

5-7-2009

Biogeography of Microorganisms Inhabiting Marine Sponges

Suzy C. Szumowski
Macalester College

Follow this and additional works at: <http://digitalcommons.macalester.edu/biogeography>

Recommended Citation

Szumowski, Suzy C. (2008) "Biogeography of Microorganisms Inhabiting Marine Sponges," *Macalester Reviews in Biogeography*: Vol. 1, Article 4.

Available at: <http://digitalcommons.macalester.edu/biogeography/vol1/iss1/4>

This Article is brought to you for free and open access by the Biology Department at DigitalCommons@Macalester College. It has been accepted for inclusion in Macalester Reviews in Biogeography by an authorized administrator of DigitalCommons@Macalester College. For more information, please contact scholarpub@macalester.edu.

Biogeography of Microorganisms Inhabiting Marine Sponges

Suzy C. Szumowski

ABSTRACT

Sponge-associated microorganisms are an intriguing system for the consideration of biogeographic patterns due to complications arising from their small size and their symbiotic relationship with sponges. Whether or not microorganisms are capable of exhibiting biogeographic patterns is a topic of heated debate in the literature. Recent studies utilizing new identification techniques have revealed an astonishing amount of diversity and spatiotemporal stability in microorganism communities. Case studies outlined in this paper suggest that some sponge-associated microorganisms represent monophyletic groups that have evolved species-specific associations with their hosts. The biogeography of these organisms is receiving increasing attention because they are one of the richest sources of novel bioactive compounds with pharmaceutical applications. Future work in this system should focus on sampling microbial distributions more thoroughly by applying methods with higher phylogenetic resolving power in more diverse habitats.

INTRODUCTION TO MICROORGANISMAL BIOGEOGRAPHY

The field of biogeography is concerned with geographic distribution patterns of taxonomic groups. Researchers often focus on distinguishing between environmental and historical causation for observed patterns of speciation, diversity, colonization, and extinction, all of which contribute to variations in biodiversity across space and time. Perhaps due to the inherent bias of human researchers, most biogeographic studies focus on relatively large, terrestrial organisms. Marine and microorganismal systems have received substantially less attention despite their disproportionate contributions to global habitat area and biomass.

Many fundamental theories of biogeography are highly sensitive to the scale of the system in question (Cox and Moore, 1999). For example, when discussing "extinction" events, it is important to specify whether the area in question is the entire globe, a certain continent, or a specific blade of grass. Thus, considering biogeography on a microorganismal scale involves several interesting twists compared to traditional studies of macroorganisms. The purpose of this paper is to consider the biogeography of marine microorganisms by using microorganisms that inhabit marine sponges as a model system.

COMPLICATIONS IN MICROORGANISMAL BIOGEOGRAPHY

Insufficient knowledge

The field of biogeography relating to microorganisms varies significantly from that of macroorganisms for several reasons, most of which can be attributed to the microscopic size of these organisms. As opposed to vascular plant and vertebrate species, of which an estimated 83% and 90% have been described, only 20% of existing protozoans and algae, and 1% of bacteria and have been characterized. However, microorganisms can comprise over 60% of the biomass in some systems (Cox and Moore, 1999). This paucity of knowledge stems from difficulties associated with studying such minuscule organisms and is but one of the factors that complicates studying their biogeography.

The dispersal debate: cosmopolitan theory

Another complication with microorganismal biogeography is the differing interpretations of dispersal capabilities that exist among researchers. There is a long-standing debate in the literature regarding whether microorganisms have distinct biogeographies, or if all microscopic organisms have a cosmopolitan distribution (Hedlund and Staley, 2004). Some researchers hypothesize that due to the small size of microorganisms, fewer constraints may act on their

distribution compared to macroorganisms. Thus, combined with high population densities and minimal range-size requirements, the small size of microorganisms may allow virtually unimpeded dispersal and eliminate biogeographic patterning. Other researchers argue that endemism and distinct biogeographies can be detected if sensitive enough analysis methods are used. These two differing interpretations of dispersal capabilities result in significantly different implications for microorganism diversity.

The dispersal debate originated in 1934, when the Baas-Becking hypothesis, also known as cosmopolitan theory, was first proposed. This theory states that “everything is everywhere and the environment selects”. In other words, microorganisms do not have biogeographies because their distribution is dependent solely on survival probability at a given location. Therefore, distribution is not limited by historical factors or dispersal capabilities. This represents a major departure from macroorganismal biogeographic theory (Finlay, 2002; Hedlund and Staley, 2004; Papke and Ward, 2004; Taylor *et al.*, 2005; Martiny *et al.*, 2006). Cosmopolitan theory also implies that the genetic unity of microorganisms can be disrupted only by adaptation, and not by allopatric speciation (Papke and Ward, 2004). Finlay is a major proponent of cosmopolitan theory, and points out that many microbial species have inactive states that enable long-term survival and thus wide ranging dispersal via random environmental processes. He cites genetically identical foraminifera from Arctic and Antarctic waters as support for universal microbe distribution. Additionally, Finlay claims that the ability of microbial eukaryotes to adapt to wide ranging ecologic factors would limit the number of species possible (Finlay, 2002). However, the popularity of cosmopolitan theory is currently declining.

The dispersal debate: existence of endemism

Countering Finlay and cosmopolitan theory are those who believe that microbial endemism exists. Many recent studies propose that microbes display complex biogeographic patterns (Hentschel *et al.*, 2002; Papke and Ward, 2004; Taylor *et al.*, 2004; Webster *et al.*, 2004; Taylor *et al.*, 2005; Hill *et al.*, 2006; Martiny *et al.*, 2006; Holmes and Blanch, 2007; Taylor *et al.*, 2007; Thiel *et al.*, 2007). All of these studies report evidence of endemic microorganisms and argue against universal dispersal. Finlay (2002) contends that seemingly endemic microbes are merely the result of insufficient sampling efforts, and that as more habitats are examined, supposedly endemic microbes will be identified around the globe.

Supporters of the existence of microbial endemism have suggested that some free-living microorganisms may even display biogeographic patterns quite similar to those of macroorganisms. Observations based on highly sensitive molecular criteria suggest that

geographic isolation exists even among seemingly homogenous communities of microorganisms (Martiny *et al.*, 2006). Papke and Ward (2004) review studies that demonstrate how free-living microbes can exhibit geographic isolation, thus arguing against the universal validity of the Baas-Becking hypothesis. Cyanobacteria from 42 hot springs in four countries showed that endemism exists between and within countries, independent of the variation of 20 chemical parameters between hot springs. Other studies have also shown that genetic variation in microorganisms can increase with increasing geographic distance and can correlate with changes in environmental factors (Martiny *et al.*, 2006). The macroorganismal species-area relationship, in which more species are found in larger areas, may also hold true for microorganisms. Several attempts have been made to estimate a body length cut-off value, beneath which organisms would be exclusively cosmopolitan (Martiny *et al.*, 2006). However, Hedlund and Staley (2004) challenge these attempts by questioning whether endemism in fact is eliminated with decreasing body size, or if our abilities to resolve species are eliminated with decreasing body size. They thus suggest emphasizing molecular approaches when attempting to distinguish biogeographic patterns. Taylor *et al.* (2005) propose a compromise to the cosmopolitanism/endemism debate, suggesting that while universal dispersal and thus decreased diversity is quite probable for free-living marine protozoans due to the interconnectedness of oceans, such trends should not be extrapolated to terrestrial or host-associated microorganisms, which have been shown to display endemism when methods utilizing high levels of genetic resolution are applied.

So far, most biogeography studies involving microorganisms simply catalog observed distributions as opposed to actively searching for a particular species in a variety of locations. As Taylor *et al.* (2004) point out, the combination of short generation times and small size may facilitate immense spatial and temporal heterogeneity in bacterial communities, which would require more intensive sampling and statistical schemes than those used in ecological studies of plants and animals. It is still debatable whether or not microorganisms exhibit patterns of biogeography similar to those of macroorganisms, patterns unique to microorganisms, or if everything is in fact everywhere, because more intensive sampling of more sensitive data is needed (Hedlund and Staley, 2004). Whether or not biogeographic patterns turn out to be shared with macroorganisms, the small size and ample potential for dispersal pose significant challenges for the study of microorganismal biogeography.

Taxing taxonomy

In addition to the difficulties presented by miniscule size and different mechanisms of dispersal compared to macroorganisms, microorganismal biogeography is also complicated by taxonomic issues. In fact, the definition of the basic unit of study for most macroorganism studies, the species, is still debated on the microorganismal scale. The taxonomic classification system in macroorganisms is based on morphological features rather than genetic differentiation. However, as microorganisms are very small and relatively unfamiliar, the application of this classification system results in the clumping of much more genetically divergent individuals under the same "species" label. For example, individuals of a single bacterial species may share only 70% of their DNA sequence; whereas chimpanzees and humans, two separate macroorganism species, share 98.6% of their DNA. If the same genetic variation criteria were used to define macro- and microorganismal species, many "species" would be revealed in the microbial world that are currently cryptic. The lack of a universal species definition complicates biogeographic comparisons between micro- and macroorganismal systems because it is difficult to pair analogous taxonomic groups (Martiny *et al.*, 2006).

Due to these complications, many studies (Webster *et al.*, 2004; Hill *et al.*, 2005; Meyer and Kuever, 2007) have switched from discussing the distribution patterns of microbial "species" to discussing the distribution patterns of "operational taxonomic units", or OTUs. OTUs are defined based on a >99% sequence identity cutoff. However, the problems of microbial taxonomy cannot be resolved by simply defining OTUs because microorganismal taxonomy is further complicated by diverse gene exchange processes. Many microbes can not only reproduce asexually, but are also capable of horizontal gene transmission (e.g. bacterial plasmid transmission and transformation, or uptake of naked DNA from the environment). These processes are especially problematic for the biological species concept, and thus ultimately affect studies of biogeography as well. Taylor *et al.* (2005) pose a very appropriate question: what level of taxonomic resolution is ecologically relevant for bacteria? However, until a consensus is reached on how to treat microbial "species" or "OTUs", such pertinent questions cannot be adequately addressed. Clearly, the small size of microorganisms complicates the study of their biogeography. Disagreements about microbial classification and their dispersal capabilities mean that researchers must take care to approach the biogeography of microorganisms somewhat differently than macroorganisms.

INTRODUCTION TO SPONGE-ASSOCIATED MICROORGANISMS

Examining the biogeography of symbiotic microorganisms adds yet another layer of complexity to study systems that must be taken into account. For example, the biogeography of the host,

the specificity of the association, and the effects of interactions between the symbiotic organisms may all affect the distribution of symbiotic microorganisms. The remainder of this paper will focus on microorganisms inhabiting marine sponges, a symbiotic relationship that poses interesting challenges to the field of biogeography and serves as a powerful model system for the often overlooked field of marine prokaryote-eukaryote associations (Taylor *et al.* 2004).

Because of the symbiotic nature of sponge-associated microorganisms, it is highly probable that they exhibit distinct biogeographic patterns from free-living microorganisms. Sponge-associated microorganisms may even exhibit different patterns from each other depending on the degree of sponge specificity. Taylor *et al.* (2004) suggest that understanding the amount and scope of host specificity is also essential for an accurate portrayal of global biodiversity because estimates of biodiversity are positively related to high occurrences of host specificity.

The association between sponges and their microorganismal communities is not trivial. Microorganisms inhabiting marine sponges can comprise 40 to 60% of host sponge biomass (Schmitt *et al.*, 2007) and may be up to four orders of magnitude more concentrated in sponge tissue than in sea water (Thakur and Müller, 2004). Studies have consistently shown that some sponges living in distant and dissimilar habitats can host very closely related sponge-associated microbial communities that are distinct from seawater communities (Hentschel *et al.*, 2002; Hill *et al.*, 2005).

In 2002, Hentschel and colleagues published what has quickly become a classic study in the field. In this report they introduced the concept of "bacterial clusters", defined as monophyletic groups of three or more bacterial 16S rRNA gene sequences that have been isolated from multiple sponges species or geographic areas. The bacteria in these clusters are by definition more similar to each other than to non-sponge-associated bacteria and are robust regardless of the phylogenetic model used in analysis. The concept of clusters has become standard in the field and is an analysis approach used in many studies to characterize the diversity of microorganisms inhabiting various species of sponges. Since Hentschel *et al.*'s landmark study, the goal of many studies has been to identify clusters in various geographic regions or associated with various species of marine sponges.

Varying degrees of specificity: generalists, sponge associates, and specialists

Members of all three taxonomic domains have been identified in association with marine sponges. In order to categorize the broad spectrum of associated archaea, eukaryotes and

bacteria, researchers have developed a classification system that avoids complications caused by the incredible diversity and uncertain taxonomy of some microorganisms. Microorganisms associated with sponges are usually grouped into one of three categories based on the degree of association with sponges. The *generalists* are found in ambient seawater and many species of marine animals, including non species-specific associations with sponges. A second group is *sponge associates*, who are always associated with some type of sponge, although the species identity of the host sponge may vary. The third group consists of *specialists*, or microorganisms that are only found on one species of host sponge (Taylor *et al.*, 2004; Meyer and Kuever, 2007; Thiel *et al.*, 2007). This classification system allows researchers to distinguish between microorganisms with varying degrees of specificity to their sponge associates.

One advantage of this system is that it allows microorganisms that are filtered from the seawater and consumed by sponges to be treated separately than microorganisms that are specifically and functionally associated with sponges (Thiel *et al.*, 2007). Although this is a useful system, it is still new, and Meyer and Kuever (2007) predict that as the available database of environmental sequences increases, many microbes currently designated as "specialists" may change their classification status to "generalists".

Specificity at smaller scales

In addition to host specificity, several microorganisms display tissue specificity within their hosts. As mentioned earlier, biogeographic investigations must clearly define the scale of the system being studied. Even within individual sponges, several studies have identified complex, yet consistent trends in microorganism distribution. The outer layers of sponges, through which light can penetrate, are dominated by photosynthetic microbes such as cyanobacteria and eukaryotic algae. However, detailed studies of cyanobacterial-dominated regions of different species of sponges have revealed significant variation within even these highly specific communities (Ridley *et al.*, 2005). Deeper in the sponge reside heterotrophic or autotrophic bacteria (Webster *et al.*, 2001; Wang, 2006; Thiel *et al.*, 2007; Meyer and Kuever, 2007). Thus, one factor shaping the fine-scale biogeography of these microorganisms seems to be their type of nutritional metabolism.

In the case of sponge-associated microorganisms, it is instructive to think of sponges as a specific type of habitat for microorganisms. Some researchers have even gone so far as to suggest that marine eukaryotes may act as habitat islands, permitting allopatric speciation of microbes inhabiting physically distinct hosts (Taylor *et al.*, 2005). Just like macroscopic habitats, some symbiont species appear to be endemic to a particular sponge habitat; some

species may inhabit sponges only at specific times or under specific conditions; and some microbial species may not inhabit sponges at all. Some species may be recent colonizers, and some symbiont species may have been evolving in association with their host sponge for millions of years. However, one must also remember that the "habitat" in this system is actually a living organism with its own distribution patterns, which significantly complicates the consideration of symbiotic biogeography as opposed to island biogeography.

THE SYMBIOTIC NATURE OF ASSOCIATION

To fully understand the degree of host specificity and thus the potential diversity associated with the sponge-microorganism system, it is important to consider the specific mechanisms of the symbiotic relationship. Although some studies, such as Hoffmann *et al.* (2005), have explicitly shown biomass transfer from associated bacteria to host sponge cells, in most cases, the details of symbiosis are still not well defined. Therefore, most studies consider symbiosis in this system as consistent but not obligate *per se* (Ridley *et al.*, 2005). While sponges are filter-feeders and consume some microorganisms for food, it has been suggested that symbiotic microorganisms avoid consumption and may perform the numerous roles for their sponge hosts. For example, microbes may contribute to sponge nutrient acquisition by assisting with phagocytosis, photosynthetic-based carbon cycling, or utilization of other organic compounds. Microorganisms may also assist with stabilization of the sponge skeleton, processing of metabolic waste, and production of secondary metabolites for use as chemical defenses or antioxidants against UV damage (Hentschel *et al.*, 2002; Ridley *et al.*, 2005; Taylor *et al.*, 2005; Sharp *et al.*, 2006; Wang, 2006). The potential of sponge-associated microorganisms to produce antibiotics is one of the major driving forces prompting and funding a better understanding of the biogeography of this system (Li and Liu, 2006). The spatial and temporal stability of sponge-microbe associations suggests that elaborate cell recognition pathways may be active to prevent the sponges from consuming their symbionts (Hill *et al.*, 2005). Cyanobacterial symbionts in shallow water sponges have been extensively studied and are known to not only photosynthesize, but also fix nitrogen and thus provide their sponges with amino acids, affect redox potential in sponge tissue, and contribute to host nutrition via extracellular lysis and phagocytosis (Thiel *et al.*, 2007).

While cyanobacteria and sponges clearly have a mutualistic relationship, the nature of symbiotic relations between most sponge-associated microorganisms and their hosts is yet to be specifically determined. It seems unlikely that all microorganisms contribute to marine sponges in the same fashion, and equally unlikely that all sponges provide equivalent habitats

for microorganisms. In fact, some genera of sponges, such as *Callyspongia* and *Stylinos*, appear to host microorganism communities that are highly variable and simply depend on the diversity in the surrounding seawater, whereas *Cymbastela concertrica* sponges in the same study proved to have specific and stable bacterial communities (Thiel *et al.*, 2007).

However, it is clear that microorganisms have the potential to play a large role in defining characteristics of their host sponges. For example, in addition to nutritional contributions, microorganisms are thought to provide sponges with distinctive coloration forms and secondary metabolite arsenals for defense against predators (Wang, 2006). Metabolic diversity of symbionts likely contributes to the survival of sponges in the ecosystems that they populate (Sharp *et al.*, 2006). Nutritional contributions to the host and mechanisms of predator defense from symbionts could easily affect the range of habitats that a given sponge species could inhabit, just as nitrogen-fixing bacteria can affect the soil types permissible for plants hosting them in their root nodules or anemones can facilitate clownfish survival in the presence of predators. Thiel *et al.* (2007) suggest that sponge-associated microorganisms may even be directly interacting with other microorganisms in the vicinity, using the sponges as a microhabitat. In this scenario, the authors suggest that members of the microbial community may be partitioning metabolic niches within sponges, and perhaps even participating in micro-scale predator-prey relationships with one another. The contribution of symbionts to the distribution patterns of their host sponges could thus depend on the varying microbial communities residing therein. If symbiosis in fact facilitates a cyclic contribution to defining distribution patterns, it would imply that microorganisms are capable of indirectly influencing their own distributions, an interesting biogeographic phenomenon.

CASE STUDIES DEMONSTRATING THE CONSISTENCY OF MICROBIAL COMMUNITIES

If the composition of symbiotic microorganismal communities do influence the distribution of their host sponges and thus define their own distribution patterns, then it would be instructive to know if these communities operate as consistent units, or if individual members of the community tend to vary independently of one another and with some other measure. There are several ways in which the identity of sponge microbial communities can vary: with time, with broad-scale environmental factors, (e.g. temperature, sunlight intensity, and salinity), or with host species identity. Although most early work involving this symbiotic system has failed to procure data across a range of these factors, the recent studies summarized below represent major progress in characterizing patterns that impact sponge-associated microorganism distribution. The most strongly supported pattern throughout these studies seems to be that

sponge species identities correlate more closely with the associated microorganism community compositions than do environmental factors.

Before delving into studies identifying how microorganism communities differ from one another, it is important to establish that stable sponge-associated communities even exist. Taylor *et al.* (2004) analyzed microbial sponge community composition across a seasonally variable temporal scale and concluded that the observed communities were stable within sponge species over the course of an entire year, and thus did not represent transient entities. This stability seems to hold true even in response to varying ocean current patterns (Taylor *et al.*, 2005). Several studies have shown that sponge-associated bacterial communities are highly consistent across distances spanning at least 10 km (Hentschel *et al.*, 2002; Webster *et al.*, 2004; Taylor *et al.*, 2005; Taylor *et al.*, 2007; Thiel *et al.*, 2007). Combined, these results suggest the existence of spatiotemporally stable sponge-associated microorganism communities.

Numerous studies examine microorganism communities either in specific geographic regions or within specific species of marine sponges. In a study conducted on three closely related species of sponges with non-overlapping global distribution patterns, it was found that within sponge species, uniform microbial communities existed between different oceans. These communities were composed of phylogenetically complex, but consistent microorganisms (Hentschel *et al.*, 2002). Webster *et al.* (2004) surveyed microbial communities of Antarctic sponges and found that 61 of 250 bacterial clones were not found in the surrounding sea water and thus were placed in the sponge-associates group. These bacterial communities proved to be consistently stable within particular sponge species, even across the entire McMurdo Sound of the Ross Sea, Antarctica. These two studies indicate microbial communities within individual sponge species can be consistent across broad geographic ranges.

A similar project surveyed the bacterial communities associated with the sponge *Chondrilla nucula* from the Ligurian and Adriatic Seas, which are on opposite sides of Italy and do not directly exchange water masses. Communities were determined to be temporally stable over three consecutive summers and consistent within the geographic range studied. Of the 14 phylogenetic clusters of sponge-associated bacteria, 4 appeared to be sponge associates, 7 were generalists, and the degree of sponge-specificity of 3 clusters was uncertain (Thiel *et al.*, 2007). Populations of the same *C. nucula* species from the Florida Keys were the focus of a study by Hill *et al.* (2005), which strongly supported the existence of specialized bacterial clusters associating with marine sponges. Of 21 OTUs isolated from *C. nucula*, none

overlapped with 53 OTUs isolated from the surrounding seawater. Based on these studies, *C. nucula* is an example of a marine sponge that hosts a specific microorganism community.

A study of the deep sea sponge *Polymastia cf. corticata*¹ by Meyer and Kuever (2007) revealed 53% of the 38 archaeal and bacterial 16S rRNA phylotypes to be monophyletic sponge-associated sequence clusters. *P. cf. corticata* samples were collected at a depth of 1,127 m from the Kahouanne Basin of the Caribbean Sea. These results indicate that the associated microbial diversity of deep sea species is quite comparable to the much more heavily studied shallow water species.

One study with findings that differed those thus far discussed was conducted by Taylor *et al.* (2005). In this work, microbial communities associated with *Cymbastela concentrica* were sampled from temperate and tropical Australia. Although communities across 500 km of temperate *C. concentrica* habitat were consistent, communities varied in temperate regions compared to tropical regions, despite relatively homogenous bacterial communities in the seawater. Three explanations for the variation between temperate and tropical sponge-associated communities were suggested. One possibility is that sponges may be acting as "islands" permitting allopatric speciation of associated communities within certain host species. Alternatively, the observed climatic community variations may simply be due to taxonomic irregularities. The authors suggest that temperate and tropical populations of *C. concentrica* may in fact be the result of cryptic speciation, implying host-specificity as a simple explanation for the varying tropical and temperate microbe communities. However, if the taxonomy of *C. concentrica* holds, this study could support a third possibility, that environmental differences such as latitudinal light or temperature clines may impact the distribution of some sponge-associated microorganism communities. Interestingly, other phylogenetic studies have shown that within a single habitat, highly diverse microbial communities exist when multiple sponge species are examined. These studies lend support to the cryptic species hypothesis outlined above, as it seems that the identity of the host sponge is of crucial importance to defining the sponge-associated microbial community (Meyer and Kuever, 2007).

Taken together, these case studies seem to suggest that, within a certain scale, the biogeography of sponge-associated microorganisms is primarily dependent on the identity of the host sponge rather than geographic location. Whether or not the associated microorganisms reciprocally influence the distribution of their hosts has not been conclusively determined. However, given the significant role that many microorganisms play in ecological nutrient cycling

¹ In taxonomy, genus cf. species indicates uncertainty as to the species-level identification

and organismal metabolism, it is easy to imagine how species-specific microorganisms could contribute to defining the inhabitable geographic range, and thus the biogeographic patterns of their host sponges. A detailed understanding of the impact of microbial communities on host habitat range, and thus on sponge-associated microorganism range, will require and a more complete picture of the identity of the members of various suites of microbial communities than is currently available. Nonetheless, an important concluding trend from these studies is that the most critical factor for predicting the distribution of microorganism communities seems to be the distribution of specific marine sponges species that host stable microbial communities.

MAINTENANCE OF SPONGE-SPECIFIC ASSOCIATIONS

Now that it is well established that sponge-specific microbial communities do exist, one of the pressing questions facing sponge-associated microorganism biogeography is the degree of coevolution that has occurred in the system. As microorganisms do not seem to be simply associated at random with sponges, several alternative possibilities exist that could explain the maintenance of this spatiotemporally stable relationship. Either sponges select their symbionts from the environment, microorganisms have specifically evolved mechanisms to avoid ingestion by the sponge, or sponges actively transmit microorganisms between generations (Sharp *et al.*, 2006).

The possibility that sponges actively transmit microorganisms to their offspring has been rigorously tested in controlled settings with follow-up studies conducted in natural marine settings. Thus, this mechanism will be the explanation of spatiotemporal stability maintenance that is focused on in this discussion (Sharp *et al.*, 2006; Schmitt *et al.*, 2007). Even if sponges require some level of reinfection with each generation, vertical transmission between generations would imply that the host sponges are capable of controlling the symbiont communities in their offspring. Such a mechanism would explain the high degree of similarity between microbial communities inhabiting the same species of sponges even under varying environmental and free-living bacterial conditions (Hill *et al.*, 2005).

Recent studies have lent extensive support to the existence of vertical transmission of microbes (Sharp *et al.*, 2006; Schmitt *et al.*, 2007). Sharp *et al.* (2006) successfully localized microbial aggregates, consisting of bacteria and archaea, within embryos of the sponge genus *Corticium*. The microorganisms identified in the embryos persisted in the adult samples that were tested over a 3-year period and 100 km range of the Palau Islands. These results indicate a sophisticated and highly evolved mechanism of intergenerationally maintained microorganism transmission. A similar study comparing the microbial communities of larval, juvenile, and adult

Ircinia felix sponges from the Florida Keys showed that vertical transmission is an important mechanism for initiating sponge-microbe associations (Schmitt *et al.*, 2007).

RECENT ADVANCES CONCERNING THE STUDY OF SPONGE-ASSOCIATED MICROORGANISMS

Research in the field of sponge-associated microorganism biogeography has experienced two major transformations in the past decade: the advent of metagenomics for microbial identification, and increased interest in the field due to pharmaceutical applications of secondary metabolites. Both of these changes have impacted biogeographic studies on this complex system.

Metagenomics: advantages and disadvantages

Marine microorganisms are hard to procure and even harder to culture in a lab, so until 2002, when Hentschel *et al.* successfully applied metagenomics to sponge-associated microorganisms, even geographic areas that had been sampled lacked a complete picture of the total diversity of microorganisms present. Metagenomics, which consists of sequencing 16S rRNA, a highly conserved region of the genome, is now a standard technique in the field that is used to identify microorganisms isolated both from the environment and from sponge tissue (Hentschel *et al.*, 2002). Researchers had previously relied on cell culture techniques to identify microbes found in sponge tissue. However, a major disadvantage of culturing was that only a very small percentage of microorganisms could be grown in laboratories. Those that could be cultured tended to exhibit differential fitness in the culture conditions that sometimes resulted in growth that was not representative of the composition of microorganisms originally present in the sponge (Wang, 2006). Metagenomics now allows researchers to identify and obtain sequence information from even unculturable microbes without distorting the identity of the microbial community present. Metagenomics has significantly increased the amount of data available on microbial distributions. Before the advent of this technology, the overwhelming majority of the microbial diversity was overlooked (Martiny *et al.*, 2006).

However, Taylor *et al.* (2007) point out two major cautions with this technique that deserve to be noted. Nonsponge-associated (free-living) organisms used as an outgroup for cluster definition must be selected thoughtfully, in order to avoid artificially increasing the number of sponge-specific organisms identified by excluding closely related free-living organisms. Secondly, researchers should focus on obtaining full-length 16S rRNA sequences

in order to provide more phylogenetically informative sequences than many of the truncated sponge-derived sequences currently published.

Echoing concerns with this technique, Papke and Ward (2004) conducted a study that applied microbial identification methods with varying degrees of sensitivity in order to determine the effect of methodology on the microbial communities identified. Results from this test suggested that the 16S rRNA gene may be too conserved to detect variation in some microorganisms with a high level of resolution. In order to determine this, the researchers compared restriction patterns from three sources of DNA. In addition to the traditional 16S rRNA analysis, Papke and Ward also analyzed the internal transcribed spacer (ITS) region of 16S-23S rRNA. The third source of DNA was from repetitive extragenic palindromic-PCR (REP-PCR), which takes into account the complexity of the entire genome rather than a single gene or spacer region. When comparing restriction patterns from these three sources in 248 species from ten sites on four continents, it was found that 16S rRNA discerned no endemism, while the ITS region found some endemism, and REP-PCR identified clades corresponding to the geographic regions that were sampled (Papke and Ward, 2004). This study illustrates the strong impact of methodology on study results.

Webster *et al.* (2004) describe a second study suggesting problems with 16S rRNA resolution in which archaeal populations yielded identical 16S rRNA gene sequences yet considerable genomic variation. A study of terrestrial thermophiles by Whitaker *et al.* (2002) revealed that the analysis of faster evolving protein-encoding genes enabled the creation of monophyletic clades of microbe strains, and were capable of resolving groups separated by only 5.7 km. This contrasted the analysis of 16S rRNA, which indicated no biogeographic patterns. Again, the clades constructed by using faster-evolving genes correlated geographic and genetic differences, but not environmental factors with genetic variation (Hedlund and Staley, 2004). These are convincing examples that demonstrate the importance of developing reliable methodologies for microorganismal identification. Clearly 16S rRNA data and the bacterial cluster analyses based on these data should be interpreted with caution in biogeographic studies.

Pharmaceutical products

In addition to the development of more efficient (though questionable) identification techniques, a second major transformation to the field involves the potential application of secondary microbial metabolites for pharmaceutical products, which has greatly increased interest in microorganismal biogeography. Of particular interest are the fungi associated with

marine sponges, some of which are known to produce bioactive compounds (Wang, 2006). Interest in finding bioactive compounds and the microorganisms that produce them is one of the primary driving forces that is motivating and funding additional studies to uncover the existence of biogeographic patterns exhibited by sponge-associated microorganisms.

GAPS IN CURRENT KNOWLEDGE AND FUTURE DIRECTIONS

Despite the amount of recent attention that sponge-microbe associations have received due to their potential as a source for pharmaceutical compounds, there are still many fundamental gaps in our understanding of this system. Much is yet to be determined about microbial diversity and the causes for varying diversity among sponge hosts. Also, the specific mechanisms of the symbiotic relationship, both its evolution and its effects on symbiont metabolism, remain unknown (Taylor *et al.*, 2007). The overall trend evident from the case studies addressed in this review seems to be that sponge-specific microbial clusters exist and seem to follow the distribution patterns of specific host-species of sponges.

However, in order to determine if patterns observed thus far in the field are in fact representative of host association and diversity, Taylor *et al.* (2004) suggest focusing more on assessing the spatiotemporal variability of microorganisms' associations with sponges. In order to accomplish this, they emphasize the importance of improving sampling methodology and experimental design by replicating tests and reporting statistical procedures used in analyses. Hedlund and Staley (2004) suggest using concatenated gene fragments for analysis rather than 16S rRNA sequences in order to take advantage of higher genetic resolving power. To better understand the evolutionary implications of sponge-associated microorganism specificity, cross-inoculation trials should be performed to determine if any examples of specific symbionts are capable of colonizing alternate host sponge species. This technique was successfully used in a study by Ashen and Goff (1999) to determine that there are coevolving systems of bacterial symbionts and red algae hosts. In addition to seeking a more complete understanding of microorganisms and their host sponges, Thiel *et al.* (2007) suggest that future studies in this field should include considerations of the interactions between the sponge-inhabiting microbial communities themselves. Regardless of which future directions are taken, new and exciting trends are bound to be uncovered in this unique and complex biogeographic system.

LITERATURE CITED

Ashen, J.B. & Goff, L.J. (2000) Molecular and ecological evidence for species specificity and coevolution in a group of marine algal-bacterial symbioses. *Applied and environmental microbiology* **66**, 3024-3030.

Cox, C.B. & Moore, P.D., eds. (1999) *Biogeography: an ecological and evolutionary approach*, 6th edn. Blackwell Science Ltd, Oxford.

Finlay, B.J. (2002) Global dispersal of free-living microbial eukaryote species. *Science* **296**, 1061-1063.

Hedlund, B.P. & Staley, J.T. (2004) Microbial endemism and biogeography. *Microbial Diversity and Bioprospecting* (ed. by A.T. Bull), pp. 227-230. ASM Press, Washington DC.

Hentschel, U., Hopke, J., Horn, M., Friedrich, A.B., Wagner, M., Hacker, J. & Moore, B.S. (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. *Applied and environmental microbiology* **68**, 4431-4440.

Hill, M., Hill, A., Lopez, N. & Harriott, O. (2006) Sponge-specific bacterial symbionts in the Caribbean sponge, *Chondrilla nucula* (Demospongiae, Chondrosida). *Marine biology* **148**, 1221-1230.

Hoffmann, F., Larsen, O., Thiel, V., Rapp, H.T., Pape, T., Michaelis, W. & Reitner, J. (2005) An anaerobic world in sponges. *Geomicrobiology Journal* **22**, 1-10.

Li, Z.Y. & Liu, Y. (2006) Marine sponge *Craniella australiensis*-associated bacterial diversity revelation based on 16S rDNA library and biologically active Actinomycetes screening, phylogenetic analysis. *Letters in Applied Microbiology* **43**, 410-416.

Martiny, J. B. H., Bohannan, B. J. M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Øvreås, L., Reysenbach, A., Smith, V.H. & Staley, J.T. (2006) Microbial biogeography: putting microorganisms on the map. *Nature reviews. Microbiology* **4**, 102-112.

Meyer, B. & Kuever, J. (2008) Phylogenetic diversity and spatial distribution of the microbial community associated with the Caribbean deep-water sponge *Polymastia cf. corticata* by 16S rRNA, *aprA*, and *amoA* gene analysis. *Microbial ecology* **56**, 306-321.

Papke, R.T. & Ward, D.M. (2004) The importance of physical isolation to microbial diversification. *FEMS microbiology ecology* **48**, 293-303.

- Ridley, C.P., Faulkner, D.J. & Haygood, M.G. (2005) Investigation of Oscillatoria spongelliae-dominated bacterial communities in four dictyoceratid sponges. *Applied and environmental microbiology* **71**, 7366-7375.
- Schmitt, S., Weisz, J.B., Lindquist, N. & Hentschel, U. (2007) Vertical transmission of a phylogenetically complex microbial consortium in the viviparous sponge *Ircinia felix*. *Applied and environmental microbiology* **73**, 2067-2078.
- Sharp, K.H., Eam, B., Faulkner, D.J. & Haygood, M.G. (2007) Vertical transmission of diverse microbes in the tropical sponge *Corticium* sp. *Applied and environmental microbiology* **73**, 622-629.
- Taylor, M.W., Radax, R., Steger, D. & Wagner, M. (2007) Sponge-Associated Microorganisms: Evolution, Ecology, and Biotechnological Potential. *Microbiology and molecular biology reviews* **71**, 295-347.
- Taylor, M.W., Schupp, P.J., de Nys, R., Kjelleberg, S. & Steinberg, P.D. (2005) Biogeography of bacteria associated with the marine sponge *Cymbastela concentrica*. *Environmental microbiology* **7**, 419-433.
- Taylor, M.W., Schupp, P.J., Dahllöf, I., Kjelleberg, S. & Steinberg, P.D. (2004) Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. *Environmental microbiology* **6**, 121-130.
- Thakur, N. L., Müller, W. E. (2004) Biotechnological potential of marine sponges. *Current Science* **86**, 1506-1512.
- Thiel, V., Leininger, S., Schmaljohann, R., Brümmer, F. & Imhoff, J.F. (2007) Sponge-specific bacterial associations of the Mediterranean sponge *Chondrilla nucula* (Demospongiae, Tetractinomorpha). *Microbial ecology* **54**, 101-111.
- Wang, G.Y. (2006) Diversity and biotechnological potential of the sponge-associated microbial consortia. *Journal of Industrial Microbiology & Biotechnology* **33**, 545-551.
- Webster, N.S., Negri, A.P., Munro, M. M. H. G. & Battershill, C.N. (2004) Diverse microbial communities inhabit Antarctic sponges. *Environmental microbiology* **6**, 288-300.
- Webster, N.S., Wilson, K.J., Blackall, L.L. & Hill, R.T. (2001) Phylogenetic Diversity of Bacteria Associated with the Marine Sponge *Rhopaloeides odorabile*. *Applied and environmental microbiology* **67**, 434-444.
- Whitaker, R.J. (2006) Allopatric origins of microbial species. *Philosophical transactions of the Royal Society of London* **361**, 1975.