

Biogeography: An Emerging Cornerstone for Understanding Prokaryotic Diversity, Ecology, and Evolution

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Abstract

New questions about microbial ecology and diversity combined with significant improvement in the resolving power of molecular tools have helped the reemergence of the field of prokaryotic biogeography. Here, we show that biogeography may constitute a cornerstone approach to study diversity patterns at different taxonomic levels in the prokaryotic world. Fundamental processes leading to the formation of biogeographic patterns are examined in an evolutionary and ecological context. Based on different evolutionary scenarios, biogeographic patterns are thus posited to consist of dramatic range expansion or regression events that would be the results of evolutionary and ecological forces at play at the genotype level. The deterministic or random nature of those underlying processes is, however, questioned in light of recent surveys. Such scenarios led us to predict the existence of particular genes whose presence or polymorphism would be associated with cosmopolitan taxa. Furthermore, several conceptual and methodological pitfalls that could hamper future developments of the field are identified, and future approaches and new lines of investigation are suggested.

Introduction

Prokaryotic biogeography can be defined as the science that documents the spatial distribution of prokaryotic taxa in the environment at local, regional, and continental scales. In a broader sense, this discipline examines variation of microbial features (e.g., genetic, phenotypic, physiological) at different spatial scales, between distantly

located sampling sites or along large environmental gradients. Its scope also encompasses the understanding of the processes generating and maintaining those distribution patterns. The ultimate goals are to propose and evaluate theories regarding the creation and evolution of such diversity patterns in the environment. Not surprisingly, this definition is identical to the one already existing for eukaryotic organisms [41], and this also may explain why current conceptual approaches share high similarities with the ones already described for eukaryotes.

The study of microbial biogeography may offer promising benefits to many research areas. Examples of application may be foreseen in the global epidemiology of human, animal, and plant pathogens; in the monitoring of the fate of inoculated biocontrol or bioremediation agents when applied at large scale; or in the identification of geographic areas where those beneficial bacteria thrive in the environment. More generally, this field will undoubtedly improve our understanding of the global microbial diversity and of the evolutionary forces shaping it.

Interest in studying spatial patterns of prokaryotes has its origin back to the beginning of the 20th century when scientists began to question the existence of different bacteria at distantly located sites [3, 5]. Microbial biogeography has recently gained renewed interest in the microbial ecology community because of new questions about microbial diversity and the availability of powerful molecular tools to describe diversity, especially at the genotype level (e.g., [43, 48, 68]). Thus, an increasing number of studies have been published on this topic as applied to different bacterial groups in terrestrial or marine ecosystems. Biogeographic patterns associated with bacterial diversity have been reviewed elsewhere [27, 29].

The aims of this article were (1) to summarize the knowledge gained from studies about prokaryotic bioge-

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ography at different taxonomic levels and (2) to review the processes generating biogeographic patterns using recent evidence. Those processes are further explored on a conceptual level to tentatively draw a synthetic portrait of prokaryotic traits associated with either cosmopolitan or endemic microbes. Finally, conceptual and methodological issues that could hamper further development of this research area are discussed and future lines of investigation are suggested.

What is Currently Known About Bacterial Biogeography?

In the following sections, “species” refers to the current criteria of the bacterial species definition [64, 73], and taxon denotes any taxonomic category or group, such as a phylum, order, family, genus, or species.

Cosmopolitan Distribution. Many lines of evidence suggest that prokaryotes have a cosmopolitan distribution and in this section we give selected examples at different taxonomic levels. At the domain level, it is now well accepted that Bacteria and Archaea are globally distributed [15]. At the class level, the β -Proteobacteria, Cyanobacteria, Actinobacteria, and Flavobacteria have been shown to display worldwide distribution in marine or terrestrial ecosystems (e.g., [4, 19, 58]). For instance, Abell and Bowman [1] identified a large, so far uncultivated, cosmopolitan phylogenetic clade of the class Flavobacteria by sampling across a latitudinal transect in the Southern Ocean and by analyzing 16S rRNA sequence diversity. That clade was not only distributed throughout the Southern Ocean, but also widely encountered from temperate to polar seawaters, e.g., off the coast of England, the North Sea, the U.S. West Coast, and the Arctic Ocean.

At the genus level, there is a general consensus that many prokaryotes have a cosmopolitan distribution in their respective habitats [27]. For instance, the same genera of hyperthermophiles inhabit remotely located hydrothermal vent materials or deep sediments, and can survive dispersal in the open ocean in metabolically inactive states [31]. Other classical examples of cosmopolitanism among free-living bacterial genera include *Polynucleobacter*, which are isolated from freshwater habitats located in various climatic zones on different continents [25], fluorescent *Pseudomonas* and *Bacillus* isolated from various regions and countries [11, 59].

Classical examples of worldwide distribution of the same bacterial species may be found for certain human, animal, and plant pathogens. Indeed, pathogenic or commensal bacterial species, such as *Escherichia coli*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*, have long been identified as cosmopolitan [45, 47], as well as

certain spore-forming bacteria such as *Bacillus mojavensis* and *B. subtilis* species [59]. In plant pathology, certain species or subspecies of plant pathogens may cause huge loss in crops and orchards on a worldwide scale. Typical examples of worldwide distributions of bacterial pathogens include the case of *Pseudomonas syringae* causing plant canker in commercial greenhouses globally [76], *Ralstonia solanacearum* causing bacterial wilt on several hundreds of plant species [78], or *Erwinia amylovora* responsible for the fire blight disease in pears and apples on different continents [16].

At the level of 16S rRNA gene resolution, the same phylotypes have often been identified in similar habitats in different geographic areas. For instance, in the marine ecosystem, certain phylotypes of planktonic archaea were found ubiquitously in most samples investigated within eight oceanic regions [44]. In that latter study, one of the two archeal groups identified was dominant throughout the water column in the Southern Ocean and below the surface in temperate regions, whereas the other group was found dominant at the surface in temperate regions. Cosmopolitan distribution of 16S rRNA sequence-based phylogenetic clusters has also been identified for freshwater bacteria using culture-independent approaches [19]. These findings are not limited to samples from water column, as high 16S rRNA sequence similarities have also been evidenced between coastal and open ocean sediments (e.g., [8]). Because of the conserved nature of the molecule, evidence of cosmopolitan distribution at this level of resolution is not surprising. In fact, it is probably an inappropriate molecule to target fine biogeographic structures in environmental studies.

Endemic Taxa. Pioneering observations of non-cosmopolitan distribution of prokaryotes may be found in the study of the cyanobacterial genus *Synechococcus* inhabiting mats in hot springs. Although certain cyanobacterial morphotypes were clearly identified in hot springs in North America, they were not observed in other hot springs around the globe, suggesting a restricted dispersal range due to physical isolation and lack of viability during transport [10, 54].

Although higher taxonomic levels have been identified as cosmopolitan, recent studies have clearly demonstrated that certain bacteria may not be uniformly distributed over the Earth’s surface. This is contrary to a nearly century-old paradigm advocating bacterial cosmopolitanism as the rule [3], which is still generating much debate among microbial ecologists [27]. The key point, however, may be at what level the difference among cells is being evaluated, as further discussed below.

Indeed, endemic genotypes have been unequivocally identified in the soil bacteria *Pseudomonas* spp. [11], in the thermophilic archeon *Sulfolobus* “*islandicus*” found in water and sediments [74], or in *Rhizobium legumino-*

sarum bv. *phaseoli* strains that live in soils and in nodules of wild and cultivated beans [70]. In those studies, target organisms were generally isolated in pure cultures and their genotypic diversity was determined based on sequence analysis of protein-coding genes [74], multi-locus enzyme electrophoresis [63], or genome-wide polymerase chain reaction (PCR) amplification of repetitive elements [11]. Pairwise genetic distances between genotypes were then inferred from DNA sequences or fingerprinting patterns between isolates, and clades obtained by cluster analyses were compared with the geographic origins of the isolates. Interestingly, most of the terminal clades consisted of isolates from specific geographic locations with little or no overlap between them, indicating the existence of spatial structure in prokaryotic communities over large geographic distances, but at a relatively fine level of genetic resolution.

Processes Generating Biogeographic Patterns

Generally, the combination of speciation, extinction, and dispersal are proposed to explain the generation of biogeographic patterns. Those processes have recently been compared between eukaryotes and prokaryotes [29]. We review those processes in an evolutionary and ecological context, in order to propose theoretical scenarios leading to the formation of biogeographic patterns in prokaryotes.

Speciation. The creation and maintenance of diversity is a fundamental process in the generation and evolution of biogeographic patterns at different spatial and temporal scales. In prokaryotes, vertical speciation through descent from a common ancestor is blurred by the existence of genetic recombinations [both homologous recombination and lateral gene transfer (LGT)] [14, 20, 37]. Natural selection is thought to subsequently act on the resulting pool of diversity by sorting out genotypes better adapted to the prevailing environmental conditions through periodic selection events [13, 14]. Recombination rates have been found to be low so that they may not alter the integrity of distinct adaptations in different ecotypic lineages [14, 18]. Hence, recombination may not hinder ecologically distinct populations to diverge even further. Allopatric divergence is thus not a prerequisite for population speciation, and sympatric divergence leading ultimately to speciation could occur as soon as new adaptive traits are present in one of the members of a given ecotype. Although it is generally expected that speciation rates may be high in bacteria because of their large population sizes, the promiscuity of gene transfers, and the breadth of environmental conditions they encounter, little is known about the magnitude of those rates in nature [29].

Besides the traditional processes of prokaryotic evolution (i.e., mutation, homologous recombination, LGT, gene loss, and genome rearrangements), physical isolation has been advocated as an additional evolutionary force shaping bacterial populations [54, 67, 74, 79]. The existence of pre- and postmating barriers to gene flow could lead to the accumulation of nonselective, neutral divergences in symbiotic and free-living bacterial populations (for review, see [53]). Hence, processes such as genetic drift (random fluctuation in allele frequencies owing to sampling effects between generations), founder effects (invasion of a new environment by only few members of a taxon, which then multiply rapidly), population bottlenecks (a marked reduction of population size often resulting in a loss of genetic diversity), and neutral evolution which are commonly described in eukaryotic population genetic studies, should be given full consideration when examining prokaryotic evolution, especially in a biogeographic context. Indeed, certain examples of endemic populations were found with little correlation between the presence of specific genotypes at a given location and environmental parameters or chemical composition associated with that geographic location (e.g., [54, 74]). This may suggest that geographic isolation and subsequent neutral divergence could have had a more prominent role than adaptation to environmental conditions in those cases. However, more needs to be done on this aspect to enable more definitive conclusions to be drawn about the role of physical isolation in the generation of biogeographic patterns.

Dispersal. This is defined as the movement of populations away from their point of origin. It not only encompasses physical transport by a vector between two locations (which may be a stochastic process in the case of free-living prokaryotes), but also the establishment in, and colonization of new geographic locations by the emigrating taxa. For free-living prokaryotes, transport over large geographic distances (i.e., between continents or oceans) may be possible through the combination of favorable climatic factors (e.g., winds, storms), disseminating vectors (e.g., oceanic currents, dust, plant seeds, surfaces of humans, birds, or insects migrating between regions), and sometimes the existence of survival or dormant stages in bacteria that would enhance their chance to endure long-distance transport (for review, see [22, 29]). Although it is thought that microbial transport may be an ongoing process [22], it is not yet known how many of those transported microorganisms may survive their journey and subsequently establish into new environments. Scarce data are available concerning the dispersal of free-living bacteria in the environment because molecular techniques have not reached the level of resolution that would enable an easy track-

ing of a given genotype in space and time (sampling issues are further discussed below).

The distribution patterns of symbionts, pathogens, or commensals may mostly be dictated by their hosts' specific biogeography. This is the case, for instance, for human pathogens that are increasingly spread worldwide due to human activity [17, 21, 47]. In an evolutionary context, dispersal may also be seen as a stochastic process enabling taxa to physically escape the purging of diversity caused by periodic selection that potentially may lead members of a same ecotypic lineage to extinction [53].

To quantify the extent of migration between spatially separated populations, migration rates have generally been inferred from molecular data (e.g., protein-coding gene sequences). In the case of two *Bacillus* species, migration rates were found to be the highest between close locations, but still prevented genetic drift to occur even among the most distant populations [59]. Therefore, genetic divergence was found to be limited between local and more distant populations, i.e., those populations displayed a cosmopolitan distribution. Similarly, for *Bradyrhizobium canariense* and *B. japonicum*, two sympatric species sharing partially the same ecological niches (i.e., the same root nodules), recombination and migration may represent significant evolutionary forces that provide internal cohesiveness, shape their population genetic structure, and may prevent their geographical differentiation [74].

Barriers to dispersal, i.e., areas where taxa cannot establish because of physical barriers (e.g., topography), physiological requirements (e.g., narrow temperature, salinity, or pH tolerance range), ecological constraints (e.g., higher competition), or because of limited resource availability between favorable patches, have been proposed to explain the existence of endemic patterns (for review, see [53]). For instance, different Flavobacteria communities seem to be selected based on both the physical barrier of the polar front and water temperature [1]. For thermophilic *Sulfolobus* inhabiting geothermal hot springs, Whitaker *et al.* [74] showed that subpopulations were spatially isolated from each other, probably as the result of their specific physiological requirements (i.e., low pH, high temperature) that would reduce their chance to colonize inhospitable habitats between geothermal regions.

Extinction. It is generally postulated that extinction may be rare in prokaryotes because of their large population sizes, high growth rates, and/or the presence of survival or dormant states when facing harsh environmental conditions [29]. However, little data exist in the literature about how common such characteristics may be in natural populations. Such generalizations therefore remain very questionable.

Protection against adverse environmental conditions has yet been observed in *Pseudomonas aeruginosa* that may undergo extensive self-generated diversification within its biofilms [6]. Interestingly, in that process, induced pleiotropic effects could lead to the appearance of genotypes with increased dissemination abilities and greater resistance to environmental stresses. Such biological "insurance" may therefore contribute significantly to the reduction of the risk of extinction, especially when colonies are transported further away from their original niches or when local resources have been depleted. However, the existence and magnitude of this mechanism in other prokaryotes is not yet known.

As for the speciation and dispersal processes, extinction rates may be difficult to measure *in situ* and indirect methods based on inference from genetic data have been proposed to estimate those parameters [49]. Thus, using simple genealogical models, theoretical studies have shown that diversity could be concentrated in small subpopulations, whose extinction could cause fluctuations of diversity even in the absence of extrinsic perturbations [57]. Those results were found to agree with biogeographic data obtained for *Pseudomonas* spp. populations [11, 57], and strengthened the idea that extinction may dramatically impact prokaryotic diversity.

Although speciation may initially occur at a small scale and dispersal is supposed to happen at a larger scale, the spatial scale relative to extinction may be more difficult to grasp and may depend on the joint effects of dispersal and speciation. Moreover, it is not known to what extent extinction is either stochastic or deterministic. Indeed, abiotic and biotic factors leading to prokaryotic population survival or extinction in the environment are still poorly understood.

Biogeographic Patterns in an Evolutionary Context. Based on the current prokaryotic evolutionary theory, we examine in this section two extreme cases, starting from parental lineages that are either cosmopolitan or endemic, and then discuss possible outcomes in an evolutionary context.

A cosmopolitan taxon may intrinsically be particularly suited to succeed in contrasting environmental conditions owing to, for instance, a high genome plasticity and metabolic versatility as may be the case for the previously mentioned *Pseudomonas* genus of the Proteobacteria. Such taxa would display a cosmopolitan distribution as long as dispersal occurs over large geographic distances in a continuous manner (see "Dispersal") and genetic divergence is limited in the same time frame. However, ecological divergence and geographic isolation may ultimately take place over time and would not be counterbalanced by recombination [13], leading local populations to be more genetically distinct from

the ancestral cosmopolitan population. As aforementioned, transport of free-living prokaryotes may be a stochastic process, which implies that a part of local populations would not disperse and would be more subject to genetic divergence. If adaptive mutants appear within the resident population and enough time is given for periodic selection to occur, they would purge the diversity locally, because they may be better adapted than their congeners for a given set of local, environmental conditions. Additionally, newly immigrating cosmopolitan ecotypes would also be outcompeted by those adaptive mutants, leading to even more differentiation of the locally vs. globally distributed populations.

It may, however, be argued that dispersal may favor the replacement of those latter adaptive mutants by other adaptive mutants coming from other locations, which would have arisen in parallel and could potentially harbor even better adaptations for the given set of local conditions. Although it is difficult to predict the outcome of those interactions in such a theoretical approach, the ultimate results would probably be that cosmopolitan taxa would tend to be more endemic, given enough evolutionary time to pass. If this scenario holds true, we may be able (1) to find genetic markers (gene presence, absence, or polymorphism) indicating the cosmopolitan nature of those organisms, and (2) to significantly correlate genetic modifications of those markers with a change in the ability of those organisms to be globally distributed.

In the case of endemic taxa, ecological and neutral divergence would lead to even more patterns of endemism, leading potentially to extinction if the environmental conditions are met only locally and dissemination is limited. However, LGT events that may be rare but promiscuous have a significant impact on the ecology and speciation of prokaryotes [14]. Acquisition of new ecological traits may offer new possibilities to the organism, such as the invasion of new niches, and colonization of new habitats, which could distantly be located from the original site of endemism. Noticeably, the chance to acquire those putative genes associated with cosmopolitan traits may be high, as those genes would logically be more frequent in nature than endemism-related ones (because cosmopolitan taxa are everywhere, they are more likely to be donors of genetic information). The final outcome would be a gradual expansion of the habitat or niche range of such taxa, ultimately leading to a cosmopolitan distribution. In this view, cosmopolitanism is clearly posited to be selected for by natural selection. However, it is not known to what extent traits associated with global distributions are selected for in natural populations. Noteworthy, the acquisition of new traits may also occur in cosmopolitan taxa, which would potentially counterbalance

their unavoidable differentiation as a result of geographic isolation and ecological divergence.

Interestingly, the second scenario consisting of a range expansion from endemic to cosmopolitan taxa may be supported by observations of the well-documented evolution of human pathogens. Acquisition of new virulence factors or intragenomic recombinations have been proposed to explain recent, global dispersal of clonal lineages (clonal expansion), which were formerly known to occur only locally (e.g., [34, 47, 62]). This illustrates that evolution in prokaryotes may lead to dramatic changes in their biogeographic patterns, because prokaryotic biology and ecology are both affected during the evolutionary process.

How long could biogeographic patterns be stable? Examples from bacterial pathogens may again offer first glimpses of the longevity of clonal lineages, although they might not be good representatives for the whole prokaryotic diversity. Depending on the level of clonality of a given species, longevity of clonal lineages have been suggested to last from few decades for rapidly diversifying and recombining *N. meningitis* clones, to thousands of years for the more clonal *Salmonella enterica*, to more than 35,000 years for *Shigella* clones [36]. Therefore, it seems that future biogeographic studies would have to take into account the variation of evolutionary rates in different lineages to better understand prokaryotic distribution patterns and their likely evolution at different spatial and temporal scales.

Summarizing, evolution in prokaryotes may potentially have two contrasting effects: either the organisms may become even more specialized to their niches and tend to be restricted to the same area, or they may acquire new ecological traits that would make them more prone to invade new niches or habitats and disperse. Although cosmopolitan and endemic distributions may be two extreme, opposite distributions, many prokaryotic taxa would probably have a distribution in between those two extremes, by being, for instance, present as patches distributed in many regions without yet displaying a cosmopolitan distribution (Ramette and Tiedje, unpublished data). This could reflect the fact that the formation of biogeographic patterns may be a dynamic process of expansion or regression of the habitat range at the genotype level, which may be governed by the complex interplay of the nature of the genes gained, lost, or modified, the extent of dispersal and extinction, and natural selection.

Conceptual and Methodological Limitations

The study of microbial biogeography must cope with several conceptual and methodological limitations, which dramatically influence each other. Approaches chosen by environmental microbiologists are intimately related to

their *a priori* conceptions about biogeography and microbial behavior at larger scales, which were mostly inherited from biogeographic studies of eukaryotic organisms.

Many of the practical issues discussed in this section are not only specific to prokaryotic biogeography, but are common to environmental microbial ecology in general. The fact that large spatial and potentially temporal scales are involved in biogeographic approaches may yet add another layer of complexity to experimental design and data analyses.

Theoretical Foundations. Because spatial and temporal scales are often too large for experimentation to be carried out, theories of biogeography are generally developed by searching for patterns, formulating theories, and testing their assumptions and predictions for a limited number of data. Such predictions may include taxa presence/absence, their interactions, their relative abundance, and their persistence in time. For prokaryotes, the finding of such biodiversity rules would be a major advance in the ability to predict the evolution of diversity patterns of natural populations in the environment, which is so far poorly understood.

It may be fruitful to question whether biogeographic theories that have been developed and refined for plants and animals would also fit for prokaryotes. The “island” model of MacArthur and Wilson [42] certainly constitutes a starting point to address that question. Indeed, recent studies (e.g., [54, 74]) showing that endemism was more common than previously thought used the “island” model that consisted of well-isolated habitats (e.g., hot springs) naturally occurring in geographically distant areas. The rationale was that, from an extremophile’s perspective, extreme environments may be seen as island-like habitats dispersed in an inhospitable environment, leading to geographic isolation and potentially to subsequent neutral divergence. Although the island-like nature of hot springs was essential to reveal endemic patterns, predictions based on island biogeographic theories have yet not been extensively tested using those prokaryotic diversity data. Indeed, the “mainland” that represents the source area of taxa that emigrate to those islands may be difficult to define, thus limiting inferences about rates of dispersal and extinction, which would be necessary to assess the validity of such theoretical predictions in the prokaryotic case.

The niche-based equilibrium view is, so far, dominant among microbiologists and constitutes the foundation of the actual evolutionary theory for prokaryotes (e.g., [14]), which aims to explain ecological divergence and ultimately speciation. Neutral processes of diversification, such as genetic drift mediated by geographic isolation, have challenged this unique view and were suggested as additional evolutionary factors to be con-

sidered in prokaryotic evolution [53]. It is thus relevant to quantify the relative importance of those different evolutionary forces, and to determine whether they could be better represented by either niche-based (i.e., taxon specific) or neutral (i.e., ecological equivalence of taxa, *sensu* Hubbell [30]) models of diversification leading to the generation of biogeographic patterns in prokaryotes. A reconciliation of those two perspectives, which should not be mutually exclusive, would also be much needed to produce a unified theory.

Although an extensive review of the existing biogeography and biodiversity theories goes beyond the scope of the present review, future lines of exploration would benefit from comparing prokaryotic biodiversity data with theoretical predictions coming from, e.g., the unified neutral theory of biodiversity and biogeography [30], and metacommunity paradigms such as the species-sorting, the mass-effect, or the patch-dynamic models (for review about their scopes, assumptions, and predictions, see, e.g., [39]). The comparison of observed vs. theoretical diversity patterns would pave the way toward developing future biodiversity and biogeography theories for prokaryotes, whose ultimate goals would be to link evolutionary and ecological perspectives into a common framework.

Species Concept and Taxonomic Level of the Study. As probably influenced by plant and animal biogeographic approaches, the species is also the most commonly found unit in microbial biogeographic studies. However, the meaning of the current bacterial species definition is still much debated [35, 60], because it does not take into account the evolutionary forces that provide cohesiveness to bacterial population structure [14, 71, 74]. Although a polyphasic approach combining both phenotypic and genotypic characters has been recommended to help classify new strains into species [64, 69], species are sometimes identified as operational taxonomic units based on the similarity of 16S rRNA gene sequences for practical reasons in environmental studies. The reliance on that unique ribosomal gene to assess similarity in microbial biogeography may lead to premature conclusions about the biogeographic distribution of the target taxa due to a lack of resolving power, and hence may lead to erroneous conclusions about the ecological significance of bacterial diversity [32].

It may however be argued that if biogeographic patterns can already be observed using 16S rRNA gene-based approaches, this may indicate even stronger spatial structure of the populations when molecular tools with finer levels of resolution are used (this rationale was used in, e.g., [28, 54]). Besides, 16S rRNA-based approaches may still be valuable as the first steps into identifying and

further describing biogeographic patterns for uncultured taxa in the environment. Indeed, few data have yet been obtained for the vast unseen majority of uncultured microorganisms that represent more than 99% of prokaryotic life in seawater, sediments, and soil [2].

Choice of the Target Taxa. It is well established that microbial diversity is unparalleled in the biological world and a large number of prokaryotic taxa present in the environment are still to be discovered [2, 66, 72]. However, most of the recent biogeographic studies dealt with only culturable species, and it may be questionable to extrapolate results based on those few selected taxa to prokaryotic biogeography in general. The choice of target organisms is often dictated by practical issues such as natural abundance in the environment or ease of isolation and storability, as may be the case for *Bacillus* or *Pseudomonas*, as previously mentioned.

Different taxa may respond differently to evolutionary and ecological processes governing the creation and maintenance of biogeographic patterns at different scales. One challenge of microbial biogeography is therefore to identify the magnitude of those underlying principles and to determine at what taxonomic levels those forces operate. In that process, it may be important to identify whether “indicator” taxa exist and to what extent their distribution patterns could be representative of those of other taxa.

Methodological Issues. The increasing number of tools to resolve microbial microdiversity has provided access to more genetic information about environmental bacterial populations and communities, which in turn has significantly helped the reemergence of the study of prokaryotic biogeography. However, several pitfalls in the methodological approaches have to be recognized and, if possible, avoided in future studies. Issues relating to the study of microbial ecology in general may be dramatically amplified when dealing with biogeographic approaches, because the latter try to draw conclusions over large spatial scales based on a limited number of samples. In the following sections, we review concerns about the sampling process in a spatial and temporal context, and discuss the central issue of resolution and saturation of molecular tools used.

Sampling Issue and Spatial Scale. Conversely to plant and animal biogeographic studies, bacterial biogeography suffers from the destructive nature of the sampling process that hinders rigorous monitoring of the evolution of diversity at the same locations. It is thus not surprising that, although clear endemic, patchy, or cosmopolitan patterns have been evidenced in several

prokaryotic taxa, scant data exist on the stability of those patterns in time or about their seasonal fluctuations.

Another critical issue is the size of environmental samples from which diversity data are derived. For instance, it is technically and practically difficult to scale up sample size from 1 g to 1 kg of soil or sediment. However, assessment of biogeographic patterns shifts with various spatial scales is essential not only for sampling reasons but also for a better understanding of the ecological principles at play. Such an approach was followed by, for instance, Nicol and colleagues [50], who examined the heterogeneity of archaeal community structure in grassland soil at two contrasting spatial scales using 16S rRNA-based analyses. At a macroscale, single core samples were found to be mostly representative of the heterogeneity across larger transects (i.e., for soil cores distant of 2-m intervals). However, microscale analyses revealed that community structure varied significantly within single cores, and more similarity was evidenced for samples consisting of 10 g soil than for smaller sample sizes (i.e., 1 and 0.1 g). Interestingly, similar results were found for cold marine sediments where community shifts were generally evidenced between different sediment layers, whereas geographical differences appeared to result in smaller differences [7]. Thus, one essential methodological step would be to determine the validity and implications of using rather small sample sizes to describe prokaryotic ecology and diversity at larger spatial scales.

Because biogeographic pattern analyses are initially a mere description of taxa presence or absence in different samples, one way to ascertain the absence of target organisms may be to perform *a posteriori* controls of all target-negative samples. For instance, lineage-specific PCR (e.g., [54]), combined or not with enrichment steps when population abundance in the samples is thought to be low (e.g., [56]), may be used to validate the presence (or absence) of rare genotypes. Alternatively, direct microscopic observations of the samples (e.g., using fluorescent *in situ* hybridization with taxon-specific probes) or the utilization of various physiological methods to identify prokaryotic activity (e.g., [2, 46, 65]) may be used.

The third dimension of the habitat being sampled, which can be either soil, sediment layers, or water columns, may constitute another challenge. Hitherto, microbial biogeographic studies have mostly focused on the Earth's surface, probably in a similar way as being done for plants and animals. Nevertheless, few studies dealing with large scales and depth may be found in the marine environment or sediments (e.g., [4, 8]). Such studies illustrate that still much is to be learned from vertical analysis of the diversity of our planet, all the more as prokaryotic life has been evidenced kilometers below the Earth's surface [75].

Although certain studies focus on microbial biogeography between sites, regions, and continents, another line of research has also been followed, which consists of examining microscale patterns of bacterial populations [23]. The rationale behind this approach is that synecological and autecological interactions between the target organisms and their surrounding environment occur in microenvironments, at a microscale [24]. The microscale and macroscale approaches that aim at better understanding spatial diversity patterns should be integrated into a common framework. Indeed, different ecological and evolutionary principles may occur at different scales. As outlined below, new analytical tools need to be explored or developed to combine such data into a coherent perspective.

Resolution and Saturation of the Methods. Molecular methods with increasing power of resolution were used to demonstrate that the choice of molecular tools has a profound impact on our final interpretation of biogeographic patterns [11]. In that study, when methods with a coarse level of resolution such as 16S rDNA and ITS restriction analyses were used, fluorescent *Pseudomonas* populations displayed a cosmopolitan and weakly endemic distribution, respectively, whereas the use of whole-genome fingerprinting methods (i.e., rep-PCR [55]) revealed that nearly all fluorescent *Pseudomonas* genotypes were endemic to one geographic area, with little overlap between regions and sites. Therefore, using molecular tools with weak resolving power could hide the existence of small communities with their particular biogeographic patterns. A combination of methods of increasing resolving power may be a good approach to study biogeography in prokaryotes, because they would offer the advantage of looking at the same organisms with different perspectives, and would target different levels of similarity.

Genetic relationships inferred from whole-genome typing (e.g., rep-PCR) approach saturation when similarity values are near or under 65% [11, 55, 70]. This indicates that it may not be possible to use such techniques to confidently compare higher taxonomic levels, and that alternate methods are therefore needed. For each methodology, the levels of saturation and resolution have to be experimentally determined to better understand the biases and limits of the chosen approach. In that respect, fingerprinting methods such as rep-PCR may be initially used to sort out clonemates, and then more targeted approaches such as sequence analysis of multiple protein-coding genes may be used to analyze phylogenetic, biogeographic, and evolutionary patterns. For instance, Whitaker and colleagues [74] showed that the concatenation of the sequences of nine protein-coding genes was necessary to obtain enough resolution to indicate endemic clades, whereas the analysis of those

gene sequences taken individually could not provide that level of resolution. Although rep-PCR and multilocus sequence analyses provide precious tools to type genotypes into clonal lineages, there is still no comparable tool to assess the genotypic diversity for uncultured prokaryotes. New methodological developments are therefore needed to explore this field.

Future Developments

Despite the existence of numerous pitfalls when dealing with prokaryotic biogeography, new developments at the conceptual and methodological levels would dramatically improve our understanding of spatial and temporal patterns of microbial life in different habitats. Here, a series of potential developments is thus proposed to stimulate future research effort.

Experimental Evaluation of Biogeography Theory for Prokaryotes. Because spatial and temporal scales are too large for mechanistic experiments to be performed *in situ*, testing biogeographic hypotheses would remain a formidable task. However, working with prokaryotes has tremendous advantages because of their small size, short generation time, and the huge availability of molecular tools and, generally, of biological and physiological information. Thus, transposition of complex ecological questions to simpler laboratory-based model systems would be extremely valuable (for review, see [33]) in future biogeographic studies. The main objectives of such approaches would be to deconstruct and simplify the effects of spatial and temporal scales on complex systems into individual parts in order to study them with high degree of experimental control and reproducibility (e.g., [67]).

Taxonomic Level, Representative Taxa, and Sampling. As mentioned above, a current question in prokaryotic biogeography is to understand at which genetic level (or taxonomic level) significant geographic differences can be observed. This approach would provide first bases for a more synthetic and predictive view of prokaryotic biogeography and its ecological meaning. Although studies based on single species may still be needed to obtain more field data on which theories and hypotheses can be built, efforts should also be directed toward describing multiple coexisting species (comparative biogeography), so that a more general picture can emerge.

A promising approach would be to systematically compare samples from various natural habitats to draw an overview about genetically coherent and incoherent areas and their scale dependency. This would help determine regions or sites of high and low diversity to be further investigated and would provide clues about

the interplay of major biotic and abiotic parameters that would lead to such situations. Areas of higher endemism would then be the necessary targets of conservation biology programs.

Beyond the mere description of diversity patterns, future research should also focus on the spatial and temporal distribution of the resulting ecological functions and properties. The biogeography of microbial functions would be a specificity of the prokaryotic model compared with that of plants and animals. It is therefore essential for microbial ecologists to undertake this line of research.

Technical Developments. The reemergence of the study of microbial biogeography was made possible by the spectacular technical breakthroughs to resolve microbial diversity at the genotype level. It may therefore be hypothesized that the future availability of methods with higher resolving power would indubitably be central in providing new concepts in prokaryotic biogeography. In that context, microarray technology and genomics (for review, see for instance [12]) may thus offer promising tools to obtain fine-scale description of the genetic background of organisms in various habitats [51]. For instance, a novel type of microarray consisting of whole genomic DNA isolated from 67 representative bacterial strains has been designed to identify and detect specifically, sensitively, and quantitatively microorganisms in environmental samples [77]. However, to our knowledge, no such applications have yet been carried out to document biogeographic distribution of prokaryotes.

Hitherto, sequencing of protein-coding genes has been shown to be an efficient method to analyze microbial biogeographic data of culturable prokaryotes (e.g., [74]). However, such approach traditionally relies on analyzing a given set of conserved genes [61]. The fact that bacterial genomes could be split into a core of housekeeping genes and accessory genes that encode for adaptive traits [20, 36, 52] offers great promise for the study of the evolution and phylogeny of those accessory genes in concord with biogeographic patterns. This could potentially shed light on a set of genes or single nucleotide polymorphisms (SNP) that would generally be associated with cosmopolitan or endemic organisms.

Development or Utilization of New Analytical Tools. Microbial biogeographic studies are *per se* multidisciplinary because of the breadth of the concepts and tools used. They require knowledge in many fields such as (microbial) ecology, (geo)statistics, mathematics, geology, phylogenetics, and population genetics. Therefore, future challenges may rely on how to constructively integrate those different fields of expertise to provide beneficial and coherent grounds for the development of a microbial biogeography theory.

As phylogenetic tools may be central to the understanding of modern environmental microbiology, more effort should be put toward integrating spatial statistical tools in the microbial ecologist toolbox. Introductory reviews illustrating the integration of spatial scale in ecology may be found in, for instance, Liebhold and Gurevitch [40] or in the classical reference book of Legendre and Legendre [38].

Other scientific fields should also be explored to develop new analytical and conceptual tools that would systematically include spatial scales in environmental microbiology studies. In that context, more interactions between microbial ecologists, mathematicians, and statisticians should necessarily be fostered to address issues specific to the prokaryotic case. It would be interesting, for instance, to determine whether the generation of biogeographic patterns in prokaryotes is either deterministic or random, or neither of the two (i.e., chaotic). Advances in other disciplines such as the science of complexity [9, 26] may thus offer new lines of investigation.

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