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Biodiversity, molecular ecology and phylogeography of marine sponges: patterns, implications and outlooks[1]

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SYNOPSIS. Marine sponges are an ecologically important and highly diverse component of marine benthic communities, found in all the world's oceans, at all depths. Although their commercial potential and evolutionary importance is increasingly recognized, many pivotal aspects of their basic biology remain enigmatic. Knowledge of historical biogeographic affinities and biodiversity patterns is rudimentary, and there are still few data about genetic variation among sponge populations and spatial patterns of this variation. Biodiversity analyses of tropical Australasian sponges revealed spatial trends not universally reflected in the distributions of other marine phyla within the Indo-West Pacific region. At smaller spatial scales sponges frequently form heterogeneous, spatially patchy assemblages, with some empirical evidence suggesting that environmental variables such as light and/or turbidity strongly contribute to local distributions. There are no apparent latitudinal diversity gradients at larger spatial scales but stochastic processes, such as changing current patterns, the presence or absence of major carbonate platforms and historical biogeography, may determine modern day distributions. Studies on Caribbean oceanic reefs have revealed similar patterns, only weakly correlated with environmental factors. However, several questions remain where molecular approaches promise great potential, *e.g.,* concerning connectivity and biogeographic relationships. Studies to date have helped to reveal that sponge populations are genetically highly structured and that historical processes might play an important role in determining such structure. Increasingly sophisticated molecular tools are now being applied, with results contributing significantly to a better understanding of poriferan microevolutionary processes and molecular ecology.

INTRODUCTION

Molecular studies in marine biodiversity and ecology have enjoyed a steady boost in popularity since the mid-1980s. Applying increasingly sophisticated molecular tools (*e.g.,* Sunnucks, 2000; Posada and Crandall, 2001), these studies have contributed much to better understanding the marine biome. The ocean has been thought to have few boundaries, and marine species have been often perceived to be panmictic (Palumbi, 1994). However, genetic studies have clearly shown that this is mostly not the case, with frequent occurrence of cryptic sibling species (Thorpe and Solé-Cava, 1994; Knowlton, 2000). Furthermore, genetic markers have allowed determination of ranges and distributions of marine taxa, and estimation of genetic diversity of marine stocks. Molecular markers have also made it possible to evaluate the degree of genetic cohesiveness between populations of marine species, as well as discrimination of the ecological and historical processes shaping their present day distributions. Genetic studies of this kind are pivotal aids to bioregional planning, fisheries management and conservation of dwindling marine resources.

Marine sponges are an essential and highly diverse component of marine benthic communities, ranging from the euryhaline estuarine, to intertidal, to the deep-sea (Hooper and Van Soest, 2002). Aside from their important role for reconstructing metazoan evolutionary relationships (*e.g.,* see Maldonado, 2004; Nichols, 2004), the commercial potential of sponges is increasingly being recognized (*e.g.,* Munro *et al.*, 1994; Faulkner, 2002), but many aspects of their basic biology and especially their biogeographic relationships remain enigmatic.

Some recent major changes were proposed for the higher systematics of Porifera (Hooper and Van Soest, 2002) and molecular data have significantly contributed to our understanding of sponge systematics (reviewed in Borchiellini *et al.*, 2000; Boury-Esnault and Solé-Cava, 2004). Despite significant progress towards the goal of a strictly phylogenetic classification of all sponge classes corroborated by robust molecular data (*e.g.,* Chombard *et al.*, 1997; Alvarez *et al.*, 2000; McCormack *et al.*, 2002; Manuel *et al.*, 2003; Erpenbeck, 2004), the systematic framework in general is still relatively poorly resolved. Similarly, knowledge of biodiversity (richness, endemism, spatial distributions, *e.g.,* Hooper *et al.*, 2002), historical biogeographic affinities (*e.g.,* van Soest and Hajdu, 1997) remains rudimentary and there are few data about genetic variation among sponge populations and spatial patterns of this variation (reviewed in van Oppen *et al.*, 2002*b*; Boury-Esnault and Solé-Cava, 2004). Even less is known about the processes responsible for shaping the genetic structure of sponge populations (Wörheide *et al.*, 2004*c*).

Better understanding of these aspects has crucial implications for evolutionary and ecological studies not only of sponges, but of most marine invertebrate spe-

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cies. As in other marine invertebrate taxa, sponge species have often been perceived to be widely-distributed, with many having near cosmopolitan distributions, assuming panmixia at least at the ocean basin scale. Many of these allegedly wide distributions are now known to result from the lumping of morphologically similar but often evolutionarily distinct lineages into single, artificially cosmopolitan morphospecies (Klautau *et al.*, 1999). Furthermore, from the data available on sponge larval biology, it appears that dispersal capabilities of propagules are limited, mostly not exceeding 72 hr in the water column before settlement (Maldonado and Young, 1996; Uriz *et al.*, 1998) (for an exception see Vacelet, 1981), and one might predict high genetic structuring of sponge populations.

Worldwide about 7,000 described species of sponges are considered to be valid, although potentially many more remain undescribed as evidenced by the huge, largely unidentified collections in the world's museums. These collections, together with molecular studies that have detected a number of cryptic sibling species, suggest that this diversity might be twice that presently recognized (Hooper and Lévi, 1994), representing a considerable body of taxonomic work still awaiting to be done. Several regional faunas are comparatively well known, including the Mediterranean, Caribbean and Australian (*e.g.,* van Soest, 1994), but most of the world's sponge faunas remain both relatively undersampled and unidentified.

Here we briefly review patterns of sponge biodiversity, with Australasian and amphi-Atlantic sponge populations as examples, and highlight a few pertinent issues where genetic studies have demonstrated their utility to contribute significantly to resolve trends where morphometric data sets alone were inadequate. We also provide an overview on recent progress in the field of poriferan molecular biodiversity and ecology, which has made sponges much more accessible as potential model research organisms.

BIODIVERSITY

Australasian tropical sponge fauna

Knowledge of the Australian regional sponge fauna commenced with the pivotal works of Lamarck in the early 1800s and continues in a greatly escalated way up to the present day. The described fauna consists of approximately 1,400 species in 313 genera and 83 families (Hooper and Wiedenmayer, 1994; ABIF-Fauna, 2004), although over 4,000 morphospecies have already been collected (with records soon to be available online at Ozcam; www.ozcam.gov.au). The estimate of 5,000 species proposed for the entire regional fauna (Hooper and Lévi, 1994) largely ignores the potentially very many cryptic and thinly encrusting species that still await discovery. Over the last two decades knowledge of this sponge fauna has received substantial attention owing to its growing potential as commercial sources of novel therapeutic compounds (*e.g.,* Munro *et al.*, 1999; Faulkner, 2002), but the majority of species are still unnamed and a significant challenge remains to reconcile living populations with often ancient and inadequate published taxonomic descriptions. Nevertheless, Australian sponge distributional data have been used with some success as an analytical tool for bioregional marine conservation planning and management (*e.g.,* Great Barrier Reef Marine Park Authority Representative Areas Program—http://www.reefed.edu.au/rap/; National Oceans Office marine bioregionalisation of Australian territorial waters—http://www.oceans.gov.au/oceans_portal.jsp), and several biodiversity analyses, based on species presence/absence data.

These latter studies have revealed some spatial trends that differ from other marine phyla in the Indo-West Pacific (*e.g.,* Hooper *et al.*, 2002), and together with molecular studies offer some clues on sponge community patterns.

Major trends from biodiversity analyses of the Australian tropical fauna, at the smaller ''intra-regional'' spatial scales, indicate that sponges frequently form spatially heterogeneous assemblages with patchy distributions, often containing high numbers of species not found in adjacent communities (termed ''apparent endemics''; *e.g.,* Hooper and Kennedy, 2002), sometimes with as little as 15% similarity in species composition between geographically adjacent reef sites (Hooper, 1998). The potential connectivity between adjacent communities is hampered by their reportedly very limited sexual reproductive dispersal capabilities and alleged preponderance of clonal dispersal and recruitment (Battershill and Bergquist, 1990; Zea, 1993; —but see Davis *et al.*, 1996; Zea, 2002).

From studies on cross-shelf distributions certain environmental variables have been linked to community heterogeneity, most notably light, depth, substrate quality and nature such as coralline *vs.* non-coralline, hard *vs.* soft substrata, local reef geomorphology indicative of the presence or absence of specialised niches, water quality and flow regimes, food particle size availability, larval recruitment and survival (Wilkinson and Cheshire, 1989; Hooper, 1994; Roberts and Davies, 1996). At larger ''landscape'' spatial scales, latitudinal gradients of species richness are absent along the tropical to warm temperate coastal and shelf faunas (Hooper *et al.*, 1999; Hooper *et al.*, 2002). Nevertheless, there are significant differences in species richness and taxonomic composition between the major Australian marine bioregions on the NE, NW, SE and S coasts and shelf faunas, the Coral Sea and subantarctic territories (*e.g.,* Hooper and Lévi, 1994). Those differences indicate large-scale community patterns that might be linked to processes such as changing current patterns resulting from significant sea level changes impacting on connectivity, the presence or absence of major carbonate platforms at the bioregional level, and historical biogeography.

Despite clear bioregionalisation of sponge distributions, most obvious at larger spatial scales, between 5% (New Caledonia fauna, Hooper and Lévi, 1994) and 15% (Sahul Shelf fauna, Hooper, 1994) of species

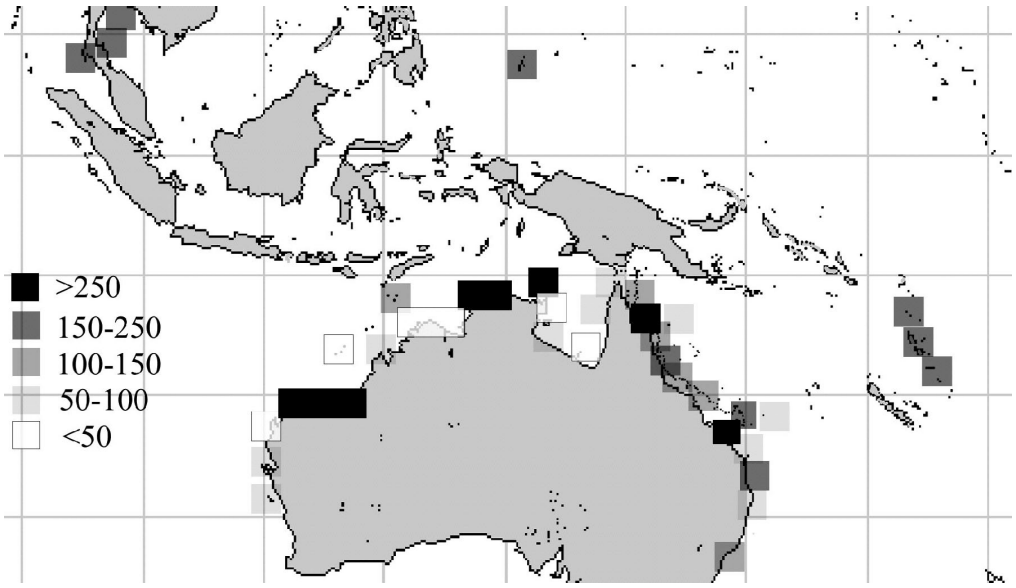

FIG. 1. Patterns of sponge species richness (numbers of species) at smaller inter-regional spatial scales across tropical Australasia, with increasing species richness indicated by darker colours, and biodiversity 'hotspots' in black (data modified from Hooper *et al*., 2002)

are reported to have extensive geographic distributions, ranging from the Red Sea to the central western Pacific islands. More recently, however, the existence of these so-called widely distributed species (*e.g., Astrosclera willeyana*, Wörheide *et al*., 2002*a*; *Chondrilla* spp. Usher *et al*., 2004) has been disputed with molecular evidence, suggesting that they may consist of several cryptic sibling species with high genetic diversity that is not clearly manifested at the morphological level across their wide geographic ranges. This makes their practical species determination difficult and perhaps justifies the adoption of the ''zoogeographic species'' concept (Mayr and Diamond, 2001) for sponge ''superspecies'' where indeterminate practical species boundaries are sidestepped.

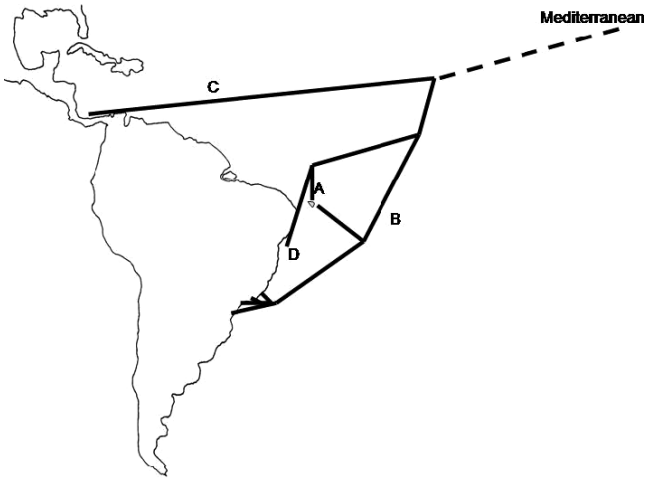

FIG. 2. Genetic relationships between populations of Amphiatlantic *Chondrilla* ''*nucula*,'' based on analyses of 10 allozyme loci (based on Klautau *et al*., 1999). A–D are cryptic species found within the ''cosmopolitan'' *Chondrilla nucula*. These species have been confirmed, and one other cryptic species found, by subsequent ITS sequence analyses (Zilberberg and Solé-Cava, unpublished results).

Determining acceptable, definable or practical spatial scales for these zoogeographic species boundaries still remains unclear, as illustrated by an analysis of larger ''landscape'' spatial scale community structure of sponges in northern Australia (Hooper *et al*., 2002). In that study five hotspots of biodiversity were evident, encompassing tropical to warm temperate waters on the coastal and continental shelf, based on gradients in species richness, taxonomic endemism and marine area relationships (Fig. 1). Of particular interest was high heterogeneity amongst Great Barrier Reef (GBR) sponge assemblages, with separate hotspots in the Southern and Far Northern regions, and a variable mosaic of diversity and species richness elsewhere. This pattern has been subsequently confirmed by a phylogeographic study of the calcareous sponge *Leucetta* '*chagosensis*' (Wörheide *et al*., 2002*b*), described elsewhere in this paper (see Fig. 3). The conclusions question the validity of some traditional Australian marine biogeographical boundaries (reviewed by Wilson and Allen, 1987) proposed to encompass all marine phyla, especially those with apparently limited dispersal capabilities including sponges. Unfortunately we cannot yet apply the same level of knowledge to the southern Australian temperate sponge faunas. Although historically better known, and believed to contain much higher proportions of endemic species with Gondwanan origins than the tropical faunas, they have not been studied in a contemporary or comparable manner to the tropics.

Tropical west-atlantic sponge fauna

Levels of regional endemism between the North- and Southwest Atlantic have been considered to be

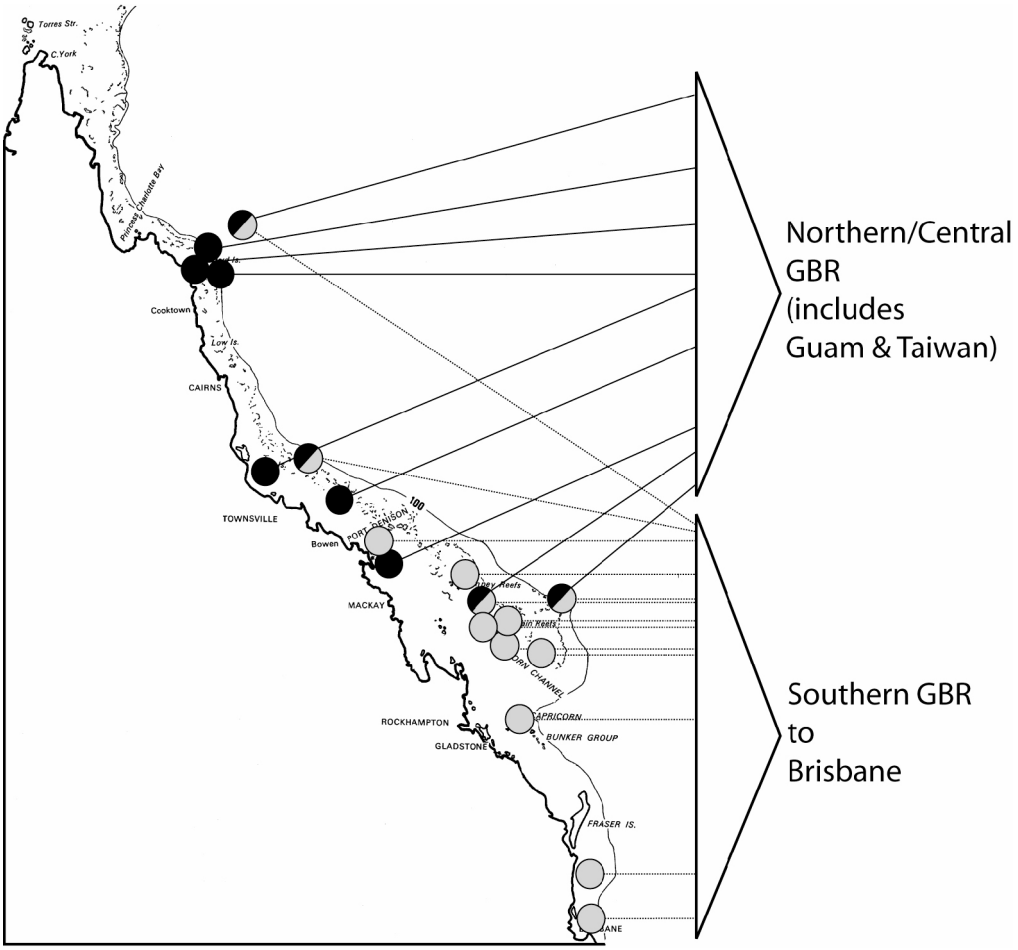

FIG. 3. rDNA ITS Sequence type distribution of *Leucetta* "*chagosensis*" on the Great Barrier Reef. Each circle represents one sampling locality. Distinct and deeply divergent northern and southern clades were found. The northern clade also contains sequence types found in Taiwan and Guam. The two clades only narrowly overlap in the central GBR; however, single specimens containing southern-clade sequence types were found at Osprey and Myrmidon Reefs (modified after Wörheide et al. 2002*b*). This structure has subsequently been confirmed with an extended data set, covering more samples from the central GBR as well as from Reefs on the Queensland Plateau (Epp, 2003).

low, although we still have only relatively few data on sponge biodiversity and biogeography in the Atlantic than the Indo-West Pacific (Hechtel, 1976) to make general statements. Nevertheless, in a recent study (Zea, 2002) on remote oceanic reefs in the Caribbean trends similar to those observed in the Pacific were observed. Patterns of species richness and taxonomic relatedness were stochastic and highly spatially heterogeneous, with only weak correlation with environmental factors, comparable to trends observed on the NW Shelf of Western Australia (Hooper, 1994). Zea's (2002) study was able to demonstrate that the majority of sponges on Caribbean reefs had marked habitat preferences, with alleged random colonization events.

Classic marine biogeography, however, regards the Western tropical Atlantic as a single biogeographic area (the Caribbean province), ranging from about 20 degrees North to 20 degrees South (Briggs, 1974; see also Paulay, 1994; van Soest, 1994). The few molecular studies available indicate that many of the species that were considered to occur both in the North and Southwest Atlantic are, in fact, differentiated, at least at the sub-species level, in those regions (Sarver *et al*., 1998; McCartney *et al*., 2000). This pattern is not unexpected considering the separate circulating cells of North/South surface currents in the Atlantic since the closure of the Tethys Sea (Vrielynck *et al*., 1997), and the strong freshwater and sediment barrier caused by the Amazon outflow (Rocha *et al*., 2002). On the other hand, a remarkably high level of genetic similarity between populations from those regions has been found in many other invertebrate species (Lessios *et al*., 1999; Williams, 2000; Vianna *et al*., 2003; Fukami *et al*., 2004; Nobrega *et al*., 2004). Genetic studies confirm that some sponge species may display little differentiation between the North and South Atlantic (*Chondrosia*, Lazoski *et al*., 2001; *Placospongia* sp1, A. Mattos and A. Solé-Cava, unpublished results). Conversely, in some species, levels of differentiation between the Caribbean and the Atlantic can be very high (Fig. 2; *Chondrilla*; Klautau *et al*., 1999; *Placospongia* sp2 and sp3; A. Mattos and A. Solé-Cava, un-

published results). Thus a mixed scenario seems to exist, with some sponge species able to cross the Amazon barrier and others not able to do so.

Given the importance of determining levels of endemism and phylogenetic uniqueness (Moritz and Faith, 1998) for conservation purposes, a large molecular study is underway to evaluate the levels of local endemism in sponges from the Tropical Western Atlantic. The large number of cryptic species found within amphi-Atlantic species (Solé-Cava *et al*., 1991, 1992; Klautau *et al*., 1994; Lazoski *et al*., 2001) indicate that currently recognized levels of endemism underestimate the real diversity in this area.

PHYLOGEOGRAPHY AND ENDEMISM

Recently, internal transcribed spacer (ITS) sequences of the rDNA have been used successfully in a few sponge phylogeographic studies. Lopez *et al*. (2002) demonstrated a deep phylogeographic break between two disjunct populations of *Axinella corrugata* from the Atlantic and Indian Oceans. Wörheide *et al*. (2002*a*) confirmed that the sclerosponge *Astrosclera* ''*willeyana*,'' is actually a species complex, each sibling species with restricted geographical distributions, as previously suspected from morphological data (Wörheide, 1998). Wörheide *et al*. (2002*b*) applied, for the first time for sponges, Nested Clade Phylogeographic Analysis (NCPA, Templeton *et al*., 1995; Templeton, 1998; Templeton, 2004*b*) to study relationships regional populations of *Leucetta* ''*chagosensis*'' in the SW and NW Pacific. Significantly, they detected a deep genetic separation between the northern and southern Great Barrier Reef (Fig. 3), with both clades more closely related to the Indonesian clade than to each other. The Indonesian clade was hypothesized to be the oldest amongst all clades found. However, in that study sample size was limited and no populations from the Indian Ocean were included. Historical processes such as fragmentation due to periodic lowering of the sea level, with subsequent recolonization from refuges, were hypothesized to be responsible for the observed structure on the GBR.

A more recent study with extended sample sizes of this species (including samples from the Maldives), using the ITS regions plus a 400 bp region of the 28S rDNA, confirmed the phylogeographic structure indicated previously (Epp, 2003). That study also made clear that rDNA sequences were not able to resolve finer population structure with high significance. Duran *et al*. (2003) reported the existence of highly structured populations of *Crambe crambe* in the Mediterranean and eastern Atlantic also based on rDNA ITS sequence types and NCPA, which appeared to result from restricted gene flow and isolation-by-distance. Further, a recent introduction from the Mediterranean Sea to the Macaronesian region via human-mediated transport was inferred. The study of Duran and coworkers was the first to report intragenomic variation in the rDNA spacer regions in sponges: previous studies on *Leucetta* (Wörheide *et al*., 2002*b*) and *Astrosclera* (Wörheide *et al*., 2002*a*) did not detect such variation. However, Duran *et al*. (2003) did not consider the implications of intragenomic variation on intraspecific phylogeny estimation. Wörheide *et al*. (2004*b*) filled this gap recently by surveying, for the first time, diverse marine sponges to determine the extent and phylogenetic implications of intragenomic polymorphisms (IGPs) exhibited at their ITS loci.

The tandemly repeated nuclear ribosomal DNA clusters may prove to be increasingly popular as markers for fine scale analyses in sponges in the future due to their ubiquitous presence and ease of PCR-amplification (see also van Oppen *et al*., 2002*b*). However, the occurrence of divergent paralogs within individual genomes, frequently found in other animal groups (*e.g.,* Hugall *et al*., 1999; van Oppen *et al*., 2002*a*), can be phylogenetically confounding and warranted a thorough investigation in poriferan taxa. Wörheide *et al*. (2004*b*) discovered that ITS IGPs varies greatly between sponge taxa (with most taxa exhibiting very few) and cannot be predicted by morphologically based taxonomic methods. Nevertheless, it was demonstrated that ITS can be phylogenetically informative between species when moderate levels of IGPs are detected, but that ITS paralogy can interfere with population level studies. Wörheide *et al*. (2004*b*) cautioned against the routine use of ITS in phylogenetic studies of sponges without 1) screening for IGPs in specimens from all populations sampled; 2) including all divergent paralogs in phylogenetic analyses; 3) testing ITS phylogenies using other single-copy, unlinked loci (such as nuclear introns and allozymes).

While mitochondrial DNA (mtDNA) sequences are most frequently employed for studies on intraspecific phylogeography in most animal taxa (Avise, 2000), especially cytochrome oxidase I (COI and COII), these sequences appear to be too conserved in sponges (Wörheide *et al*., 2000, Wörheide, unpublished data; Shearer *et al*., 2002; Duran *et al*., 2003) to provide adequate information to resolve population level relationships.

Microsatellite loci, potentially more variable nuclear markers, are only available for two sponges (*Crambe crambe*: Duran *et al*., 2002; *Halichondria panicea*: Knowlton *et al*., 2003). Surprisingly, variation in microsatellite alleles in *Halichondria* was relatively low. Conversely, six microsatellite loci of *C. crambe* were sufficiently polymorphic to be used to compare Mediterranean and Atlantic populations (Duran *et al*., 2004). They evidenced a high level of population structuring ($F_{ST} = 0.18$), as well as a significant ($P < 0.02$) correlation between geographic distance and pair-wise F_{ST} values, showing that they can be useful for fine-scale studies of sponge populations. However, many heterozygote deficiencies were found, and there was a lack of geographical consistency in pairwise F_{ST} comparisons among sample localities (recalculated from the gene frequency tables presented by Duran *et al*., 2004).

From the limited data that we have to date, very

few if any general patterns can be deduced. However, it appears that sponge populations are genetically highly structured; even the previously so-called genetically homogeneous Great Barrier Reef was found to harbor deeply divergent ITS clades of *Leucetta* (Wörheide *et al*., 2002*b*) (see Fig. 3), and the Western Atlantic area presents at least five different and highly divergent species within what was considered to be only one species of *Chondrilla* (Klautau *et al*., 1999) (Fig. 1). Historical processes, such as changed current systems during sea level low stands, might play an important role in structuring sponge populations (Wörheide *et al*., 2002*b*), although recent ecological processes like human-mediated transport may also contribute significantly, with the apparent restricted dispersal capabilities of sponge larvae most likely contributing to patterns of restricted gene flow and isolation-by-distance (Duran *et al*., 2003). Clearly, more studies are needed, and the development of new and more variable single-copy molecular markers that promise higher resolution is currently topical (van Oppen *et al*., 2002*b*; Wörheide *et al*., 2004*c*).

Implications and Outlook

As highlighted in the previous sections, biodiversity analyses have shown that at smaller spatial scales, Australasian sponges frequently form heterogeneous assemblages, with no apparent latitudinal diversity gradients at larger spatial scales.

Molecular studies promise to contribute significantly to our understanding of sponge biodiversity patterns where the power of species presence/absence data clearly fades. Such studies have already helped to reveal that sponge populations are genetically highly structured and that historical processes might play an important role in determining such structure. While allozyme studies are important to determine population-level differences in allele frequencies, and have contributed much to discover cryptic species, they have acknowledged limitations. Studies using allele frequencies (like those of allozymes, intron length polymorphisms and microsatellites) also ignore the information about how alleles are related phylogenetically. Here DNA sequence markers are better suited, because phylogenetic relationships of alleles can be presented in allele (gene) trees. To date, however, only a few DNA sequence markers have been applied to study sponge populations. The most frequently used of these, the internal transcribed spacers of the rDNA, successfully resolved larger (gamma) scale phylogeographic patterns, but have limited value at finer spatial scale applications. Due to limitations resulting from their molecular biology (*e.g.,* Gerbi, 1985; Hillis and Dixon, 1991), *i.e.,* they are repetitive multi-copy markers not behaving in a Mendelian fashion (Álvarez and Wendel, 2003; Wörheide *et al*., 2004*b*), they are not suitable for population genetic analyses (van Oppen *et al*., 2002*b*). Mitochondrial DNA (mtDNA), the most frequently used DNA sequence marker for phylogeographic studies in most animal taxa, apparently shows little variation on the population scale in sponges (Duran *et al*., 2003; Wörheide, unpublished data). However, only cytochrome oxidase I and II have been investigated so far, and it is probably too early to rule out mitochondrial DNA as an informative molecule for phylogeographic analyses in sponges. No complete sponge mtDNA genome has been published to date (although preliminary data were presented at the 2004 SICB meeting, Lavrov and Lang, 2003), and mtDNA may contain regions variable enough for population level analyses.

The lack of available gene systems for the study of sponge populations is critical (van Oppen *et al*., 2002*b*), because patterns observed from the study of single *loci* may only be relevant for the evolutionary patterns of those, but have little to do with the underlying biogeographical problems of interest. One obvious way of reducing the errors associated with the use of single-gene trees is the simultaneous use of multiple, unlinked genes (Edwards and Beerli, 2000). The time for alleles to coalesce can be very long, so that incomplete lineage sorting can be an important source of homoplasy in phylogeographic analysis (Rosenberg, 2003; Ballard and Whitlock, 2004).

Although nuclear gene genealogies have proven potential for phylogeographic studies (Hare, 2001), and are pivotal for comparative multi-locus phylogeography (Bermingham and Moritz, 1998) and cross-validation of phylogeographic hypotheses (Templeton, 2004*a*), their universal application is hampered by several difficulties, such as recombination, paralogy due to gene duplication, length heterozygotes (reviewed, *e.g.,* in Hare, 2001) and their three-times longer coalescent times compared to mitochondrial loci (Palumbi *et al*., 2001). In addition, it is usually necessary to develop such markers *de novo,* especially in lesser-known phyla such as sponges (see Lessa, 1992; Lessa and Applebaum, 1993; Palumbi and Baker, 1994 for approaches and how to overcome technological difficulties). In sponges in particular, due to the fact that they harbour a diverse fauna of commensals and symbionts (*e.g.,* Pawlik *et al*., 1995; Duffy, 1996), it is mandatory to carefully establish the true taxonomic nature of sequenced alleles. This can be done efficiently by comprehensive sub-cloning and sequencing of PCR products amplified with conserved primers, and subsequent database searches or cladistic analyses (Erpenbeck *et al*., 2002). The analysis of intron polymorphisms may be the way forward in molecular ecology studies of sponges, because they can be numerous and unlinked. Such markers are currently under development and preliminary results presented during the symposium on an ATPSβ intron allele-phylogeny of *Leucetta* "*chagosensis*" populations, amplified with conserved primers (Jarman *et al*., 2002), are promising (Wörheide *et al*., 2004*a*).

Microsatellite loci, although published for only two sponge species as yet, have already demonstrated their usefulness for fine-scale studies. Even though their analysis represents clear progress, they also need to be

analyzed and interpreted very carefully. The high variances associated with microsatellite loci mean that sample sizes must be high (typically >50 samples per locality, Ruzzante, 1998). Furthermore, microsatellite systems suffer from low cross-species ubiquity, so they often need to be designed from scratch for each species studied. The constraint of having to design new primers for each species and the need to use high sample sizes ultimately translate into high cost in human and financial resources when studying marine populations, often limiting their use to specific cases where other markers have failed to produce results.

The array of molecular and analytical methods currently available for the study of ecological and evolutionary genetics of sponges is relatively large. Much progress has been made since the earlier genetic studies, when only allozymes were available (*e.g.,* Solé-Cava and Thorpe, 1986; Sarà *et al*., 1988). However, few if any general patterns can be deduced as yet, and issues to be investigated more broadly include detecting how sponge populations are spatially structured in different ecological settings, what factors are responsible for the patterns observed, and how different life histories, clonal reproduction and recruitment influence those patterns and lead to speciation. Determining the spatial scales and actual boundaries of biological and zoogeographical species, the connectivity between populations, investigating phylogeographic relationships and the timing of population divergences will be pivotal.

Methodological choices now are numerous: frequency methods are suited for detection of cryptic species and for fine-scale population analyses, and sequence approaches are useful for everything else; sequence data can be analysed by distance or discrete character methods, and the computing resources currently available in most laboratories permit powerful evolutionary analyses including maximum likelihood, Bayesian and Coalescence that were unavailable 20 years ago. The association of cheaper-by-the-day DNA sequencing and the sound theoretical framework of these approaches, resulting in testable phylogeographic hypotheses, is starting to shape the future of sponge microevolutionary studies. Now is the time to wisely choose the good problems to study. The methodology is finally ready to efficiently deal with them.

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