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High Levels of Genetic Variation in Marine Sponges

Abstract

Levels of genetic polymorphism are estimated for natural populations of the sponges *Halichondria panicea*, *Mycale macilenta*, *Suberites subereus*, and two species of *Suberites "ficus"* from the Irish sea. The animals were analyzed by horizontal starch gel electrophoresis and staining for specific enzymes. A total of 86 enzyme loci covering 18 different enzymes were detected over the five populations studied. Between 50 and 75% of the enzyme loci were polymorphic in each species, and levels of mean heterozygosity varied from 0.168 *(S. ficus)* to 0.335 *(S. subereus)*. These levels are high when compared with values normally obtained for most animals or plants, but compare well with heterozygosities found in marine cnidarians and some mollusks. Possible relationships between levels of genetic variation and random genetic processes or environmental factors are discussed.

A basic premise of the theory of evolution is that genetic variation exists within natural populations. Without this variation there would be little scope for the action of natural selection, genetic drift, or other evolutionary processes. However, the real magnitude of that variation was not fully appreciated until researchers began to use electrophoretic techniques to estimate genetic polymorphism. The main advantage of electrophoresis over other (non-biochemical) techniques in this application is that electrophoresis makes it possible to directly detect variation in products of single chosen gene loci in individual animals or plants (Lewontin, 1974; Ayala, 1976; Ferguson, 1980). Furthermore, samples of populations or groups of individuals can be readily investigated over a substantial number of loci; the estimates obtained are statistically fairly robust and not much affected by the sample size (Nei and Roychoudbury, 1974; Nei, 1978; Gorman and Renzi, 1979). Useful results can therefore be obtained even from uncommon species for which only small sample sizes may be available.

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Data on the levels of genetic variability in natural populations of a wide variety of organisms have been available for many years. A notable feature of findings to date is that, irrespective of the species involved, levels of genetic variability are generally similar, within a limited range, in the vast majority of populations (see reviews, e.g., of Powell, 1975; Selander, 1976; Nevo, 1978; Burton, 1983). Among both animal and plant populations, the proportion of loci polymorphic *(P)* generally falls within the range 0.10–0.50 while mean heterozygosity per locus *($\bar{H}$)* varies from about 0.02 to 0.15. The comprehensive survey by Nevo (1978: table 1) covering 277 populations spread over 243 species of vertebrates, invertebrates, and plants records only four heterozygosity estimates greater than 0.25, the highest being 0.309 (from the bisexual parthenogenic weevil *Otiorrhynchus scaber,* Soumalainen and Saura, 1973). Very low $\bar{H}$ values are uncommon and are (predictably) frequently linked to very low population size (e.g., Selander and Kaufman, 1975; Bonnell and Selander, 1974). However, $\bar{H}$ values greater than 0.3 are considerably rarer, and we know only of three published studies giving such data: the recent work of Ritte and Pashtan (1982) on Red Sea populations of two species of the marine gastropod *Cerithium* (in which $\bar{H}$ values are around 0.6), of Beaumont and Beveridge (1984) on the bivalve *Chlamys distorta* ($\bar{H}$ = 0.321), and of Solé-Cava et al. (1985) on sea anemones of the genus *Urticina* ($\bar{H}$ = 0.41–0.44).

No heterozygosity estimates are available for Porifera; the few electrophoretic studies that have been carried out on sponges to date either do not include a genetic interpretation of the results (e.g., Connes et al., 1974; Urbaneja and Lin, 1981; Hooper, this volume) or use too few loci for meaningful heterozygosity estimates, although the numbers are adequate for biochemical taxonomic comparison (e.g., Sará, this volume).

In this paper we provide estimates of levels of genetic variation in five common species of marine sponge.

Materials and Methods

Halichondria panicea (Pallas) was collected from low in the intertidal zone at Port St. Mary Ledges, Port St. Mary, Isle of Man. *Suberites* cf. *ficus* (Johnston) species *A*, *S.* cf. *ficus* (Johnston) species *B*, *S. subereus* (Johnston), and *Mycale macilenta* (Bowerbank) were collected by dredging at 10–30 m off Port Erin, Isle of Man (northern Irish Sea) by the Liverpool University research vessel *Cuma* (for further information on the three *Suberites* species, see Solé-Cava and Thorpe, 1986).

Samples were analyzed immediately after collection. To avoid diluting the enzymes during extraction, we homogenized the samples without any buffer. Homogenized samples were analyzed by horizontal starch (12.5%) gel electrophoresis. Several buffer systems were tried, but the best results in terms of enzyme activity and resolution were all obtained using a tris-citric acid pH 8.0 system from Ward and Beardmore (1977). (Composition of the electrode buffer was 30.3 g tris + 12.0 g citric acid + 1000 ml distilled water; the gel buffer consisted of 38.5 ml of electrode buffer diluted to 1000 ml with distilled water.)

The staining of the gels followed standard procedures (Shaw and Prasad, 1970; Harris and Hopkinson, 1978). Enzyme nomenclature follows that of Harris and Hopkinson (1978).

All samples were analyzed for 23 enzymes, but no activity could be obtained for octanol dehydrogenase (E.C. 1.1.1.1), aldolase (E.C. 4.1.2.13), leucine aminopeptidase (E.C. 3.4.1.1), superoxide dismutase (E.C. 1.15.1.1), or aconitase (E.C. 4.2.1.3). The other 18 enzymes (Table 1) each gave useful results for at least one of the populations studied.

Observed heterozygosity for each locus was calculated as the proportion of individuals in the sample heterozygous for that locus (i.e., number of heterozygotes/total number of genotypes). Expected heterozygosity per locus was calculated as $1 - \Sigma x^2_{ij}$, where x is the frequency of the i^{th} allele in the locus j. Heterozygosities for each species

Table 1. Calculated heterozygosities for each of the studied loci in five sponge species (na = no activity observed, He = the mean calculated heterozygosity for each species)

Locus	*Halichondria panicaea*	*Mycale macilenta*	*Suberites ficus* A	*Suberites ficus* B	*Suberites subereus*
ak	0.210	na	na	na	na
aldox	0	0.444	0	0.069	0
cat	0.198	0	0.375	0.180	0.451
est.1	na	0	0.219	0.291	0.720
est.2	na	0	0.180	0.204	na
fum	0	0.245	0.219	0	0.117
gdh	na	na	na	na	0.561
got	0	0	0	0	na
gpd.1	0.375	0.245	0.117	0	na
gpd.2	0	na	na	na	na
hk.1	0.401	0.278	0	0.124	0.627
hk.2	0.475	na	na	na	na
idh	na	0	0.219	0.444	0.531
mdh	0	na	0	0	0.225
me	0.153	0.459	na	na	na
mpi	na	0	0.305	0.293	0.461
pep.1	0.500	0.245	0	0.180	0
pep.2	0.153	na	na	na	na
pgd	0.424	0	0.111	0.305	0.492
pgi.1	na	0.459	0	0	0
pgi.2	na	0	0.420	0.451	0.490
pgm.1	0.647	0.520	0.570	0.420	0.061
pgm.2	na	0.500	0.170	0.549	0.398
xod	0.204	0	0.117	0	0.227
He	0.234	0.189	0.168	0.190	0.335

Enzymes analyzed are: adenylate kinase (ak, E.C. 2.7.4.3), aldehyde oxidase (aldox, E.C. 1.2.1.3), catalase (cat, E.C.1.11.1.6), esterases (est, E.C. 3.1.1.1), fumarase (fum, E.C. 4.2.1.2), glutamate dehydrogenase (gdh, E.C. 1.4.1.3), glutamate–oxaloacetate transaminase (got, E.C. 2.6.1.1), glycerophosphate dehydrogenase (gpd, E.C. 1.1.1.8), hexokinase (hk, E.C. 2.7.1.1), isocitrate dehydrogenase (idh, E.C. 1.1.1.42), malate dehydrogenase (mdh, E.C. 1.1.1.37), malic enzyme (me, E.C 1.1.1.40), manosephosphate isomerase (mpi, E.C. 5.3.1.8), peptidase (pep, E.C. 3.4.11–17.–), 6-phosphogluconate dehydrogenase (pgd, E.C. 1.1.1 43), phosphoglucose isomerase (pgi, E.C 5.3.1.9), phosphoglucomutase (pgm, E.C. 2.7.5.1), and xanthine oxidase (xod, -E.C. 1.2.3.2).

are presented as the arithmetic means of the values obtained over all loci studied for that species (see Nei, 1975).

Another measure of genetic variation used here is the proportion of loci polymorphic, P, expressed as a percentage of loci for which the frequency of the commonest allele was lower than 95% ($P_{0.95}$) or lower than 99% ($P_{0.99}$).

Results

A total of 86 enzyme loci as detected over the five species studied (Table 1). At least 16 loci were analyzed for each species, and the mean number of individuals studied per locus for each species ranged from 7 to 18 (Table 2). The numbers of loci and individuals were based on the numbers recommended by Gorman and Renzi (1979) for the estimation of genetic variation in natural populations.

Discussion

The heterozygosity levels (Table 2) observed for the five species of sponges studied here were very high in comparison with values normally observed for other animals and plants (see, e.g., Nevo, 1978). Interestingly, all the sponges studied–even the more polymorphic species of *Cerithium*, *Chlamys*, and *Urticina* (Ritte and Pashtan, 1982; Beaumont and Beveridge, 1984; Solé-Cava et al., 1985)–are marine invertebrates. Nevo (1978) lists 14 (out of 243) studies in which heterozygosity values exceeded 0.2: one of these is on a plant, seven are on insects (mainly *Drosophila*), and six on marine invertebrates (Ayala et al., 1973, 1975; Manwell, 1975; Campbell et al., 1975; Valentine and Ayala, 1976). If our data and those of Ritte and Pashtan (1982), Beaumont and Beveridge (1984), and Solé-Cava et al., (1985) are included, most species known to have heterozygosities greater than 0.2 and all those considerably higher than 0.3 appear to be marine invertebrates. This is surely remarkable in that only a minority (29/243 from table 1 in Nevo, 1978) of the vast number of published estimates of heterozygosity are for marine invertebrate species.

Table 2. Genetic variation in five sponge species (ni = mean number of individuals analyzed per locus, nl = number of loci analyzed, $P_{(0.95)}$ = proportion of polymorphic loci in the sample, Ho = mean observed heterozygosity, He = mean expected heterozygosity; $\bar{x}$ and σ = mean and standard deviation of $P_{(0.95)}$, Ho and He over all species studied)

Species	ni	nl	$P_{(0.95)}$	Ho	He
Halichondria panicea	18	16	0.688	0.227	0.234
Mycale macilenta	7	18	0.500	0.246	0.189
Suberites ficus A	13	18	0.667	0.179	0.168
Suberites ficus B	11	18	0.611	0.215	0.190
Suberites subereus	16	16	0.750	0.365	0.335
$\bar{x}$			0.643	0.265	0.223
σ			0.094	0.071	0.067

Note, too, that among the marine invertebrates crustaceans on average appear to account for the lowest levels of heterozygosity (see Nelson and Hedgecock, 1980), whereas more primitive organisms with limited mobility—such as actiniarians (Bucklin, 1985; Solé-Cava et al., 1985), brachiopods (Hammond and Poiner, 1984), and mollusks (Ritte and Pashtan, 1982; Beaumont and Beveridge, 1984)—account for the highest heterozygosities.

Selander and Kaufman (1973) have suggested that heterozygosity may be inversely correlated with both the mobility and the degree of complexity of an organism. They note that more mobile species are better able to avoid environmental fluctuations while more complex organisms may be expected to have a greater capacity for homeostatic control. Therefore the enzymes of such species will generally have less need for genetic variability, which would enable the enzymes to function successfully under more widely fluctuating internal environmental conditions. This idea is supported, for example, by a negative correlation between vagility and heterozygosity in decapod crustaceans (Nelson and Hedgecock, 1980). Moreover, there is evidence among teleosts, that demersal, specialized, and less mobile (i.e., narrow niche) species generally show higher levels of genetic variation than do pelagic and more generalist species (Smith and Fujio, 1982).

Thus, heterozygosity could be related to environmental grain (i.e., the way one species "sees" the environment; Levins, 1968), as suggested by Valentine (1976) and supported, with slight modifications, by Nevo (1978), Nelson and Hedgecock (1980), and Smith and Fujio (1982). Many benthic invertebrates seem to have adopted a "coarse grain" strategy; that is, they experience the environment as a mosaic of niche opportunities rather than a homogeneous entity. For example, marine invertebrates can be very specialized when it comes to the microhabitat selected by the larva during settlement (Campbell, 1974; Shroeder and Hermans, 1975). In groups where the larval settlement is less specific, as observed in the Porifera (Fell, 1974; Sará and Vacelet, 1973; Bergquist, 1978), differential larval mortality is another way of producing differential microhabitat colonization (Fell, 1974). In a heterogeneous environment this differential mortality could produce a selective pressure for diversification of genotypes specialized to particular microclimatic conditions and thus could lead to higher levels of gene polymorphism in the population as a whole.

Nonselectionist "neutralist" hypotheses are also available to explain the levels of heterozygosity in natural populations (Kimura, 1968, 1983). According to neutralist concepts, population size, genetic drift, and divergence time are expected to be the main determinants of levels of

gene polymorphism. Precise estimates of population size are not available for the sponges studied. *Halichondria panicea* is very common under a wide range of ecological conditions in all temperate seas and probably represents large populations (Vethaak et al., 1982); *Suberites* cf. *ficus A* and *B* are normally epizoic upon the shells of *Chlamys* spp. (Bloom, 1975; Solé-Cava et al., 1985). *Chlamys* are fished commercially and have large populations in the Irish Sea and elsewhere, and therefore population sizes for these two *Suberites* species may also be large (note that the species of *Chlamys* that act as hosts to *Suberites* are among the molluscan species with highest heterozygosities; Beaumont and Beveridge, 1984). *S. subereus* and *Mycale macilenta*, however, seem to have far smaller populations, at least in the Irish Sea (Solé-Cava and Thorpe, unpublished results), yet their levels of heterozygosity are as high as those observed for the other sponge species (Table 2).

According to the neutralist hypothesis, levels of heterozygosity may be related to the phylogenetic "age" (Soule, 1972) and to the "conservativeness" (Gorman and Kim, 1977) of the taxonomic group—old and conservative groups being expected to show higher levels of genetic variation than younger or more speciose ones. In fact, if the average expected heterozygosities are plotted against levels of polymorphism for all the groups of organisms cited by Nevo (1978), we find that the more "primitive" groups tend to show the highest levels of gene variation (Figure 1). However, we should not overlook the possibility that heterozygosity is related to other factors as well, such as differences in the size or homeostatic efficiency of each group.

Conclusions

As can be seen from the above discussion and some recent reviews (Nevo, 1978; Smith and Fujio, 1982; Burton, 1983; Kimura, 1983), various, often conflicting, hypotheses, have been proposed to explain observed variations in the levels of naturally occurring genetic polymorphism. Much can be said for and against each of these hypoth-

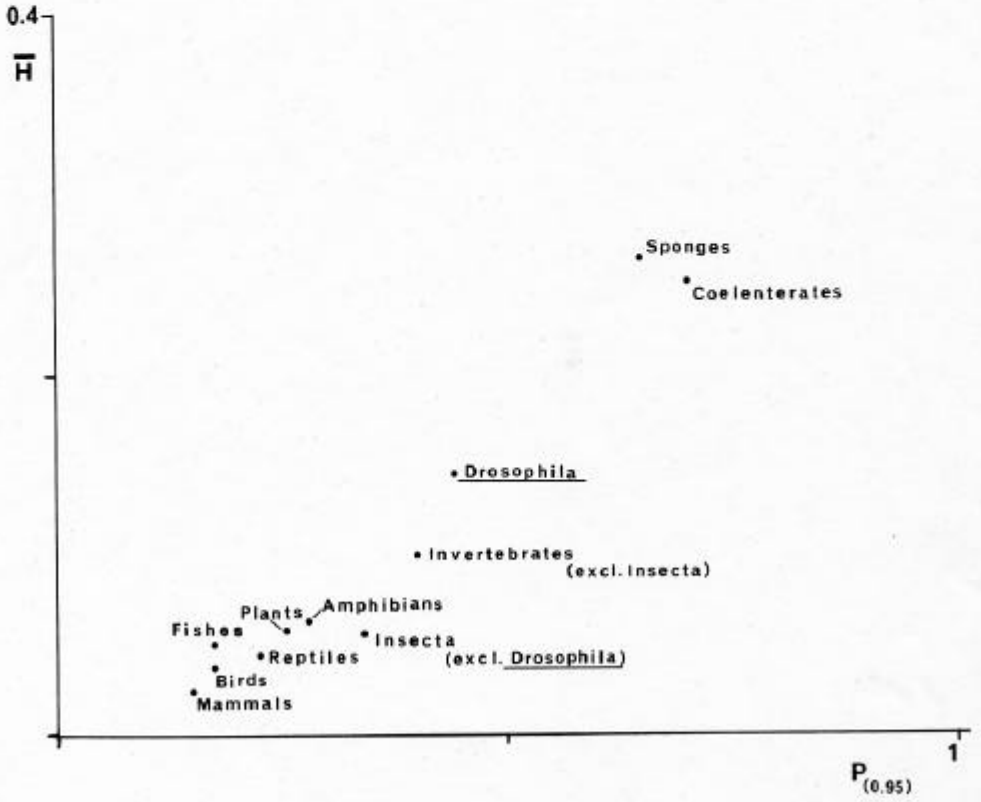

Figure 1. Levels of genetic variation, expressed as a percentage of polymorphism (P) and average heterozygosity ($\bar{H}$) in different groups of organisms. Data obtained from Nevo (1978) and Solé-Cava et al. (1985).

ceses, but it is very difficult, because of the nature of the problem, to test each suggestion objectively. In the case of marine sponges, a great deal of work still needs to be done, particularly with regard to population dynamics and genetics, before any conclusions can be drawn concerning the likely reasons for the observed high levels of genetic variation.

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