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SPATIAL SCALING OF MICROBIAL BIODIVERSITY

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Spatial Scaling of Microbial Biodiversity

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1. Introduction

One of the key goals of ecology is to understand the spatial scaling of species diversity. Spatial patterns of species diversity provide important clues about the underlying mechanisms that regulate biodiversity and are central in the development of biodiversity theory (Brown 1995, Gaston and Blackburn 2000, Holyoak et al. in press, Hubbell 2001, MacArthur and Wilson 1967, Rosenzweig 1995). Asssumptions regarding the spatial scaling of biodiversity are a fundamental component of conservation biology and are frequently used to identify local- and global-scale priority conservation areas (Desmet and Cowling 2004, Ferrier et al. 2004) and to predict extinction risk due to climate change (Thomas et al. 2004) and habitat loss (Gaston et al. 2003). Although scaling patterns have been documented in hundreds of studies of plant and animal diversity, such patterns in microbial species (i.e., microeukarya, bacteria and archaea) have not been well documented. This is a serious omission, given that microorganisms may comprise much of Earth's biodiversity (Torsvik et al. 2002, Whitman et al. 1998) and play critical roles in biogeochemical cycling and ecosystem functioning (Balser 2000, Morin and McGrady-Steed 2004, Wardle 2002). Furthermore, microbial biodiversity is a major source of novel pharmaceuticals and other compounds of industrial importance, and an understanding of the scaling of microbial biodiversity is crucial to the search for such compounds (Bull 2004).

There are both technical and conceptual reasons for our lack of understanding of the scaling of microbial biodiversity. Technically, it has been very challenging to quantify microbial biodiversity. Prokaryotic and many eukaryotic microorganisms cannot be identified morphologically, and must either be identified using traits that require culture in the laboratory (e.g., the utilization of specific substrates) or identified via biochemical markers extracted from environmental samples (e.g. phospholipid fatty acids or DNA sequences from indicator genes; (O'Donnell et al. 1994). Even at small scales it has proven impractical to exhaustively inventory microbial communities. Conceptually, it has long been assumed that microorganisms have cosmopolitan distributions (Bass-Becking 1934, Fenchel and Finlay 2004). The small size and high abundance of microbes (as well as other aspects of their biology) has been assumed to increase the rate and geographic distance of dispersal to levels where dispersal limitation is essentially nonexistent. It has been argued that such continuous large-scale dispersal would result in fundamentally different biodiversity scaling relationships for microbes, relative to those observed for other forms of life (Fenchel and Finlay 2004).

Should microbes have cosmopolitan distributions? Do they have cosmopolitan distributions? In this chapter, we discuss the evidence for cosmopolitan distributions of microbes. We argue that the assumption of a lack of dispersal limitation among microbes is based on a confusion of hypotheses for facts, and that the actual evidence for microbial cosmopolitanism is mixed, often misinterpreted, and likely an artifact of broad taxonomic definitions for microorganisms.

2. Should microbes have cosmopolitan distributions?

It has been argued that due to their unique biology, organisms smaller than 1mm

in diameter should have cosmopolitan distributions. These biological differences include very large population sizes, a high capacity for dispersal, and low extinction rates (Table 1). Although these differences have been assumed to be universally characteristic of microbes, the evidence for this is scant, as described below.

2.1 Population sizes of microbes. The most commonly claimed mechanism underlying a cosmopolitan distribution of microbes is that their large population sizes result in high rates of dispersal (Fenchel and Finlay 2004). The probability of chance dispersal (e.g. via an accidental vector such as a bird or mammal) is increased when abundance is high. Certainly microbes are very abundant. A gram of soil may contain 10^9 individual bacteria and perhaps ten thousand ciliates (Fenchel and Finlay 2004, Torsvik et al. 2002). Overall abundance, however, does not necessarily suggest that the population sizes of individual species of microbes are large; what matters is how these abundant individuals are distributed among taxa. The size of a given species population will depend on how one defines "species" (or whatever taxonomic classification is used). A broader definition of a taxon will result in a larger estimated taxon population. For example, if one defined a taxon as "all plants" that definition would result in a very large estimate of taxon population size, a large potential rate of dispersal, and a high probability of cosmopolitanism (which is what we observe).

How are taxa usually defined? For many eukaryotic microorganisms, an approach is used that is roughly similar to that used for the taxonomy of macroorganisms. Morphological traits are used to group individuals into species, with the underlying assumption that shared morphological traits reflect interbreeding, although this is rarely tested. It is unclear whether microbial species defined via morphological traits are comparable in genetic diversity, evolutionary relatedness or ecological breadth to macroorganism species. In general, the smaller the organism, the more limited the morphological information. It has been argued that some protists present as many morphological traits as macroorganisms (Fenchel and Finlay 2004) but this is debatable (Coleman et al. 2002, Foissner 1999, Hedlund and Staley 2004). It is certainly not true for some protists (such as amoebae and flagellates) nor for bacteria or archaea, which have very few morphological features, even when magnified with electron microscopy. It is likely that due to reduced morphological information, microbial morphospecies represent a coarser taxonomic resolution than plant or animal species (Hedlund and Staley 2004).

Phenotypic traits other than morphology (such as the utilization of specific substrates) have been used historically to identify microorganisms that lack sufficient morphological traits. Determining such phenotypic traits usually requires that the organism be cultured in the laboratory. It has become apparent that the vast majority of prokaryotic microorganisms (as well as many eukaryotic microorganisms) cannot yet be cultured (Amann et al. 1995) and thus an alternative approach is needed.

One alternative approach is to use biochemical markers to define taxa. The most common of these techniques use ribosomal gene sequences as indicators of microbial diversity, although other genes, including protein-coding genes, have also been used. The use of these molecular techniques and their drawbacks and biases has been reviewed in detail elsewhere (e.g. (Wintzingerode et al. 1997). These approaches have enabled the detection of non-culturable species and allowed a more complete and detailed picture of

bacterial communities (reviewed in Head et al. 1998, Mlot 2004). Taxa are usually defined as sequence similarity groups using this approach. For bacteria and archaea, 97% DNA sequence similarity of 16S rDNA is the most common definition of taxa and is considered to approximate the species level of resolution defined using culture-dependent methods (Stackebrandt and Rainey 1995). Taxa defined using such approaches are likely not comparable to macroorganism species. If this definition was applied to animals, all the primates (from lemurs to humans) would be considered the same species (and would be cosmopolitan; (Staley 1997). A number of studies have demonstrated that substantial ecological diversity is often hidden within taxa defined in this manner (e.g. Moore et al. 1998, Ward et al. 1998).

Even if it was possible to define "species" of microbes in a manner comparable to macroorganisms, we would still not be able to predict the dispersal rates of microbes without an understanding of the taxon-abundance relationships among microbes. If we could determine that the abundance of bacteria was distributed among a limited number of species (defined in some way comparable to that of plants and animals) this would not necessarily suggest that population sizes were universally large unless we also knew that the taxon-abundance relationship was very even (i.e. that individuals were distributed relatively evenly among taxa). Little is known about microbial rank-abundance relationships, and both highly skewed and very even distributions have been reported (e.g. Dunbar et al. 2002, Zhou et al. 2002).

It is not possible to claim that microbes have larger population sizes than macroorganisms if different definitions of taxa are used for microbes and macroorganisms, and without an understanding of taxon-abundance relationships. Finding an equivalent taxon definition for both microbes and macroorganisms may not be possible, rendering this claim (and the debate over microbial cosmopolitanism) meaningless. It is more interesting (and tractable) to ask: at what level of taxonomic resolution does cosmopolitanism break down for microbes? And what is the ecological relevance of this level of taxonomy (e.g. can we detect differences among groups of this resolution in ecological function, environmental optima, or response to environmental change)? Although these questions were essentially suggested over a decade ago (Tiedje 1993), they are just beginning to be addressed by microbial ecologists (e.g. Cho and Tiedje 2000b, Horner-Devine et al. 2004b, Whitaker et al. 2003).

2.2 Low rates of extinction and speciation. Another common a priori argument for a cosmopolitan distribution of microbes is that microbes have lower extinction rates than macroorganisms (Dykhuizen 1998, Fenchel and Finlay 2004, Torsvik et al. 1990). This argument is based on the assumption that microbes have larger population sizes than macroorganisms, which makes stochastic extinction events less likely (Dykhuizen 1998, Finlay and Clarke 1999). Microbes do not necessarily have larger population sizes, as argued above. Extinction rates may also be relatively low because some microbes have traits that allow them to reduce the risk of catastrophic losses typical of extinction events in plants and animals. Some microbes are known to form life stages that can survive harsh environmental extremes, reducing the probability that chance fluctuations in environmental conditions drive them to extinction. High dispersal rates over large distances may also reduce the chance that local environmental change results in extinction (see 2.3 below). Finally, some microbes are also able to avoid the negative effects of

competitive interactions; for example, resistance to starvation has been documented for some species in the laboratory (Finkel and Kolter 1999). It is not clear how widespread the traits discussed above are among microbial taxa (and thus how likely it is that extinction rates are actually lower for microbes), and there are no direct measures of extinction rates for microbes in the field.

It has also been argued that microbes have low rates of speciation and that such low rates contribute to cosmopolitanism by reducing local diversification (Finlay and Fenchel 2004, Martin et al. 2004). One mechanism that could lower speciation rates among microbes is parasexuality. Some eukaryotic microbes and most if not all prokaryotic microbes are "parasexual"; i.e. they exchange genetic material "rarely but promiscuously" through a variety of mechanisms (Gogarten 2003, Ochman et al. 2000). Genetic exchange through parasexuality, if it occurs at a high rate, could lower the rate of speciation by acting as a homogenizing force, spreading key innovations among a number of different microbial lineages. If exchange occurs at a low rate (at or below the rate of mutation) it can have the opposite effect, increasing the rate of speciation. This can occur because rare exchange enables genetic variation associated with niche diversification to lead to speciation, eliminating the need for geographic isolation (Cohan 2002). In addition, parasexuality may also increase speciation rates by introducing genetic novelty. Parasexuality has only been studied in detail in a few microorganisms, and the extent and rate of parasexual exchange among the vast majority of microbes is unknown.

It has also been suggested that speciaton rates among microbes are likely low due to a lack of barriers to dispersal. A lack of dispersal barriers would prevent the geographic isolation necessary for allopatric speciation. Whether a reduction in the rate of allopatric speciation would lower the overall speciation rate of microbes relative to macroorganisms would depend on the balance between allopatric and sympatric speciation among microbes. A high rate of sympatric speciation (due for example to niche diversification as described above) could compensate for the reduction in allopatric speciation.

Despite the claims outlined above, there is little direct evidence that rates of speciation are low among microbes. Measuring rates of speciation is difficult and is usually done through studies of the fossil record (which is scant for most microbes). It may be possible to infer rates from the dynamics of cladogenesis, via lineage-through-time plots (Nee et al. 1994a, Nee et al. 1994b). The only study we are aware of that has attempted this is that of Martin et al. (Martin et al. 2004). Martin et al. (2004) created lineage-per-time plots for eukaryotic and prokaryotic microorganisms based on ribosomal gene sequences amplified from the same alpine soil. They observed that there was a tendency for eukaryotic lineages to exhibit more variability in the rates of cladogenesis, although neither eukaryotic nor prokaryotic microorganisms displayed the highly variable rates characteristic of most plant and animal lineages (Figure 1). The lack of variability could be due to a lack of sensitivity of cladogenesis to environmental change, which could reflect low rates of speciation and extinction due to dispersal barriers or to the homogenizing effects of parasexuality.

2.3 High capacity for dispersal. If microbes have cosmopolitan distributions it must ultimately be due to a high capacity for dispersal. Although a high abundance of

microbes suggests that microbes in general can disperse at high rates, whether a given taxon enjoys a high rate of dispersal depends on the absolute abundance of that particular population (which is dependent, among other things, on how we define the taxon, as described above), and the taxon-abundance relationship (a highly uneven distribution would result in high dispersal of only a few types). Some microbes have the potential to have very high rates of dispersal; it is known that bacteria can be dispersed passively in the atmosphere (e.g. Gage et al. 1999, Lighthart 1997) and through water (e.g. Leff et al. 1998, McNair et al. 1997) due to their small size. In addition to high rates of dispersal, the ability to disperse over long distances is also necessary for cosmopolitan distributions. The distance over which a given taxon can disperse is a function of the specific characteristics of the taxon (dispersal adaptations). Some bacteria, such as members of the bacterial genus *Bacillus*, can form hardy life stages (spores) that are highly resistant to environmental stresses such as desiccation; such stages could allow these organisms to disperse widely. It is not known how widespread dispersal adaptations are among microbes, and few studies have been able to quantify even relatively small-scale dispersal and colonization rates of individual microbial taxa.

The most frequently cited evidence for a high capacity for dispersal of microbial taxa is the wide distribution of protist morphospecies. A number of studies have demonstrated that many protist morphospecies are found worldwide (Fenchel and Finlay 2004, Finlay and Fenchel 2004). Whether it is valid to directly compare protist morphospecies to species as defined for plants and animal is debatable (Coleman et al. 2002, Foissner 1999, Hedlund and Staley 2004); despite this, protist morphospecies distributions and the high capacity for dispersal they imply have been accepted by many microbiologists as representative of all microorganisms (Fenchel and Finlay 2004, Finlay and Fenchel 2004). It is not clear that this is a valid assumption, as described below.

There is evidence that some bacterial taxa may have wide distributions, suggesting that the capacity for bacterial dispersal is also high (e.g. Brandao et al. 2002, Glöckner et al. 2000, Ward and O'Mullan 2002, Zwart et al. 2002). Brandao et al. isolated identical *Rhodococcus* 16S rDNA sequences from Argentinian soil, the ocean floor near Japan, lake bottoms in Antarctica, bogs in England and Indonesian swamps. Most studies (although not all, e.g. (Ward and O'Mullan 2002) reporting cosmopolitan distributions of bacteria used taxon definitions based on sequence similarity of the 16S ribosomal gene, a very conservative definition (Papke et al. 2003).

Protein coding sequences may provide greater resolution and be more appropriate for inferring rates of dispersal (Palys et al. 1997, Rotthauwe et al. 1997). Studies of proteins and protein-coding genes suggest that dispersal rates vary widely among prokaryotic microorganisms. Roberts and Cohan (Roberts and Cohan 1995) used sequence data from three genes to estimate the migration rates among different populations of two closely related species of the bacterial genus *Bacillus*. The populations were sampled at a range of geographic scales, ranging from 30 to 10,000 km apart. The magnitude of the migration rate was generally associated with geographic scale (migration was highest among the closest sites). However, even at the largest scale, where migration rates were lowest, the rate of exchange was sufficient to prevent neutral geographical evolutionary divergence (i.e. the most distant populations were not isolated enough to exhibit genetic drift). *Bacillus*, as described above, forms hardy life stages and thus it is not surprising that it may have high dispersal rates. Studies of nine loci in the bacterium *Rhizobium*

(Souza et al. 1992) and the archaean *Sulfolobus* (Whitaker et al. 2003) at spatial scales ranging from meters to tens of thousands of kilometers also found that the magnitude of the migration rate was generally associated with geographic scale. In contrast to the *Bacillus* study, these studies found that dispersal of *Rhizobium* and *Sulfolobus* was not sufficient to prevent divergence of geographically distant populations.

2.4 Habitat turnover. Even if we assume that most microbes are globally dispersed, this does not imply that their diversity does not scale with space. Spatial scaling relationships (e.g., the species-area relationship) do not depend solely on limitations to dispersal. These relationships can also be driven by the relationship between the size of an area and the number of individuals (larger areas contain more individuals and thus are likely to contain those types that are rare) and the relationship between the size of an area and the number of different habitats it contains (which usually increases as area increases). If microbes do indeed have shallow species-area relationships, as often claimed (Fenchel and Finlay 2004), then this would require that either abundance not scale with area sampled (an implausible scenario) or that microbial habitats turn over very slowly with area. Given the small size of microbes and the existence of steep environmental gradients at very small spatial scales, slow turnover of microbial habitats in space requires that microbes have very broad environmental tolerances and resource requirements. The existence of extreme specialization among at least some microbes (Tankere et al. 2002) suggests that broad ecological tolerances are not a universal trait among microbes.

Estimates of habitat turnover are vulnerable to the same weakness as estimates of population size, extinction/speciation rates and capacity for dispersal - namely that they can be sensitive to taxonomic definitions. A habitat is usually defined as the particular combination of resources and conditions required by a particular a taxonomic group to persist (Looijen 1998, Tiedje 1993). Since habitats are defined relative to a particular taxonomic definition, habitat measurements can be just as prone to artifacts of taxonomic "lumping" as are the other attributes of microorganisms described above. For example, the range of conditions and resources required for the persistence of a particular species (i.e., its habitat) would likely be much narrower than that required for the persistence of a genus or a family. Very broad taxon definitions would result in broadly defined habitats and estimates of relatively low habitat turnover in space, and very narrow definitions would lead to narrowly defined habitats and estimates of relatively high habitat turnover in space.

The only study of microbial habitat turnover in space that we are aware of is that of Horner-Devine et al. (Horner-Devine et al. 2004a). They observed that bacterial taxa (defined as 16S rDNA sequence similarity groups) turned over in space at a much lower rate than plant species, and that this turnover was driven primarily by turnover in habitats with distance, rather than distance alone. Furthermore, they observed that the rate of turnover of taxa was higher as one increased taxonomic resolution (i.e. went from taxa defined as 95% sequence similarity groups to those defined as 97% or 99% groups). They also observed that turnover varied with taxonomic focus; taxa within the Betaproteobacteria changed more slowly with distance than did other groups of bacteria sampled. Since the turnover of taxa was driven primarily by turnover of habitats in this study, these results suggests that habitat turnover is slower for bacteria than plants in this system, that it varies with taxonomic resolution, and that it differs among different

bacterial lineages.

3. Do microbes have cosmopolitan distributions?

The discussion above suggests that there is not strong evidence that microbes SHOULD have cosmopolitan distributions. But do they? In the discussion below we provide an overview of the direct evidence for microbial cosmopolitanism. We focus on three spatial patterns of microbial biodiversity often cited as evidence for cosmopolitanism: the local/global taxa richness ratio, the taxa-area relationship, and the relationship between taxa similarity and geographic distance.

3.1 Local/global taxa richness ratios. If microorganisms are cosmopolitan, then they will show a higher relative local taxa richness compared to the global taxa pool than larger organisms. In other words, for a specified habitat type, microbes should have a higher local/global taxa richness ratio than macroorganisms. The most compelling evidence of this pattern comes from research on protist morphospecies. In a study of the flagellate genus *Paraphysomonas*, 80% of the known global species were found in <0.1 cm^2 of sediment collected from Priest Pot, a 1-ha freshwater pond in England (Finlay and Clarke 1999). Data compiled by Fenchel and Finlay across a wide range of eukaryotic taxonomic groups (e.g. amoebae, diatoms, mollusks, etc.) in Priest Pot suggest a more general relationship between body size and global distribution (Fenchel and Finlay 2004). They found that the local/global species ratio, expressed as a percentage of the global number of freshwater species, consistently decreased with mean body size. A parallel analysis of data collected from NivÂ Bay, a 2-ha marine shallow-water habitat in Denmark, revealed the same pattern, indicating that small organisms (less than 1 millimeter in length) tend to have a cosmopolitan distribution (Fenchel and Finlay 2004). Data on polar surveys for testate amoeba assemblages also support this hypothesis (Wilkinson 2001).

These studies are misleading for at least two reasons. First, they assume that for a given habitat type, the magnitude of microbial eukaryote global species richness is known, or as least as well known as that of macroorganisms. Some researchers claim that for particular groups of microbial eukaryotes, such as ciliated protozoa, the number of described species globally is unlikely to increase in the future (Finlay et al. 1996), while others claim that a large number remain undiscovered (Foissner 1997). Due to unequal relative sampling effort, the probability of underestimating species richness at the global scale is significantly higher than the probability of underestimating species richness in a local sample. Underestimating global species richness will inflate projected local/global species ratios, and hence distort reported patterns of microbial eukaryote biogeography.

A second point to consider is that almost all data on protist species richness rely on morphological species concepts (as described above). The proposed cosmopolitan distribution of microbes based on morphospecies has been repeatedly criticized (Coleman et al. 2002, Foissner 1999, Hedlund and Staley 2004, Hillebrand et al. 2001). A major question is whether the resolution of morphospecies is poorer for smaller than for larger organisms. It has been suggested (although see Finlay and Fenchel 2004) that at some body size or morphological complexity limit more sensitive and less subjective

taxonomic criteria (e.g. criteria based on genetic similarity) may be more appropriate (Hedlund and Staley 2004). Higher resolution taxonomic criteria for microbial eukaryote species would likely lead to increased global species pool estimates and decreased local/global species ratio estimates.

Studies using molecular techniques to examine spatial patterns of prokaryote diversity suggest that the perceived spatial distribution of microbes depends on the taxonomic criteria used. Studies using a wide array of methods including 16S rDNA sequencing, pairwise DNA/DNA hybridization, repetitive extragenic palindromic (REP) genomic fingerprinting, and amplified ribosomal DNA restriction analysis (ADRA) suggest that prokaryotic genera are widely distributed in their respective habitats (Fulthorpe et al. 1998, Hagstrom et al. 2000, Hedlund and Staley 2004, Staley and Gosink 1999). However when methods offering finer genetic resolution are employed, bacteria appear to have endemic geographical distributions (Cho and Tiedje 2000a, Fulthorpe et al. 1998, Papke et al. 2003, Whitaker et al. 2003). Below (section *Similarity – geographic distance relationships*), we provide more empirical evidence supporting the idea that microbial diversity patterns depend on the level of phylogenetic or taxonomic resolution assumed.

3.2 Taxa-area relationships. The relationship between species richness and sampled area – the 'species-area relationship' (SAR), is one of the most widely cited and studied patterns in ecology. Evidence that the number of species tends to increase with increasing area was reported as early as 1855 (DeCandolle 1855). In his dissertation, Olof Arrhenius was the first to propose a mathematical description of the SAR, which he later simplified to a general power-law of the form:

$$S = cA^z, \qquad (1)$$

where S is species number, A is area, and z and c are constants (Arrhenius 1921). Although many functional forms of the SAR have been proposed (Connor and McCoy 1979, He and Legendre 1996), the power-law form has withstood the test of time relatively well (May 1975, Rosenzweig 1995). Empirical evidence suggests that for large organisms (i.e. plants and animals) within continental habitat patches, z is generally in the range of 0.1 – 0.3. There is evidence for slightly steeper SARs between islands in archipelagos ($0.25 < z < 0.35$) and the steepest SARs when whole biotas are compared ($0.5 < z < 1.0$) (Rosenzweig 1995).

Although species-area relationships have been observed repeatedly for plants and animals, little is known about the relationship between microbial taxonomic richness and area (the taxa-area relationship, or TAR). Advocates of microbial cosmopolitanism have suggested that in addition to high local/global species ratios, microbes should be characterized by relatively flat taxa-area curves, with z values an order of magnitude lower than those reported for macroorganisms. We discuss below some of the few published observations of microbial TARs.

3.2.1 *Protozoan diversity: estimating z for free-living ciliate species.* To our knowledge, the first reported microbial TARs were for free-living protists in marine interstitial and freshwater benthos (Finlay et al. 1998; Figure 2). Using data on the

numbers and abundance of ciliate species from field samples, Finlay and colleagues applied novel extrapolation techniques to estimate the global species richness of these microbial communities. They concluded that the ciliate TAR exponent $z = 0.043$ from local to global scales, and that this slope successfully estimates the number of ciliate species recorded globally. This low value of z has been repeatedly cited to characterize microbial eukaryote biogeography (Finlay 2002), and has been assumed to represent the z value of other microorganisms. However it is based on a number of unsupported assumptions, as described below.

(1) The marine interstitial data were collected from beaches off the coast of Denmark and Sweden. A total of 79,342 individuals and 151 ciliate species were recorded. Finlay and colleagues suggest that the sample data is adequately fit by a lognormal species-abundance distribution (Preston 1962), with standard deviation $\sigma = 0.22$ (Figure 1a in Finlay et al. 1998). However when plotting the same data in terms of a rank-abundance relationship, they find that the best fit has an exponential relation of the form

$$\log N = A - Bi \qquad (2)$$

Here, N is the abundance of a species, i is the rank with respect to the abundance (i.e. $i = 1$ for the highest abundance, $i = 2$ for the second highest abundance, etc.), and the parameters A and B are constants (Figure 2a in Finlay et al. 1998). A lognormal species-abundance distribution does not correspond to an exponential rank-abundance relationship (May 1975). The remainder of Finlay et al.'s analysis rests upon the assumption of an exponential rank-abundance relationship.

(2) Finlay et al. assume that an aggregate sample consisting of 151 ciliate species, comprised of subsamples drawn from disparate beaches in Denmark and Sweden, is representative of the total species richness sampled from one location of comparable size. They sampled a total of 87 cm^2 of sediment from all regions, and thus assume there are 151 ciliate species per 87 cm^2 at local scales. In studies of macroorganisms, it has long been established that the number of species counted in scattered subplots will be greater than the species surveyed in one contiguous subplot of equivalent area (Gleason 1922, Rosenzweig 1995). This is due to clumping, or heterogeneity in the spatial distribution of species within the contiguous subplot. If the spatial distribution of ciliate species is more clustered than random at the geographic scale of the sampling (i.e., Denmark and Sweden), there will be less than 151 ciliate species per 87 cm^2 at local scales.

(3) The next major assumption made by Finlay et al. is that an exponential rank-abundance curve (Equation 2) holds from the 87 cm^2 scale to the 'global' scale (which they assume is 2×10^6 km^2). Further, they assume that the slope of the exponential rank-abundance curve, B, is independent of spatial scale. Little is known about the spatial scaling of species abundance distributions for either macro- or micro-organisms. To our knowledge, there is no empirical or theoretical basis for assuming that the functional form of the ciliate species-

abundance distribution remains constant across such a large range of spatial scales.

(4) Finally, Finlay et al. assume that the relative abundance of ciliate species scales linearly with sampling area, such that a species represented by one individual in 87 cm^2 will harbor $10^4/87 = 115$ individuals per m^2. Note that this assumption does not directly follow from the assumed scale-invariant exponential rank-abundance curve.

(5) Using the framework and assumptions outlined in (1) – (4), Finlay et al. project a total of 597 ciliate species at the 'global scale' (2×10^6 km^2). Assuming a power-law TAR with constant z from 1 m^2 to 2×10^6 km^2, it follows from Equation 1 that

$$z = \frac{\log\left(\frac{151}{597}\right)}{\log\left(\frac{87}{2\times10^{16}}\right)} = 0.042 \qquad (3)$$

In summary, Finlay et al. (1998) make a large number of assumptions to obtain the estimate z = 0.04 from local (87 cm^2) to global scales for free-living ciliated protozoa in marine benthic interstitial habitats. They use a similar approach to estimate z for freshwater benthos. The validity of these assumptions is questionable, and relaxing these assumptions generally increases the value of z. The conclusion that the TAR slope is "flat" for ciliates is thus premature.

3.2.2 *Size-dependent taxa richness patterns*. Microbial TARs have more recently been studied in the context of body size-dependent patterns. Using original and previously-published data on morphospecies richness, Azovsky (Azovksy 2002) compared the species-area relations for 5 different classes of Artic benthos with body sizes spanning four orders of magnitude (0.01 mm to 10 mm in diameter). Data collected from overlapping or nested samples were pooled together from areas covering a wide range of scales, from cm^2 (single samples) to thousands of km^2 (regional surveys or synopses of whole seas). Azovsky found that the power-law SAR slope increased significantly with body size, ranging from z = 0.066 at the smallest size group (diatoms) to z = 0.152 in the largest size group (invertebrate "macrofauna"; Figure 3). In a similar meta-analysis of original and published data, Hillebrand and colleagues (Hillebrand et al. 2001) also found that small organisms (protozoans and microalgae) tended to have small z values in comparison to larger organisms, although low z values (<0.09) were reported for some macroscopic organisms (i.e. birds and insects). Unfortunately, Hillebrand et al. did not report the spatial scale over which z was calculated for each group of organisms, rendering comparison between size classes difficult.

It is important to note that both of these studies estimated microbial SAR slopes using a nested, rather than a complete nested, sample design. As with the local/global taxa richness ratios, the probability of underestimating species richness at large scales is significantly higher than the probability of underestimating species richness at small

scales due to unequal relative sampling effort. Underestimating large-scale species richness will reduce the projected SAR slope z. It is not possible to detect and identify all microbial species in large areas by identifying all individuals in the whole area, and methods for extrapolating microbial community structure from small scales to landscape and global scales are needed to critically evaluate the hypothesized species:body-size:area relationship.

3.3 Similarity – geographic distance relationships. Ecologists studying macroorganisms have long recognized that beta-diversity (how community composition changes across a landscape) is central to understanding the forces responsible for the magnitude and variability of biodiversity (Condit et al. 2002, Harrison 1997, Schluter and Ricklefs 1993, Whittaker 1960). Although it is widely accepted that the similarity in plant and animal community composition decays with increasing distance between samples, data on microbial community turnover remains sparse.

The assumption of global microbial dispersal by a combination of randomizing forces (e.g. wind, water, animal vectors, etc.) would lead to random primary spatial distributions, followed by subsequent population growth in nonrandom spatial niches (Finlay 2001). According to this cosmopolitan view of the microbial world, spatial patterns of microbial diversity are primarily driven by environmental heterogeneity. Thus, one might expect to find similar microbial communities in similar habitats, and differentiated microbial communities along an environmental gradient. In other words, within a given habitat type lacking strong environmental gradients, the similarity of microbial assemblages should be independent of the distance between the two sites.

To our knowledge, Hillebrand and colleagues (Hillebrand et al. 2001) were the first to report on the relationship between microbial taxa similarity and geographic distance. They gathered morphospecies data on diatoms, ciliate, corals and polychaetes sampled from similar habitats and environmental conditions (e. g., temperature, light, salinity) that were separated by distances ranging from 1 km to 1000 km. For each group of organisms, they estimated the similarity of species composition between samples in terms of the widely-used Jaccard Index, which is based on presence/absence data. Finally, for each group they quantified the rate at which similarity decayed with increasing distance between samples, or the slope of the "distance-decay relationship" (Nekola and White 1999). They found that for all groups, species similarity decayed significantly with distance, which contradicts the hypothesis of ubiquitous dispersal. Metazoan species were characterized by substantially steeper slopes than the diatom and ciliate species, suggesting that body-size may influence spatial biodiversity patterns.

A recent study of ascomycete fungi (Green et al. 2004) provides further evidence that, as with plants and animals, the similarity in microbial community composition decays with increasing distance between samples (Figure 4). Green and colleagues characterized soil microbial fungal community structure by automated ribosomal RNA intergenic spacer analysis (ARISA), a commonly used DNA-based community fingerprinting method (Ranjard et al. 2001). Using over 1,500 soil samples collected from four distinct desert habitats using a spatially explicit nested design, Green et al. found that community taxonomic similarity decayed significantly with distance across distances ranging from 1 m to ~100 km. Applying theoretical techniques based on scaling and dimensional analyses (Harte et al. 1999, Krishnamani et al. 2004), they also

showed how microbial turnover patterns can be used to project taxa-area relationships up to whole continents. Unlike the previously discussed studies that assumed a power-law taxa-area relationship (Azovksy 2002, Finlay et al. 1998, Hillebrand et al. 2001), Green et al. tested multiple distance-decay models and found that the power-law model was best fit to the data, implying a power-law taxa-area relationship. Their predicted power-law taxa-area slope of 0.074 (across the scales 1 m^2 to 10^{10} m^2) was consistent with those reported for microbial eukaryote species in Arctic benthos (Azovksy 2002) and in freshwater habitats (Finlay et al. 1998). This is remarkable, given the number of questionable assumptions (described above) that underlie the latter estimates. The study of Green et al. suggests that despite high local diversity, microbial eukaryotes may have only moderate spatial turnover and hence moderate regional diversity. This conclusion, however, is based on taxa defined using the ARISA method, and it is not clear how such taxa compare with traditionally defined species for plants and animals, although it is likely that ARISA-defined taxa are of coarser resolution than traditional plant/animal species. Perhaps if the micro-eukaryote taxa in the Green et al. study were defined in a manner comparable to that of plants and animals, a higher value of z would be obtained.

Horner-Devine and colleagues (Horner-Devine et al. 2004a) uncovered a more complex relationship between measured patterns of beta-diversity, the TAR, and the taxonomic definitions used (Figure 5). They collected salt marsh sediment cores sampled in a nested manner over a scale of centimeters to hundreds of meters, and identified the presence of proteobacteria within each core by PCR-amplifying, cloning, and sequencing regions of 16S rDNA. They defined taxa based on 95%, 97%, and 99% sequence similarity. They found significant distance-decay curves for all taxonomic resolutions. By applying the distance-decay approach developed by Harte et al., they concluded that the TAR was well modeled by a power-law, and that the slope of the TAR relationship z varied with taxonomic resolution, ranging from $z = 0.019$ at 95% sequence similarity to $z = 0.04$ at 99% sequence similarity. Their data clearly indicate that spatial biodiversity patterns depend on the defined taxonomic resolution.

4. Conclusions

It has long been assumed that microorganisms have cosmopolitan distributions. The evidence, however, is inconclusive. The fundamental processes assumed to underlie microbial cosmopolitanism (large populations sizes, low extinction rates, etc.) are merely hypotheses, although they are frequently assumed to be facts, and there is little evidence that these are universal attributes of microbial life. It is clear that some microbial taxa do have cosmopolitan distributions, but these conclusions are very sensitive to how taxa are defined, and there is evidence that microbial taxa as they are now defined are of much lower resolution than the typical plant or animal species. If this is true, then the controversy surrounding microbial cosmopolitanism may be merely the result of taxonomic “lumping” of microorganisms.

It has also been repeatedly argued that microbial cosmopolitanism results in fundamentally different biodiversity scaling relationships for microbes, relative to those observed for other forms of life. These differences include low local:global taxa richness ratios, shallow taxa-area relationships, and a lack of decay of community similarity with distance. These claims are premature. Not only is the evidence for microbial

cosmopolitanism inconclusive, but the evidence for fundamentally different scaling relationships is scant as well. Low local:global richness ratios have been reported, but they depend on an accurate estimate of global richness, which is unlikely even for relatively well studied protists. Furthermore, they are subject to the same sensitivity to taxa definitions described above. Shallow taxa-area relationships have also been reported. These require a number of unsupported assumptions, are subject to undersampling artifacts, and/or are sensitive to taxon definitions. Finally, there is evidence of decay in microbial community similarity with distance within habitats that appear to lack strong environmental gradients. These results suggest that limits to dispersal may play a pivotal role in restricting the spatial distribution of microbes.

We suggest, as have others, that the debate over microbial cosmopolitanism be recast. Rather than ask the unanswerable question "do microbes have fundamentally different scaling relationships from those of plants and animals?" we suggest that the debate focus instead on the question "is there a spatial scale and a level of taxonomic resolution at which microbial biodiversity scaling relationships approach those of macroorganisms?" This is a tractable question, and one that avoids the impossible task of identifying equivalent taxonomic definitions for microbes and macroorganisms. To answer this question, microbial ecologists would need to use multiple taxonomic definitions based on a variety of genetic markers (and biochemical and morphological traits if accessible). Such a polyphasic approach to studies of microbial biogeography is just beginning to be applied.

Biodiversity scaling rules have been suggested to be universal to all life. The universality of such rules has been called into question by the conclusion of some microbiologists that microbial biodiversity obeys fundamentally different rules. This conclusion is extremely premature. Determining rigorously the validity of this conclusion will not only increase our understanding of microbial ecology, but it will also provide ecologists with a true understanding of the universality of spatial scaling rules.

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Table 1. Differences assumed by some microbiologists to exist between macroorganisms and microorganisms (modified from Finlay and Esteban 2004). These differences are unproven hypotheses, although they are often treated as facts.

Characteristic	Plants&Animals	Microorganisms
Species abundance	Low	High
Migration rate	Low	High
Speciation rate	High	Low
Extinction rate	High	Low
Relative number of endemics	High	Low
Global number of species	High	Low
Local:global species richness	Low	High

Figure 1. Lineage-per-time plots for prokaryotes and microeukaryotes in alpine soil. From Martin et al. 2004, copyright Evolution, used with permission.

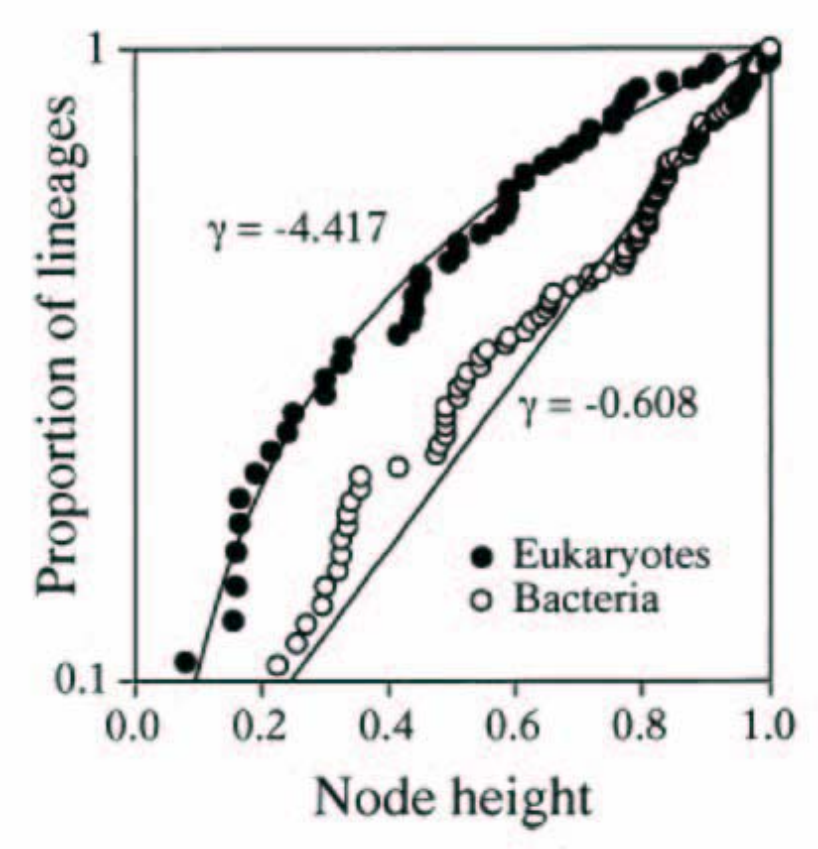

Figure 2. Extrapolated species-area relationships for ciliates and insects. From Finlay et al. 1998, copyright Protist, used with permission.

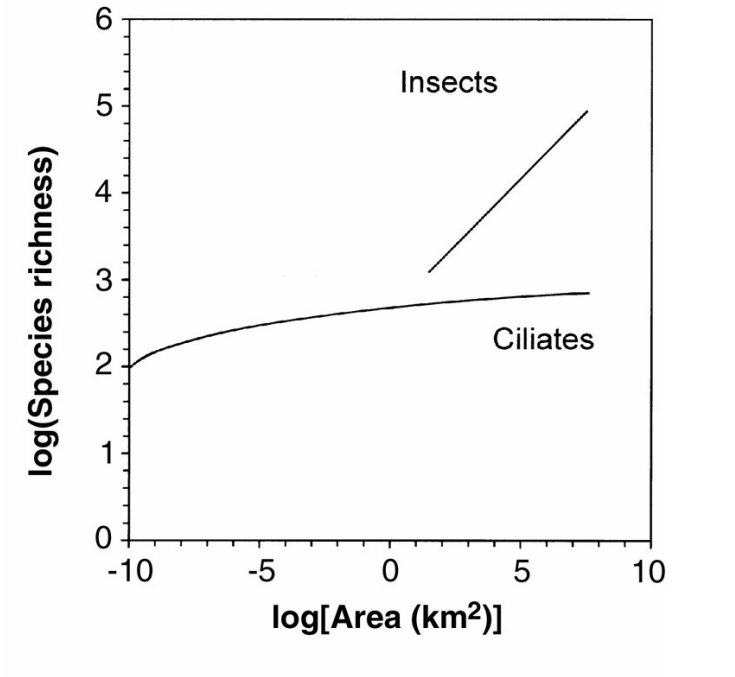

Figure 3. Species-area curves for Artic benthos. From Azovsky 2002, copyright Ecography, used with permission.

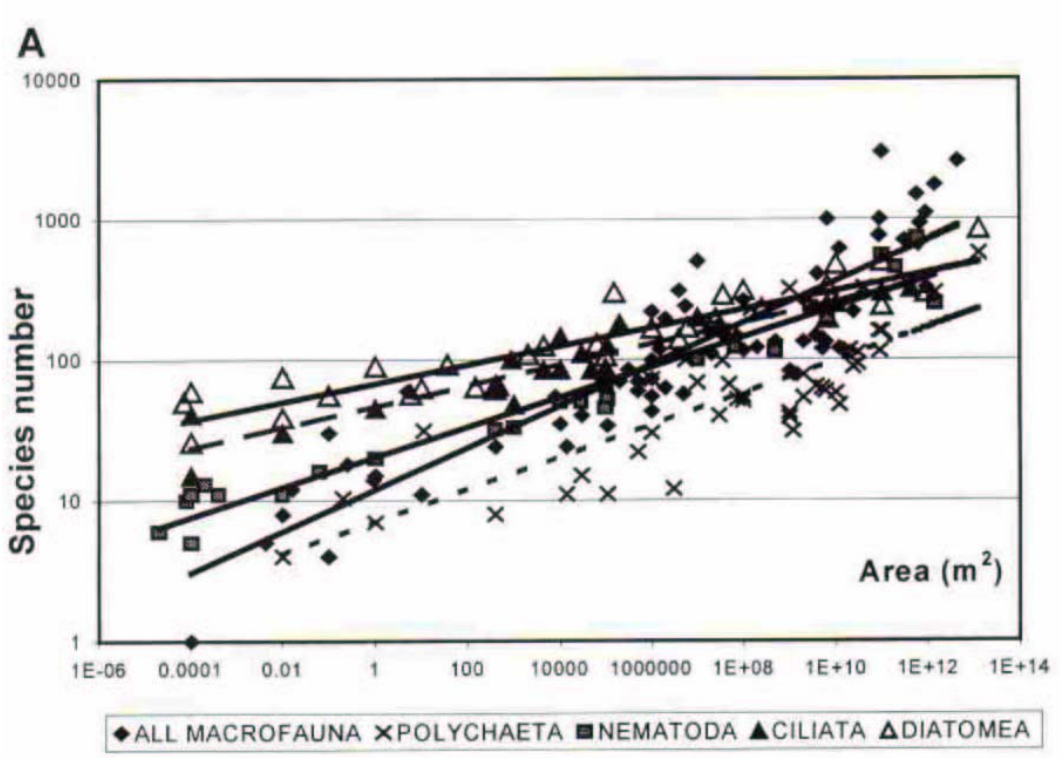

Figure 4. The similarity-geographic distance relationship for microbial fungi. Shown are the average Sørensen similarity values for within land system data (open circles) and between land system data (dots). Data correspond to different desert land systems (a) Pulgamurtie - stony foothills below silcrete ridges, (b) Rodges - sand plains with dominant mulga trees (*Acacia aneura*), (c) Olive Downs - stone covered rolling downs, and (d) Corner - sand dunes with scattered sandhill wattle. (From Green et al. 2004, copyright Nature, used with permission).

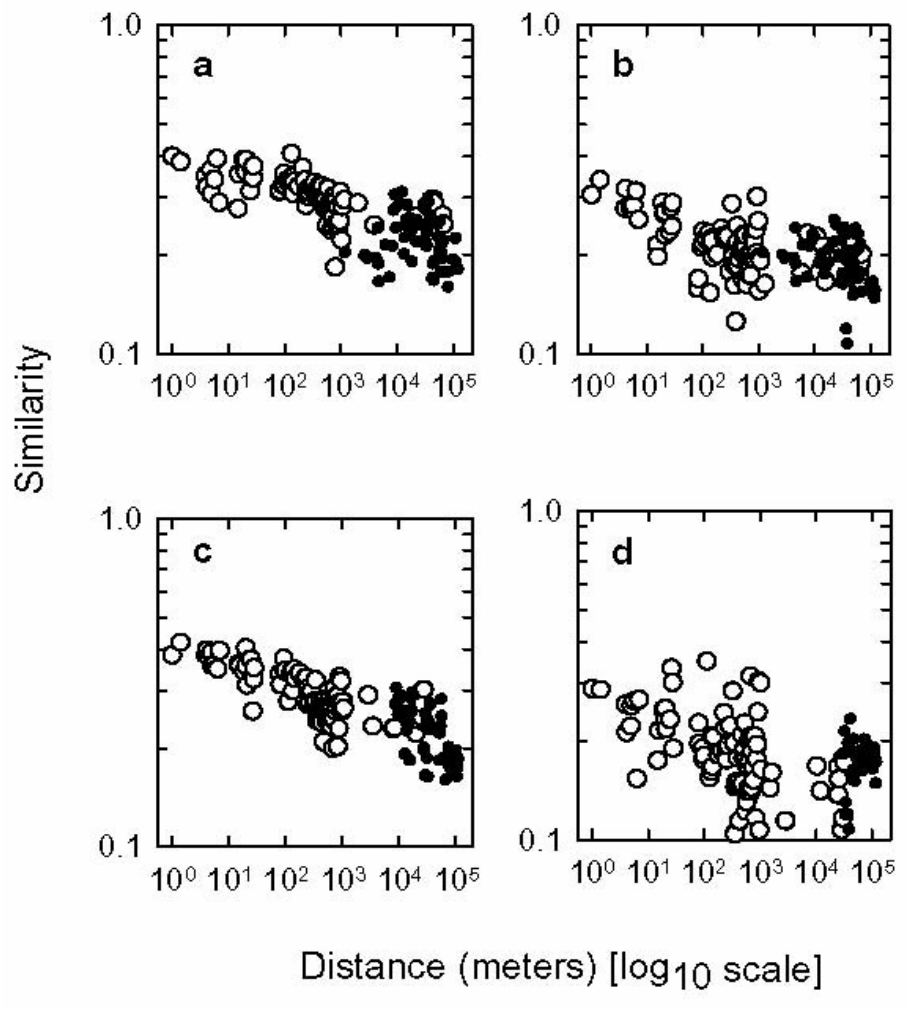

Figure 5. The taxa-area relationship for salt marsh bacteria varied with taxonomic resolution (from Horner-Devine et al. 2004, copyright Nature, used with permission).