Genetic differentiation between morphotypes of the marine sponge *Suberites fuscus* (Demospongiae: Hadromerida)

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Abstract

Sponges of three morphotypes of *Suberites fuscus* (Johnston, 1842) were collected during February and March 1985 off the south-west of the Isle of Man, and were compared by using spicle size distributions and genetic allele frequencies of isozyme loci. The populations did not show any significant differences of spicle size or type, but could be easily differentiated into three separate species based on isozyme patterns. Samples of pale orange *S. fuscus* growing on gastropod shells inhabited by hermit crabs (*Pagurus* spp.) were reproducibly isolated from the red-orange and the pale yellow colour morphs encrusting the bivalve *Chlamys opercularis*. These latter two colour morphs were genetically similar, but significant differences were observed at two of the 19 gene loci assayed. All the sponges studied were sympatric, and therefore the genetic differences, indicating reproductive isolation, are strong evidence for separate gene pools and, hence, that they are different species. The genetic identity between the two colour morphs of *S. fuscus* on *C. opercularis* shells was 0.977, whilst between each of these and *S. fuscus* on hermit crabs it was about 0.65. In all three species genetic variability was high, with mean expected and observed heterozygosity values per locus ranging from 0.17 to 0.36.

Introduction

Sponges of the genus *Suberites* (Nardo, 1833) are common worldwide, living epilithically or epizoically in both intertidal and subtidal environments (Ridley and Dendy, 1887; Hartman, 1958). Most are morphologically highly polymorphic but, since the spicules are simple and show little variation, the genus has constantly presented systematic problems to taxonomists and ecologists. Synonymic lists of the species are usually large (e.g. De Laubenfels, 1936; Burton, 1953; Hartman, 1958). *Suberites fuscus* (Johnston, 1842) (= *Halichondria fuscus* Johnston, 1842) is a typical species of this genus, and is taxonomically problematic: this sponge has been reported under a number of synonyms in the North Atlantic and North Pacific Oceans, the Irish Sea, the Bering Sea, the Mediterranean and the North Sea (Hartman, 1958). Vosmaer (1933) and Burton (1953) considered *S. fuscus* to be a synonym of *S. domunculus* (Oliv., 1792), a species which is morphologically similar and shows an overlap in the size of the macroscleres (mainly tylostyles). However, Bowerbank (1858, 1866), De Laubenfels (1936, 1949), Hartman (1958) and, more recently, Hiscock et al. (1983) regard the presence of centrotyloite microscleres (mainly microtylostyles) as diagnostic of the more widespread northern *S. fuscus*, and consider *S. domunculus*, which lacks microscleres, to be solely Mediterranean.

The most recent major taxonomic work to include *Suberites fuscus* remains that of Hartman (1958). Following a detailed study of the considerable morphological and ecological variation found within the "species" he suggested that *S. fuscus* itself could be a species complex. The subspecific taxonomy of *S. fuscus* is the subject of immense confusion and many names of species as well as subspecies and varieties have been misunderstood and have become badly compromised over periods of time (S. M. K. Stone, personal communication, 1986). To use names of previously described nominal taxa will result in a serious danger of the creation of homonyms and, therefore, following taxonomic advice (S. M. K. Stones, personal communication, 1986), it is proposed, in the present work, to create new taxa for putative species or subspecies. As long as descriptions are given, the creation of new synonyms is likely to cause fewer subsequent problems than would the possible misuse of ill-defined pre-existing names. The subspecific name *fuscus* is not used, because to do so would designate a type for the species group. The present work is concerned with only three putative subspecies and all of these are from a single locality. Without
further research into the taxonomic literature and a detailed examination of specimens from more localities it is, at present, undesirable to designate a type.

In the waters of the Northern Irish Sea, around the Isle of Man, at least three distinct variants of *Suberites ficus* occur. Two of these are colour morphs (one red-orange, the other yellow) found only encrusting the shells of pectinid bivalves [e.g. *Chlamys opercularis* (L.), *Pecten maximus* (L.)]. The other variant is pale orange and apparently grows only upon gastropod shells inhabited by hermit crabs (*Pagurus* spp.). More detailed descriptions of all three variants, under new subspecific names, are given below.

The object of the present work is to examine in some detail the genetic structure of sympatric populations of the three variants of *Suberites ficus*. The approach used was the genetic analysis of isozyme patterns, a technique widely used to solve taxonomic problems in a range of groups (for reviews see e.g. Ferguson, 1980; Thorpe, 1982, 1983; Ayala, 1983), but which has not previously been used to solve taxonomic problems in the Porifera. Electrophoresis of a small number of isozymes from sponges has been reported previously (Urbanaeja and Lin, 1981), but no attempt at genetic interpretation of the isozyme patterns was made by those authors. Connes *et al.* (1974) used serological and electrophoretic techniques to differentiate two morphotypes of *S. massa* (Nardo), but again no genetic interpretation of the results was possible (mainly because these authors worked only on total proteins, which are not easily analysed in genetic terms). The study of the genetic structure of sponge populations is of additional interest because of the recent findings of very high levels of genetic variation in some other marine invertebrates (Nevo, 1978; Ritte and Pashtan, 1982; Beamont and Beveridge, 1984; Bucklin, 1985, Solé-Cava *et al.*, 1985) and the possible evolutionary implications of such results. The study of genetic distance between species is also important and potentially valuable in a group like Porifera, where the morphological characters used in taxonomy are still under evaluation, and where the definition of species presents considerable problems.

**Materials and methods**

**Samples**

The sponges were collected during February and March 1985, by the University of Liverpool research vessel “Cuma” dredging off the south-west of the Isle of Man (54°00'N; 4°49'W) at depths from 15 to 30 m. The three variants of *Suberites ficus* (Johnston, 1842) could be easily and unambiguously distinguished. For taxonomic reasons, stated above, these are given new subspecific names.

*Suberites ficus* ssp. *rubrus*, ssp. nov. Colour red-orange (Colour No. 8A8, Kornerup and Wanscher, 1978). Sponge thinly encrusting, friable and compressible. Found en-crusting (commonly completely covering) upper shell valves of *Clamys opercularis*. Pores slightly oval, 20 to 60 μm largest diameter, osicular sizes 1 to 2 mm. Surface smooth to microhispidate. Small, yellow gemulne, about 800 μm in diameter were found in some specimens, particularly in autumn. These gemulne are devoid of spicules and occur in the thickest part of the sponge, usually in contact with the bivalve shell. Spicules: tylostyles 250×7 μm, usually with deformed heads; centrotylotes 31×2.2 μm. Name from Latin: *rubrus*, red.

*Suberites ficus* ssp. *luridus* ssp. nov. Colour pale yellow (Colour No. 4A8, Kornerup and Wanscher, 1978). Sponge thinly encrusting (generally somewhat thinner than ssp. *rubrus*). Found on upper valves of *Clamys opercularis*, but never completely covering the valve. Pores slightly oval, 20 to 60 μm in largest diameter, osicular sizes 0.6 to 1.8 μm. Surface microhispidate. Again, small aspiculate gemulne were found in some specimens, particularly in autumn. Spicules: tylostyles 249×8 μm; centrotylotes 30×2 μm. Name from Latin: *luridus*, pale yellow.

*Suberites ficus* ssp. *pagurorum* ssp. nov. Colour pale orange (Colour No. 6A6, Kornerup and Wanscher, 1978). Sponge massive (1 to 2 cm in thickness) and compressible. All specimens found were completely covering gastropod shells inhabited by *Pagurus bernhardus* (L.). Pores 80 μm, usually in groups of 4 to 6, osicular sizes 0.8 to 2.0 mm. Surface smooth, gemulnae absent. Spicules: tylostyles 252×8 μm; centrotylotes 29×3 μm. Name from Latin: *pagurorum*, of hermit crabs.

**Spicule analysis**

Prior to electrophoresis, spicule preparations were made for every sponge and, from these, ten measurements were made of each kind of spicule in each of ten different sponges of each of the three subspecies. Possible differences in spicule size were tested by two-way analysis of variance (Sokal and Rohlf, 1981). Samples of the material collected were also compared with type and other specimens in the collections of the British Museum (Natural History).

**Electrophoresis**

Horizontal electrophoresis was performed by standard methods (reviews by Brewer, 1970; Harris and Hopkinson, 1978; Ferguson, 1980; Gaal *et al.*, 1980) using 12.5% starch gels (Sigma Chemical Company, Poole, Dorset, England). To avoid possible reduction in enzyme activity or staining artifacts which might have resulted from the freezing of the samples (Scozzani *et al.*, 1980), only fresh, live animals were used. The buffer used was a continuous Tris-citrate system (pH 8.0) (Ward and Beardmore, 1977; Solé-Cava *et al.*, 1985). Gels were stained for 19 different enzymes, using standard methods (see Brewer, 1970; Harris and Hopkinson, 1978).
Table 1. _Suberites ficus_ ssp. Mean and (standard deviation; n=100) for length measurements (µm) of the two types of spicules from _S. ficus_ ssp. _rubras_, _luridus_ and _pagurom_. _F_ (columns) calculated from two-way analysis of variance (Sokal and Rohlf, 1981)

<table>
<thead>
<tr>
<th>Spicule</th>
<th>rubras</th>
<th>luridus</th>
<th>pagurom</th>
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<tr>
<td>Tylostyles</td>
<td>249.9 (25.5)</td>
<td>249.0 (40.0)</td>
<td>251.5 (41.5)</td>
</tr>
<tr>
<td>Centrotylete</td>
<td>30.8 (4.9)</td>
<td>29.5 (6.1)</td>
<td>28.8 (7.3)</td>
</tr>
</tbody>
</table>

_F_ (columns) = 0.3527  P = 0.261 NS

Results

No significant differences were found between the sizes of the spicules of any of the three subspecies of _Suberites ficus_ (Table 1). All individuals analysed contained microstrongyles, which are considered by Hartman (1958) to be diagnostic of _S. ficus_ sensu lato.

The allele frequencies for the 19 enzyme loci which produced useful results are presented in Table 2 (see also Fig. 1). Nomenclature of enzymes and loci follows that of Harris and Hopkinson (1978). Three further enzymes, CK (creatine kinase, EC 2.7.3.2), SOD (superoxide dismutase, EC 1.15.1.1) and ME (malic enzyme, EC 1.1.1.40) gave at best very faint activity although run on several different buffers. It was concluded that quantities of these enzymes present in the tissue were too low to give adequate results with the extraction and staining methods used.

Discussion

Spicules are generally the main morphological character used in sponge taxonomy (Levi, 1973; Bergquist, 1978; Bergquist and Wells, 1983). Qualitative differences of spicule types are considered to be particularly important in sponge identification and, in certain groups, provide clear cut criteria in presence/absence matrices or in dichotomous identification keys. Differences in spicule size, however, are much more difficult to use, particularly since intraspecific variation is frequently very large (Fry, 1970; Jones, 1984): spicule size, like many other morphological and meristic characters, is influenced by the environment, and can show geographical or seasonal variation (Steen, 1970a, b; Jones, 1984). This variability can produce an overlap of spicule size-frequency distributions of populations from closely related species and, therefore, may mask small interspecific differences (Jones, 1984). The difference in effectiveness between qualitative and quantitative spicule characters is well demonstrated in _Suberites_ spp., where the presence or absence of a microsclere (microstrongyles) is useful in distinguishing _S. ficus_ from _S. domunculus_ (Hartman, 1958; Hiscock et al., 1983), although the differences in spicule sizes do not discriminate between the two species (Hartman, 1958). It is clear from the results of the present work (Table 1) that there are no
taxonomically useful spicule differences between *S. ficus* ssp. *rubrus*, *luridus* or *pagurorum*.

When interpreting the results of the enzyme electrophoresis, it should be borne in mind that all the samples used were collected from the same place and at the same time and therefore may be regarded as being sympatric. If sympatric, conspecific organisms should be freely interbreeding and, therefore, within the limits of sampling errors, they should have the same allele frequencies at each genetic locus. Significant genetic differentiation occurring at any locus will indicate the existence of a barrier to gene flow. Clearly, from biological definitions of species, sympatric morphs with a barrier to gene flow between them cannot be conspecific.

The results of the electrophoresis show clearly that at several enzyme loci (e.g. *Mdh, Fum, Xod*, see Fig. 1) there are large differences in gene frequencies between *Suberites ficus* ssp. *pagurorum* and the other two subspecies. At any

<table>
<thead>
<tr>
<th></th>
<th>rubrus</th>
<th>luridus</th>
<th>pagurorum</th>
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<tbody>
<tr>
<td>rubrus</td>
<td>-</td>
<td>0.900</td>
<td>0.593</td>
</tr>
<tr>
<td>luridus</td>
<td>0.977</td>
<td>-</td>
<td>0.584</td>
</tr>
<tr>
<td>pagurorum</td>
<td>0.671</td>
<td>0.656</td>
<td>-</td>
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</table>

Table 3. *Suberites ficus* ssp. Pairwise comparisons based on isozyme date from "subspecies" *rubrus*, *luridus* and *pagurorum*. Above diagonal, values for genetic similarity (Thorpe, 1979); below diagonal, values for genetic identity (Nei, 1972)

of these loci taken alone, the differences are highly significant (*P* ≤ 0.01) and the results overall show beyond reasonable doubt that ssp. *pagurorum* is genetically differentiated and cannot be conspecific. Over all the loci used, the genetic differentiation between two species may be reduced to a single figure by any of several published measures of genetic distance or genetic similarity. Between *pagurorum* and the other subspecies, values of Nei's (1972) genetic identity (*I*) or of Thorpe's (1979) genetic similarity (*S*) are between 0.5 and 0.7 (Table 3). Such levels of differentiation are typical of congeneric species, where values about 0.35 to 0.8 maybe expected (see Thorpe, 1982, 1983; Ayala, 1983) and are well outside the usual range for conspecific populations.

Possibly more surprising is the degree of genetic differentiation between the orange and yellow colour morphs, ssp. *rubrus* and *luridus*. Over most of the loci examined, differences in gene frequency were not significant for any single locus. However, at both *Pgm-1* and *Pgm-2* there were clear and substantial differences in gene frequency (Table 2 and Fig. 1). The analysis of *Pgm-1* by the chi-square test is difficult because of the small sample sizes, but observed numbers of each allele can be easily compared binomially. Differences between the two frequencies for *Pgm-1* Allele 3 are moderately significant (*P* = 0.031). For *Pgm-2* Allele 3 the difference is highly significant (*P* = 4.65 × 10⁻³) and, for this locus, if Alleles 1 and 3 are pooled to increase sample size, the overall difference, using contingency table analysis is highly significant (χ² = 16.33, df = 1, *P* = 5.32 × 10⁻³). These differences are most unlikely to have occurred by chance alone and, therefore, it must be concluded that sympatric populations of *S. ficus* ssp. *rubrus* and *luridus* show gene frequency differences at some enzyme loci. This leads to the conclusion that they are reproductively isolated and therefore not conspecific. Genetic differentiation between these two "subspecies" is surprisingly small (both *I* and *S* > 0.9) compared to the usual range of interspecific values and, indeed, is more typical of values usually associated with allopatric conspecific populations. However, since the two colour morphs are sympatric (even to the extent that some of the samples were found growing upon the same shells), it is clear from the results that speciation has occurred.

Fig. 1. *Suberites ficus* ssp. Banding patterns for five polymorphic enzyme loci (*Mdh, Fum, Xod, Pgm-1, Pgm-2*) following electrophoresis of eight random samples of each of the three subspecies. 1-4 and 13-16: *pagurorum*; 5-8 and 17-20: *rubrus*; 9-12 and 21-24: *luridus*. All the enzyme molecules migrate anodically.
The main overall conclusion from this study, therefore, is not only that *Suberites ficus* ssp. *pagurorum* is reproductively isolated and hence not conspecific, but also that the two colour morphs found on *Chlamys opercularis* shells are different species. Any possible distortion of gene frequencies by the presence of clones or even by inbreeding can be discounted, since all three species showed good fits of allele frequencies to Hardy-Weinberg expectations and no two sponges were of identical genotype over all the loci examined. Although tests of goodness of fit to Hardy-Weinberg frequencies are generally weak unless sample sizes are large (Lewontin, 1958; Fairbairn and Roth, 1980), these results indicate that *Suberites* species are likely to be at least generally, if not totally, outbreeding and also show that no two sponges came from a single clone. Thus, our results also provide evidence for asexual reproduction in *Suberites* spp. Some sponge species are known to show fusion of sponges derived from different larvae (Fry, 1971). Clearly such fusion could lead to problems in the interpretation of zymograms. However there was no evidence from any of the banding patterns for intraspecific fusion in *Suberites ficus*. Given the very high levels of genetic polymorphism at many of the loci (Table 2), if more than one genotype was present in some of the samples, single samples would be expected to show three or possibly even four alleles per locus in some cases. No such results were observed. Also if fusion occurred to any significant extent, large excesses of heterozygotes should have been found at polymorphic loci. As stated above, no locus deviated significantly from Hardy-Weinberg expectations.

Expected and observed values for mean heterozygosity per locus in all three *Suberites* species are surprisingly high (range 16.8 to 36.5%; Table 4). These are considerably higher than "typical" values for most other eukariotic species (generally about 5 to 15%, see e.g. Selander, 1977; Nevo, 1978). It is possibly of note that our data (mainly not yet published, but see Haylor et al., 1984; Solé-Cava et al., 1985) for levels of genetic variability in nine species of sea anemones from around the Isle of Man show a similar range of very high heterozygosity values from 15% (*Actinia prasina*) to 44% (*Urticina eques*), with an average value of 26% (for comparison, Nevo's (1978) very large survey of just about all animals or plants for which adequate data were then available covered 243

Table 4. *Suberites* spp. Estimates of genetic variation in populations of three species. *H*<sub>0</sub> and *H*<sub>0</sub>: mean expected and observed heterozygosity per locus, respectively; *P*<sub>0.05</sub> and *P*<sub>0.95</sub>: proportions of polymorphic loci with frequency of most common allele < 0.95 or < 0.99, respectively. n: mean effective number of alleles per locus. Allele frequency data are given in Table 2.

<table>
<thead>
<tr>
<th></th>
<th><em>S. rubrus</em></th>
<th><em>S. luridus</em></th>
<th><em>S. pagurorum</em></th>
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<tr>
<td><em>H</em>&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.168</td>
<td>0.195</td>
<td>0.335</td>
</tr>
<tr>
<td><em>H</em>&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.179</td>
<td>0.215</td>
<td>0.365</td>
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<tr>
<td><em>P</em>&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>0.667</td>
<td>0.611</td>
<td>0.750</td>
</tr>
<tr>
<td><em>P</em>&lt;sub&gt;0.95&lt;/sub&gt;</td>
<td>0.667</td>
<td>0.667</td>
<td>0.813</td>
</tr>
<tr>
<td>n</td>
<td>1.259</td>
<td>1.360</td>
<td>1.732</td>
</tr>
</tbody>
</table>

species of which only 13 had heterozygositites above 20%, the highest being 30.9%). Clearly, ranges of heterozygosity values in sponges, and also apparently in sea anemones, are very different from those of most other groups for which data are available. However, at present it is easy to speculate but very difficult to draw any conclusions as to why this should be so. Among the more obvious common features of the biology of the sponges and sea anemones are a sessile adult with probably much restricted larval dispersal and a very limited ability to adapt to or to counteract, behaviourally or otherwise, any fluctuations in environmental conditions. It may also be of significance that Porifera and Coelenterata are two of the phylogenetically oldest and most primitive extant animal phyla.

The present work has various implications for sponge taxonomy. Firstly it indicates that there are at least three species of *Suberites ficus*, two of which (the two "subspecies" *rubrus* and *luridus*) are very closely related to each other, whereas the third (*pagurorum*) is considerably less closely related. Using estimates of Nei's genetic distance, *D*, between the three species as a "molecular clock" (see Wilson et al., 1977; Thorpe, 1982) and the calibration that one *D* unit indicates about 18 million years (m.yr) since evolutionary divergence, the divergence time between the two closely related species may be estimated at about 0.4 m.yr, and that between these two species and the "subspecies" *pagurorum* as about 7 to 8 m.yr.

Considerable research into often obscure and frequently almost unobtainable early taxonomic literature is needed to establish valid names for the three *Suberites* species covered in the present work. Such work is likely to be included in the substantial taxonomic revision of *Suberites* and related genera, which is currently being undertaken by Miss S. M. K. Stone and Mrs P. Fry. However, pending the publication of this very important and necessary work, it is desirable that the three species investigated here should have some names. It is proposed therefore, that our newly created subspecific names should be raised to specific status. Thus, the subspecies *pagurorum* found upon hermit crab shells becomes *S. pagurorum*, the red-orange colour morph from *Chlamys opercularis* shells becomes *S. rubrus* and the pale yellow colour morph from *C. opercularis* shells becomes *S. luridus*. To continue to call these "subspecies" would be misleading, since they are clearly not conspecific.

It is likely that, eventually, at least some of these names will prove to be synonyms, but meanwhile they will enable marine ecologists and others to give a "label" to the three species described and identified here. In this particular case, as pointed out earlier when discussing subspecies, it is clearly preferable to create new, but easily rejected synonyms rather than to add further confusion to the already chaotic taxonomy of the genus *Suberites* by possibly misusing existing nominal taxa. Also, for reasons previously outlined, the name *ficus*, which would designate a new type, is not used.

Of existing names, that which corresponds most closely to our *Suberites pagurorum* would appear to be the *S. ficus*
“var. suberbus” of Hartman (1958) (= *S. domunculus sensu* Hanitsch, 1890; Bruce *et al*., 1963). Our *S. rubrus* and *S. lirudus* both probably would have been considered by Hartman (1958) to fall within his concept of *S. fuscus* “var. farinarius” (= *S. fuscus sensu* Hanitsch, 1890; Bull, 1963). However, at present, we do not wish to draw any conclusions as to possible synonyms of the three species which we have identified within *S. fuscus*.

A further implication of the results of the present work is that, for distinguishing between fairly closely related sponge species, spicules are possibly not of such great value as is frequently supposed. Indeed, if other sponge species are similar to *Suberites fuscus*, it could be that many “varieties” and “colour morphs” are not conspecific and that sponge species are more numerous, more specific in ecological requirements, and less morphologically variable than would appear to be the case for currently accepted “species”. Clearly, there is a need for further use of isozymes to investigate the taxonomy and population structure of sponge species.


**Acknowledgements.** The authors are grateful to Miss S. M. K. Stone, Mrs P. Fry, Dr W. D. Hartman and Professor M. Sarà for helpful discussions and to Professor T. A. Norton for the provision of facilities. A. M. Solè-Cava was supported by Grant CAPES 4334/82 from the Brazilian Government.

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Date of final manuscript acceptance: July 11, 1986.
Communicated by J. Mauchline, Oban