Microbial biogeography: patterns in microbial diversity across space and time

Noah Fierer*
Department of Ecology & Evolutionary Biology
Cooperative Institute for Research in Environmental Sciences
University of Colorado
Boulder, CO 80305
Phone: 303-492-5615
Fax: 303-492-1149
Email: 805-729-1657

Introduction

Biogeography is a science that attempts to describe and explain spatial patterns of biological diversity and how these patterns change over time (Ganderton and Coker, 2005; Lomolino et al., 2006). In other words, biogeographers seek to answer the seemingly simple question: Why do organisms live where they do? While biogeography has traditionally focused on macro-organisms, i.e. plants and animals, microbiologists have studied biogeographical questions for many decades and there has been a recent resurgence in interest in microbial biogeography (Green and Bohannan, 2006; Martiny et al., 2006; Ramette and Tiedje, 2007). This resurgence has been led, in part, by advancements in molecular tools that allow us to survey uncultivated microbes in the environment and a growing recognition that microbial taxa are the most biologically diverse taxa on earth.

At present, the study of microbial biogeography is in its infancy. Even the existence of microbial biogeography has been recently called into question ["*There is no biogeography for anything smaller than 1 millimeter*", Bland Finlay quoted in Whitfield (2005)]. If this statement was correct, this chapter would be very brief. However, we do know that a wide variety of microbial taxa exhibit biogeographical patterns; microbial communities are not homogeneous across habitat-types, and within a given habitat, microbial diversity can vary between locations separated by millimeters to thousands of kilometers. If microbial biogeography did not exist, there would be no spatial or temporal heterogeneity in microbial communities and global patterns in microbial diversity could be predicted by studying the microbial community in a single location at a single point in time. Unfortunately this is not the case; documenting and understanding patterns in microbial biogeography is not so simple.

Probably the best summary of microbial biogeography as a research field was provided by an unlikely source, D. Rumsfeld, ex-Secretary of Defense of the United States who said "… *there are things we know we know. We also know there are known unknowns; that is to say we know there are some things we do not know. But there are also unknown unknowns - the ones we don't know we don't know*" (Feb. 12, 2002. Dept. of Defense News Briefing). While he was not referring to biogeography when he uttered this phrase, it is a useful framework for thinking about microbial biogeography in that the 'things we know we know' are relatively few, the 'known unknowns' are abundant, and in coming decades we are likely to discover many phenomena that are currently 'unknown unknowns'. While reading this chapter, it will become readily apparent that the science of

microbial biogeography is currently as mature as 'macro'-bial (i.e. plant and animal) biogeography was in the 19th century. Just as early naturalists set out on voyages to document the diversity of plants and animals in uncharted lands, we are attempting to document "uncharted" microbial diversity and we currently lack a comprehensive understanding of how (and why) microbial diversity changes across space and time.

The immaturity of the field of microbial biogeography is not due to lack of interest in the topic. The first paradigm in microbial biogeography, "*everything is everywhere, but, the environment selects*" was offered by Baas Becking (1934) more than 70 years ago and his adage continues to be cited in nearly every recent publication on microbial biogeography (de Wit and Bouvier, 2006). Although the field of microbial biogeography is not new, we now have the methods available to survey a large portion of the microbial diversity on earth and to quantify the biogeographic patterns exhibited by microbes living in a wide range of environments. As these techniques and methodologies continue to improve at a nearly exponential rate, the field of microbial biogeography is poised for significant advances.

In this chapter, I will not attempt to summarize everything that is known about microbial biogeography. The field of biogeography encompasses a wide breadth of research topics and covering all topics related to microbial biogeography would be a Sisyphean task. In addition, microbes inhabit a wide range of habitats from hot springs to the deep subsurface and it is highly improbable that we would observe similar biogeographical patterns across the full range of possible microbial habitats. At the same time, it is also unlikely that all microbial taxa share similar biogeographical patterns as the term 'microbe' encompasses a broad array of taxa (e.g. bacteria, fungi, archaea, viruses, and protists) that are phylogenetically distinct and distinct with respect to their morphologies, physiologies, and life histories. For these reasons, this chapter should not be considered a comprehensive review of 'microbial biogeography', as there is unlikely to be a common set of concepts and patterns unifying the field of microbial biogeography. Instead, I will primarily focus on selected topics that are particularly relevant to researchers studying uncultivated microbes in natural environments in order to illustrate what we do, or do not, currently know about their biogeography. Most of the examples will be drawn from research on bacteria as bacterial biogeography has received far more attention than the biogeography of other microbial groups.

Microbial dispersal and colonization

From work on plants and animals, we know that dispersal is likely to be one of the key processes shaping microbial biogeography and macroecological patterns (Hubbell, 2001; Lomolino et al., 2006). There is currently some debate regarding the extent of microbial dispersal. Finlay (2002) has argued that any organism less than 1 mm in size is likely to be ubiquitous due to an essentially unlimited capacity for long-distance dispersal. This speculation is primarily based on the assumption that the high local abundance of microbes (the large number of individuals per unit area) increases the probability that individual microbes may travel a long distance and successfully colonize a remote location simply by chance (Fenchel, 2003; Finlay, 2002; Martiny et al., 2006). If we combine a high probability of dispersal with the ability to survive the long-distance transport, we would expect few geographic constraints on microbial dispersal (Figure 1). In contrast, Papke and Ward (2004) have argued that geographic barriers to microbial dispersal are relatively common and physical isolation is an important driver of microbial evolution. They cite a handful of studies as evidence for the occurrence of microbial endemism, including work on hot spring microbes (Papke et al., 2003; Whitaker et al., 2003) and soil pseudomonads (Cho and Tiedje, 2000).

Unfortunately, the debate surrounding microbial dispersal is not likely to be resolved any time soon as there is limited information on actual rates of microbial dispersal. In a recent meta-analysis of published literature, Jenkins et al. (2007) concluded that: "claims that microbes disperse widely cannot be tested by current data". However, they did find that the distance-mass relationship for passive dispersers was essentially random. In other words, the small size of microbes, in and of itself, does not necessarily mean that microbes have average dispersal distances that differ from those of larger plants or animals. In addition, both Jenkins et al. (2007) and Martiny et al. (2006) have speculated that the dispersal distances can vary considerably between microbial taxa. Such differences could arise from differences in the mode of transport, habitat characteristics, population densities, and the ability of the microbe to survive the transport process itself (Figure 1).

At larger spatial scales, the active dispersal (self-propulsion) of microbes should be severely constrained (Jenkins et al., 2007; Martiny et al., 2006). However, passive dispersal may occur via a variety of mechanisms, including transport in the atmosphere, water currents, or transport on or within larger plants and animals. Likewise, microbes that can go dormant for extended periods of time and survive harsh environmental

conditions are more likely to be transported long distances (Figure 1). This is particularly true for those microbes that are aerially dispersed as the atmospheric environment poses a unique set of challenges due to the high levels of UV radiation, low moisture levels, and extremely oligotrophic conditions (Jones and Harrison, 2004; Lighthart, 1997; Madelin, 1994). Microbes inhabiting certain habitats, such as surface soils, plant leaf surfaces, or streams, are more likely to be dispersed longer distances than those in other habitats (such as subsurface soils and deep-sea sediments) where the potential for long-range transport is likely to be more limited. The same pattern should hold for microbes associated with plants or animals that can move (or be moved) long distances (such as whales, agricultural crops, and migratory birds) versus those microbes that are free-living or associated with organisms of more-limited dispersal abilities. Of course, this is a fundamental concept in epidemiology, illustrated most recently by the rapid intercontinental dispersal of avian flu by migratory birds (Rappole and Hubalek, 2006). All other factors being equal, those microbes that are more abundant in a given area are likely to be transported further as high densities may effectively broaden the dispersal distribution (Figure 1).

Dispersal itself will not alter biogeographical patterns unless dispersal is accompanied by successful establishment (or colonization) of the new environment. If colonization rates are very low, we would expect to observe high levels of endemicity at the community-level (Papke and Ward, 2004). A variety of biotic and abiotic processes may influence the frequency of successful colonization. Microbes that are generalists, i.e. those that are able to grow in a wide range of environments, are more likely to colonize new habitats than those microbes that can only grow under very specific conditions. Likewise, microbes that need to live in close association with other organisms (such as syntrophic microbes, specific pathogens, or species-specific mycorrhizae) are less likely to successfully colonize a "new" habitat than free-living microbes. We would expect habitats with more challenging environmental conditions to support lower colonization rates than those that are more hospitable. The amount of available niche space should also regulate the suitability of a habitat for colonization; if there is no "room" for an introduced microbe, it will not survive for long. Perhaps one example of this is the protective influence that healthy gut microflora can have against gastrointestinal pathogens. After prolonged antibiotic usage, the microbial community is disrupted and out of equilibrium, rendering the gastrointestinal system more susceptible to colonization by harmful pathogens (Guarner and Malagelada, 2003).

The processes associated with dispersal and colonization can be elucidated by research on community development in a previously lifeless environment, the process of primary succession. This has been elegantly demonstrated by work on plant community development on Indonesian islands sterilized by the eruption of Krakatau in 1883 (Whittaker et al., 1989). Patterns of primary succession have been documented in a variety of microbial systems including water pipes (Martiny et al., 2003), lake biofilms (Jackson et al., 2001), and recently-deglaciated soils (Nemergut et al., 2007), but it is not clear if microbial succession follows similar patterns as those documented for plant communities (Jackson, 2003). With more studies on microbial community assembly during primary succession and careful analyses of the successional patterns, we can begin to estimate microbial dispersal/colonization rates and, possibly, determine how these rates are influenced by habitat type, phylogenetic characteristics, and environmental conditions.

Why are microbial communities so diverse?

There is no question that microbial communities can be amazingly diverse. While some "extreme" environments, such as acid mine drainage (Baker and Banfield, 2003), harbor relatively few microbial taxa, the microbial diversity found in individual environmental samples is often very high. Small-subunit rRNA gene surveys, even those that are relatively large, rarely encompass the full-extent of microbial diversity found in a sample (Figure 2) making it difficult to accurately estimate the total taxonomic richness (Curtis and Sloan, 2005; Curtis et al., 2002; Hughes et al., 2001). This is particularly true for studies conducted in soil and sediment environments, where it has been estimated that individual samples are likely to harbor many tens of thousands of bacterial phylotypes (Gans et al., 2005; Hong et al., 2006; Torsvik et al., 2002; Tringe et al., 2005). Recent evidence suggests that bacteria are not unique in this regard as other microbial groups (including protists,viruses, archaea, and fungi) may also exhibit very high levels of local phylogenetic diversity (Breitbart et al., 2002; Fierer et al., In Press; O'Brien et al., 2005; Walsh et al., 2005) (Figure 2). The statement by E.O. Wilson, "microbial diversity is beyond practical calculation" (Wilson, 1999), is likely to be accurate in many environments and for a variety of microbial taxa.

The term 'diversity' can be confusing in that it encompasses two very different components of community structure, richness and evenness. Richness is simply the number of unique operational taxonomic units (OTUs) in a given sample, area, or in a

given community. In contrast, evenness describes the distribution of individuals among the OTUs (the proportional abundances of OTUs) and evenness is maximized when all OTUs have the same number of individuals (Magurran, 2004). In most terrestrial and aquatic environments, microbial communities appear to have both high levels of richness and evenness. This is clearly evident if we examine taxa-accumulation curves, otherwise known as rarefaction curves, generated by plotting the cumulative number of unique OTUs against the size of the sampling effort (Figures 2 and 3). The taxa-accumulation curves are often close to linear for soil and sediment microbial communities (Figures 2 and 3) indicating that these communities are very even and any attempt to survey the full-extent of microbial richness in a given sample would be a difficult (and expensive) effort given current sequencing technologies. For example, Schloss and Handelsman (2006) have estimated that a complete census of the unique bacteria (those with more than 3% divergence in their 16S rRNA gene sequences) in a single gram of Alaskan soil would require sampling more than 480,000 sequences.

The near-linearity of many taxa-accumulation curves (Figures 2 and 3) indicates that microbial communities commonly have a large number of rare OTUs, a so-called "long tail" distribution (Figure 2). We can model the taxon-abundance distribution of microbial communities using a variety of mathematical functions, including types of lognormal, logarithmic, or power-law functions (Angly et al., 2005; Curtis et al., 2002; Dunbar et al., 2002; Fierer et al., In Press; Hong et al., 2006; Schloss and Handelsman, 2006). The question of which mathematical function is most appropriate for describing bacterial community structure is subject to some debate. This debate is not likely to be resolved any time soon; plant and animal communities have been surveyed far more comprehensively than most microbial communities yet macro-ecologists have been arguing for decades over the choice of models used to describe species-abundance distributions (Hughes, 1986; Magurran, 2004). While we may not be able to accurately identify the specific taxon-abundance distribution in a given microbial community, we do know that bacterial communities are typically dominated by a few, more abundant taxa and many taxa that are relatively rare, a classic example of the "long tail" phenomenon well-studied by economists (Anderson, 2006).

Given that most environments harbor diverse microbial communities, the obvious question is: "Why are microbial communities so diverse?" Perhaps every researcher has his or her own hypothesis for the high diversity of microbial communities and many of these hypotheses have not been tested or can not be tested. What follows is a general

overview of the various categories of explanations that have been used to explain why many environments harbor highly diverse microbial communities. It is important to recognize that these hypotheses are not mutually exclusive as some of the mechanisms and processes may act synergistically to affect levels of microbial diversity.

Environmental complexity

At the scale at which microbes perceive their environment, most microbial habitats are spatially heterogeneous due to either biotic or abiotic factors (Kassen and Rainey, 2004) and a given sample can contain a large number of potential niches. For example, a 10 cm^2 sediment core may encompass a range of redox conditions with obligate aerobes, facultative anaerobes, and obligate anaerobes living in close proximity to one another. Likewise, a 1 g soil sample is likely to support microbes with a broad array of physiologies, including autotrophs (such as nitrifiers and methane oxidizers), aerobic heterotrophs that are either copiotrophic or oligotrophic, and anaerobes (such as denitrifiers and sulfate reducers). Even environments that appear to be relatively homogeneous can harbor a number of distinct microenvironments. For example, numerous *Pseudomonas* genotypes can arise from a single, ancestral genotype due to the availability of multiple ecological niches in different locations of an unshaken culture vessel (Kassen et al., 2000). Laboratory studies have demonstrated that there is a positive correlation between habitat heterogeneity ("patchiness") and the phylogenetic diversity of bacteria (Korona et al., 1994; Rainey et al., 2000) but such patterns have been more difficult to confirm in the field. Zhou et al. (2002) found that saturated subsurface soils contained less diverse bacterial communities than unsaturated soils and they attributed this difference to the increased patchiness of the unsaturated soils. Their hypothesis has been supported by laboratory experiments (Treves et al., 2003), but it remains to be determined if there is a direct correlation between habitat complexity and microbial diversity, partly because it is so difficult to quantify environmental complexity given the large number of biotic and abiotic factors that interact to shape microbial habitats.

Body size and spatial scaling

Robert May (1988) and others (Azovsky, 2002; Morse et al., 1985; Ritchie and Olff, 1999) have hypothesized that smaller organisms should have a higher local diversity than larger organisms due to their ability to partition a given environment more

finely. In other words, a decrease in body size increases the apparent number of habitats in a given environment as there is more of a fine-grained perception of environmental heterogeneity and a corresponding increase in the number of different ways the environment can be utilized by organisms. Of course, this hypothesis is similar to the "environmental complexity" hypothesis described above in that both hypotheses suggest that the high levels of microbial diversity are driven by the large number of potential niches in a given microbial habitat. However, it is important to recognize that the high levels of microbial diversity may be a direct result of our scale of inquiry; the samples analyzed by microbiologists are relatively small from our perspective, but incredibly large compared to the size of individual microbes living in that sample.

More specifically, surveying microbial diversity in individual environmental samples may be similar in magnitude to surveying the diversity of macro-organisms at continental scales. To illustrate this point, consider an environment that has 10^4 unique bacterial species in a 100 m^2 area, a reasonable estimate for soil and sediment samples (Gans et al., 2005; Hong et al., 2006; Torsvik et al., 2002). In order to directly compare the bacterial richness in this 100 m^2 area to bird species richness, we would have to survey bird species richness across the entire globe which has approximately 10,000 bird species (Howard and Moore, 1991). This calculation is based on the assumption that species richness is correlated with the abundance of a taxon in a given area (Diamond, 1988; Siemann et al., 1996), which is largely a function of body size (May, 1988; Oindo et al., 2001), and birds (assume a body size of 10^{-3} m^3) are nearly 10^{15} times larger than an average bacteria. While this calculation is an obvious oversimplification, it does demonstrate that estimating microbial diversity in a relatively small area is analogous to estimating plant and animal diversity at much larger spatial scales. While body size alone is not likely to account for the high diversity of soil microbes, once we reconcile differences in spatial scale, the local richness of microbes may be more comparable to the observed levels of plant and animal richness.

Speciation and extinction rates

High levels of microbial diversity could also be driven high rates of speciation, low rates of extinction, or some combination of these two processes. Unfortunately, we do not have good estimates of microbial speciation and extinction rates in the field (Horner-Devine et al., 2004; Ramette and Tiedje, 2007), so we can only speculate on the importance of these competing processes. We would expect bacteria to have lower rates

of extinction than most metazoans because bacteria are probably less likely to die of starvation or harsh environmental conditions, bacteria do not typically die of old age, and they are capable of rapid, asexual reproduction (Dykhuizen, 1998; Horner-Devine et al., 2004; Ramette and Tiedje, 2007). Likewise, the large number of individuals present in most bacterial populations and high rates of dispersal may effectively buffer microbial taxa from changes in the environment or other stochastic processes that could lead to extinction. One could also speculate that speciation rates should be higher for microbes than for plants and animals because bacteria often have short generation times, high rates of horizontal gene transfer, large population sizes, an ability to finely partition a given environment into distinct niches, and bacteria often engage in direct inter-species interactions (both positive and negative) that may contribute to ecological specialization (Dykhuizen, 1998; McArthur, 2006; Papke and Ward, 2004). We know from experimental studies that rates of speciation can be very rapid for laboratory strains of bacteria grown under controlled conditions (Elena and Lenski, 2003; Lenski et al., 1991; Rainey et al., 2000), but we do not know if speciation rates are also rapid for the majority of bacteria living in more natural conditions. If bacteria really do have high rates of speciation and low rates of extinction, than this combination of processes could contribute to the high levels of bacterial richness observed at both local and global scales.

The "storage effect"

Peter Chesson and colleagues have outlined a hypothesis, termed the "storage effect", to explain the maintenance of species coexistence (Chesson, 1994; Chesson and Huntly, 1989; Chesson and Warner, 1981). Although the "storage effect" has not been explicitly applied to microbes, the hypothesis may provide an elegant explanation for the high levels of local microbial diversity. The "storage effect" hypothesizes that temporal fluctuations in recruitment rates among species can lead to the stable coexistence of competitors. More specifically, species will remain in a community and not become extinct as long as three conditions are met: 1) competition is an important factor regulating community structure, 2) environmental conditions vary and there are species-specific responses in recruitment rates to this variability, giving species (even rare species) the capacity to increase in population size on occasion, and 3) organisms have a long-lived life stage to survive periods of poor recruitment when environmental conditions are less favorable. All other factors being equal, the "storage effect" predicts

that we would observe maximum levels of diversity in communities of long-lived, fecund organisms living in environments that experience high levels of temporal variability.

In all likelihood, most bacterial communities probably satisfy the conditions outlined above. Microbial habitats are often temporally variable, bacteria are likely to compete for limited resources (or space), and many bacteria are capable of rapid growth rates given the appropriate environmental conditions. In addition, distinct phylogenetic groups of bacteria often have distinct environmental requirements (with respect to redox levels, substrate preferences, pH, and light availability, for example), and many bacteria can survive in a dormant or semi-dormant state for prolonged periods of time. Testing the applicability of the "storage effect" hypothesis to microbial communities would not be easy given our limited knowledge of microbial life histories. Nevertheless, the "storage effect" hypothesis may provide a comprehensive set of mechanisms to explain, and predict, levels of diversity in microbial systems.

Are microbes globally as well as locally diverse?

We can assess microbial diversity at a variety of spatial scales, ranging from the diversity in an individual environmental sample to the diversity measured across large geographic regions. Typically local diversity is referred to as alpha diversity, while the total species richness over continents and biomes is referred to as gamma diversity (Lomolino et al., 2006; Magurran, 1988; Whittaker, 1975). As described above, we know that most microbial communities have a high local (alpha) diversity, however there is currently some debate regarding the gamma diversity of microbes. In particular, Tom Fenchel and Bland Finlay have speculated in a series of papers (Fenchel, 1993; Fenchel et al., 1997; Finlay and Clarke, 1999; Finlay, 2002) that the global richness of microbes is not significantly higher than their local richness and a large percentage of the microbial taxa on earth can be found in an individual sample collected from a single habitat. If correct, the global richness of a given microbial group could be calculated by surveying a handful of habitats as distinct habitats would not necessarily harbor distinct species assemblages. The competing hypothesis is that gamma diversity far exceeds local diversity, there is minimal overlap in species assemblages between habitats, and the number of unique microbial taxa on earth is enormous. The two opposing "sides" of this debate are graphically represented in Figure 4.

Fenchel and Finlay's hypothesis that microscopic-sized organisms are locally very diverse, but globally species-poor, is based on the idea that "smaller organisms

tend to have wider or even cosmopolitan distribution, a higher efficiency of dispersal, a lower rate of allopatric speciation and lower rates of global extinction than do larger organisms" (Fenchel, 1993). Support for their hypothesis comes from the observation that a large proportion of the global richness of protozoa can be found in a given local area (Fenchel et al., 1997; Finlay, 2002). In particular, they found that 80% of the global species total in the genus *Paraphysomonas* was found in <0.1 cm^2 of sediment from a single freshwater pond (Finlay and Clarke, 1999). They conclude from this research that, at the global scale, microscopic-sized organisms should have lower levels of taxonomic richness than organisms that are of intermediate size (Fenchel, 1993). This conclusion is supported by species-area curves generated for different size classes of organisms living in Arctic marine sediments (Azovsky, 2002).

There have been a number of direct criticisms of Fenchel and Finlay's conclusions. Foissner (2006) has argued that their work is fundamentally flawed since they assume that the global richness of protozoa is a known quantity. Foissner speculates that the global diversity of protozoa is likely to be far higher than Fenchel and Finlay have estimated and therefore their estimate of the local:global diversity ratio is unreasonably high. He argues that Azovsky's work (2002) suffers from a similar flaw. There are a number of other specific criticisms of Fenchel and Finlay's work (and their conclusions) including their reliance on morphospecies definitions (Coleman, 2002), their undersampling of rare taxa (Foissner, 2006), and the unsupported extrapolation of their work on one taxonomic group to all microscopic organisms (Lachance, 2004). Perhaps more damning are the large number of studies, reviewed here and elsewhere (Horner-Devine et al., 2004; Martiny et al., 2006; Ramette and Tiedje, 2007), that have documented considerable spatial heterogeneity in microbial community composition. If Fenchel and Finlay are correct, then microbes should have no biogeography, and the discovery of new microbial taxa should be far less common than has been observed.

Unfortunately, the debate surrounding the magnitude of global microbial richness has been fueled by speculation and a scarcity of hard data. Fortunately, sequencing efforts have been increasing at an exponential rate and public databases (such as GenBank, http://www.ncbi.nlm.nih.gov/Genbank/index.html) are now filled with sequences of microbial small-subunit rRNA genes from a wide range of habitats and locations. We should be able to use these sequence data to quantify the degree of overlap in microbial assemblages between habitats and estimate (or roughly approximate) the lower bounds of microbial richness on earth. Until this is done, the

global richness of bacteria, fungi, and other microbial taxa will remain a question mark and the validity of these competing hypotheses can not be assessed.

Taxa-area relationships

One of the cornerstones in the field of modern biogeography is the equilibrium theory of island biogeography developed by R.H. MacArthur and E.O. Wilson (1967). Put simply, their model represents the number of species inhabiting an island as an equilibrium between rates of immigration (colonization) and extinction. While there have been a number of criticisms leveled against MacArthur and Wilson's theory (Lomolino et al., 2006), their simple model is elegant in that it qualitatively predicts whether species numbers and species turnover rates will increase or decrease with changes in island (patch) size and the degree of isolation. In particular, MacArthur and Wilson's theory provides a conceptual explanation for the species-area relationship, one of the most-studied and best-documented patterns in plant and animal biogeography. The species-area relationship describes the pattern that species numbers tend to increase with increasing area and is generally expressed with the equation:

$S = cA^z$

where S is species richness; c is a fitted constant; A is area and z represents the slope when S and A are plotted on logarithmic scales (the slope of the species-area relationship). This equation, referred to as the Arrhenius equation or the power model, is commonly used to model species-area relationships with the steepness of the species-area relationship describing the rate at which communities differentiate in space (Figure 5). The exponent z has been the focus of much inquiry and there has been considerable debate surrounding the biological relevance of the z value (Lomolino et al., 2006). For plant and animal taxa, z values generally range from 0.1 - 0.2 in contiguous habitats and 0.25 – 0.35 across discrete island habitats (Horner-Devine et al., 2004; Rosenzweig, 1995). Recent studies, reviewed in Green and Bohannan (2006), Prosser et al. (2007) and Woodcock et al. (2006), have shown that microbes also demonstrate a positive species-area relationship with the taxonomic richness of microbes increasing with the amount of area surveyed (Figure 5). However, it is unclear if microbial z values are comparable to those observed for plant and animal taxa. Studies of the taxa-area relationship for saltmarsh bacteria (Horner-Devine et al., 2004), marine diatoms

(Azovsky, 2002), soil fungi (Green et al., 2004), and soil bacteria (Fierer and Jackson, 2006) have found z values lower than those generally observed for comparable studies of plants and animals. However, studies of bacteria inhabiting sump tanks (van der Gast et al., 2005), tree holes (Bell et al., 2005), and forest soils (Noguez et al., 2005), have yielded z values that are similar (0.25 – 0.45) to those observed for plant and animal taxa. In a particularly elegant study of the microbial species-area relationship, Peay et al. (2007), examined the diversity of ectomycorrhizal fungi across "tree islands" and also found a species-area slope ($z \approx 0.2$) similar to that reported for macro-organisms (Figure 5).

Although microbial taxa-area relationships have received considerable attention over the past few years, it is important to recognize that direct comparisons of z values from microbial taxa-area relationships must be considered carefully. One reason for this is that z values will vary with taxonomic resolution (Horner-Devine et al., 2004) and the different methods used to assess microbial diversity (e.g. fingerprinting techniques, morphological analyses, and direct analyses of gene sequences) quantify community-level diversity at varying levels of taxonomic resolution. This issue becomes particularly problematic if we are trying to compare taxa-area relationships between micro- and macro-organisms since there is no uniform and consistent definition of what constitutes a microbial "species". Likewise, microbial communities are highly diverse and it is often difficult to survey the full extent of microbial diversity in a given sample. Under-surveying a community will lead to an underestimation of z and this may explain why the z values reported for microbial taxa are often lower than those reported for plant and animal taxa (Woodcock et al., 2006). It is also important to recognize that, since a number of different mechanisms may generate the apparent taxa-area curves, z values (in and of themselves) do not tell us what specific process, or processes, are driving the spatial differentiation in microbial communities.

Is microbial biogeography shaped by environmental factors or history?

We know that microbial communities often vary across space and we can confidently ignore Bland Finlay's speculation that "there is no biogeography for anything smaller than 1 millimeter" (Whitfield, 2005). The question then becomes: What process or processes are responsible for generating the observed biogeographical patterns?

From an oversimplified perspective, there are two general factors that may contribute to the formation of the biogeographical patterns: environmental heterogeneity and dispersal limitation. The idea that environmental heterogeneity drives biogeographic patterns is best summarized by the Baas Becking hypothesis "*everything is everywhere, but, the environment selects*" (Baas Becking, 1934; de Wit and Bouvier, 2006). In other words, there is effectively no dispersal limitation, biogeographic patterns solely reflect contemporary environmental conditions, and similar environments will harbor similar microbial taxa regardless of the geographic distance between the environments. The opposing hypothesis is that spatial variability in microbial communities is a product of historical events, namely dispersal limitation and (possibly) past environmental conditions (Martiny et al., 2006). If dispersal limitation is the primary driver of biogeographical patterns, then geographic distance should be the best predictor of genetic divergence between communities and habitats in close proximity are more likely to share similar microbial taxa. Obviously these two hypotheses represent opposite ends of the spectrum and microbial biogeography, like the biogeography of plants and animals (Lomolino et al., 2006), probably reflects some combination of both environmental heterogeneity and dispersal limitation (i.e. history). Nevertheless, it is worth considering these two processes independently and examining the limitations associated with using this strict dichotomy to understand the biogeographical patterns exhibited by microbes.

There is no shortage of evidence that environmental heterogeneity can, to some extent, directly influence the spatial heterogeneity in microbial communities. To cite just a few examples, pH has been found to be the best predictor of the continental-scale patterns exhibited by soil bacteria (Fierer and Jackson, 2006), estuarine bacterioplankton communities change along a salinity gradient (Crump et al., 2004), and shifts in hot spring cyanobacterial communities correspond to temperature (Ward et al., 1998). These patterns can also be observed in experimental studies where changes in environmental conditions, e.g. substrate availability, redox potential, light intensity, and wide range of other factors, can induce shifts in microbial community composition (Buckley and Schmidt, 2002; Horner-Devine et al., 2004; McArthur, 2006). Of course a correlation between one or more environmental factors and community composition does not, in and of itself, indicate that environmental heterogeneity is the sole factor influencing the observed biogeographical patterns.

Even in cases where environmental heterogeneity directly influences the spatial structure of microbial communities, it may still be difficult to determine the specific

environmental factors that are directly responsible for generating the observed biogeographical patterns. First, the physical environment in most microbial habitats is highly variable and it can be difficult to measure environmental characteristics at the fine levels of resolution that will be most relevant to microbes. Second, environmental influences are going to be highly dependent on the taxa in question and the scale of inquiry (Ganderton and Coker, 2005). Individual taxa may respond to different environmental factors and those factors that correlate with the taxonomic structure of entire microbial communities may be unrelated to the taxonomic structure of subsets of the microbial community. For example, communities of marine cyanobacteria may shift with changes in light intensity, while non-photosynthetic heterotrophs in the same environment may be more responsive to gradients of organic carbon bioavailability. Likewise, those factors that drive biogeographical patterns within a single habitat may not necessarily be important when we look across habitats. This is the so-called 'paradox of scale' (Ganderton and Coker, 2005), the idea that different environmental factors will affect the same microbial community or population if we change our scale of inquiry. As an example, consider the spatial patterning of soil bacterial communities which may be strongly correlated with plant presence/absence within an individual plot (Kuske et al., 2002), but may appear to be correlated with soil pH at the continental scale (Fierer and Jackson, 2006). Third, microbes can exert a significant influence on their local environment making it difficult to distinguish between environmental effects on the community and community impacts on the environment. This phenomenon is particularly evident in biofilm communities where microbes can effectively alter the environmental characteristics of their habitat.

There is some evidence the dispersal limitation may also influence biogeographical patterns, but oftentimes the effects of dispersal limitation can be difficult to distinguish from the effects of environmental heterogeneity. We know that microbial taxa can exhibit some degree of endemism (Cho and Tiedje, 2000; Papke and Ward, 2004), but only a handful of studies have specifically examined the effects of dispersal (i.e. geographic distance) versus environmental heterogeneity on microbial biogeography. Perhaps, the most widely-cited study is that of Whitaker et al. (2003) where they examined *Sulfolobus* strains isolated from hot spring habitats and found that the geographic distance between hot springs, not the environmental characteristics of the hot springs, explained the biogeographic patterns. This study is not alone; other studies (Green et al., 2004; Papke et al., 2003; Reche et al., 2005) have also reported a

negative correlation between geographic distance and the genetic similarity of microbial taxa with little to no influence of measured environmental heterogeneity on microbial community composition. We would expect that the influence of dispersal limitation on biogeographic patterns may be more apparent at finer levels of taxonomic resolution (Cho and Tiedje, 2000) where small phylogenetic differences between populations or communities can be more readily observed. Likewise, the influence of dispersal limitation may be more apparent at continental or global-scales than in studies that examine spatial structure over smaller scales (Martiny et al., 2006).

While dispersal limitation is likely to have an important influence on microbial biogeography, designing studies to distinguish between the effects of environmental heterogeneity and dispersal limitation is difficult. Some of the studies that are frequently cited as evidence that dispersal limitation (i.e. geographic distance) exerts a major influence on microbial biogeography (see above) may be flawed in that they have not directly measured the environmental characteristics of the collected samples (e.g. Green et al., 2004) or they measured only a limited number of environmental characteristics (e.g. Reche et al., 2005). Without a thorough assessment of environmental characteristics at each sampled location and the temporal heterogeneity in the environmental characteristics, it cannot be assumed that dispersal limitation has a stronger influence than environmental heterogeneity on the observed biogeographical patterns. A correlation between geographic distance and genetic distance does not necessarily indicate that dispersal limitation drives biogeographical patterns. There is always a strong possibility that an unmeasured environmental characteristic may explain more of the variance in community structure than geographic distance, especially when we consider that habitats in close proximity are often similar with respect to their environmental characteristics. Even if we use statistical methods to separately quantify the influence of geographic distance and habitat heterogeneity (Martiny et al., 2006), we are still assuming that the degree of environmental similarity between samples has been sufficiently assessed.

Given the inherent difficulties associated with adequately characterizing microbial habitats, the most robust approach for quantifying the influence of dispersal limitation on microbial biogeography is to compare microbial communities across identical habitats in different geographic regions. Of course, finding identical habitats is nearly impossible as they would have to be the same with respect to their size, age (to allow establishment of microbial communities), and abiotic conditions (which may be influenced by the

characteristics of the microbial assemblages). For this reason Foissner (2006) has argued that Baas Becking's hypothesis ("*everything is everywhere, but, the environment selects*") is not a falsifiable hypothesis and is more valuable as a metaphor than as a scientific hypothesis.

How much of the spatial variation in microbial communities is driven by environmental heterogeneity versus dispersal limitation? It depends. It depends on a number of factors including the taxonomic group in question, the scale of inquiry, habitat characteristics, and the level of taxonomic resolution. Microbial biogeography is likely to be driven by both environmental heterogeneity and dispersal limitation but distinguishing between these two factors is not trivial and may be impossible given our inability to adequately assess micro-scale environmental characteristics and the difficulties associated with finding identical, but spatially separated, habitats. Even when we can assess the contributions of environmental heterogeneity and dispersal limitation to biogeographical patterns, it is highly likely that we will still find a large amount of unexplained variation due to unmeasured environmental heterogeneity, spatial structure, or ecologically neutral processes (Ramette and Tiedje, 2007). Of course these concerns are not unique to microbial biogeography. Even though we know far more about the natural history and spatial distribution of plants and animals, 'macro'-bial biogeographers still struggle to predict (and explain) the spatial structure of macro-organisms.

Future directions in the study of microbial biogeography

The field of microbial biogeography is on the cusp of rapid advancement. New tools and methods are emerging that will give us unprecedented abilities to survey individual microbial communities and document changes in microbial communities across space and time. There is also growing recognition that microbes do exhibit biogeographical patterns and that, by studying these patterns, we may be able to develop biogeographical theories and hypotheses that apply across the entire tree of life, not just the small portion of the tree of life where we find macro-organisms. However, it is important to recognize that the "unknown unknowns" and "known unknowns" in microbial biogeography currently outnumber the "known knowns". For this reason, I will conclude this chapter by highlighting some key topics where the gaps in our knowledge of microbial biogeography are particularly apparent. This list is neither unbiased nor exhaustive I have simply highlighted a few research topics that may be ripe avenues for future research.

<u>Taxa-time relationships</u>

Although the field of biogeography principally focuses on the spatial distribution of organisms (Ganderton and Coker, 2005), the temporal aspects of microbial biogeography may be particularly important. Species turnover, the changes in species composition as new species arrive at a location and older species go extinct, is usually measured across years, decades, or centuries by plant and animal biogeographers (MacDonald, 2003). In microbial systems, turnover may be much more rapid, especially if we consider that the process of microbes leaving or entering a dormant state (thereby entering or leaving the active microbial community) may be akin to immigration/extinction processes. If the temporal turnover in microbial communities is rapid, there may be little consistency in the composition of the "active" microbial community across sampling dates. Likewise, if turnover rates are very low, there may be a weak correlation between microbial community composition and the environmental characteristics measured at the time of sampling. Unfortunately, turnover rates in the field are difficult to quantify as there are no robust methods for measuring generation times of individual microbial cells *in situ* and it can be difficult to distinguish between "active" microbes and microbes that are in a dormant or semi-dormant state (even with RNA-based surveys of microbial communities). Turnover rates are likely to be highly variable within a given community as some populations may have generation times on par with *E. coli* strains growing in the laboratory (< 1 hour) while other populations may remain viable, but non-reproductive, for months if not centuries (Kennedy et al., 1994; Kieft and Phelps, 1997). Likewise, we would expect microbial community turnover rates to vary across habitats. Those communities that experience relatively static environmental conditions, limited predation, and are resistant to disturbances should have particularly low turnover rates. For example, we would expect microbial communities residing in deep sea sediments to have lower turnover rates (possibly weeks to months) than the planktonic communities found in surface waters (possibly hours to days). Just as taxa-area curves can provide a useful metric for comparing the spatial heterogeneity in microbial communities (see above), future research on taxa-time curves (Rosenzweig, 1995) may be useful for estimating microbial turnover rates and assessing those biotic and abiotic factors that influence the temporal heterogeneity in microbial community composition.

Viruses

Although viruses are ubiquitous and abundant in many environments, the biogeographical patterns exhibited by viruses have received little attention. Recent work in both terrestrial and aquatic environments suggests that the taxonomic diversity of viral communities is likely to be very high (Breitbart et al., 2004; Breitbart et al., 2002; Williamson et al., 2005). It has been hypothesized that the composition of viral communities is relatively invariant across distinct locations and habitat types (Breitbart et al., 2004; Breitbart and Rohwer, 2005) with viruses from one biome able to survive (and propagate) in other biomes (Sano et al., 2004). If these observations are confirmed with additional studies, they would suggest that viral biogeography is distinct from the biogeography of other microbial groups. Now that we are able to survey viral diversity in the environment using metagenomic tools (Edwards and Rohwer, 2005), we can begin to integrate viruses into the field of biogeography.

Incorporating phylogenetics into microbial biogeography

Throughout this chapter I have emphasized how studies in microbial biogeography are more difficult to conduct than comparable studies of plant or animal biogeography, largely due to the problems associated with surveying microbial communities. However, because unculturable microbes are difficult to identify, microbial biogeographers (by necessity) often rely on nucleic acid sequence data to examine the spatial structure in microbial populations. This puts microbial biogeographers at a distinct advantage as their community surveys can directly incorporate information on evolutionary history to understand and explain observed biogeographical patterns. While plant and animal biogeographers may also use phylogenetic approaches to examine biogeographical patterns, there is generally less of an incentive to conduct sequence-based surveys as species-level identification is (often) more straightforward.

Sequence-based surveys of microbial diversity (the most common being small-subunit rRNA-based surveys) yield a wealth of information but this information is rarely mined to its full potential. Most studies in microbial biogeography compare diversity patterns by grouping sequences into one, or several, operational taxonomic units (OTUs). The OTU-based approach is problematic because there is no consensus OTU definition. For example, a study that groups sequences at the 97% similarity level is not comparable to a study that groups sequences at the 99% similarity level. In addition, the

OTU-based approach ignores evolutionary history, treating all OTUs equivalently even though some may be closely related and some distantly related. Phylogenetic approaches for analyzing the diversity of microbial communities are now available (Jones and Martin, 2006; Martin, 2002) and these methods can reveal patterns in the phylogenetic structure of microbial communities that would essentially be hidden with the standard OTU-based approach. For example, lineage per time plots can reveal shifts in number of divergent microbial lineages across an elevation gradient (Martin, 2002), shifts that would be difficult to discern if applying standard ecological statistics to OTU distributions. We can also use phylogenetic methods to quantify the pairwise distances between microbial communities (Lozupone et al., 2006; Lozupone and Knight, 2005). Since such methods incorporate information on the phylogenetic relationships between sequences, they are more sensitive than OTU-based approaches. For example, there may be zero overlap between two communities if we group sequences at the 97% sequence similarity level, however, these two communities could either be very similar (if all the sequences are from the same bacterial taxon), or markedly distinct (if the sequences from the two communities represent distinct phylogenetic lineages). The utility of applying phylogenetic-distance based approaches to examine microbial biogeography is illustrated in two recent studies by Catherine Lozupone (Lozupone et al., 2007; Lozupone and Knight, In Press).

Latitude and other diversity gradients

The latitudinal diversity gradient, whereby diversity tends to increase with decreases in latitude, is one of the most fundamental patterns in 'macro'-bial biogeography. A wide range of plant and animal taxa exhibit an increase in diversity from the poles to the equator and many competing hypotheses have been proposed to explain the pattern (Lomolino et al., 2006). Although the existence of these patterns in plant and animal taxa has been documented and studied for centuries, relatively few studies have explicitly tested whether microbial taxa also exhibit a latitudinal gradient in diversity. Hillebrand and Azovsky (2001) found that latitudinal gradients in richness are largely absent for diatoms and they hypothesize that the strength of the latitudinal gradient is positively correlated with organism size. Other studies that have looked for latitudinal trends in microbial diversity have either found no relationship (Fierer and Jackson, 2006) or a reverse pattern whereby richness increases with latitude (Buckley et al., 2003). These results are intriguing, but additional studies are needed before we can

confidently conclude that microbes, unlike plants and animals, do not generally exhibit an increase in diversity with a decrease in latitude. More importantly, such studies can inform biogeographical theory by testing the universality of the latitudinal diversity pattern and the validity of the various hypotheses that have been offered to explain the pattern.

Of course, the latitudinal diversity gradient is not the only diversity gradient to be frequently studied (and debated) by 'macro'-bial biogeographers. Diversity-productivity and diversity-disturbance relationships have received considerable attention with many studies having observed maximum levels of plant and animal diversity in habitats that have intermediate frequencies of disturbance or intermediate productivity levels (Connell, 1978; Mittelbach et al. 2001; Rosenzweig, 1995). A number of experimental studies have observed that some (but not all) microbial taxa exhibit similar patterns (Buckling et al., 2000; Floder and Sommer, 1999; Horner-Devine et al., 2003; Li, 2002) highlighting the utility of using microbial taxa to test macroecological hypotheses.

Characterizing the microbial habitat at the microbe-scale

The study of microbial biogeography is constrained by the enormous discrepancy between our scale of inquiry and the scale at which microbes live in their environment. We typically survey microbial communities in samples that are cm^3 to m^3 in size, but these individual samples represent a wide array of distinct microbial habitats. As a result, we often lack detailed knowledge of the *in situ* characteristics of the microbial microenvironment and information on where specific microbial taxa are living in a given environment. Although some techniques are now employed to study microbes at the microbe-scale (e.g. Crawford et al., 2005; Huang et al., In Press; Kuypers and Jørgensen, 2007; Teal et al., 2006), we often have to ignore the micron-scale complexity and use indirect methods in order to understand how microbes influence, and are influenced by, their environment. These constraints face all microbiologists studying microbes outside of the laboratory (Madsen, 1998) and represent a set of conceptual and methodological barriers that are not typically encountered by biogeographers focusing on macro-organisms.

Conclusion

The study of microbial biogeography will help us move beyond anecdotal studies and observations to build a predictive understanding of microbial diversity and the

factors influencing this diversity across space and time. Microbiologists may be able to use pre-existing concepts in biogeography to understand microbial systems, or we may find that such concepts, which are largely derived from studies of plants and animals, are not directly applicable. Either way, the incorporation of microbiology into the field of biogeography promises to be a fruitful endeavor as many fundamental questions remain unanswered. Microbiologists can test biogeographical theories that are difficult, if not impossible, to test with plant and animal communities, and by studying microbial biogeography, we will move closer to understanding the full breadth of biological diversity on earth.

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Figure 1: Hypothetical dispersal capabilities of microbes that differ in population densities and stress tolerances. A = high population density, stress tolerant, B = high population density, stress intolerant, C = low population density, stress tolerant, D = low population density, stress intolerant. Across larger spatial scales, microbial dispersal rates should be directly related to population densities in the source population and the ability to withstand biotic and abiotic stresses associated with dispersal. Figure based on Martiny et al. (2006).

Figure 2: Comparison of rarefaction curves (A) and rank-abundance curves (B) for bacterial, archaeal, and fungal clone libraries targeting the small-subunit (16S, 18S) rRNA gene. Libraries constructed from a single desert soil sample collected in Joshua Tree, CA. Operational taxonomic units (OTUs) are defined at the ≥ 97% sequence similarity level. For the rank-abundance curve (B), only the 50 most abundant OTUs are shown. All three rarefaction curves fail to asymptote indicating that we have not surveyed the full extent of taxonomic richness in the sample. The differences in the slopes of the rarefaction curves (Fig. 2A) are a result of differences in community evenness (evident in Fig. 2B), not necessarily differences in overall richness. Data from Fierer et al. (In Press).

Figure 3: Comparison of rarefaction curves from bacterial communities found in different environments. All data are from bacterial clone libraries targeting the 16S rRNA gene with operational taxonomic units (OTUs) defined at the ≥ 97% sequence similarity. Data from Vasanthakumar et al. (2006) for the beetle gut-associated bacteria, Wani et al. (2006) for the soda lake sediment, Lawley et al. (2004) for the Antarctic soil, and Fierer et al. (In Press) for the stream sediment. The total number of clones (*n*) in each library is indicated in the legend.

Figure 4: Hypothetical changes in the total number of unique microbial taxa identified from surveys of different spatial scales. The grey line represents the predictions of Fenchel and Finlay (Fenchel, 1993; Fenchel et al., 1997; Finlay, 2002), the black line represents the competing hypothesis that there is minimal overlap in species assemblages across habitats. The dashed line and the question mark indicate the high degree of uncertainty.

Figure 5: A comparison of published taxa-area relationships (TARs) from contiguous habitats (arctic diatoms and salt marsh bacteria) and non-contiguous (island) habitats (treehole bacteria and ectomycorrhizal fungi). The TAR for arctic diatoms is from Azovsky (2002) and represents the number of diatom species in Arctic sediments versus area (m^2). The TAR for treehole bacteria is from Bell et al. (2005) and represents bacterial genetic diversity (determined by DGGE fingerprinting) versus the volume (mL) of water-filled treeholes. The TAR for salt marsh bacteria is from Horner-Devine et al. (2004) and represents the number of bacterial OTUs in a salt marsh (99% sequence similarity) versus area (cm^2). The TAR for ectomycorrhizal fungi is from Peay et al. (2007) and represents the number of ectomycorrhizal fungal species in 'tree islands' of a given area (m^2).