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Evolutionary genetics of marine sponges

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ABSTRACT: For many years the application of formal genetic techniques to sponges has been inhibited by the great problems involved in carrying out breeding work. More recently new, mainly molecular, methods have begun to be applied and already these are giving valuable insights into evolutionary genetics, systematics and speciation within the group. Sponges in general have been found to be remarkable for the very high levels of genetic variability found within populations. In the taxa examined to date a surprisingly high number of cryptic species have been found and there are also suggestions that many allegedly cosmopolitan species may be merely a result of a failure to recognise species because of inadequate taxonomy in groups of sponges with a particular paucity of useful taxonomic characters. There have also been major advances in the understanding of histocompatibility, larval and adult fusion and modes of reproduction of sponge species. The relative advantages and disadvantages of the available molecular techniques are discussed particularly in relation to current problems in sponge genetics and systematics, and suggestions are made for future work and how some of these problems may be tackled.

1 INTRODUCTION

For a geneticist sponges are very interesting organisms: they are possibly close to the root of the Metazoa lineage; they present sexual and asexual reproduction and they are morphologically quite simple (and therefore can have relatively simpler ontogeny). In addition, sponges display features, such as allogenic larval fusion, highly relevant to fundamental questions about the evolution of individuality and the differentiation of somatic and germinal lineages. Until recently, most genetic studies depended upon formal genetic analysis, which usually involved the crossing of individuals and the observation of their offspring. This process was (and still is) very difficult to carry out with marine sponges, so our knowledge of sponge genetics remained very limited (for example, in the major books about sponge biology - Brien et al., 1973; Bergquist, 1978 - there were no chapters about sponge genetics). However, with the advent of molecular techniques such as gel electrophoresis and nucleic acid sequencing, it is has become possible to gain a new insight into the genetics and evolutionary biology of marine (or freshwater) sponges. The aim of this paper is to briefly review the recent literature on the evolutionary genetics of these organisms, and to use the limited knowledge presently available to pose new questions and suggest ideas for future work in this field.

2 GENE VARIATION

Natural populations display some degree of phenotypic variability, which is usually the result of the interaction between the environment and the genome. This has often led to problems in the evaluation of the relative importance of these two factors for the interpretation of observed variation in morphological characters (Jones, 1984). The recent use of genetic markers, which are less affected by the environment, has led to a better understanding of the actual levels of genetic variation in populations. So far, most of these estimates have been obtained through the genetic interpretation of isozyme electrophoresis patterns, this method having been favoured because of its sensitivity and the relative simplicity. Other molecular markers, such as nucleic acids, could be also used, but to date no data on genetic variation for DNA markers have been published for marine sponges (or, for that matter, for most organisms). The levels of genetic variation can be studied at two different levels: within and between populations.

2.1 *Within populations*

Heterozygosity estimates indicate that sponges are among the most genetically variable organisms (Table 1). There has been much debate about the possible reasons for this. To some the levels of allozyme gene

Table 1. Levels of genetic variation in marine sponges, estimated through allozyme electrophoresis. nl = number of loci analyzed; He = mean expected heterozygosity; Ho = mean observed heterozygosity; $P_{0.95}$ = Proportion of polymorphic loci; * = these low values of observed heterozygosity are possibly due to sampling of asexual populations. Ref. = bibliographical source of the data, as given below:

1- Solé-Cava & Thorpe, 1991
2 - Solé-Cava et al., 1991a
3 - Solé-Cava et al., 1991b
4 - Solé-Cava et al., 1992b
5 - Solé-Cava & Thorpe, 1990
6 - Boury-Esnault et al., 1992
7 - Bavestrello & Sarà, 1992
8 - Balakirev & Manchenko , 1985
9 - Solé-Cava & Thorpe, 1986
10 - Sarà et al., 1989

Species	nl	He	Ho	$P_{0.95}$	Ref
Agelas oroides	18	0.215	0.192	0.611	1
Axinella damicornis	8	0.087	-	0.250	2
Axinella verrucosa	8	0.125	-	0.250	2
Chondrilla nucula	16	0.187	0.189	0.563	1
Chondrosia reniformis	12	0.335	0.336	0.833	1
Clathrina clathrus	11	0.165	0.166	0.166	3
Clathrina cerebrum	7	0.398	0.399	0.857	3
Clathrina aurea	11	0.095	0.100	0.455	3
Clathrina brasiliensis	7	0.170	0.176	0.423	3
Corticium candelabrum	16	0.180	0.170	0.440	4
Halichondria panicea	15	0.234	0.227	0.688	5
Mycale macilenta	18	0.189	0.246	0.500	5
Oscarella lobularis	14	0.123	0.128	0.710	6
Oscarella tuberculata	14	0.028	0.028	0.154	6
Petrosia ficiformis (sphe)	9	0.312	0.305	0.778	1
Petrosia ficiformis (cyl)	9	0.059	-	0.111	7
Petrosia ficiformis (spher)	9	0.205	-	0.333	7
Suberites domuncula	28	0.137	-	0.344	8
Suberites luridus	18	0.195	0.215	0.611	9
Suberites pagurorum	16	0.335	0.365	0.750	9
Suberites rubrus	18	0.167	0.175	0.667	9
Tethya aurantium	10	0.000	0.000*	0.000	10
Tethya citrina (Porto Pozzo)	10	0.028	0.010*	0.200	10

variation are maintained by natural selection (the Selectionist hypothesis); to others the main effect of natural selection is an erosion of genetic variation and, therefore, only genes that are selectively equal (i.e., neutral) will accumulate over time (the Neutralist hypothesis). Selectionists usually relate levels of genetic variation to feeding diversity, to ecological specialisation, or to environmental grain, stability or predictability; neutralists relate heterozygosity to effective population size and neutral mutation rates (for reviews see e.g. Kimura, 1983, 1991; Nevo et al., 1984; Nei, 1987; Solé-Cava & Thorpe, 1991). Levels of genetic variation are apparently similar in sponges living in cold or warm waters, or at different depths. This indicates that, at least for sponge species, many of the selectionist predictions do not hold, and hence that the neutral theory may be more adequate to explain the levels of gene variation observed in their populations. However, some alternative selectionist explanations, such as the environmental grain hypothesis of Levins (1968) still cannot be ruled out (Solé-Cava & Thorpe, 1991).

2.2 *Between populations*

The heterozygosity estimates discussed above describe the overall level of gene variation within morphologically locally homogeneous populations of a given species. However, if the population is genetically structured this overall gene variation can be further split into local levels of gene variation. We still have no idea about the levels of population structure in marine sponges. In other sessile marine invertebrates, levels of population structure can be very high. This is principally due to inbreeding resulting from the tendency of the larvae of some species to settle close to their parents (philopatry; Shields, 1982), even when they have the capability, at least in the laboratory, of swimming for long periods of time (Knowlton & Keller, 1986; Grosberg & Quinn, 1986; for a discussion applied to marine sponges see Zea, 1993). Other species can be genetically homogeneous over very large distances, and there seems to be a strong correlation between predominant mode of reproduction and levels of population structure (Solé-Cava et al., 1992a; Russo et al., 1993). This could be because the sexually reproduced dispersing propagules (eggs, larvae) released by

sessile invertebrates that are facultative sexual/asexual generally have reduced powers of dispersal when compared to those produced by exclusively sexually reproducing species (Jackson, 1986). The relative contributions of sexual and asexual reproduction to sponge population structure are still not well known. Sponge larvae can swim for quite long periods in the laboratory (reviewed in e.g. Jackson, 1985), but how effective they are at maintaining gene flow over long distances in natural conditions remains to be determined.

3 HISTORECOGNITION

The first description of intraspecific discrimination by individual sponges was that of Van de Vyver (1970) who detected, through the fusion/non-fusion of larvae, different strains of freshwater sponges. The demonstration of the presence of an immune system in marine sponges, through transplant experiments with *Hymeniacidon* and *Callyspongia* (Curtis, 1979; Hildeman et al., 1979) initiated a fruitful and controversial period in the study of sponge histocompatibility (reviewed, for freshwater sponges, by Van de Vyver, 1988). It was suggested that the allogenic rejection of grafts could be used to infer the relative contributions of sexual and asexual reproduction in marine sponges (Jokiel et al., 1982; Neigel & Avise, 1983; Wulff, 1986). However, since the exact mode of genetic inheritance of the histocompatibility system in sponges is not known (Wulff, 1986), many conflicting results appeared indicating that graft acceptance and rejection could not be used without a critical assessment of its limitations (Buscema & Van de Vyver, 1983; Stoddart et al., 1985; Ilan & Loya, 1990a; Feldgarden & Yund, 1992; Grosberg, 1992). All studies so far show that the allogenic graft reaction in marine sponges is transitive, i.e. the reaction of sponge A to sponge B and of sponge B to sponge C can be used to predict the reaction between sponges A and C (Neigel & Avise, 1985). This indicates that, if there is a Mendelian genetic system for allorecognition, then a total identity will be required for graft acceptance. In this sponges differ from some colonial ascidians, where the sharing of one allele out of several is enough to make colonies histocompatible (Grosberg & Quinn, 1986). The precision with which histocompatibility reactions can be used to identify individual clones will depend, thus, on the number of histocompatibility loci and the alleles segregating at them. If these numbers are high, then each individual will be more or less unique, due to the frequency distribution of different genotypes. If these numbers are low a reaction of graft rejection will demonstrate (other things being equal) a genetic difference, but graft acceptance will only indicate a lack of difference at the histocompatibility loci.

4 SEXUAL / ASEXUAL REPRODUCTION

Sponges are among the many groups of organisms that can reproduce asexually (Simpson, 1980; Battershill & Bergquist, 1993). The relative advantages and disadvantages of sexual reproduction for a species, and the ultimate influence of mode of reproduction on its evolutionary fate, has always been one of the favourite topics of discussion among evolutionary biologists (Shields, 1982; Hughes, 1989; Maynard-Smith, 1989). In species living on hard substrata in ecologically stable environments, asexual reproduction is considered to be more advantageous than sexual reproduction, since it allows a faster growth of the individual and the multiplication of successful genotypes, without the energetic requirements of producing gametes (Jackson, 1985, 1986). A problem with sexual reproduction in stable environments is the cost of meiosis, i.e., the segregation and dissolution of well-adapted genotypes. On the other hand, sexually produced propagules generally have enhanced powers of dispersal when compared to those which are asexually produced (Jackson, 1986), and the gene shuffling effect of meiosis allows the production of many different genotypes, leaving scope for adaptation in spatially or temporarily varying conditions (Maynard Smith, 1989). It is to be expected, thus, that asexually reproducing sponges should be more common in temporarily stable regions, such as coral reefs, than in more variable environments, such as in temperate regions. A preliminary comparison of the relative contributions of asexual reproduction to sponge colonization of hard substrata of different regions seems to confirm this prediction (Jackson, 1985). However, studies on a larger number of species in different environments, using more sensitive techniques for the detection of genetically identical clone mates ("ramets" sensu Harper, 1977) are necessary before we can properly estimate the importance of asexual reproduction to sponge population structure.

4.1 *Larval fusion*

In many invertebrate species living on hard substrata size is considered to be a major factor governing the survival of recruits (Ayling, 1980; Ivan & Loya, 1990). Consequently, larval fusion can be advantageous for the species, specially under high levels of interspecific competition. When the larvae that fuse are genetically identical, the fusion is the equivalent of a very fast growth of one single genetic individual ("genet" sensu Harper, 1977). In this case, the evolutionary advantages of larval fusion are obvious, and it is expected that many species capable of asexually producing larvae or other types of propagules like gemmulae will make use of some form of fusion in the early phases of settlement. However, the fusion of genetically different individu-

als, be it in the larval phase or in the adult phase, is more difficult to explain in evolutionary terms. This is because in organisms like sponges, which lack a clear separation between somatic and germinative cell lineages, after the fusion of two or more individuals a special type of intraspecific competition can occur, when one of the genetic individuals invests more energy into reproduction (therefore increasing its fitness), at the expense of the other individual. This process has been called "somatic cell parasitism" by Buss (1982, 1990), and is considered to be one of the driving forces for the evolution of allorecognition and rejection systems in invertebrates (Grosberg, 1988; Buss, 1990; Feldgarden & Yund, 1992). The evolution of allogenic larval fusion mechanisms, thus, would seem to be the result of a trade off between the ecological advantage during settlement and the disadvantage of somatic cell parasitism during reproduction (Grosberg & Quinn, 1988). It has been argued that allogenic larval fusion could be evolutionary advantageous in organisms with limited larval dispersal and highly structured populations (Grosberg, 1988). In this case, the probability that fusing larvae will be genetically related would be higher, and hence would leave scope for kin selection which may counteract the disadvantages of somatic cell parasitism (Ilan & Loya, 1990; Grosberg, 1992). Others argue that natural selection for allorecognition systems alone cannot produce a high polymorphism in histocompatibility alleles, and that allorecognition is a pleiotropic expression of other genetic systems (Crozier, 1986; Amano, 1990). Most of these arguments are based on theoretical analysis of evolutionary models, and it is imperative that more empirical data be obtained, principally about the fate of the different genotypes in the chimeras formed after allogenic larval fusion. If these ontogenetically and re productively stable chimeras really exist, and if they represent a true mixing of different genotypes, then the whole discussion of individuality in sponges, once considered more or less settled as "specimens surrounded by a continuous pinacoderm" (Borojevic et al., 1967; Bergquist, 1978), would have to be readdressed. At least from the evolutionary geneticist's point of view, an individual is a genotypically unique evolving unit (or "genet"), and if genotypes in a chimera can contribute their genetic material to the next generation then these chimeras must not be considered individual organisms.

5 MOLECULAR SYSTEMATICS

Molecular systematics is the use of informational macromolecules for the identification of organisms, and the formulation of phylogenetic hypotheses on the relationships between them (reviewed in e.g. Hillis & Moritz, 1990). The use of macromolecules (unlike the use of secondary metabolites employed in chemotaxonomy) for systematics has the advantage that these molecules are all synthesised de novo by the organisms, and are linked, directly or through gene expression, to the genomic composition of the species. Molecular systematics can be used to separate species (alpha-systematics) or to cluster them into evolutionary linked units (phylogeny).

5.1 *Species level*

Cosmopolitanism and the actual number of sponge species: Many of the sponge species that are considered to have wide geographical distribution (e.g *Chondrosia reniformis*, *Clathrina primordialis*, *Dysidea fragilis*, *Oscarella lobularis*, *Suberites domuncula*) have few taxonomically useful characters and consequently are also taxonomically poorly defined. Thus it can be argued that for many of these species the apparent cosmopolitanism is merely a result of the failure to distinguish geographically differentiated species because of inadequate taxonomic resolution (Solé-Cava et al., 1991a). The objectivity of molecular systematics makes this the best approach to this type of taxonomical problems (Solé-Cava & Thorpe, 1987; Hillis & Moritz, 1990), and molecular methods have been successfully used to solve taxonomic problems in many sponge genera (*Suberites* - Solé-Cava & Thorpe, 1986; *Tethya* - Sarà et al., 1990; *Clathrina* - Solé-Cava et al., 1991a; *Axinella* - Solé-Cava et al., 1991b; *Oscarella* and *Corticium* - Solé-Cava et al., 1992; Boury-Esnault et al., 1992; *Petrosia* - Bavestrello & Sarà, 1992). If, as many of the molecular systematics studies have demonstrated, allegedly cosmopolitan species may be simply the result of weak systematics, then probably the actual number of extant sponge species must be much higher than that usually assumed. It is also clear from genetic data that even in studies of local populations significant numbers of cryptic species may be present in many sponge morphospecies. The problem of hidden variation within nominal species is not exclusive to the Porifera: molecular systematics has disclosed a much finer level of specific differentiation in many other marine invertebrate phyla (reviewed in e.g. Hillis & Moritz, 1990). The picture that is emerging from much of the molecular systematics work is that the taxonomic frontier that separates intraspecific from interspecific variation may have to be shifted from where it stands at the moment. Many of the present species may well be "species groups" and subtle discontinuities in morphological, ecological or cytological characters, classically dismissed as intraspecific variation in fact may be the result of specific differentiation.

5.2 *Phylogeny*

The Porifera is one of the very few phyla in which the classification at higher taxonomic levels is still not

well established (as can be seen from many papers in this volume). This lack of a consensus for the classification of sponges even after many years of research illustrates the need for the acquisition of additional and independent data to corroborate or contradict competing phylogenetic hypotheses. Genetic data are ideal for studying this type of problem, and their application in the next few years is likely to lead to the clarification of many of the current problems in the higher taxonomy of sponges.

5.3 *Allozymes and DNA sequencing*

The type of genetic technique to be used will depend on the taxonomic level being studied (Solé-Cava & Thorpe, 1987). Basically, the evolutionary conservativeness of the molecules studied will determine the level at which they can be most effectively applied: every character accumulates modifications (through the generation of new alleles by mutation and their maintenance or elimination by selection or genetic drift) with time. However, some molecules or regions of molecules evolve far more rapidly than others. One suggestion is that molecules that are evolutionary constrained, i.e. molecules that are under the effect of strong normalising selection, will evolve more slowly than molecules which are evolutionary more "neutral" (i.e., less constrained; Nei, 1987). For molecular systematics the consequence of this property of the genetic material is that some molecules will accumulate (through effective mutation) and lose (through saturation of mutation sites) phylogenetic information much faster (in evolutionary terms) than others. So, for genus or species level systematics, it will be important to work with fast evolving molecules (such as most structural genes like allozymes), whereas research on higher systematic levels will require slow evolving genes (such as ribosomal or some very constrained structural genes such as those coding for histones or collagen). The importance of the correct choice of molecules (or part of molecules) and their analysis can be seen in recent papers reporting conflicting results from the analysis of sequence data of ribosomal nucleic acids of sponges (Kelly-Borges et al., 1991; Lafay et al., 1992; Rodrigo et al., 1993). This problem is not exclusive of the study of sponges: similar discrepancies have been encountered with the phylogenetic analysis of, for example, the Coelacanth, where the phylogenetic analysis of 18S and 28S rDNA sequence data produced conflicting phylogenetic hypotheses (Hillis et al., 1991; Stock et al., 1991). Sequence data analysis is a very promising field for phylogenetic inference, and future work in this area will certainly contribute much to our understanding of the relationships within the Phylum. However, until a larger number of species and nucleotide sites have been analysed caution will have to be exercised in the interpretation of such data.

Allozymes are usually assumed to be useful only for the study of populations at the intraspecific or intrageneric level (Solé-Cava & Thorpe, 1987; Hillis & Moritz, 1990). However, in some cases it has been shown that they may be also used to infer relationships between confamilial genera (Stoddart, 1989; Solé-Cava et al., 1992b, 1993), provided they are analysed with methods able to detect the accumulation of homoplasies due to allelic convergence and to take these into account when formulating phylogenetic hypotheses.

It is the analysis of protein or nucleic acid sequences, though, that offers the most useful tools for the study of evolutionary relationships at all levels of taxonomic resolution. At the moment, because of the high costs still involved in DNA/RNA sequencing, it is best to use such data for problems that cannot be approached through other methods. Also because of cost, it is more useful to work at higher taxonomical levels, thus using highly conserved molecules, with the assumption of very low levels of intraspecific variation, so that sample sizes can be extremely small (usually only one individual) for each species. With faster evolving molecules larger sample sizes may be needed to take account of intraspecific genetic variation.

Another advantageous feature of sequence data is that (unlike some allozyme data) they are comparable between laboratories, and hence our knowledge about the studied species steadily accumulates over time. Sequence data can then be deposited in one of the genetic banks currently available, and used by other workers for sequence comparisons. It is essential, of course, that due care is taken in the identification of the species studied, so that data entering the "genetic banks" are really useful in future work.

6 FUTURE WORK

Genetic studies on sponges are just beginning, but many very interesting questions can already be tackled with the new wealth of techniques currently available to the evolutionary biologist (reviewed in Hoelzel, 1992). Below we give a brief sketch of some of these questions and some possible ways of trying to obtain answers to them:

6.1 *How common is true cosmopolitanism in marine sponges?*

This question is of great importance, since species considered cosmopolitan will often be those that are most studied in ecological, chemical or physiological work. If cosmopolitanism in marine sponges is the result of an over conservative classification of morphologically (but not necessarily physiologically) similar species, then much work will be wasted in studying badly defined species. It is paramount, then, that genetic comparisons of sponge species over their geographical range be made, particularly when their

diagnosis is based on weak characters. The technique of choice for such work is allozyme electrophoresis, principally because of the wealth of empirical data on species comparisons already available, which provides a baseline indicating expected levels of genetic similarity associated to different taxonomic levels (Thorpe, 1982). Other molecular techniques (sequencing, RAPDs, RFLPs) can be difficult to interpret.

6.2 *What is the level of genetic variation for neutral genes in sponge populations?*

Allozymes have classically been used to estimate levels of genetic variation in natural populations, and sponges are among the most variable organisms for allozyme genes (Solé-Cava & Thorpe, 1991). However, allozyme genes only represent a very limited part of the genome of the organism, and because they are structural genes they are likely to be more strongly influenced by natural selection than, say, pseudogenes or introns (Nei, 1987). It would be very interesting to see whether sponges are more variable than other organisms also at these more neutral parts of the genome. The most comprehensive way of assessing levels of polymorphism at these neutral parts of the genome would be, of course, DNA sequencing of homologous regions in many individuals of many sponge populations, principally because of the possibility of comparing levels of gene variation between "neutral" and "constrained" regions of the genome (Nei, 1987). This, however, would still require a prohibitively large investment both in terms of time and resources. Alternative useful sources of information about neutral genetic polymorphism would be the same techniques described for the question below (6.3), reinterpreted as expressions of overall levels of genetic variation.

6.3 *What are the levels of gene flow between populations of marine sponges?*

I.e. how effective is dispersal in sponge populations, and how much genetic cohesion are we to expect in sponge species? The traditional technique of allozyme electrophoresis is still the simplest and most cost-effective approach here, since large number of individuals per population can be analysed and formal population genetics methods can be applied (Solé-Cava & Thorpe, 1987; Hillis & Moritz, 1990). Three main techniques could be used for that: 1) the use of restriction fragment length polymorphisms (RFLPs), either through total DNA digests that can be detected very efficiently using homologous or heterologous probes (Aquadro et al., 1992), or through digest of PCR (polymerase chain reaction) amplified fragments and direct visualization with ethidium bromide (Brufford et al., 1992); 2) the use of length variation of simple sequence regions (variation in number of repeats of simple nucleotide themes, like ATAT..., also known as "microsatelites"), amplified through PCR (Brufford et al., 1992); and 3) the use of PCR to amplify random segments of DNA using arbitrary primers (the "RAPD" technique, Welsh & McClelland, 1990; Williams et al., 1990). All these techniques are moderately expensive (but quicker and cheaper than gene cloning or sequencing), and can produce useful data for population genetics analysis.

6.4 *How natural is the present Class/Sub-Class/Ordinal classification of marine sponges?*

Nucleic acids are particularly useful for phylogenetic analysis (Hillis & Moritz, 1990; Olsen, 1990; Kelly-Borges et al., 1991; Lafay et al., 1992), principally because sequence information will accumulate over time and each new set of data will permit a more comprehensive overall analysis. These techniques are relatively new, and it is very important that they be only applied together with a critical analysis of the tree construction methods used and an adequate evaluation of the quality of the molecular data obtained, principally in terms of their effective phylogenetic information (Nei, 1987; Rodrigo et al., 1994). The use of other molecular techniques, such as immunological systematics (Custódio et al., 1993), can also be promising, principally after adequate calibration in relation to evolutionary rates (Hillis & Moritz, 1990).

6.5 *What are the relative contributions of sexual and asexual reproduction to the composition of sponge populations?*

The ideal technique to approach such questions is DNA fingerprinting analysis, which is particularly suitable because of its high precision and relative simplicity (Bruford et al., 1992). DNA fingerprinting has been successfully used to assess clonal structure in reef cnidarians (Coffroth et al., 1992), but has not been used on sponge populations. Alternative methods, such as allozyme and histocompatibility analyses are also useful, but their discriminatory power is smaller.

6.6 *What happens to the different genotypes after allogenic larval fusion?*

Firstly it must be verified whether true allogenic larval fusion occurs in sponges at all, but if it does, it would be clearly of widespread interest to determine the fate of individual genotypes after fusion, in order to test directly the hypothesis of somatic cell parasitism. The use of PCR allows now the amplification of tiny amounts of DNA, and their comparison by single locus fingerprinting or RFLP analysis. Hence it should be possible to amplify the DNA of the gametes or larvae

produced by chimaeric sponges. This would allow the estimation of the relative contribution, to the next generation, of each of the individual genotypes that originally fused to form the sponge.

7 CONCLUSIONS

"When one has a new hammer, everything looks like a nail"(old Russian proverb)

Genetic methods are obviously not a panacea that will solve all problems in the study of sponge biology. However, they represent a welcome addition for the limited set of tools available to the study of sponge populations, particularly for studying reproductive biology and systematics. The basic material for genetic studies - genetic variation - is abundant in sponges, and although there have been few studies so far these are encouraging since they demonstrate that molecular and population genetic techniques generally do work for sponge species. The potential rewards of applying the right methods to the right problems in sponge biology are great. Surely the time is ripe for close collaboration between geneticists and sponge biologists?

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REFERENCES

Amano, S. 1990. Self and non-self recognition in a calcareous sponge, *Leucandra abratsbo*. *Biol. Bull.* 179: 272-278.

Aquadro, C.F., W.A. Noon & D.J. Begun 1992. RFLP analysis using heterologous probes. In: A.R. Hoelzel (ed.), *Molecular genetic analysis of populations*: 115-158. Oxford: Oxford University Press.

Ayling, A.L. 1980. Patterns of sexuality, asexual reproduction and recruitment in some intertidal marine Demospongiae. *Biol. Bull.* 158 : 271-282.

Balakirev, E.S. & G.P. Manchenko 1985. Intraspecies genetic variation in the sponge *Suberites domuncula*. *Biol. Morya* 5: 36-40.

Battershill, C.N. & P.R. Bergquist 1993. Sex in the sea and how to avoid it. In: *Book of Abstracts. 4th International Porifera Congress*. Amsterdam: University of Amsterdam.

Bavestrello, G. & M. Sarà 1992. Morphological and genetic differences in ecologically distinct populations of *Petrosia* (Porifera, Demospongiae). *Biol. J. Linn. Soc.* 47 : 49-60.

Bergquist, P.R. 1978. *Sponges*. London: Hutchinson.

Borojevic, R., W.G. Fry, W.C. Jones, C. Lévi, R. Rasmont, M. Sarà & J. Vacelet 1967. Mise au point actuelle de la terminologie des éponges. *Bull. Mus. nation. Hist. nat. Paris* 39 : 1224 - 1235.

Boury-Esnault, N., A.M. Solé-Cava & J.P. Thorpe 1992. Genetic divergence between morphs of the Mediterranean sponge *Oscarella lobularis* Schmidt (Porifera, Demospongiae, Oscarellidae). *J. nat. Hist.* 26: 271-284.

Brien, P., C. Lévi, M. Sarà, O. Tuzet & J. Vacelet 1973. Spongiaires. In: P.P. Grassé (ed.), *Traité de Zoologie. Anatomie, Systematique, Biologie. 3*. Paris: Masson et Cie.

Bruford, M.W., O. Hanotte, J.F.Y. Brookfield & T. Burke 1992. Single-locus and multilocus DNA fingerprinting. In: A.R. Hoelzel (ed.), *Molecular genetic analysis of populations*: 225-270 Oxford: Oxford University Press.

Buss, L.W. 1982. Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. nation. Acad. Sci. USA*. 79: 5337-5341.

Buss, L.W. 1990. Competition within and between encrusting clonal invertebrates. *Trends Ecol. Evol.* 5: 352-356.

Buscema, M. & G. Van de Vyver 1983. Variability of allograft rejection processes in *Axinella verrucosa*. *Developm. Comp. Immunol.* 7: 613-616.

Coffroth, M.A., H.R. Lasker, M.E. Diamond, J.A. Bruenn & E. Bermingham 1992. DNA fingerprints of a gorgonian coral: a method for detecting clonal structure in vegetative species. *Mar. Biol.* 114: 317-325.

Crozier, R.H. 1986. Genetic clonal recognition abilities in marine invertebrates must be maintained by selection for something else. *Evolution* 40: 1100-1101.

Curtis, A.S.G. 1979. Individuality and graft rejection in sponges. In: G. Larwood & B.R. Rosen (eds.), *Biology and systematics of colonial organisms*: 29-48. London: Academic Press.

Custódio, M.R., M. Klautau & R. Borojevic 1993. Antigenic properties of sponge collagen and their potential use in taxonomy. In: *Book of Abstracts. 4th International Porifera Congress*. Amsterdam: University of Amsterdam.

Feldgarden, M. & P.O. Yund 1992. Allorecognition in colonial marine invertebrates: does selection favor fusion with kin or fusion with self? *Biol. Bull.* 182: 155-158.

Grosberg, R.K. 1988. The evolution of allorecognition specificity in clonal invertebrates. *Quart. Rev. Biol.* 63 : 377-412.

Grosberg, R.K. 1992. To thine own self be true? an addendum to Fledgarden and Yund's report on fusion and the evolution of allorecognition in colonial marine invertebrates. *Biol. Bull.* 182: 454-457.

Grosberg, R.K. & J.F. Quinn 1986. The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate. *Nature* 332: 456-459.

Grosberg, R.K. & J.F. Quinn 1988.The evolution of allorecognition specificity. In: R.K. Grosberg, D. Hedgecock & K. Nelson (eds.), *Invertebrate historecognition*: 157-167. New York: Plenum Press.

Harper, J.L. 1977. *Population biology of plants*. London: Academic Press.

Hildeman, W.H., I.S. Johnston & P. Jokiel 1979. Immunocompetence in the lowest Metazoan Phylum: transplantation immunity in sponges. *Science* 204: 420-422.

Hillis, D.M., M.T. Dixon & L.K. Ammerman 1991. The relationships of the coelacanth *Latimeria chalumnae*: evidence from sequences of vertebrate 28S ribosomal RNA genes. *Environm. Biol. Fish* 32: 119-130.

Hillis, D.M. & C. Moritz 1990. *Molecular systematics*.

Sunderland, Massachusetts: Sinauer Associates.
Hoelzel, A.R. 1992. *Molecular genetic analysis of populations. A practical approach.* Oxford: Oxford University Press.
Hughes, R.N. 1989. *A functional biology of clonal organisms.* London: Chapman and Hall.
Ilan, M. & Y. Loya 1990a. Ontogenetic variation in sponge histocompatibility responses. *Biol. Bull.* 179: 279-286.
Ilan, M. & Y. Loya 1990b. Sexual reproduction and settlement of the coral reef sponge *Chalinula* sp. from the Red Sea. *Mar. Biol.* 105: 25-32.
Jackson, J.B.C. 1985. Distribution and ecology of clonal and aclonal benthic invertebrates. In: J.B.C. Jackson, L.W. Buss & R.E. Cook (eds.), *Population biology and evolution of clonal organism*: 297-356. London: Yale University Press.
Jackson, J.B.C. 1986. Modes of dispersal of clonal benthic invertebrates: consequences for species' distributions and genetic structure of local populations. *Bull. mar. Sci.* 39: 588-606.
Jokiel, P.L., W.H. Hildemann & C.H. Bigger 1982. Frequency of intercolony graft acceptance or rejection as a measure of population structure in the sponge *Callyspongia diffusa*. *Mar. Biol.* 71: 135-139.
Jones, W.C. 1984. Spicule dimensions as taxonomic criteria in the identification of haplosclerid sponges from the shores of Anglesey. *Zool. J. Linn. Soc.* 80: 239-259.
Kelly-Borges, M., P.R. Bergquist & P.L. Bergquist 1991. Phylogenetic relationships within the Order Hadromerida (Porifera, Demospongiae, Tetractinomorpha) as indicated by ribosomal RNA sequence comparisons. *Biochem. Syst. Ecol.* 19: 117-125.
Kimura, M. 1983. *The neutral theory of molecular evolution.* London: Cambridge University Press.
Kimura, M. 1991. The neutral theory of molecular evolution: a review of recent evidence. *Japan. J. Genet.* 66: 367-386.
Knowlton, N. & B.D. Keller 1986. Larvae which fall far short of their potential: highly localized recruitment in an alpheid shrimp with extended larval development. *Bull. mar. Sci.* 39: 213-223.
Lafay, B., N. Boury-Esnault, J. Vacelet & R. Christen 1992. An analysis of partial 28S ribosomal RNA sequences suggests early radiations of sponges. *Biosystems* 28: 139-151.
Levins, R. 1968. *Evolution in changing environments.* Princeton, New Jersey: Princeton University Press.
Maynard Smith, J. 1989. *Evolutionary genetics.* Oxford: Oxford University Press.
Nei, M. 1987. *Molecular evolutionary genetics.* New York: Columbia University Press.
Neigel, J.E. & J.C. Avise 1983. Histocompatibility bioassays of population structure in marine sponges. *J. Hered.* 74: 134-140.
Neigel, J.E. & J.C. Avise 1985. The precision of histocompatibility response in clonal recognition in tropical marine sponges. *Evolution* 39: 724-732.
Nevo, E., A. Beiles & R. Ben-Schlomo 1984. The evolutionary significance of genetic diversity: ecological demographic and life-history correlates. In: G.S. Mani (ed.), *Evolutionary dynamics of genetic diversity*: 13-212. Heidelberg: Springer Verlag.
Olsen, J.L. 1990. Nucleic acids in algal systematics. *J. Phycol.* 26: 209-214.
Rodrigo, A., P.R. Bergquist, P.L. Bergquist & R.A. Reeves 1994. Are sponges animals? an investigation into the vagaries of molecular phylogenetic inference. In: R.W.M. van Soest , Th.M.G. van Kempen & J.-C.Braekman (eds.), *Sponges in time and space. Proc. 4th Int. Porifera Congr.* Rotterdam: Balkema.
Russo, C.A.M., A.M. Solé-Cava & J.P.Thorpe 1993. Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actiniidae) with different reproductive strategies. *Mar. Biol* (in press)
Sarà, M., P. Mensi, R. Manconi & E. Balletto 1989. Genetic variability in Mediterranean populations of *Tethya* (Porifera, Demospongiae). In: J.S. Ryland & P.A. Tyler (eds.), *Reproduction, genetics and distribution of marine organisms*: 293-298. Berlin: Springer Verlag.
Shields, W.M. 1982. *Philopatry, inbreeding, and the evolution of sex.* Albany: State University of New York Press.
Simpson, T.L. 1980. Reproductive processes in sponges: a critical evaluation of current data and views. *Int. J. Invert. Reprod.* 2: 251-269.
Solé-Cava, A.M., C.A.M. Russo, M.E. Araujo & J.P. Thorpe 1993. Cladistic and phenetic analysis of allozyme data for nine species of sea anemones of the family Actiniidae (Cnidaria: Anthozoa). *Biol. J. Linn. Soc.* (in press).
Solé-Cava, A.M., C.A.M. Russo & E.P. Silva 1992a. Fluxo gênico e estruturação em populaçães de invertebrados marinhos bênticos. *Braz. J. Genet.* 15: 274-279.
Solé-Cava, A.M. & J.P. Thorpe 1986. Genetic differentiation between morphotypes of the marine sponge, *Suberites ficus* (Demospongiae, Hadromerida). *Mar. Biol.* 93: 247-253.
Solé-Cava, A.M. & J.P. Thorpe 1987. The uses of electrophoresis in sponge taxonomy. In: J. Vacelet & N. Boury-Esnault (eds.), *Taxonomy of Porifera.NATO-ASI G13:* 243-258. Berlin: Springer Verlag, .
Solé-Cava, A.M. & J.P. Thorpe 1991. High levels of genetic variation in natural populations of lower marine invertebrates. *Biol. J. Linn. Soc.* 44: 65-80.
Solé-Cava, A.M., M. Klautau, N. Boury-Esnault, R. Borojevic & J.P. Thorpe 1991a. Genetic evidence for cryptic speciation in allopatric populations of two cosmopolitan species of the calcareous sponge genus *Clathrina*. *Mar. Biol.* 111: 381-386.
Solé-Cava, A.M., J.P. Thorpe & R. Manconi 1991b. A new species of Mediterranean *Axinella* (Porifera - Demospongiae) detected by isozyme electrophoresis. In: J. Reitner & H. Keupp (eds.), *Fossil and recent sponges*: 313-321. Berlin: Springer Verlag.
Solé-Cava, A.M., N. Boury-Esnault, J. Vacelet & J.P. Thorpe 1992b. Biochemical genetic divergence and systematics in sponges of the genera *Corticium* and *Oscarella* (Demospongiae: Homoscleromorpha) in the Mediterranean Sea. *Mar. Biol.* 113: 299-304.
Stock, D.W., K.D. Moberg, L.R. Maxson & G.S. Whitt 1991. A phylogenetic analysis of the 18S ribosomal DNA sequence of the coelacanth *Latimeria chalumnae*. *Environm. Biol. Fish* 32: 99-117.
Stoddart, J.A. 1989. Foliose Dictyoceratida of the Australian great barrier reef. III. Preliminary electrophoretic systematics. *Mar. Ecol.* 10: 167-178.
Stoddart, J.A., D.J. Ayre, B. Willis & A.J. Heyward 1985. Self-recognition in sponges and corals? *Evolution* 39: 461-463.

Thorpe, J.P. 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. *Ann. Rev. Ecol. Syst.* 13: 139-168.

Van de Vyver, G. 1970. La non confluence intraspécifique chez les spongiaires et la notion d'individu. *Ann. Embryol. Morph.* 3: 251-262.

Van de Vyver, G. 1988. Histocompatibility responses in freshwater sponges: a model for studies of cell-cell interactions in natural populations and experimental systems. In: R.K. Grosberg, D. Hedgecock & K. Nelson (eds.), *Invertebrate historecognition*: 1-13. New York: Plenum Press.

Welsh, J. & M. McClennand 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18: 7212-7218.

Williams, J.K.G., A.R. Kubelick, K.J. Livak, J.A. Rafalski & S.V. Tingey 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.

Wulff, J.L. 1986. Variation in clone structure of fragmenting coral reef sponges. *Biol. J. Linn. Soc.* 27: 311-330.

Zea, S. 1993. Recruitment of Demosponges (Porifera, Demospongiae) in rocky and coral reef habitats of Santa Marta, Colombian Caribbean. *Mar. Ecol.* 1 : 1-22.