POPULATION GENETICS AND PHYLOGEOGRAPHY OF SPONGES - A WORKSHOP SYNTHESIS

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ABSTRACT

We provide a synthesis of a workshop on sponge population genetics and phylogeography, held at the 6th International Sponge Conference in Rapallo. The main issues discussed were: sponge population structure and larval dispersal, whether the high levels of gene variation observed in allozymes occur with other molecular markers, and the development of novel markers. Current data for microevolutionary processes in sponges lag behind other more well-studied marine taxa in some areas, especially regarding the development and application of up-to-date molecular markers. Some of the pertinent problems were identified and several areas of innovative future research approaches are outlined.

KEYWORDS

Population genetics, phylogeography, Porifera, population structure, dispersal, molecular markers.

INTRODUCTION

Investigations at the population level using molecular markers in sponges remain highly topical, despite numerous population genetic studies using allozyme electrophoresis (reviewed in SOLÉ-CAVA & BOURY-ESNAULT, 1999; BORCHIELLINI *et al.,* 2000; VAN OPPEN *et al.,* 2002). Population level studies remain a research focus due to the enormous biodiversity of sponges (HOOPER *et al.,* 2002), their pharmaceutical and biotechnological potential (MUNRO *et al.,* 1994), and because many microevolutionary aspects as well as their basic biology, systematics and taxonomy remain enigmatic (HOOPER & VAN SOEST, 2002). A workshop was held at the 6th International Sponge Symposium in Rapallo to discuss three pertinent issues relating to sponge population genetics and phylogeography: a) how sponge population structure and dispersal capabilities correlate with genetic variation, b) whether there is a parallel between the high levels of gene variation observed in allozymes with other molecular markers; c) how to find and develop novel molecular markers for population genetics and phylogeography. Here, we provide a synthesis of this workshop. Citations are given in the text either to published data or to direct contributions of the cited persons during the workshop.

DISCUSSION AND CONCLUSIONS

Sponge population structure and larval dispersal

One of the main issues discussed was the putative relationship between high genetic variation and frequent occurrence of cryptic speciation in sympatric and allopatric sponge populations. In addition, how do the alleged low dispersal capabilities of sponge larvae contribute to these population characteristics?

It was noted that in the laboratory larvae may survive from two to seven days, but in their natural habitat most sponge larvae do not swim for long periods (URIZ *et al.,* 1998). Under normal circumstances, a bias against long distance dispersal was thought to be likely, with a requirement for larvae to settle quickly (van Soest). Many species will settle within 12 to 24 hours given a suitable substrate, and the life span of larvae appears to be short, resulting in poor survival of juveniles if settlement occurs beyond a viable pre-settlement phase (Leys).

A few exceptions to rapid local settlement have been reported in the literature, with juveniles of calcareous sponges found on plates 7 km off the Mediterranean coast (VACELET, 1981), and a report of long-lived planktonic propagules in *Alectona* (VACELET, 1999). Preliminary laboratory experiments appear to suggest that sponge larvae may be able to metamorphose in a pelagic environment (Ilan), but unfavourable sea conditions *e.g.* wave action, are likely to prevent extended pelagic survival in natural circumstances (Boury-Esnault). A different strategy to prolong larval life, such as developmental arrest, may occur in extreme situations, *e.g.* hydrothermal vents (Leys). However, even larvae that are capable of prolonged viability or dispersal may settle near their origin given suitable habitat and cues, as observed in many other marine invertebrates (Solé-Cava).

It is also important to determine if the colonization of different areas was episodic or continuous, which could be investigated using fast evolving markers like microsatellites (Solé-Cava). Drifting of sponge fragments and rafting on ocean debris may play an important role in asexual episodic recruitment (Wörheide). Further, sponge larvae have been found to survive and disperse in fragmented adult tissue (MALDONADO & URIZ, 1999). Cyanobacterial symbionts that are transferred in the eggs and larvae of *Chondrilla australiensis* (USHER *et al.,* 2001) may help prolong larval life, and thereby contribute to the widespread distribution of this species (Usher).

Finally, we need to consider sponge dispersal in geological time frames, as some populations may have evolved over long time frames (Wörheide). For example, populations of *Chondrosia* from Bermuda and Brazil are genetically quite similar (allozyme electrophoresis data, LAZOSKI *et al.,* 2001), although geographically distant. Possibly there has not been sufficient elapsed time for these populations to diverge genetically and accumulate mutations (Solé-Cava). Once separated, sponge populations may require long time spans to reach migration/drift equilibrium, so that high levels of genetic homogeneity should not be interpreted as unambiguous indications of current levels of gene flow (BOSSART & PROWELL, 1998). In addition, overlapping generations are frequently observed in sponges due to the occurrence of asexual dispersal (fragmentation) again suggesting that sponge populations may not reach equilibrium rapidly. As a consequence, highly variable molecular markers are required, and other models such as nearest neighbour and parametric likelihood analyses (GUINAND *et al.,* 2002) or non-equilibrium models should be applied (*e.g.* WÖRHEIDE *et al.,* 2002b) (Solé-Cava).

More studies combining field observations on larval biology, including length of life and dispersal capabilities coupled with genetic approaches (application of high resolution / fast evolving markers like microsatellites) are necessary to elucidate these important aspects of sponge-microevolutionary processes and biology.

Genetic variation – allozyme results and other molecular markers

Although allozyme electrophoresis has been the method of choice with great success (*e.g.* THORPE & SOLE-CAVA, 1994) for population genetics in marine invertebrates, including sponges, there is a need for the development of new markers that provide sufficient resolution at or below the species/population level. Interpretation of allozyme gels is very difficult for the inexperienced (Solé-Cava) and the requirement for fresh tissue is a disadvantage. Contamination by bacterial symbionts does not appear to be a problem in allozyme electrophoresis (MILLER *et al.,* 2001), as bacterial cells are not damaged by the preparation procedures.

In contrast, DNA-based methods, such as sequencing, are more straightforward, and as for allozyme electrophoresis, contamination by bacterial symbionts is theoretically not a problem in PCR experiments if metazoan primers are used.

Contamination with exogeneous DNA can be problematic in sponges, especially if designing and developing new markers such as microsatellites (Wörheide). Similarily, if "universal primers" (*e.g.* FOLMER *et al.,* 1994) are used, these primers might amplify exogeneous DNA (*e.g.* nematode) (Nichols) and it is mandatory to always check sequences by BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST /) to verify poriferan origin (Wörheide). However, BLAST searches are not always infallible, *e.g.* if deposited sequences are wrongly identified (Solé-Cava). If new markers like microsatellites are being developed (*e.g.* DURAN *et al.,* 2002), large exon-containing regions adjacent to the microsatellites could be amplified initially (about 3-5kb), as those regions are likely to contain genes whose poriferan identity could be verified (Müller). Gene density in the sponge genome is about 10x higher than in humans (Müller), because sponge introns are mostly short (< 2000 bp) but located in conserved positions (MÜLLER *et al.,* 2002) - a fact that could be beneficial for developing EPIC primers (exon priming-intron crossing; LESSA & APPLEBAUM, 1993; PALUMBI & BAKER, 1994) for nuclear intron phylogenies.

Novel molecular markers

Presently, the limited information available suggests that genes of mitochondrial DNA do not appear to provide the appropriate resolution for the investigation of microevolutionary processes in sponges, although only a few genes have been examined so far (COI, COII, reviewed in SHEARER *et al.,* 2002; see also DURAN *et al.,* 2003). However, if future studies further support the limited data about slow evolution in sponge-mtDNA, which appears to be similar to rates in anthozoan cnidarians (reviewed in KNOWLTON, 2000; SHEARER *et al.,* 2002), sponge mitochondrial DNA sequences could be a useful marker for medium level phylogenies (*e.g.* ERPENBECK *et al.,* 2002) in lineages that diverged up to 200 MYA ago (Müller).

The putative control region, apparently the fastest evolving region of animal mitochondrial DNA (PESOLE *et al.,* 1999), has not been investigated in sponges, so we do not presently know if one exists in the sponge mitochondrial genome (Solé-

Cava). Complete mitochondrial genome sequences are needed, but all attempts at mitogenome sequencing have failed so far (Müller). The fact that many fungi, protists and cnidarians have linear mtDNA molecules (BRIDGE *et al.,* 1992; NOSEK *et al.,* 1998) should also be taken into account if approaches like whole-mt PCR are to be attempted.

Other DNA markers currently used for phylogeography are internal transcribed spacer sequences (LOPEZ *et al.,* 2002; WÖRHEIDE *et al.,* 2002a,b), which also have disadvantages as they are a multi-copy marker (VAN OPPEN *et al.,* 2002). They are not highly variable, *e.g.* individuals of *Spirastrella* from both sides of the Isthmus of Panama show little divergence (Nichols). The development of variable single-copy nuclear markers (HARE, 2001) is likely to be the most promising future avenue for genealogical approaches.

The need for unifying the presentation of data

One of the big advantages of DNA sequences over allozyme data is the possibility of inter-laboratory comparisons of data, particularly because of the existence of large, world-wide sequence-databases like Genbank (http://www.ncbi.nlm.nih.gov/) (Müller). However, in order for this advantage to be fully realized, it is necessary that care be taken in the submission of sequences, particularly in the identification of the specimens from which they were obtained (Solé-Cava). Ideally, the same specimens from which the sequences were produced should be deposited in museums, and their voucher numbers should be included in the descriptors of the sequences at the time of submission to the databases. In addition, whenever large numbers of sequences are produced and analysed, their alignments should be deposited in alignment bases such as that of the European Molecular Biology Laboratories (EMBL: ftp://ftp.ebi.ac.uk/pub/databases/embl/align/). It is also very important that the approaches used for the analyses of sequence data are clearly stated in each paper, particularly regarding the treatment of insertions/deletions and the assumptions of the models used (POSADA & CRANDALL, 1998; MCGUIRE *et al.,* 2001; WHELAN *et al.,* 2001; WANG *et al.,* 2002).

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