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Review Paper

An ecological perspective on bacterial biodiversity

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Bacteria may be one of the most abundant and species-rich groups of organisms, and they mediate many critical ecosystem processes. Despite the ecological importance of bacteria, past practical and theoretical constraints have limited our ability to document patterns of bacterial diversity and to understand the processes that determine these patterns. However, recent advances in molecular techniques that allow more thorough detection of bacteria in nature have made it possible to examine such patterns and processes. Here, we review recent studies of the distribution of free-living bacterial diversity and compare our current understanding with what is known about patterns in plant and animal diversity. From these recent studies a preliminary picture is emerging: bacterial diversity may exhibit regular patterns, and in some cases these patterns may be qualitatively similar to those observed for plants and animals.

Keywords: bacteria; biodiversity; productivity; disturbance; biogeography; habitat heterogeneity

1. INTRODUCTION

Humans have long been fascinated by the extraordinary diversity of life on Earth. Not only is the sheer diversity of living creatures intriguing, but there are also striking patterns in their distribution over space and time. However, most of what we know about the origin, maintenance and distribution of biodiversity stems from research on plants and animals. Although there may be millions of species of bacteria, we are only beginning to investigate patterns in their diversity and the forces that govern these patterns (Ward *et al.* 1998; Tunlid 1999). Understanding patterns of bacterial biodiversity is of particular importance because bacteria may well comprise the majority of the Earth's species diversity, they mediate many of the environmental processes that sustain life on Earth and their diversity is of great applied importance in bioremediation (the biological degradation of pollutants) and bioprospecting (the search for novel biochemicals for use in medicine and industry).

In this review, we discuss what is currently known about patterns of bacterial diversity and the fundamental processes underlying these patterns in the environment. We focus on free-living bacteria (rather than symbionts or obligate pathogens), as they are estimated to comprise the vast majority of bacterial biomass (Whitman *et al.* 1998), and on communities in the field, rather than in the laboratory. Much of what we discuss here may also apply to archaea, the other domain of prokaryotic life. However, even less is known about archaeal diversity than about bacterial diversity, and we have thus chosen to focus on bacteria in this review. We begin our discussion by examining briefly what is known with relative certainty about bacterial diversity and abundance. We follow this with a discussion of a preliminary picture of bacterial-diversity patterns that is emerging from recent studies and of fundamental underlying processes leading to these patterns. Finally, we discuss challenges that face the study of bacterial biodiversity and suggest future research priorities.

Although there are many unique aspects to microbial life (e.g. parasexuality), micro-organisms and macro-organisms also share many fundamental aspects of their biology (Andrews 1991). In this review, we focus on ecological patterns and principles that may be common to micro-organisms and macro-organisms. Microbiologists and ecologists alike have touted the benefits of such a unifying approach. For example, the microbiologist John Andrews (1991) argued that a synthetic view of microbial, plant and animal ecology was not just possible, but crucial to the development of microbial ecology and the general study of ecology. Similarly, the ecologist John Lawton (1999) has argued that, although the details of individual organisms and ecological systems matter, ecologists would profit most from trying to uncover underlying patterns, rules and laws. Furthermore, he has argued that such generalizations would be most likely to be discovered at very small scales (e.g. populations) and at very large scales (e.g. aggregate patterns in the distribution of biodiversity).

In this review, we take such an approach and look for patterns at aggregate scales, as such patterns may give us insight into the underlying unity of the patterns and process of biological diversity. The subject of bacterial diversity has been reviewed from a microbiological perspective by others (e.g. Dworkin 1992; Nold & Zwart 1998; Kent & Triplett 2002); we emphasize an ecological perspective on bacterial diversity and place what is known in the context of relatively well-studied macro-organisms and classical ecological theory. Throughout the paper we use 'diversity' in its simplest sense: the number of taxonomic groups.

2. WHAT WE KNOW: BACTERIA ARE UBIQUITOUS AND DIVERSE

One of the most striking ecological aspects of bacteria is their ubiquity. Bacteria inhabit an extraordinary array

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Table 1. Prokaryotic abundance and diversity. Abundance was determined by fluorescence microscopy; diversity was estimated from the reassociation rate of DNA isolated from samples of the habitats indicated. Modified from Torsvik *et al.* (2002).

habitat	abundance (cells cm^{-3})	diversity (genome equivalents)
forest soil	4.8×10^9	6000
forest soil (cultivated isolates)	1.4×10^7	35
pasture soil	1.8×10^{10}	3500–8800
arable soil	2.1×10^{10}	140–350
pristine marine sediment	3.1×10^9	11400
marine fish-farm sediment	7.7×10^9	50
salt-crystallizing pond, 22% salinity	6.0×10^7	7

of habitats, from those that offer ideal conditions for most living creatures to those too extreme to support most life forms. They inhabit the relatively benign and nutrient-rich environments of soils, lakes, oceans and other organisms, but they are also found in extreme environments such as hot springs (at temperatures near and at boiling; Brock 1978), nearly saturated salt brines (Anton *et al.* 2000), acid mine waters at pHs near zero (Baker & Banfield 2003), deep in Antarctic ice (Price 2000; Christner *et al.* 2001) and kilometres below the Earth's surface (White *et al.* 1998). In fact, bacteria (and their fellow prokaryotes, the archaea) define the outer environmental limits of life. For example, *Thermus aquaticus*, the bacterium renowned for its contribution of Taq polymerase to molecular biology, has an optimum growth temperature between 70 °C and 79 °C, a temperature lethal to most plant and animal life (Brock & Freeze 1969).

Not only are bacteria everywhere, but they are also incredibly abundant (table 1). The total number of bacteria on Earth may be as high as $4–6 \times 10^{30}$, with the largest proportion of bacterial cells possibly residing in the oceanic and terrestrial subsurfaces (3.5×10^{30} and $0.25–2.5 \times 10^{30}$, respectively; Whitman *et al.* 1998). Perhaps most notably, bacterial cells are estimated to contain, in total, 350–550 Pg of carbon, up to 60–100% of the total carbon found in plants, as well as large amounts of nitrogen and phosphorous (Whitman *et al.* 1998). Despite their modest size as individuals, as a group these organisms not only contribute to the flow of nutrients worldwide, but may also constitute a significant proportion of the nutrients in living biomass.

The diversity of bacteria is also unparalleled in the biological world. Until recently, it was impossible to detect the vast majority of bacterial species. Owing to the limitations of traditional detection techniques that require growth of organisms in the laboratory, less than 1% of all bacterial species have been described to date (Torsvik *et al.* 1990; Ward *et al.* 1990; Amann *et al.* 1995). However, recent biomarker-based techniques, such as DNA analyses, have enabled the detection of non-culturable species and have allowed a more complete and detailed picture of bacterial communities to be developed (reviewed in Head *et al.* 1998). The most common of these techniques uses ribosomal gene sequences as indicators of bacterial diversity, although other genes, including protein-coding genes, have also been used. The use of these molecular techniques (as well as their drawbacks and biases) has been reviewed in detail elsewhere (e.g. Wintzingerode *et al.* 1997). Current culture-independent estimates of the number of prokaryotic species in a gram of soil range from several hundred to nearly nine thousand, orders of magnitude greater than estimates based on culture-dependent approaches (Torsvik *et al.* 2002). Dykhuizen (1998) has speculated, based on these estimates, that there could be 10 billion species of bacteria on Earth. These bacteria represent a significant proportion of all evolutionary diversity (Woese 1987; Woese *et al.* 1990).

The metabolic diversity of bacteria is perhaps as remarkable as their taxonomic and evolutionary diversity. Although culture-based studies are limited in their ability to estimate bacterial diversity, they have demonstrated that bacteria have a variety of modes of energy conversion, wide ranges of substrate use and unique metabolic pathways. For example, bacteria can use kerogen and long-chain alkanes, compounds thought to be refractory, as growth substrates under anaerobic conditions (Brock 1987). The flexibility of bacterial metabolism is perhaps best illustrated by the ability of these organisms to degrade xenobiotic compounds such as malathion (an insecticide) and 2,4,5-trichlorophenoxyacetic acid (a herbicide), which are toxic to many other organisms (Brock 1987).

3. WHAT WE ARE BEGINNING TO UNDERSTAND: PATTERNS IN THE DISTRIBUTION OF BACTERIAL BIODIVERSITY

The advent of biomarker-based techniques has enabled microbial ecologists to ask fundamental questions about the distribution and abundance of bacterial diversity. Microbial ecologists have begun to examine whether bacterial communities are distinct in different environments, whether bacterial diversity changes with habitat heterogeneity and how patterns in the diversity of bacteria compare to those of plants and animals.

(a) *Habitat type and bacterial diversity*

It has long been assumed that major environmental variables, such as vegetation type and temperature, influence bacterial-community composition (the identity of taxa in a community). Recent studies using culture-independent techniques, a select few of which we cite here, have confirmed this assumption. The composition of bacterial communities has been shown to vary with land-use type (Borneman & Triplett 1997; Nusslein & Tiedje 1999), plant species (Marschner *et al.* 2001; Kuske *et al.* 2002), agricultural growing practice (McCaig *et al.* 1999; Buckley & Schmidt 2001), temperature (Ward *et al.* 1998), nutrient status (Broughton & Gross 2000), salinity (Nubel *et al.* 2000), contamination with pollutants (Muller *et al.* 2001), predation (Jurgens & Matz 2002) and other environmental variables.

We understand less about how these environmental variables influence bacterial *diversity*, although, like the diversity of plants and animals, bacterial diversity is thought to be heterogeneously distributed across the Earth. For example, there is a growing consensus that aquatic environments support fewer bacterial taxa than do soil and sediment environments (Nold & Zwart 1998; Curtis *et al.* 2002; Torsvik *et al.* 2002). It is not clear which factors are responsible for these differences, but the high heterogeneity of the soil environment relative to that of aquatic environments may play a role.

(b) *Habitat heterogeneity and bacterial diversity*

Habitat heterogeneity has long been posited as one of the main determinants of biological diversity and is thought to underlie the seemingly universal species–area relationship, which states that the number of plant and animal species observed increases with an increase in area sampled (Rosenzweig 1995). This heterogeneity can be thought of as taking two forms: (i) structural heterogeneity, such as discontinuities in space and time (i.e. 'patchiness'); and (ii) complexity in resources, conditions and/or interacting populations. There are many reasons to expect that bacterial diversity will increase in response to increases in heterogeneity; however, observations of such patterns have been rare.

Both laboratory (Korona *et al.* 1994; Rainey & Travisano 1998; Treves *et al.* 2003) and field studies (Haubold & Rainey 1996; Zhou *et al.* 2002) suggest that environmental patchiness may play a role in the maintenance of bacterial diversity. Zhou *et al.* (2002) observed that soils which were saturated with water had both fewer bacterial taxa and a more uneven distribution of bacterial taxa than unsaturated soils (figure 1). They suggested that this difference was the result of patchiness in the unsaturated soil (i.e. the lack of water reduced migration between soil particles), which allowed the coexistence of a larger number of bacterial taxa and produced a more uniform community composition.

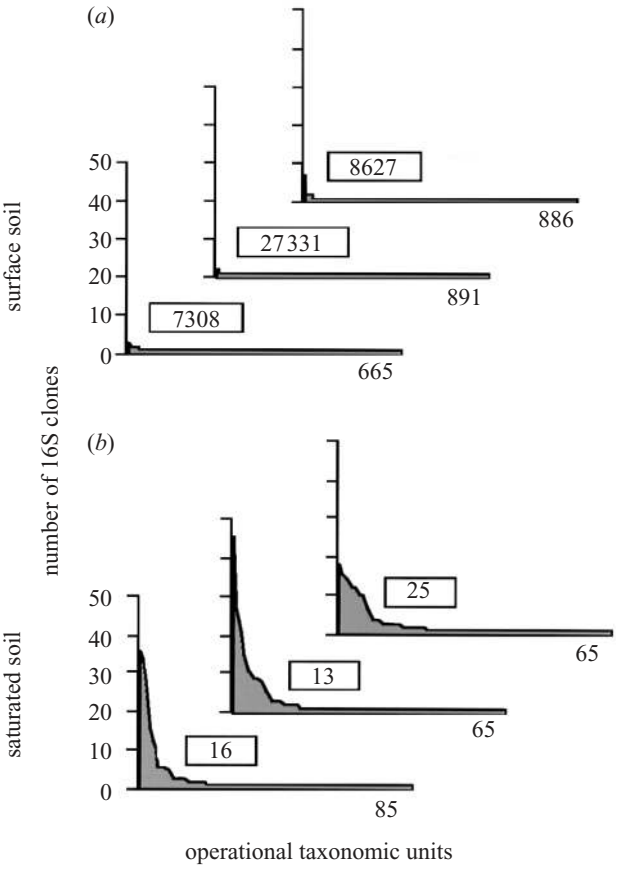

Figure 1. The effect of environmental patchiness on bacterial diversity in (*a*) surface and (*b*) saturated soils. Environmental patchiness is assumed to be higher in the surface soils than in the saturated soils. Representative rank abundance plots are depicted. The values in boxes are the reciprocals of Simpson's index (a measure of the relative distribution of individuals among taxa), and the number beneath the *x*-axis of each plot is the number of unique taxa in each sample. (Modified from Zhou *et al.* (2002). Copyright 2002, the American Society for Microbiology. Used with permission).

Complexity in environmental conditions can vary at multiple spatial scales and could potentially influence bacterial diversity. Microscale heterogeneity (e.g. at the scale of a soil particle) can be very high and potentially allows for high microbial diversity in a relatively small area. For example, within a single soil particle, oxygen concentrations can range from 21% to 0% over only a few millimetres (Madigan *et al.* 2000). Such steep environmental gradients can also be present in aquatic and marine systems, for example at the interface of oxic and anoxic layers in stratified lakes or at the surface of the lake sediments (Coyne 1999; Madigan *et al.* 2000). Heterogeneity in soil characteristics (Grundmann & Normand 2000), bacterial density (Nunan *et al.* 2002) and the distributions of some bacterial taxa (Grundmann & Debouzie 2000) have also been observed at the microscale (from micrometers and up to 65 cm) in soils. However, to our knowledge, despite these observations, relationships between spatial distance and bacterial genetic or taxonomic diversity have not been observed at this scale (Vogel *et al.* 2003).

At larger spatial scales, the bacterial-community composition in soil has been observed to be heterogeneous in some studies and homogeneous in others. For example, Webster *et al.* (2002) found that soil bacterial communities showed significant spatial heterogeneity in composition at the scale of *ca.* 1 m, the spatial scale over which both pH and ammonium concentration varied. However, other studies have observed homogeneity in community composition at this spatial scale (e.g. Felske & Akkermans 1998; Piceno *et al.* 1999). Observed homogeneity may be real (for example, the result of high rates of dispersal), or it may be an artefact of undersampling (less common species that vary at this spatial scale may not have been sampled). Although community composition has been observed to vary at this spatial scale in some studies, the relationship between bacterial *diversity* and heterogeneity at this spatial scale is unknown. Despite observations that bacteria are heterogeneously distributed in space and that environmental heterogeneity can alter their distribution, the species–area relationship has not been examined explicitly for bacteria. It is possible that differences in characteristics such as dispersal ability and survival under harsh conditions may result in a qualitatively different species–area relationship for bacteria from that for plants and animals. Alternatively, bacteria may exhibit a similar

relationship to that of plants and animals but over different spatial scales.

(c) *Primary productivity and bacterial diversity*

Primary productivity (the rate of energy capture and carbon fixation by primary producers) is thought to be a key determinant of plant and animal biodiversity (Rosenzweig 1995). Many studies of plants and animals have reported a positive quadratic or hump-shaped relationship between productivity and diversity, where diversity peaks at intermediate productivities (Rosenzweig 1995), although other patterns have also been observed (Abrams 1995; Mittelbach *et al*. 2001). There is evidence from laboratory studies that productivity can influence bacterial diversity (Bohannan & Lenski 2000; Kassen *et al.* 2000); however, we are aware of only four studies that attempt to document the relationship between primary productivity and bacterial diversity in field systems (Benlloch *et al.* 1995; Torsvik *et al.* 1998; Schafer *et al.* 2001; Horner-Devine *et al.* 2003).

Benlloch *et al.* (1995) surveyed two lagoons with different levels of primary productivity. More unique bacterial ribosomal gene sequences were retrieved from the more productive lagoon than from the less productive lagoon, suggesting that bacterial taxonomic richness increased with primary productivity. In contrast, Torsvik *et al.* (1998) observed that pristine aquatic sediments had much higher bacterial genetic diversity than sediments below fish farms (which receive a substantial input of nutrients via fish feed), suggesting that increased energy decreased diversity. Schafer *et al.* (2001) found that, over a period of 13 days, nutrient addition both decreased and increased bacterial diversity. Finally, Horner-Devine *et al.* (2003) observed that increasing productivity both increased and decreased taxonomic diversity of bacteria in aquatic mesocosms and that the shape of the relationship between productivity and diversity differed for different taxonomic groups of bacteria. For example, they observed that the diversity of the Cytophaga–Flavobacteria–Bacteroides group, the most common taxon in their study system, exhibited a significant hump-shaped relationship with primary productivity (figure 2). By contrast, they observed a significant U-shaped relationship between primary productivity and diversity for the α-proteobacteria, the second most common group, and no discernible relationship between primary productivity and diversity for the β-proteobacteria, the third most common group. These initial results suggest that bacterial diversity can vary with primary productivity and that the nature of the relationship can, in some instances, resemble that of plants and animals.

(d) *Disturbance and bacterial diversity*

Disturbance, commonly defined as 'any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment' (Pickett & White 1985, p. 7), is known to be an important influence on the diversity of plants and animals. It has been suggested that diversity may peak at intermediate intensities or frequencies of small-scale disturbance (Connell 1978; Sousa 1979). Ecologists have tested this hypothesis for a variety of taxa in a number of different environments and have found that, in general, this intermediate-disturbance hypothesis holds (e.g. Brown & Hutchings 1997; Floder & Sommer 1999).

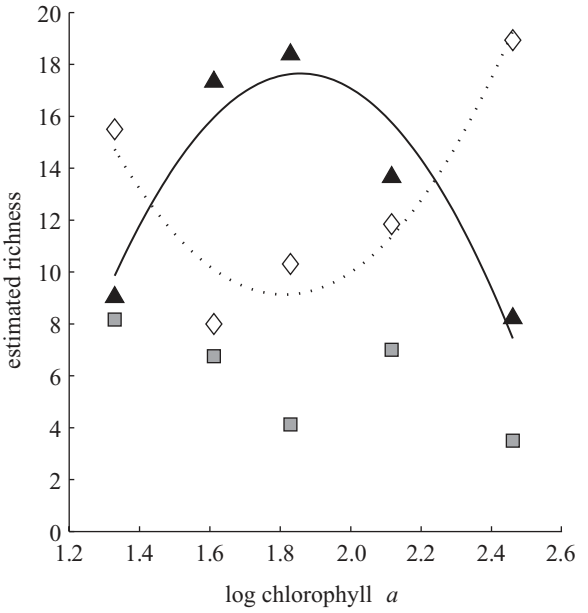

Figure 2. The relationship between primary productivity and taxonomic diversity of bacteria in five aquatic mesocosms. Primary productivity is estimated as chlorophyll *a*; taxonomic diversity is estimated from observed richness via the Chao1 approach (Hughes *et al.* 2001). α-Proteobacterial diversity (open diamonds) exhibited a U-shaped relationship with productivity significant at the $p = 0.10$ level (dotted line; $r^2 = 0.91$, $p = 0.090$). Cytophaga–Flavobacteria–Bacteroides diversity (solid triangles) has a unimodal relationship with productivity (solid line; $r^2 = 0.905$, $p = 0.095$), and β-proteobacterial diversity (solid squares) shows no discernible response to increasing productivity ($r^2 = 0.515$, $p = 0.172$). (From Horner-Devine *et al.* (2003). Copyright 2003, Blackwell Publishing. Used with permission).

Laboratory studies suggest that the hypothesis can, in fact, be applied to bacteria (Buckling *et al.* 2000). Several field studies have also examined the relationship between disturbance and bacterial diversity (reviewed in Johnsen *et al*. 2001; Kozdroj & Van Elsas 2001; Kent & Triplett 2002), most of which addressed the effect of either chemical or physical disturbance of soil on bacteria. For example, Muller *et al.* (2001) examined bacterial diversity along a gradient of mercury contamination in fields and found that sequence diversity decreased with increasing exposure to mercury. Bruce *et al.* (1995) examined the diversity of mercury-resistance genes in sedimentary bacteria at sites with varying levels and histories of mercury contamination. They found that the sites exposed to intermediate levels of mercury had the highest genetic diversities, while the most heavily contaminated site and pristine sites had low diversity. Although these studies seem to suggest a relationship between disturbance and bacterial diversity, disturbance in these studies is always confounded with other factors (e.g. vegetative cover, land history, soil structure) that may influence bacterial composition and diversity. Furthermore, few studies systematically examine how the frequency, intensity or size of disturbance affect bacterial diversity (but see Ibekwe *et al.*

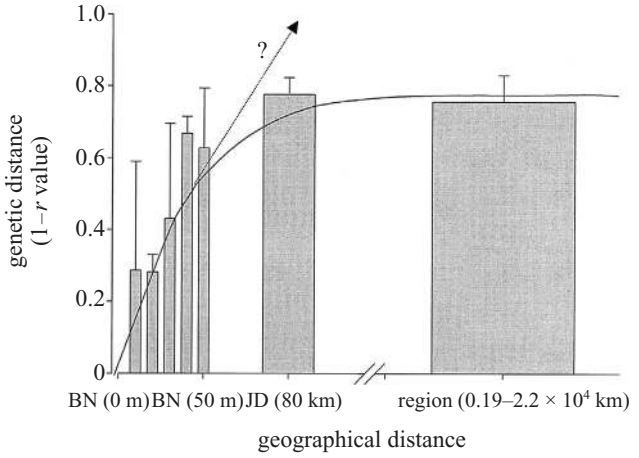

Figure 3. Relationship between geographical distance and genetic distance for fluorescent *Pseudomonas* isolates. Geographical distances are based on the distance from a reference site (BN or JD). Error bars indicate the range of the values of genetic distance. The question mark indicates that genetic distance may have continued to diverge if the method's resolution did not saturate. Grey bars, observed values; dotted line, suggested values. (From Cho & Tiedje (2000). Copyright 2000, the American Society for Microbiology. Used with permission).

2002; Fierer *et al.* 2003). Bacterial-community composition and diversity may respond to gradients in disturbance, but it is currently difficult to assess the applicability of the intermediate-disturbance hypothesis to bacteria outside the laboratory.

(e) *Biogeographical patterns in bacterial diversity*

Biogeographical patterns (i.e. the geographical distributions of organisms over the Earth in both space and time) have been relatively well documented for plants and animals. Whether bacteria exhibit biogeographical patterns has been controversial for much of the last century (reviewed in Hedlund & Staley 2003). The traditional view among microbiologists, dating back to at least the 1930s, is that high dispersal rates result in cosmopolitan distributions and a lack of biogeographical patterns for free-living micro-organisms (Baas-Becking 1934). This view has been reinforced by some field studies of eukaryotes (e.g. Finlay *et al.* 1999). However, recent research suggests that at least some bacterial taxa can exhibit biogeographical patterns (Souza *et al.* 1992; Fulthorpe *et al.* 1998; Cho & Tiedje 2000; Papke *et al.* 2003). For example, Cho & Tiedje (2000) analysed 258 isolates of the bacterial genus *Pseudomonas* from 10 sites on four continents. They analysed the isolates using three molecular methods that provided different levels of resolution. At the finest level of resolution, there was a statistically significant association between genotype and geographical distance, with evidence of migration within sites (within *ca.* 200 m) but not between sites (across more than 200 m; figure 3). Similarly, a recent survey of the genetic diversity of cyanobacterial communities from thermal springs in North America, Japan, New Zealand and Italy showed that geographical isolation was present at both global (between continents) and local (km) spatial scales (Papke *et al.* 2003). The strongest evidence to date for biogeographical patterns in prokaryotic organisms comes from a recent study of the hotspring archaean *Sulfolobus*. Whitaker *et al.* (2003) surveyed the genetic diversity of *Sulfolobus* isolates from five geographically distinct regions and found a significant correlation between genetic distance and geographical distance.

What might determine whether or not biogeographical patterns are present for a given bacterial taxon? The rates of speciation, extinction and dispersal—the three fundamental processes responsible for producing biogeographical patterns—relative to the other factors are likely candidates. A preliminary understanding of these three processes for bacteria is just beginning to emerge from recent research.

(i) *Dispersal*

Some bacteria have the potential for very high rates of dispersal. It is known that bacteria can be dispersed passively in the atmosphere (e.g. Lighthart 1997; Gage *et al.* 1999) and through water (e.g. McNair *et al.* 1997; Leff *et al.* 1998) owing to their small size. In addition, some bacteria, such as members of the genus *Bacillus*, can form hardy life stages that are highly resistant to environmental stresses such as desiccation; such stages could allow these organisms to disperse widely. However, few studies have been able to quantify even relatively small-scale dispersal and colonization rates. There is some evidence that some bacterial taxa may have wide distributions, suggesting that rates of dispersal are high. However, most of these studies use taxon definitions based on sequence similarity of the 16S ribosomal gene, a very conservative definition (Papke *et al.* 2003); sequences from protein-coding genes may provide greater resolution and be more appropriate for inferring rates of dispersal (Palys *et al.* 1997; Rotthauwe *et al.* 1997). We are aware of only one study that used protein-coding genes to infer dispersal rates in free-living bacteria (Roberts & Cohan 1995). This study used data from three genes to estimate the migration rates between different populations of two closely related species of the bacterial genus *Bacillus*. The populations were sampled at geographical scales ranging from 30 to 10 000 km. The magnitude of the migration rate was generally associated with geographical scale (migration was highest between 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 demonstrate genetic drift).

(ii) *Speciation*

The influx of new species in a given area is the product of both dispersal and local speciation. Speciation requires variation in both ecologically relevant traits and ecological opportunity, and the rate of speciation may be substantially higher in bacteria than in larger organisms because of differences in the generation of both variation and opportunity. Some bacteria exist in large populations and have rapid growth rates under favourable conditions. As the production of novel mutations scales with population size and growth rate, this can generate significant genetic variation. In addition, the breadth of physiologies present in bacteria, coupled with their small size, enables them to

exploit a wide range of ecological opportunities for diversification. It has also been argued that speciation rates may be high in bacteria because of the relatively low rate of genetic exchange between individual organisms. For example, recombination rates at or below the rate of mutation (rates typical of many bacteria) enable genetic variation associated with niche diversification to lead to speciation, and thus ecological divergence is sufficient for speciation to occur (Cohan 2002). Although rates of genetic exchange may be low overall in bacteria relative to sexual organisms, some ecologically important traits, such as antibiotic resistance (Shoemaker *et al.* 2001) and mercury resistance (Bogdanova *et al.* 1998), are acquired through gene transfer. It is possible for bacteria to acquire genes from distantly related organisms (organisms that differ by 25% or more in their DNA sequences; Duncan *et al.* 1989), which also has the potential to accelerate the rate of speciation. For all of these reasons, we might expect speciation rates of bacteria to be high relative to those of eukaryotes. Although there is some evidence for rapid diversification in the laboratory (e.g. Rainey & Travisano 1998), there is no evidence for rapid speciation in bacteria in the field. Measuring rates of speciation is difficult and is usually done through studies of the fossil record (which is scant for bacteria). Whether rapid speciation can alter the magnitude and distribution of bacterial diversity, however, depends not only on how widespread rapid speciation rates are among bacteria, but also on the rates of extinction and dispersal.

(iii) *Extinction*

Bacterial diversity may also be high owing to potentially low extinction rates (Dykhuizen 1998; Torsvik *et al.* 2002). The large population sizes assumed for many micro-organisms may make extinction less likely (Dykhuizen 1998; Finlay & Clarke 1999). Extinction rates may also be relatively low for bacteria because some bacteria have traits that allow them to reduce the risk of catastrophic losses typical of extinction events in plants and animals. For example, as noted in § 3e(i), some bacteria are known to form life stages that can survive harsh environmental extremes, reducing the probability that chance fluctuations in environmental conditions will drive them to extinction. High dispersal rates over large distances may also reduce the chance that local environmental change will result in extinction. Some bacteria 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 & Kolter 1999).

It is not clear how widespread the traits discussed in the previous paragraph are among bacterial taxa (and thus how likely it is that extinction rates are actually lower for bacteria than for other organisms such as plants and animals), and there are no direct measures of extinction rates for bacteria in the field. However, it should be possible to determine whether the relative magnitude of speciation and extinction is different for bacteria relative to other organisms. For example, the difference between speciation and extinction can be estimated from gene phylogenies via lineage-through-time plots (Nee *et al.* 1994*a*,*b*). To our knowledge, this comparison has not yet been attempted.

4. CHALLENGES AND FUTURE DIRECTIONS

From the recent studies of bacterial diversity that we discuss here, a picture is emerging: bacterial diversity may exhibit regular patterns, and in some cases these patterns may be qualitatively similar to those observed for plants and animals. These conclusions are, of course, very preliminary as the study of biodiversity patterns in bacteria is in its infancy. Significant theoretical and practical constraints have, until recently, hindered the quantification of bacterial diversity and have prevented comprehensive studies of bacterial-biodiversity patterns. These constraints include the small proportion of bacterial species that can be cultured (Brock 1987), the large number of individuals that may be present per environmental sample (Torsvik *et al.* 1990), the high diversity that may be present at a small scale (Klug & Tiedje 1993) and the difficulty of defining a bacterial species (Goodfellow & O'Donnell 1993; Rossello-Mora & Amann 2001).

This last point has been particularly problematic in studies of bacterial biodiversity. Most studies of plant and animal biodiversity focus on the species as the unit of measurement (but see Balmford *et al.* 1996), but defining a bacterial species has been far from straightforward (Claridge *et al.* 1997), in part because of their genetics (reviewed in Cohan 2002). In addition, operational definitions of bacterial species are not consistently applied. However, the use of seemingly arbitrary species definitions (Staley 1997) is a problem that is not limited to microbial organisms; debate about species definitions in eukaryotic organisms has persisted for decades and is ongoing (Dobzhansky 1935; Ehrlich 1961; Masters & Spencer 1989; Coyne 1994; Wu 2001; Lee 2003).

One way to avoid the problems associated with the use of different operational taxonomic unit (OTU) definitions is to emphasize relative comparisons of diversity: if the unit of diversity is defined within a particular study and is consistently applied, an investigator is able to examine trends in diversity, even if it is not possible to determine the 'true' magnitude of diversity in any given sample (Hughes *et al.* 2001). Furthermore, by using multiple OTU definitions and a variety of molecular techniques that reveal diversity at different scales of resolution, it may be possible to determine the most ecologically relevant measure of bacterial diversity (Nubel *et al.* 1999; Bohannan & Hughes 2003). The operational definitions commonly used in molecular studies of bacterial diversity are extraordinarily broad; for example, if the most commonly used operational species definition for bacteria (70% genome homology) were applied to humans, we would be considered members of the same species as chimpanzees and lemurs (Sibley *et al.* 1990). The effect of taxon definition on our ability to detect bacterial-biodiversity patterns is beginning to be addressed; in some cases bacterial-biodiversity patterns are sensitive to taxon definition (e.g. Cho & Tiedje 2000), while in others they are robust (e.g. Horner-Devine *et al.* 2003).

Another reason that studies of bacterial diversity have lagged behind studies of plant and animal diversity is that

the disciplines of microbiology and general ecology (i.e. plant, animal, ecosystem and theoretical ecology) have been isolated from one another since early in their respective histories. Microbial ecology has developed as a distinct subdiscipline of microbiology, separate from general ecology and with a different approach and perspective. The ultimate result of this separation is that microbial ecology today lacks a solid theoretical foundation, and general ecology has been largely prevented from studying the ecology of micro-organisms and interactions between micro-organisms and macro-organisms. However, both general ecologists and microbial ecologists have become increasingly interested in bridging the gap between these disciplines, especially in the area of biodiversity studies. Examples include the development of robust statistical approaches for the study of bacterial diversity, based on approaches first applied in plant and animal studies (Hughes *et al.* 2001; Curtis *et al.* 2002), an increase in the use of microorganisms as model systems to explore biodiversity theory (Bohannan & Lenski 2000; Rainey *et al.* 2000; Kerr *et al.* 2002; Jessup *et al.* 2003) and the application of ecological theory to microbial communities in the field (Jackson *et al.* 2001; Zhou *et al.* 2002). To apply biodiversity theory developed by plant and animal ecologists successfully to bacteria, an understanding of the fundamental differences and similarities between bacteria and other organisms is necessary, as is a basic description of how bacterial diversity is distributed along environmental gradients. As discussed in § 3, this understanding is just beginning to develop.

Despite these challenges, we now have the tools and understanding necessary to look for patterns in bacterial biodiversity and to begin to determine the processes underlying these patterns. We envision four areas that are likely to be particularly fruitful in this regard. First, research designed to increase our understanding of how bacterial diversity changes along gradients in environmental factors such as energy and disturbance is likely to be fruitful. Such research has played a major role in the study of plant and animal biodiversity. Understanding the primary drivers of bacterial diversity and how community composition changes along these gradients can lend insight into basic processes such as speciation, extinction and dispersal that are harder to study *in situ*. In addition, understanding how bacterial-diversity patterns along gradients differ from those of plants and animals will aid in appropriately applying ecological theory developed for plants and animals to bacteria. Second, an understanding of the relationships between bacterial diversity, area (or volume) and habitat heterogeneity is fundamental; such efforts are underway in several research groups. Third, patterns in the topology of phylogenetic trees (e.g. lineage-through-time patterns) could help us understand whether the relative magnitudes of speciation and extinction rates differ between bacteria and other organisms, and whether there are fundamental differences in the processes that underlie biogeographical patterns. Finally, although recent studies have begun to make some strides in understanding the relationship between bacterial diversity and ecosystem function, both in laboratory (e.g. McGrady-Steed *et al.* 1997; Naeem & Li 1997; Hodgson *et al.* 2002) and in field communities (e.g. Cavigelli & Robertson 2000; Griffiths *et al.* 2000), increased effort in this subject is warranted given the many critical processes that these organisms mediate.

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