrazolium oxidase activity was present at three zones (Fig.4), each exhibiting a single band. Within the slow zone (TO-3) a single band TO-3^{92} was observed only in Whitstable population and also in two, out of four individuals from Lancaster population. Other samples lacked this locus. The fast zone (TO-1) was observed only in those six worms which were collected from Whitstable locality. There were two presumed alleles at this locus. Three worms had TO-1^{116} and the other three had TO-1^{119}. No heterozygote was observed. The mid zone (TO-2) exhibited a single band which stained heavily. This locus TO-2^{103} was observed in all the samples of 33 individuals.

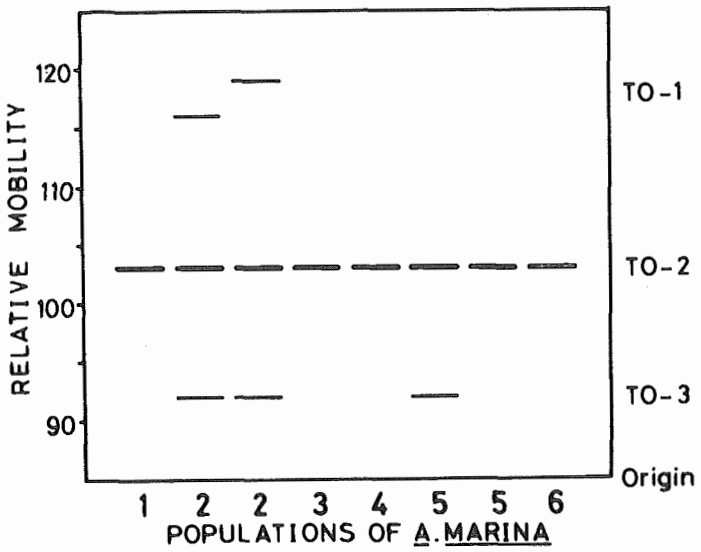

Fig.4. Diagram of tetrazolium oxidase zymogram of different populations of *A. marina*: 1.St. Andrews, 2.Whitstable, 3.Plymouth, 4.Swansea, 5.Lancaster and 6.Millport.

DISCUSSION

The results presented here are based on small samples. Hence, the allele frequencies or genetic identity were not calculated. The main objective of this work was to provide some preliminary information about enzyme electrophoresis in *A. marina* and the results indicate that a good deal of genetic variability occurs among the individuals of the same population as well as among different populations. All the enzyme systems studied here have frequently been utilized in taxonomic studies of marine invertebrates (Chambers, 1978; Gooch and Schopf, 1970, 1971; Grassle and Grassle, 1974, 1976; Marcus, 1977; Turner and Lyerla, 1980 and Mustaquim, 1988).

A total of 13 presumed loci were detected, of these 10 loci were monomorphic. The three loci which appeared to be polymorphic are; LAP-1, EST-2 and TO-1. However, no heterozygote was observed, which may be attributed to the small sample size.

The sample of St. Andrews and Whitstable populations exhibited greater variability. The two monomorphic loci; LAP-3 and LAP-2 were observed only in St. Andrews population while populations from other localities lacked these. Similarly, TO-1 locus was observed only in those worms which were collected from Whitstable, whereas all other worms lacked this. It is well known that a variety of factors other than genetic polymorphism can result in band variation. However, single-locus genetic polymorphism can be distinguished from other sources of band variation by analysing a large number of individuals. If banding pattern is consistent with the suspected quaternary structure for the enzyme (that is a two-banded heterozygote for monmeric enzyme, and three-banded heterozgote with 1:2:1 staining intensity for dimers etc) and if the genotype frequencies are in approximate to Hardy-Weinberg expected proportion, at least to the extent that genotypes expected to be in high frequency are not totally absent, then the banding variation could be attributed to the genetic polymorphism. These two criteria were suggested by Selander *et al.* (1970) and have gained general acceptance among population geneticists. *A. marina* can be collected in large numbers from suitable beaches. Further light could be thrown on the problem by more extensive study of electrophoretically separable isozymes.

This priliminary study has, however, established that, the technique of gel electrophoresis can be of some utility in studies of genetic variation of lugworm. While further studies are clearly needed, the results presented here indicate genetic difference among local populations of *A. marina*.

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