THE USE OF ELECTROPHORESIS IN SPONGE TAXONOMY

A.M. SOLE-CAVA & J.P. THORPE

Department of Marine Biology
The University of Liverpool, Marine Biological Station
Port Erin - Isle of Man - U.K.

SYNOPSIS

Enzyme electrophoresis detects products of individual gene loci and allows the calculation of gene frequencies and the estimation of exchange rates between populations. Speciation and subsequent evolution result in genetic divergence between populations and can therefore be studied. Electrophoresis has been used to distinguish sibling species and to establish the taxonomic status of dubious sub-species and colourmorphs of a wide range of organisms. The Porifera are a taxonomically difficult group, offering many possible applications for such methods. Preliminary results of electrophoretic studies on sponges are discussed and used to evaluate the potential for future work.

INTRODUCTION

The advancement of knowledge in science is achieved, principally, by the use of well established techniques to solve new problems or by the use of new techniques to solve old problems. Originally, the biological and genetic uses of enzyme electrophoresis were typical examples of the second case : after the association between gel electrophoresis and histochemical stains (Hunter & Markert, 1957), geneticists realised the potential of the technique to study the genetic structure of natural populations and the first studies, mainly on man and *Drosophila*, were published in the mid-sixties (e.g. Hubby, 1963 ; Hubby & Lewontin, 1966 ; Johnson *et al.*, 1966 ; Lewontin & Hubby, 1966 ; Harris, 1966). These works, which gave the first really useful estimates of levels of genetic variation in natural popula-

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tions, had a major impact on the evolutionary ideas of the time (for reviews see e.g. Dobzhansky, 1970 ; Lewontin, 1974 ; Dobzhansky *et al.*, 1977). Since then, biochemical genetics has been successfully used in a variety of fields such as the identification of juvenile stages of fish (e.g. Mork *et al.*, 1983), the identification of stocks for aquaculture (e.g. Moav *et al.*, 1976 ; Cruz *et al.*, 1982), physiological ecology (e.g. Zouros *et al.*, 1980 ; Beaumont *et al.*, 1985 ; Mallet *et al.*, 1986) and the use of genetic markers for fisheries (e.g. Simonarsen & Watts, 1969 ; Child, 1984).

Thus, it can safely be said that enzyme electrophoresis has revolutionised evolutionary and ecological genetics. Furthermore, this technique has transcended the field of pure genetics and also become a very important tool for the study of taxonomy and population dynamics. In taxonomy it has helped to identify and separate dubious or sibling species in a variety of groups (reviewed in Avise, 1974 ; Gotlieb, 1977 ; Ferguson, 1980 ; Ayala, 1983 ; Berlocher, 1984 ; Innes, 1984), and also provided the data for phylogenetic reconstructions of taxonomic groups, the later based on the "molecular clock hypothesis" (see e.g. Fitch, 1973, 1976 ; Wilson *et al.*, 1977 ; Vawter *et al.*, 1980 ; Thorpe, 1982, 1983 ; Avise, 1983).

THE TECHNIQUE

Electrophoresis is the migration, in response to an electric field, of electrically charged molecules through a solid or liquid medium. The rate of migration of these molecules is influenced mainly by the net charge and molecular weight. This property is used to separate proteins, the separation being regulated by the use of buffers with specific pH and of different support media, such as cellulose acetate, starch and polyacrylamide gels. The proteins (including enzymes) thus separated can then be stained by specific staining mixtures, and the banding patterns obtained interpreted genetically. For a full review of the techniques, see Brewer (1970), Harris and Hopkinson (1978), Ferguson (1980) and Gaal *et al.* (1980).

The interpretation of the banding patterns depends partially upon the knowledge of how different enzymes, with different tertiary structures, may be expected to appear in the gel for samples from different homozygous and heterozygous individuals. Monomeric enzymes, for example, will appear as two bands in heterozygotes, whereas dimeric enzymes will present three bands

(Fig.1). For further explanation of banding patterns see for example Harris & Hopkinson (1978), Ferguson (1980).

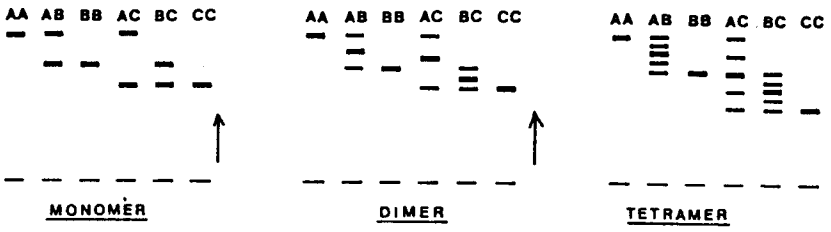
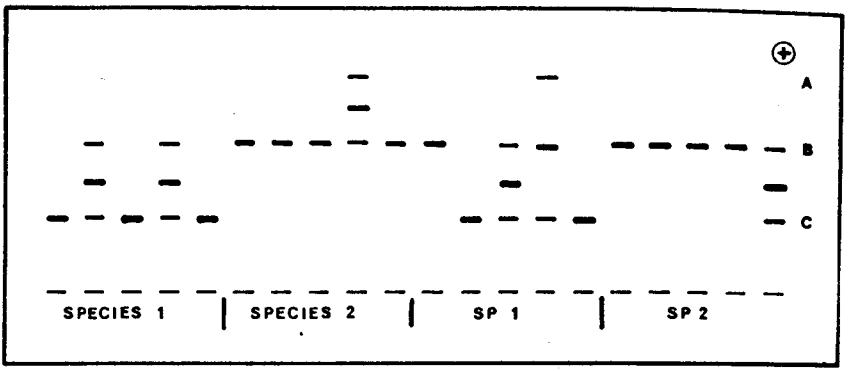


Figure 1 - Typical banding patterns for monomeric, dimeric and tetrameric enzymes. *AA*, *AB*, *BB*, etc. are the genotypes of the individuals for the given enzymes. The arrows indicate the direction of migration.

After genotype frequencies have been obtained from the banding patterns, gene frequencies can be calculated for each locus (Fig. 2) , and the populations can be compared by the use of genetic identity (Nei, 1972) or similarity (Thorpe, 1979) indices. Other parameters, such as the mean levels of polymorphism and heterozygosity can also be calculated, providing an insight into the population structure and biology of the species.

APPLICATIONS

Electrophoresis can be employed for various genetic, zoological and ecological purposes (Fig. 3). The majority of the biochemical genetic studies have been carried out on terrestrial animals, principally vertebrates (see Ward, 1978 and references therein) and insects (Berlocher, 1984). This is understandable since we are terrestrial animals, and our first interests lie in understanding phylogenetically close or economically important animals. For the same reasons, the marine animals most studied genetically are fish (reviewed by Smith & Fujio, 1982) and commercially important crustaceans (reviewed by Nelson & Hedgecock, 1980) and molluscs (e.g. Koehn & Gaffney, 1984 ; Beaumont *et al.*, 1985). Other marine invertebrates are less well studied, however, and only now is a significant amount of work starting to be produced on such organisms.



	Species 1	Species 2
AA	0	0
AB	0	1
BB	1	8
AC	1	0
BC	3	1
CC	5	0
Total	10	10

	Species 1	Species 2
fA	0.05	0.05
fB	0.25	0.90
fC	0.70	0.05
H_o	0.40	0.20
H_e	0.45	0.19

Figure 2 - A putative gel for the analysis of 10 individuals from two populations, belonging to two different species analysed for a dimeric enzyme. *A*, *B* and *C* are the three alleles studied. The genotype and allele frequencies for both populations are presented.

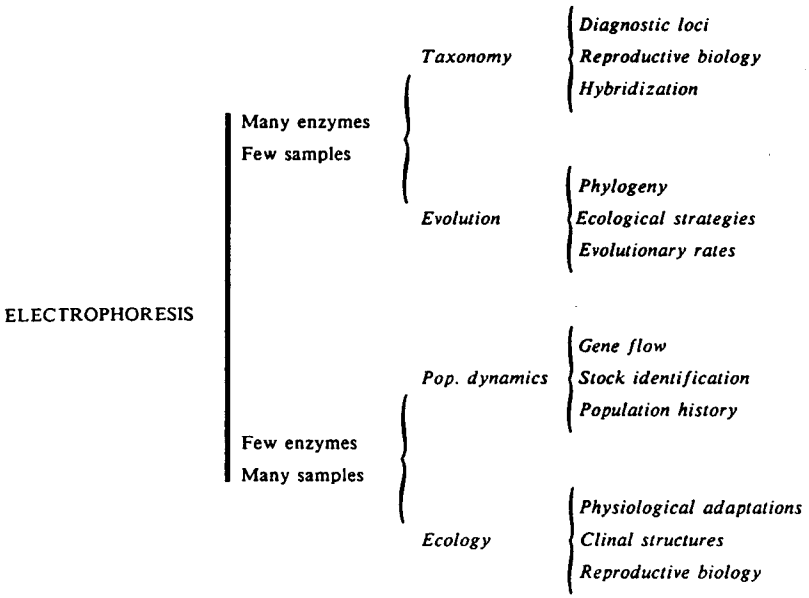


Figure 3 - Different possible uses of enzyme electrophoresis in biology.

Among marine invertebrates, benthic species have received a moderate amount of attention. These are of interest because they include many "primitive" organisms, which frequently show unusual lifestyles and very often also present rather complicated taxonomic problems. Studies published so far include organisms such as sipunculids (Balakirev & Manchenko, 1983), tropical bivalve molluscs (Ayala *et al.*, 1973 ; Campbell *et al.*, 1975), gastropods (Snyder & Gooch, 1973 ; Gresham & Tracey, 1975 ; Johnson & Black, 1984) nudibranchs (Havenhand *et al.*, 1986), phoronids (Ayala *et al.*, 1974), brachiopods (Valentine & Ayala, 1974 ; Hammond & Poiner, 1984), pycnogonids (King *et al.*, 1986), bryozoans (Thorpe *et al.*, 1978 ; Thorpe & Ryland, 1979), polychaetes (Beckitt, 1980 ; Guérin & Kerambrun, 1983), echinoderms (Ayala *et al.*, 1975 ; Marcus, 1977 ; Bisol *et al.*, 1984), tunicates (Schimdtke *et al.*, 1977 ; Fisher *et al.*, 1980) and coelenterates (McCommas & Lester, 1980 ; Carter & Thorpe, 1981 ; Solé-Cava *et al.*, 1985).

Despite their great ecological importance as major components of the benthic fauna of temperate and tropical seas, sponges have been largely

overlooked in biochemical genetics studies. This is particularly surprising given the highly problematic nature of sponge taxonomy. One of the major problems for taxonomists is to find characters which are at the same time conservative enough to be stable in the species, and variable enough to diverge following speciation events as shared derived (synapomorphic) characters. In animals like sponges, the number of different morphological characters which can be analysed is rather restricted, and taxonomists are often faced with the problem of having to choose not the characters which seem to be the most significant biologically, but, instead, characters which are the only ones available. In the case of sponges the skeletal structures are the most commonly used taxonomic features because these are often the only pratically useful part which remains in the common, "dry" collections in museums. This situation is, obviously, far from ideal, and several new techniques such as chemical (Bergquist *et al.*, 1980 ; Bergquist & Wells, 1983) and immunological (Van de Vyver, 1971 ; Connes *et al.*, 1974 ; Neigel & Avise, 1983) taxonomy have been tried with varying degrees of success. These techniques produce valuable information about the overall similarity between species or higher taxonomic ranks, and should be used, together with morphological, reproductive, cytological and ecological data to try to achieve more biologically meaningful schemes of classification. However, both chemical taxonomy and immunological taxonomy suffer from the same major drawback : they do not lead to any clear genetic interpretation of differences between taxa. It is obvious that, ultimately, almost all characters will have a genetic origin, which is modified in varying degrees by the environment. For example, new secondary metabolites, produced *de novo* by the sponge require specific enzymes (and hence specific genes) to cause their synthesis. Similarly, the strength of an immunological reaction is related to the molecular divergence in the primary structure of the antigen (again, genetically determined). However, because with such results it is generally completely unknown what proportions of the observed differences are genetically or environmentally determined, such results must be interpreted with extreme caution.

Enzyme electrophoresis produces results which can be genetically interpreted and, therefore, allows a high level of objectivity in the study of natural populations. The biological definition of species (Mayr, 1963 ; Genermont & Lamotte, 1980) is operationally useful, especially for the comparison of sympatric populations. When biochemical taxonomic methods are associated with the biological species concept, they become very powerful.

for they produce results which are generally less open to arguments and which contribute, consequently, to a more stable classification than that obtained by more conventional morphological criteria. In other cases, however, electrophoretic studies can contribute little to the solution of specific problems. It is important, therefore, that the limitations and advantages of the technique be understood, in order to avoid the creation of false hopes and, with them, of possible frustrations for the sponge taxonomist.

Limitations

1/ Preservation of the material — Enzymes are proteins, and as such are very sensitive to the conditions of preservation. Therefore, studies of biochemical genetics have to be restricted to fresh or well (and freshly) frozen samples. This is the major limitation of the technique, since this means that alcohol fixed material, museum collections and fossils cannot be studied by enzyme electrophoresis. Even with frozen samples, special care must be taken. Artifact bands have been shown to be produced by the repeated freezing and thawing of samples (Scozzani *et al.*, 1980). In any case, all samples to be analysed electrophoretically should be preserved in the same way and it is always preferable that they should be fresh. By using fresh samples potentially misleading artifacts can be avoided or easily detected ; also levels of enzyme activity are likely to be generally higher in fresh material.

2/ Cost — The price of setting up an electrophoresis laboratory can be prohibitive to some zoology departments. However, the running costs are not so high if the quantity and discriminatory power of the data which can be obtained are taken into account. Typically, an electrophoresis laboratory should be able to run four gels simultaneously, which would mean that up to sixty to eighty individuals could be analysed for about twenty different enzymes (for slices per gel) in one week (five working days). Considering that sample sizes of twenty are normally sufficient for most work in biochemical taxonomy (Gorman & Renzi, 1979), electrophoresis can actually be highly cost-effective when compared to the many samples and large amounts of time required to carry out a comprehensive morphological study.

3/ Small sample of the genome — Commonly in biochemical taxonomy about ten

to thirty enzyme loci are analysed. This is a very small number if compared with the total number of structural gene loci in one organism. This means that, although the technique is decisive about differences between populations, it is not so for similarities between them. In other words, if two sympatric populations show significant differences in allele frequencies at one or more of the loci analysed it can be positively said that they are reproductively isolated (i.e. they belong to different species), whereas if those populations show no differences at any of the enzyme loci analysed this does not provide conclusive evidence that they are interbreeding and therefore conspecific, since the two populations concerned may simply not have diverged at any of the loci studied.

4/ **Small amount of enzymes in the tissues** — Many sponges have tissues containing large amounts of siliceous or calcareous spicules. This means that the actual amount of biomass per unit of weight is effectively much smaller than in most of the other soft-bodied organisms ; also sponges generally are not highly active and therefore will probably have lower metabolic rates than many higher animals. An obvious consequence is that the enzymes will be present at low concentrations, and many will be difficult to stain after electrophoresis. There is no simple solution to this problem. Using fresh samples, homogenizing with a minimum amount of buffer and increasing the concentration of the staining reagents can be effective sometimes, and experimenting is the only way of finding the most appropriate methodology for each particular case.

Advantages

Some advantages of the technique, such as the objectivity and the production of direct genetic information have already been stated. However, three other important advantages of electrophoresis should be mentioned :

1/ **Unbiased results** — The major criterion governing the choice of enzymes for electrophoretic work is availability, that is, whether the reagents for the staining of the enzymes are available in the laboratory or whether the animals show any detectable activity for the enzymes assayed. This means that the results obtained are likely to be unbiased and therefore can be analysed in terms of statistical probability. For example, if two populations do not show any differences at twenty enzyme loci it can be said that

these populations are probably similar over more than 95 % of their structural genome.

2/ Analogy implies homology — Most enzyme loci studied belong to key biochemical pathways. Therefore, the possibility that a given enzyme locus has been replaced by another, different locus in the course of evolution is extremely unlikely (Avise, 1974).

3/ Easy detection of unusual reproductive strategies — Sponges can present unusual life strategies, such as asexual reproduction (Korotkova, 1979 ; Bergquist, 1980) and larval fusion (Fry, 1971). By means of electrophoretic methods, the genetic structure of populations can be easily analysed, and the extend (if any) of asexual reproduction or fusion estimated. In sea-anemones, for example, electrophoresis has been used to show that the small "brooding" anemones found in the coelenteron of their parents had been produced asexually (Ottaway & Kirby, 1975 ; Orr *et al.*, 1982). In three species of *Suberites* enzyme electrophoretic data indicated that the incidences of both allogenetic fusion and asexual reproduction were likely to be negligible or zero (Solé-Cava & Thorpe, 1986).

Biochemical polymorphism and sponge taxonomy

The first published studies on the enzyme electrophoresis of sponges were basically concerned with the description of banding patterns, without any attempt to interpret the results genetically (Pakhomov *et al.*, 1974 ; Schoots *et al.*, 1977 ; Baden & Corbett, 1979 ; Urbaneja & Lin, 1981). The first work to use elctrophoresis as a tool in sponge taxonomy was that of Connes *et al.* (1974), who used stains only for total (soluble) proteins. These give banding patterns which are pratically impossible to interpret genetically. Enzyme biochemical genetic studies have started only very recently in sponges (Balakirev & Manchenko, 1985 ; Sarà, in press ; Solé-Cava & Thorpe, 1986, in press). The overall pattern apparently emerging from these few published studies (table 1) is that sponges seem to have very high levels of genetic variation (about three times larger than the average for vertebrates), in a similar way to coelenterates (see e.g. Bucklin, 1985 ; Solé-Cava *et al.*, 1985 ; Solé-Cava, 1986). The reasons for this are still unclear, but it is not unreasonable to suppose that these results are probably related to the particular, sessile lifestyle of these organisms or to

their very old evolutionary history (Solé-Cava, 1986 ; Solé-Cava & Thorpe, in press).

Table 1 - Levels of genetic variation in sponges. ni = number of individuals analysed ; nl = number of enzyme loci analysed ; P(0.95) = proportion of polymorphic loci ; Ho = mean observed heterozygosity ; He = mean expected hetrozygosity. References : 1 - Solé-Cava & Thorpe (in press). 2 - Balakirev & Manchenko (1985). 3 - Solé-Cava & Thorpe (1986). 4 - Sarà (in press).

Species	ni	nl	$P_{(0.95)}$	H_o	H_e	Ref
Halichondria panicea	18	16	0.688	0.227	0.234	1
Mycale macilenta	7	18	0.500	0.246	0.189	1
Suberites domuncula	12	28	0.393	0.146	0.137	2
Suberites luridus	13	18	0.667	0.179	0.168	1,3
Suberites pagurorum	11	18	0.611	0.215	0.190	1,3
Suberites rubrus	16	16	0.750	0.365	0.335	1,3
Tethya aurantium	30	8	0.125	?	0.050	4
Tethya citrina	30	8	0.125	?	0.020	4

The great power and usefulness of the technique for taxonomic work in sponges has been shown in the separation of very close, sibling species of *Suberites* from the Irish Sea (Solé-Cava & Thorpe, 1986), and in the identification of sympatric species of *Tethya* (Sarà, in press).

It can be concluded, thus, that electrophoresis can be used successfully in resolving taxonomic problems in sponges, at least for well defined problems, such as the detection of sibling species amongst sympatric populations. It can be used as well for the analysis of the divergence between allopatric populations ; it has been shown (Thorpe, 1982, 1983) that values of genetic identity between different taxonomic levels have a tendency to be similar. For example, more than 98% of intra-specific population comparisons (out of 7000 vertebrate populations) give values of genetic identity (Nei, 1972) above 0.90, whereas only 2% of inter-specific (congeneric) population comparisons (out of 900 vertebrate populations) gave values above 0.85

(Thorpe, 1983). The results for the smaller amount of published data on invertebrates suggest that they follow the same trend (Thorpe, 1982, 1983). This means that if two populations show a genetic identity of 0.80, for example, they are very likely to belong to different species.

Electrophoresis must be used in association with classical taxonomy, particularly for the selection of reliable taxonomic characters and determination of taxonomic status of "morphs", "sub-species" and "species". Taxonomy is an indispensable science, and systems of classification are operationally important structures. Electrophoresis, together with ecology, chemistry and reproductive biology is helping taxonomy to become biologically more meaningful, and this will hopefully lead to a more stable classification of a traditionally problematic group like the Porifera.

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