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Allopatric origins of microbial species

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Although allopatric divergence is a well-accepted mechanism of speciation for eukaryotic macro-organisms, the importance of geographical barriers to divergence in microbial populations is a subject of great debate. Do geographically separated populations of micro-organisms diverge independently, or does their structure fit the often quoted Bass-Becking description 'everything is everywhere; the environment selects'? Aided by high-resolution genetic and genomic tools, the search for 'microbial marsupials' has revealed that in fact both are true; some species of micro-organisms demonstrate allopatric divergence, while others do not. This discovery opens the door for comparative analyses, where questions about the differences in evolutionary and ecological mechanisms that drive divergence and speciation in different microbial species can begin to be explored. Investigating these differences in evolutionary mechanisms will greatly enhance interest in, and understanding of, the dynamic processes that create and maintain the vast diversity of the microbial world.

Keywords: allopatric speciation; micro-organisms; biogeography; genomics

1. INTRODUCTION

The mention of species and speciation in micro-organisms has fallen out of favour, because much of the discussion on the topic has focused on identifying a single species recognition tool (i.e. a level of sequence or genome divergence) that defines a species boundary for all microbial taxa (Rossello-Mora & Amann 2001; Konstantinidis & Tiedje 2005*b*). This focus on species definitions rather than mechanisms of speciation necessarily excludes the intrinsic and the extrinsic evolutionary factors that differ between micro-organisms—the very differences that make the study of species and speciation an interesting empirical science. Without knowledge of the mechanisms that drive divergence among microbial lineages (i.e. evolutionary species concepts; Rossello-Mora & Amann 2001), 'species' can seem like nothing more than labels. It is no wonder, then, that many microbiologists who respect diversity have lost interest in the discussion of species and speciation.

Studying mechanisms of speciation involves identifying patterns of variation that develop within and between populations as well as barriers that partition this variation into discontinuous lineages over time. It is now possible to analyse patterns of individual-level variation in micro-organisms, as high-throughput and high-resolution tools (DNA sequencing, proteomics and expression analysis) become feasible for larger sample sizes. With the development of these tools, a new appreciation for population genetics and mechanisms of speciation in Bacteria and Archaea is emerging. The ability to decipher these population-level patterns of diversity will soon allow microbiologists to address many of the questions about speciation that have long interested evolutionary biologists studying diversity in macro-organisms.

Although there is little consensus about the relative importance of different speciation mechanisms, even in macro-organisms that have been studied for hundreds of years, allopatric speciation has emerged as the least controversial and technically the easiest to identify. As stated by Coyne & Orr (2004) in their text on species and speciation, 'most evolutionists accept allopatric speciation as the most common mode'. Allopatric speciation results from divergent evolution of geographically isolated populations. Because micro-organisms are so small, recognizing phenotypic differentiation among isolated populations and identifying potential geographical barriers that prevent gene flow and migration have been especially challenging, and the importance of allopatric speciation to microbes is still hotly debated (Whitfield 2005).

Identifying the importance of allopatric speciation to micro-organisms has great relevance across a spectrum of disciplines, from community ecology to microbial evolution. On the scale of community ecology, the presence of allopatric speciation mechanisms contributes to estimates of global species richness (Finlay 2002). Understanding the evolution of allopatric species has practical relevance in controlling the spread of emerging pathogens (Maynard Smith 1995), forensic analysis of sources of bacterial strains (Easterday *et al.* 2005), engineering dependable bioremediation communities (Curtis *et al.* 2002) and designing conservation strategies to protect essential endemic microbial populations (Stepkowski *et al.* 2005; Souza *et al.* 2006). The recognition of allopatric species will allow for comparative estimates of intraspecies nucleotide diversity, which are needed to identify the origins of genome complexity across the domains of life (Lynch & Conery 2003; Daubin & Moran 2004). In addition, allopatric divergence will be important to

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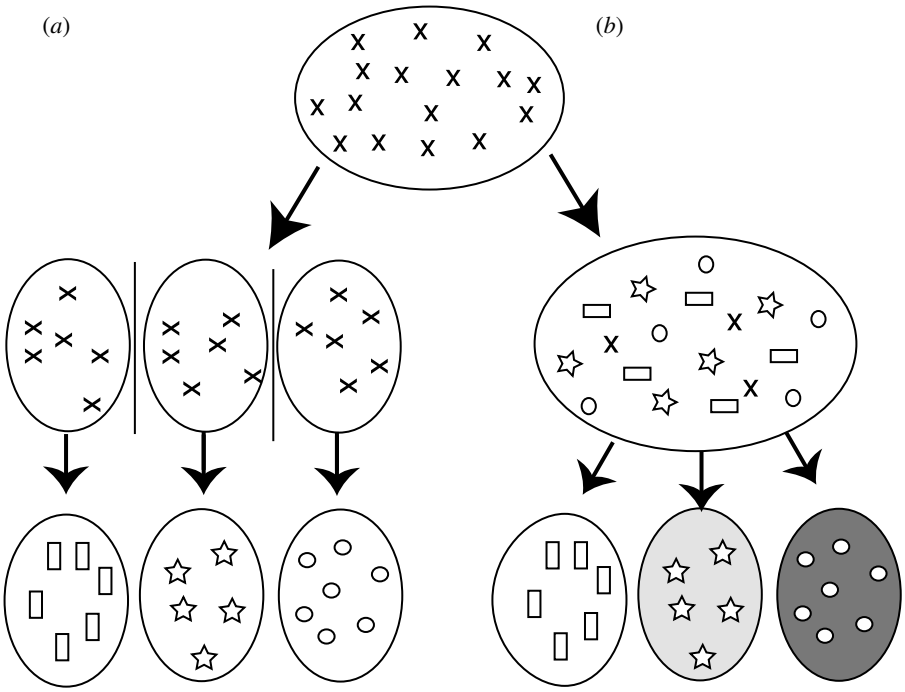

Figure 1. Two models explain biogeographic differentiation. Both begin with a single homogenous population. (*a*) Three populations become isolated by geographical barriers and diverge independently. (*b*) Different genotypes evolve to form a heterogeneous, freely mixing population from which three different environments selected for different types.

experimental microbiologists who expect consistency among similar strains cultured from different locations.

The question of whether allopatric speciation can occur in micro-organisms is not a new one (Bass Becking 1934). However, the recent claim that geographical barriers do not exist for any micro-organism (Finlay 2002) has renewed the search for examples of 'microbial marsupials' (Fenchel 2003). As the number of studies of microbial biogeography increases, it has become clear that some species show evidence of allopatric divergence (Papke *et al.* 2003; Whitaker *et al.* 2003; Kim *et al.* 2004) and others do not (Pringle *et al.* 2005; Rydholm *et al.* 2006; Zaballos *et al.* 2006). The interesting question now is not 'do microbial species have allopatric origins?', but 'what are the evolutionary parameters that lead to geographical barriers in some microbial species, but not in others?'

Here, I review methods for identifying biogeographic differentiation and allopatric speciation in micro-organisms, with attention to how the degree of resolution provided by different genetic tools reveals different patterns among different microbial species. Then, I contrast the population level mechanisms that result in biogeographic patterns driven by environmental selection (ecological speciation) to those driven by local evolution in geographically isolated populations (allopatric speciation), and suggest that comparative studies of both biogeographic patterns and population parameters are necessary to identify the basis of biogeographic diversity among different species. I conclude with a discussion of the unanswered questions specific to microbial biogeography, and how genomic tools combined with population genomic analyses will answer some of these questions.

2. SIGNATURES OF DIFFERENTIATION AMONG POPULATIONS

Biogeographic differentiation refers to the partitioning of variation into unique groups that may result from environmental factors (ecological speciation) or physical barriers to dispersal (allopatric speciation; Lomolino *et al.* 2005; figure 1). Testing for mechanisms of allopatric speciation in micro-organisms begins by identifying how variation is partitioned among geographically associated populations.

DNA sequence analysis has become the most powerful tool for resolving events in microbial evolution. The most common method used to elicit biogeographic patterns involves analysis of phylogenetic relationships among sequences derived from different locations. Biogeographically distinct populations are evident when variable gene sequences cluster by geographical region. Because discontinuities separating clusters of gene sequences can occur randomly through stochastic evolutionary processes, conclusively resolving biogeographic patterns requires phylogenetic concordance among multiple loci (Kuo & Avise 2005). Multilocus assessments of biogeographic patterns can also exclude the effects of natural selection on sequence divergence, which can misleadingly identify geographical patterns or lack thereof (Elder 1977).

Recognizing divergence between isolated populations depends upon using tools designed to detect independent evolution at the appropriate time-scale. The slowly evolving 16S rRNA gene is the most commonly used molecular marker to survey and catalogue microbial diversity. Because this gene evolves slowly over geological time, it is appropriate for detecting ancient evolutionary events. The 16S rRNA marker has revealed ancient biogeographic differentiation among populations of

Table 1. Summary of studies using high-resolution markers to test for geographical or environmental sources of biogeographic differentiation. (G, geographical; E, environmental and N, no differentiation identified. ITS, internal transcribed spacer; PCR, polymerase chain reaction.)

G	E	N	markers used	species name	habitat	spatial scale	metabolism	reference
		X	16S rRNA and one protein-coding locus	*Nitrosococcus oceani*	marine	global	aerobic chemo-heterotroph	Ward & O'Mullan (2002)
X			16S rRNA and eight protein-coding loci	*Sulfolobus islandicus*	geothermal hot springs	global	aerobic heterotroph	Whitaker *et al.* (2003)
X			16S rRNA, ITS	*Synechococcus* sp.	geothermal hot springs	global	aerobic photo-autotroph	Papke *et al.* (2003)
X			16S, ITS and three protein-coding loci	*Mastigocladus laminosus*	geothermal hot springs	local (50 km)	aerobic photo-autotroph	Miller *et al.* (2006)
		X	26 517 bp of overlapping sequence	*Thermotoga* sp.	hydrothermal hot springs	global	anaerobic heterotroph	Nesbo *et al.* (2006)
		X	five protein-coding loci	*Photobacterium phosphoreum*	marine	global	aerobic heterotroph	Ast & Dunlap (2005)
	X		three protein-coding loci and RAPDs	*Bacillus simplex*	soil	local (40 km)	aerobic heterotroph	Sikorski & Nevo (2005)
X			three protein-coding loci, two symbiotic loci	*Rhizobium gallicum*	soil, root nodules	global	aerobic heterotroph	Silva *et al.* (2005)
	X		Multilocus enzyme electrophoresis	*Burholderia cepacia*	Freshwater stream	local (50 m)	aerobic heterotroph	Wise *et al.* (1995)
X			16S–23S rRNA, ITS and box-PCR	*Pseudomonas* sp.	soil	global	aerobic heterotroph	Cho & Tiedje (2000)
X			rep-PCR	*Thioalkalivibrio* sp.	haloalkaline lakes	global	chemolitho-autotroph	Foti *et al.* (2006)

some microbial species, but not others (Glockner *et al.* 2000; Hentschel *et al.* 2002; Hewson & Fuhrman 2004; Pommier *et al.* 2005; Wu *et al.* 2006). While differences among species are apparent on this scale, there are very few comparative analyses to establish the mechanistic basis for different patterns.

Recently, a number of studies have shown that the use of slowly evolving 16S rRNA makes it difficult to recognize recent events in the evolutionary history of a species, such as those associated with incipient speciation (Stackebrandt *et al.* 2002). For microbial species in which biogeographic patterns have emerged on more recent time-scales, resolving differentiation requires sequencing more rapidly evolving non-coding or protein-encoding loci. Table 1 shows results from several studies of non-pathogenic microbial species that have addressed biogeographic patterns using high-resolution tools such as multilocus sequence typing of protein-coding genes. Again, at this scale, not all studies tell the same story, and the defining differences among species have not emerged.

It is important to note that not all protein-coding loci perform equally in population analysis of biogeographic differentiation. Several studies have concluded that, owing to a lack of variation in protein-coding markers among distant locations, biogeographic differentiation does not exist for a given species (Ward & O'Mullan 2002; Ast & Dunlap 2005). Because molecular markers evolve on different time-scales, conclusive evidence for panmixia or differentiation requires sampling multiple individuals from each location to identify markers that can vary.

Phylogenetic analyses depend upon fixed differences between populations to accurately resolve branching patterns. Even with rapidly evolving protein-coding loci, fixation of differences between populations may occur long after the formation of geographical barriers. Differentiation on more recent time-scales can be resolved when variable sites differ in frequencies among populations. F_{st} statistics, which compare the level of genetic variation within and between populations are useful for identifying these more recently evolved levels of differentiation (Hudson *et al.* 1992). Large sample sizes are required to resolve biogeographic patterns at this scale. Therefore, again, studies that find a lack of geographical isolation using a single sequence from each locale may overlook significant differentiation.

Differentiation among populations has also been recognized using genome fingerprinting metrics, such as random amplified polymorphic DNA markers (RAPDs), box-polymerase chain reaction (box-PCR; Oda *et al.* 2002; Foti *et al.* 2006) or the position of repetitive elements (rep-PCR; Fulthorpe *et al.* 1998; Cho & Tiedje 2000). These PCR-based genome fingerprints change as a result of nucleotide sequence divergence at priming sites, or with the movement of transposable elements, insertion of novel genetic elements or large-scale genome rearrangements. At least some of these genome changes can occur at rates that outpace nucleotide sequence divergence. Therefore, genome fingerprints have the potential to resolve even more recent differentiation than the sequence methods described previously (Rademaker *et al.* 2000).

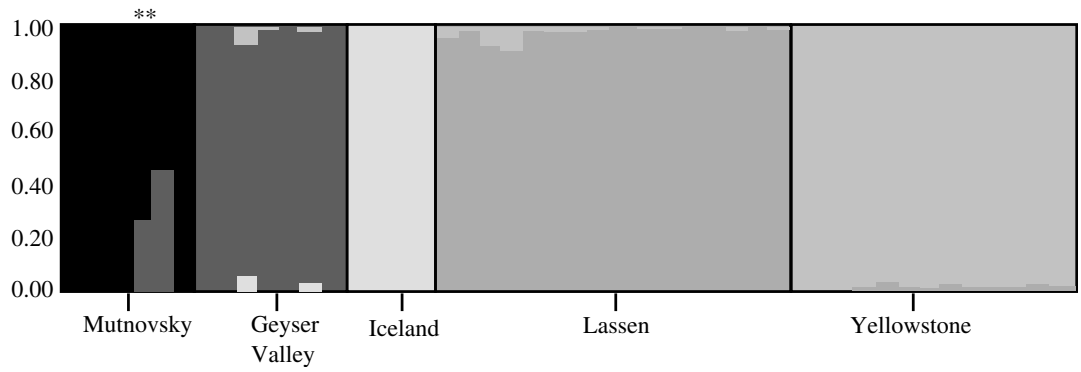

Figure 2. Ancestral population structure of *Sulfolobus islandicus*. Results from STRUCTURE analysis show assignment of 47 individuals to five populations associated with five geographical regions. Each vertical bar represents a single *S. islandicus* strain. Bold lines separate the five different populations, shown in different colours. For each strain, the *Y*-axis shows the proportion of the variable nucleotide sites in the seven genes that are assigned to each of the five ancestral populations. Stars highlight two individuals from Mutnovsky, for which a significant proportion of the variable sites are assigned to the ancestral Uzon/Geyser Valley population.

3. GEOGRAPHICAL ISOLATION OR 'THE ENVIRONMENT SELECTS'?

Both environmental selection and geographical isolation can lead to differentiation among populations (figure 1). To conclusively identify allopatry as a speciation mechanism, we must answer the fundamental question: do endemic populations diverge through neutral drift and local adaptation (allopatric speciation), or do they represent particular genotypes from a panmictic pool that can survive in a given environment (ecological speciation)?

Table 1 lists a selection of empirical studies of environmental micro-organisms that have tested for evidence of geographical isolation using high-resolution molecular tools. This summary shows that evidence for geographical isolation has been identified among diverging populations at both the global and the local spatial scales in microbial species with different habitats and metabolic characteristics. Environmental differentiation has been observed primarily at the local scale. In addition, several studies have found no evidence for geographical patterns even using high-resolution tools. This selection of studies used a variety of tests to distinguish between environmental and geographical factors where differentiation among populations was identified.

The most powerful method to parse geographical isolation and environmental selection mechanisms is to partition the effect of isolation by distance from other environmental variables. Tests of isolation by distance assume that migration decreases as a function of spatial distance between sites (Wright 1943; Slatkin 1993). Decreased migration results in greater isolation, and consequently greater genetic divergence between distant sites. To test whether there is significant correlation between genetic and geographical distance, a nested sampling strategy including samples taken at different spatial scales is needed. Whitaker *et al.* (2003) tested for a correlation between genetic and geographical distance in a combined analysis of nine genomic loci from thermophilic Archaea collected from globally distant geothermal regions. This study found a significant distance effect that shows a relationship between genetic and geographical distance among populations, but no relationship to measured environmental parameters.

When multiple genetic loci are used, distance relationships may also be resolved based on the number of shared alleles. For example, figure 2 shows the results derived from the programme STRUCTURE, which implements a Bayesian approach to reconstructing ancestral population structure from multilocus sequence data (Falush *et al.* 2003). For this analysis, STRUCTURE was run using variable positions from seven genetic loci for 46 strains of the thermoacidophile *Sulfolobus islandicus* collected from two isolated regions of Kamchtaka, Russia and populations from Iceland, and Yellowstone and Lassen National Park in North America. Using the no admixture model, which assumes that each individual derives ancestry from only one population, STRUCTURE inferred that a model with five populations best fit the data: one ancestral population from each geographical region. After evidence for rapid recombination within populations was identified (Whitaker *et al.* 2005), further analysis allowing for admixture among populations showed the proportion of nucleotides from each ancestral population identified by STRUCTURE for each genotype (figure 2). STRUCTURE identified evidence of admixture between the Uzon/Geyser Valley and Mutnovsky regions of Kamchtaka (stars in figure 2) at two of the seven loci. This is consistent with either incomplete sorting of alleles between two recently isolated populations or gene flow between geographically isolated populations. Gene flow, or lack of complete sorting of alleles between the Uzon/Geyser Valley and Mutnovsky populations of Kamchatka, which, compared with the other populations, are in relatively close proximity (250 km), is consistent with the conclusion that the five populations are isolated by distance (Whitaker *et al.* 2003).

When testing for isolation by distance, the potentially confounding effects of environmental selection may be removed through statistical analysis. However, while several authors have attempted to resolve the influence of fine-scale environmental variables on population structure, this is difficult to establish conclusively (Papke *et al.* 2003; Glaeser & Overmann

2004). One confounding factor is temporal and spatial variation in microbial environments that obscure measurement and resolution of critical environmental parameters. To correct for such spatial and temporal variation in environmental parameters, sampling strategies that allow assessment of overall diversity by sampling at different sites and times in each location are essential to identify shared characters within geographical regions and distinguish the effects of isolation by distance on biogeographic patterns.

Without a nested sampling strategy, several authors have tested for correlations between the distribution of diversity and geographical location or environmental variables. A recent study by Sikorski and Nevo explicitly sought to distinguish geographical and environmental factors on population structure of *Bacillus simplex* in the Evolution Canyons of Israel using both multilocus genealogies and genome-fingerprints generated with RAPDs (Sikorski & Nevo 2005). This study showed significant differentiation between habitat types, but very little with regard to geographical location. The authors attribute this differentiation to rapid adaptation and sympatric speciation in these spore-forming microorganisms that have been shown to have few migration barriers (Roberts & Cohan 1995).

Recently, a number of studies have tested for distance effects at the microbial community scale (Huynen & Bork 1998; Green *et al.* 2004; Horner-Devine *et al.* 2004; Yannarell & Triplett 2005; Fierer & Jackson 2006; Telford *et al.* 2006). While these approaches do not explicitly test for the population level process of allopatric speciation, they can resolve the effects of allopatric speciation on the larger scale of community composition. Just as the correlation between geographical and genetic distance results from allopatric divergence among populations, a positive relationship between area size and number of different taxa is predicted when communities are geographically isolated from each other (MacArthur & Wilson 1967). Several studies have established this relationship using low-resolution tools such as 16S rRNA or the length of the internal ribosomal RNA spacer to identify overall community fingerprints and diversity metrics. Because both an increase in environmental heterogeneity and allopatric speciation could produce this pattern, effects of geography and environment must again be disentangled. Where these studies have explicitly tested for the effects of geographical distance, they have shown that biogeographic patterns are driven by either environmental factors (Huynen & Bork 1998; Horner-Devine *et al.* 2004; Yannarell & Triplett 2005; Fierer & Jackson 2006; Telford *et al.* 2006) or geographical isolation (Green *et al.* 2004; Telford *et al.* 2006).

4. POPULATION PARAMETERS

Mechanistically, the relative rates of local *in situ* evolution (neutral mutation, drift and adaptation) and dispersal (migration) will determine whether biogeographic patterns are established by geographical barriers or environmental selection. In fact, these processes directly counteract each other. Relatively high migration rates prevent local adaptation and vice versa (Via & Lande 1985). In order to understand how the balance between *in situ* evolution and dispersal affects different species, we must describe the population genetic parameters that are involved in both the processes. Below, I describe some of the population dynamics that determine rates of *in situ* evolution and migration that result in allopatric speciation. While interesting evolutionary dynamics occur in metapopulations in which several populations in different environments are linked by migration, here I will focus only on dynamics that can lead to complete isolation and eventually, to speciation.

5. POPULATION PARAMETERS THAT DETERMINE RATES OF *IN SITU* EVOLUTION

When migration rates are relatively low, divergence among populations results from neutral genetic drift and local adaptation driven by natural selection. Although genetic drift is theoretically important to the process of evolution across adaptive landscapes (Wright 1932), its importance in allopatric speciation has rarely been demonstrated empirically, and is considered less important than the effect of natural selection (Coyne & Orr 2004). Divergence through genetic drift is most likely to occur when small founder populations become isolated from a larger source population by geographical barriers, so that low-frequency alleles become fixed by chance (Mayr 1963). Therefore, we might predict that geographical structures driven by neutral divergence will evolve in microbial species when new niches are colonized by small founder populations.

Both theoretical and empirical population genetic analyses have described a migration–selection balance that defines biogeographic population structure driven by natural selection (Slatkin 1987). This balance is determined by the rate of adaptive response of a population to natural selection in its local environment. The rate at which a population becomes locally adapted to its environment depends upon the selective regime as well as the evolutionary potential of the population. Evolutionary potential is determined by the rate at which adaptive traits are introduced into the population and adaptive mutations are distributed through the population to become fixed (Capelle & Neema 2005). In microbial populations, adaptations are introduced through random mutation, genetic exchange among individuals of the same population, horizontal gene transfer of genetic material from other species in the environment and rare migration. It has been suggested that horizontal transfer is the primary mechanism through which Bacteria and Archaea acquire adaptive alleles (Konstantinidis & Tiedje 2005*a*; Coleman *et al.* 2006). If this prediction is true, microbial species that are prone to horizontal gene transfer will acquire locally advantageous alleles at a faster rate and local adaptation will drive divergence among them. Similarly, increased rates of local adaptation resulting from gene transfer between species have been shown to occur in macro-organisms. For example, Grant (1993) showed that introgression of genetic variation leads to faster local adaptation in island finch populations.

The rate at which adaptive mutations become fixed in a population depends upon interrelated factors including the strength of selection, rate of gene transfer between individuals (i.e. whether they are recombining or clonal; Barraclough *et al*. 2003), population size, population growth rate and environmental stability (Katz *et al*. 2005). For example, microbial species that recombine frequently have a greater rate of adaptation over clonal populations in a harsh environment where selection is strong (Goddard *et al*. 2005). A review of 47 studies of rates of local adaptation in macro-organisms showed that in the majority of species, rapid local adaptation was associated with a colonization event of a novel unoccupied environment (Reznick & Ghalambor 2001). Colonization of a new unoccupied niche can lead to rapid fixation of alleles of large selective effect. Therefore, microbial species that colonize novel environments, such as anthropomorphic contaminant sites may be more likely to exhibit local adaptation and geographical isolation than others.

6. POPULATION PARAMETERS THAT DETERMINE RATES OF DISPERSAL

Global microbial populations result when dispersal rates are high relative to rates of local evolution. Dispersal rates depend upon physical transport between environments and survival of the propagule, or at least some of its genetic material, once it has arrived. Differences among macrobial species that determine rates of physical transport include active or passive dispersal mechanisms, population density and body-size. Although body-size is the defining characteristic between macro- and micro-organisms, there is a relatively small range of variation in cell size among microbial species. Therefore, while allometry may be a useful structure to compare macrobial and microbial patterns (Martiny *et al*. 2006), it is less useful for distinguishing differences among microbial species. Similar to macro-organisms, microbial species that have mechanisms for active movement (such as flagella) may exhibit biogeographic patterns on a larger scale than those without these mechanisms. Also, those that have acquired mechanisms to facilitate passive movement, such as resistant endospore formation, association with rapidly migrating macrobes (Hubalek 2004) or the formation of protective biofilms (Hall-Stoodley & Stoodley 2005), are more likely to be ubiquitous. Of course, dispersal may also be facilitated by anthropogenic means, such as agriculture or human disease. Therefore, it is not surprising that several studies have shown panmictic populations of several microbial human pathogens (Maynard Smith *et al*. 1991, 2000; Feil & Spratt 2001).

The rate of migration between sites is also related to the population density of microbial species, because the probability of a propagule being physically transferred between sites increases with the number of propagules. Recently, neutral models of microbial communities have been developed and empirically tested to show that migration rates scale with population size (Sloan *et al*. 2006).

An additional component that determines the probability of physical transport is the spatial structure of the microbial habitat. For example, micro-organisms adapted to island habitats separated by great distances have lower migration rates than those in common environments that are more continuously distributed (Staley & Gosink 1999). In addition, the identification of geographical barriers depends upon the spatial scale being assessed. While some microbial species demonstrate geographical barriers on the scale of centimetres, others may only be apparent over thousands of kilometres. Vos *et al*. found evidence for local adaptation, but were unable to recognize spatial structuring at the centimetre scale in the heterotrophic, social, fruiting bacteria *Myxococcus xanthus* (Vos & Velicer 2006). Differences in the metabolic capacities of microbial species may be important determinants of environmental distribution. For example, heterotrophic micro-organisms capable of growth on organic compounds may show higher rates of migration because they are less restricted in their habitats than those optimized for specific metabolic pathways where resources are less evenly distributed.

7. FUTURE DIRECTIONS

Comparative analysis of population parameters in combination with assessment of biogeographic population structure will ultimately identify the ecological or evolutionary basis for differences among microbial species. There are very few microbial species for which both biogeographic patterns and populations parameters have been described, so the primary drivers of these patterns are not known. Some of the parameters, such as the spatial structure of the environment, are straightforward to recognize and record. New methods to model and measure recombination, selection and mutation rates, and their influence on speciation are presently being developed and discussed by several others in this issue (Falush *et al*. 2006; Hanage *et al*. 2006). Using these methods to estimate within- and between-population values of these parameters in populations, where biogeographic patterns have been identified, will be instrumental in determining mechanisms through which allopatric speciation develops. However, several questions unique to micro-organisms remain to be addressed.

One of the most important unanswered questions relates to mechanisms of gene flow between populations. Besides migration of propogules between isolated environments, extra chromosomal carriers of genes, such as plasmids, viruses and transposable elements may contribute to gene flow between populations (Sano *et al*. 2004). These elements have been shown to carry the so-called auxiliary genes, which are relatively transient in microbial genomes and can be transferred easily between organisms, often as genomic islands (Coleman *et al*. 2006).

In a study of the biogeographic structure of the legume symbiont *Rhizobium gallicum*, Silva *et al*. (2005) found differences in biogeographic structure when chromosomal loci were compared with plasmid-borne loci. This study showed significant geographical isolation among Mediterranean, Mexican and Chinese populations using both phylogenetic and population measures of differentiation for two chromosomal loci.

Interestingly, the authors note that these patterns were not resolved using two symbiosis genes known to be carried on plasmids. The mechanism of transfer of plasmid genes between geographical regions is unknown; however, this and other studies provide a needed incentive to examine the importance of extra-chromosomal elements to gene-flow among the otherwise isolated populations.

Another question crucial to understanding the importance of allopatric speciation to microbial species is, what factors maintain species boundaries in sympatry? The strictest application of the biological species concept for sexual macro-organisms requires that, in order to be recognized as different species, allopatric populations will no longer be able to merge through mating. Barriers to gene flow and the maintenance of independent lineages in sympatric microbial populations are not well understood. In fact, recognizing sympatric speciation in microbial populations can only be done once the potential effects of allopatric divergence are excluded (Barluenga *et al.* 2006; Savolainen *et al.* 2006). One process in recombining microbial species that is analogous to biological speciation in sexual macro-organisms is the breakdown in homologous recombination frequency with neutral sequence divergence (Cohan 1995). It has been suggested that this mechanism results from mismatch repair enzymes and the properties of RecA-mediated recombination (Cohan 1995). In microbial species with these mechanisms, neutral divergence over time among allopatric populations might result in true biological speciation and permanent lineage divergence. Other mechanisms that have been suggested to limit recombination between sympatric species are differences in restriction enzymes which do not allow importation of sequences that are highly divergent (Cohan 2002), loss of genome synteny through acquisition of novel genome fragments (Vetsigian & Goldenfeld 2005), and movement of transposable elements or genome rearrangements. While intriguing as intrinsic mechanisms of speciation, the impact of these barriers on speciation is yet to be quantified in any microbial species.

Populations of macro-organisms that have diverged in allopatry may remain isolated if they have evolved by natural selection to occupy different ecological niches. This type of ecological differentiation has been proposed as a sympatric speciation mechanism for purely clonal micro-organisms which diverge into unique clonal 'ecotypes' through the recurrent action of periodic selection (Cohan 2005). Even in micro-organisms that can recombine, adaptive changes can create ecological isolation among populations that maintain lineage boundaries. Recognizing these adaptive differences, and demonstrating that they maintain species, has been difficult in micro-organisms. Experiments that identify persistent coexistence and genetic isolation may be possible; however, establishment of microcosms in the laboratory that replicate local environmental conditions is difficult for microbial systems.

8. POPULATION GENOMICS

The exponential increase in the accessibility of DNA-sequencing technology promises to provide the tools for microbial biogeographers to answer some of the critical remaining questions described previously. Interrogating the genomes of populations may be done through sequencing multiple closely related isolates. In addition, assemblies of community genome data can reveal patterns of sequence variation across reconstructed genome sequences of microbial populations (Tyson *et al.* 2004; Allen & Banfield 2005; Whitaker & Banfield 2006). Moving beyond multilocus sequence analyses to comparisons of large genome fragments or even full genome sequences promises to provide higher resolution analyses of both biogeographic patterns and genetic mechanisms that promote them.

Identifying the evolutionary history of different genes throughout the genome will show where different genomic elements demonstrate different geographical patterns. Full genome sequencing of multiple individuals from geographically isolated populations will reveal the differential distribution of auxiliary genes and genomic islands, and their association with extra-chromosomal genetic elements. Comparisons among full genome sequences will greatly enhance our ability to detect differences in gene content resulting in horizontal gene transfer within or between geographically isolated populations.

Full genome analysis may also point to mechanisms of genetic isolation that maintain species barriers in sympatry. For example, comparative genome analysis among multiple genomes from the same population will identify whether barriers to recombination are associated with changes in positions of mobile elements, genome rearrangements and levels of neutral sequence divergence. Full genomes of multiple individuals from the same population contain information about variation across the genome that can be used to identify loci that are responsible for niche differentiation and ecological isolation among sympatric species (Whitaker & Banfield 2006). Understanding mechanisms that maintain isolation among sympatric species will allow predictions about whether allopatric divergence will result in the evolution of truly isolated microbial species if sympatry is restored.

9. CONCLUSIONS

The debate over how to define microbial species and whether they exist has a surprisingly limited audience in environmental microbiology. Although many seminars begin with a reverent acknowledgement of microbial diversity, they often proceed with a disclaimer about how we do not really know how to parameterize that diversity and are not sure it really matters. Interestingly, these same seminars usually conclude with some mention of an evolutionary mechanism important to the study of speciation (horizontal gene transfer, recombination, selection or geographical isolation), but very few acknowledge the connection between these mechanisms and the process of speciation.

Many important mechanisms of speciation identified in macro-organisms (biological, allopatric and ecological) can be recognized in the microbial world. Here, I have described some of the evidence for allopatric speciation and the evolutionary parameters that will generate allopatric divergence in micro-organisms.

As with macro-organisms, each of these speciation mechanisms and evolutionary processes will not equally apply to all microbial species. Given the diversity of speciation mechanisms described for macrobes, there is no reason for microbiologists to expect a single concept or definition to apply to all micro-organisms, which represent greater than two-thirds of metabolic and genetic diversity of the planet. Excitement in species and speciation will be rekindled by a search for the underlying physical and biological population parameters that drive speciation across the diversity of the microbial world.

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REFERENCES

Allen, E. E. & Banfield, J. F. 2005 Community genomics in microbial ecology and evolution. *Nat. Rev. Microbiol.* **3**, 489–498. (doi:10.1038/nrmicro1157)

Ast, J. C. & Dunlap, P. V. 2005 Phylogenetic resolution and habitat specificity of members of the *Photobacterium phosphoreum* species group. *Environ. Microbiol.* **7**, 1641–1654. (doi:10.1111/j.1462-2920.2005.00859.x)

Barluenga, M., Stolting, K. N., Salzburger, W., Muschick, M. & Meyer, A. 2006 Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* **439**, 719–723. (doi:10.1038/nature04325)

Barraclough, T. G., Birky Jr, C. W. & Burt, A. 2003 Diversification in sexual and asexual organisms. *Evol. Int. J. Org. Evol.* **57**, 2166–2172.

Bass Becking, L. G. M. 1934 *Geobiologie of Inleiding Tot de Milieukunde*. The Hague, The Netherlands: Van Stockum & Zoon.

Capelle, J. & Neema, C. 2005 Local adaptation and population structure at a micro-geographical scale of a fungal parasite on its host plant. *J. Evol. Biol.* **18**, 1445–1454.

Cho, J.-C. & Tiedje, J. M. 2000 Biogeography and degree of endemicity of fluorescent *Pseudomonas* strains in soil. *Appl. Environ. Microbiol.* **66**, 5448–5456. (doi:10.1128/AEM.66.12.5448-5456.2000)

Cohan, F. M. 1995 Does recombination constrain neutral divergence among bacterial taxa? *Evolution* **49**, 164–175. (doi:10.2307/2410302)

Cohan, F. M. 2002 Sexual isolation and speciation in bacteria. *Genetica* **116**, 359–370. (doi:10.1023/A:1021232409545)

Cohan, F. M. 2005 Periodic selection and ecological diversity in bacteria. In *Selective sweep* (ed. D. Nurminsky), pp. 78–93. New York, NY: Kluwer Academic.

Coleman, M. L., Sullivan, M. B., Martiny, A. C., Steglich, C., Barry, K., Delong, E. F. & Chisholm, S. W. 2006 Genomic islands and the ecology and evolution of *Prochlorococcus*. *Science* **311**, 1768–1770. (doi:10.1126/science.1122050)

Coyne, J. A. & Orr, H. A. 2004 *Speciation*. Sunderland, MA: Sinauer Associates.

Curtis, T. P., Sloan, W. T. & Scannell, J. W. 2002 Estimating prokaryotic diversity and its limits. *Proc. Natl Acad. Sci. USA* **99**, 10 494–10 499. (doi:10.1073/pnas.142680199)

Daubin, V. & Moran, N. A. 2004 Comment on "the origins of genome complexity". *Science* **306**, 978. (doi:10.1126/science.1098469)

Easterday, W. R., Van Ert, M. N., Simonson, T. S., Wagner, D. M., Kenefic, L. J., Allender, C. J. & Keim, P. 2005 Use of single nucleotide polymorphisms in the plcR gene for specific identification of *Bacillus anthracis*. *J. Clin. Microbiol.* **43**, 1995–1997. (doi:10.1128/JCM.43.4.1995-1997.2005)

Elder, J. A. 1977 *Geographic variation, species and clines*. Princeton, NJ: Princeton University Press.

Falush, D., Stephens, M. & Pritchard, J. K. 2003 Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567–1587.

Falush, D., Torpdahl, M., Didelot, X., Conrad, D. F., Wilson, D. J. & Achtman, M. 2006 Mismatch induced speciation in *Salmonella*: model and data. *Phil. Trans. R. Soc. B* **361**, 2045–2053. (doi:10.1098/rstb.2006.1925)

Feil, E. J. & Spratt, B. G. 2001 Recombination and the population structures of bacterial pathogens. *Annu. Rev. Microbiol.* **55**, 561–590. (doi:10.1146/annurev.micro.55.1.561)

Fenchel, T. 2003 Microbiology. Biogeography for bacteria. *Science* **301**, 925–926. (doi:10.1126/science.1089242)

Fierer, N. & Jackson, R. B. 2006 The diversity and biogeography of soil bacterial communities. *Proc. Natl Acad. Sci. USA* **103**, 626–631. (doi:10.1073/pnas.0507535103)

Finlay, B. J. 2002 Global dispersal of free-living microbial eukaryote species. *Science* **296**, 1061–1063. (doi:10.1126/science.1070710)

Foti, M., Ma, S., Sorokin, D. Y., Rademaker, J. L. W., Kuenen, J. G. & Muyzer, G. 2006 Genetic diversity and biogeography of haloalkaliphilic sulphur-oxidizing bacteria belonging to the genus *Thioalkalivibrio*. *FEMS Microbiol. Ecol.* **56**, 95–101. (doi:10.1111/j.1574-6941.2006.00068.x)

Fulthorpe, R. R., Rhodes, A. N. & Tiedje, J. M. 1998 High levels of endemicity of 3-chlorobenzoate-degrading soil bacteria. *Appl. Environ. Microbiol.* **64**, 1620–1627.

Glaeser, J. & Overmann, J. 2004 Biogeography, evolution, and diversity of epibionts in phototrophic consortia. *Appl. Environ. Microbiol.* **70**, 4821–4830. (doi:10.1128/AEM.70.8.4821-4830.2004)

Glockner, F. O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A. & Amann, R. 2000 Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of *actinobacteria*. *Appl. Environ. Microbiol.* **66**, 5053–5065. (doi:10.1128/AEM.66.11.5053-5065.2000)

Goddard, M. R., Godfray, H. C. J. & Burt, A. 2005 Sex increases the efficacy of natural selection in experimental yeast populations. *Nature* **434**, 636–640. (doi:10.1038/nature03405)

Grant, P. R. 1993 Hybridization of Darwin's finches on Isla Daphne Major, Galapagos. *Phil. Trans. R. Soc. B.* **340**, 127–139.

Green, J. L., Holmes, A. J., Westoby, M., Oliver, I., Briscoe, D., Dangerfield, M., Gillings, M. & Beattie, A. J. 2004 Spatial scaling of microbial eukaryote diversity. *Nature* **432**, 747–750. (doi:10.1038/nature03034)

Hall-Stoodley, L. & Stoodley, P. 2005 Biofilm formation and dispersal and the transmission of human pathogens. *Trends Microbiol.* **13**, 7–10. (doi:10.1016/j.tim.2004.11.004)

Hanage, W. P., Spratt, B. G., Turner, K. M. E. & Fraser, C. 2006 Modelling bacterial speciation. *Phil. Trans. R. Soc B* **361**, 2039–2044. (doi:10.1098/rstb.2006.1926)

Hentschel, U., Hopke, J., Horn, M., Friedrich, A. B., Wagner, M., Hacker, J. & Moore, B. S. 2002 Molecular evidence for a uniform microbial community in sponges from different oceans. *Appl. Environ. Microbiol.* **68**, 4431–4440. (doi:10.1128/AEM.68.9.4431-4440.2002)

Hewson, I. & Fuhrman, J. A. 2004 Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. *Appl. Environ. Microbiol.* **70**, 3425–3433. (doi:10.1128/AEM.70.6.3425-3433.2004)

Horner-Devine, M. C., Lage, M., Hughes, J. B. & Bohannan, B. J. 2004 A taxa–area relationship for bacteria. *Nature* **432**, 750–753. (doi:10.1038/nature03073)

Hubalek, Z. 2004 An annotated checklist of pathogenic microorganisms associated with migratory birds. *J. Wildl. Dis.* **40**, 639–659.

Hudson, R. R., Slatkin, M. & Maddison, W. P. 1992 Estimation of levels of gene flow from DNA sequence data. *Genetics* **132**, 583–589.

Huynen, M. A. & Bork, P. 1998 Measuring genome evolution. *Proc. Natl Acad. Sci. USA* **95**, 5849–5856. (doi:10.1073/pnas.95.11.5849)

Katz, L. A., McManus, G. B., Snoeyenbos-West, O. L. O., Pirog, K., Griffin, A. & Foissner, W. 2005 Reframing the microbial 'everything is everywhere' debate: evidence for high gene flow and diversity in ciliate morphospecies. *Aquat. Microb. Ecol.* **41**, 55–65.

Kim, E., Wilcox, L., Graham, L. & Graham, J. 2004 Genetically distinct populations of the dinoflagellate *Peridinium limbatum* in neighboring Northern Wisconsin lakes. *Microb. Ecol.* **48**, 521–527. (doi:10.1007/s00248-004-0219-z)

Konstantinidis, K. T. & Tiedje, J. M. 2005*a* Genomic insights that advance the species definition for prokaryotes. *Proc. Natl Acad. Sci. USA* **102**, 2567–2572. (doi:10.1073/pnas.0409727102)

Konstantinidis, K. T. & Tiedje, J. M. 2005*b* Towards a genome-based taxonomy for prokaryotes. *J. Bacteriol.* **187**, 6258–6264. (doi:10.1128/JB.187.18.6258-6264.2005)

Kuo, C. H. & Avise, J. C. 2005 Phylogeographic breaks in low-dispersal species: the emergence of concordance across gene trees. *Genetica* **124**, 179–186. (doi:10.1007/s10709-005-2095-y)

Lomolino, M. V., Riddle, B. R. & Brown, J. H. 2005 *Biogeography*. Sunderland, MA: Sinauer Associates.

Lynch, M. & Conery, J. S. 2003 The origins of genome complexity. *Science* **302**, 1401–1404. (doi:10.1126/science.1089370)

MacArthur, R. H. & Wilson, E. O. 1967 *The theory of island biogeography. Monographs in population biology*. Princeton, NJ: Princeton University Press.

Martiny, J. B. *et al*. 2006 Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* **4**, 102–112. (doi:10.1038/nrmicro1341)

Mayr, E. 1963 *Animal species and evolution*. Cambridge, MA: Harvard University Press.

Maynard Smith, J. 1995 Do bacteria have population genetics? In *Population genetics of bacteria: fifty-second symposium of the Society for General Microbiology held at the University of Leicester, January 1995* (ed. S. Baumberg, J. P. W. Young, E. M. H. Wellington & J. R. Saunders), pp. 1–12. New York, NY; Cambridge, UK: Cambridge University Press.

Maynard Smith, J., Dowson, C. G. & Spratt, B. G. 1991 Localized sex in bacteria. *Nature* **349**, 29–31. (doi:10.1038/349029a0)

Maynard Smith, J., Feil, E. J. & Smith, N. H. 2000 Population structure and evolutionary dynamics of pathogenic bacteria. *Bioessays* **22**, 1115–1122. (doi:10.1002/1521-1878(200012)22:12<1115::AID-BIES9>3.0.CO;2-R)

Miller, S. R., Purugganan, M. D. & Curtis, S. E. 2006 Molecular population genetics and phenotypic diversification of two populations of the thermophilic cyanobacterium *Mastigocladus laminosus*. *Appl. Environ. Microbiol.* **72**, 2793–2800. (doi:10.1128/AEM.72.4.2793-2800.2006)

Nesbo, C. L., Dlutek, M. & Doolittle, W. F. 2006 Recombination in *Thermotoga*: implications for species concepts and biogeography. *Genetics* **172**, 759–769. (doi:10.1534/genetics.105.049312)

Oda, Y., Wanders, W., Huisman, L. A., Meijer, W. G., Gottschal, J. C. & Forney, L. J. 2002 Genotypic and phenotypic diversity within species of purple nonsulfur bacteria isolated from aquatic sediments. *Appl. Environ. Microbiol.* **68**, 3467–3477. (doi:10.1128/AEM.68.7.3467-3477.2002)

Papke, R. T., Ramsing, N. B., Bateson, M. M. & Ward, D. M. 2003 Geographical isolation in hot spring cyanobacteria. *Environ. Microbiol.* **5**, 650–659. (doi:10.1046/j.1462-2920.2003.00460.x)

Pommier, T., Pinhassi, J. & Hagström, Å. 2005 Biogeographic analysis of ribosomal RNA clusters from marine bacterioplankton. *Aquat. Microb. Ecol.* **41**, 79–89.

Pringle, A., Baker, D. M., Platt, J. L., Wares, J. P., Latge, J. P. & Taylor, J. W. 2005 Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus *Aspergillus fumigatus*. *Evol. Int. J. Org. Evol.* **59**, 1886–1899.

Rademaker, J. L., Hoste, B., Louws, F. J., Kersters, K., Swings, J., Vauterin, L., Vauterin, P. & de Bruijn, F. J. 2000 Comparison of AFLP and rep-PCR genomic fingerprinting with DNA–DNA homology studies: *Xanthomonas* as a model system. *Int. J. Syst. Evol. Microbiol* **50**, 665–677. Pt 2

Reznick, D. N. & Ghalambor, C. K. 2001 The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* **112–113**, 183–198. (doi:10.1023/A:1013352109042)

Roberts, M. S. & Cohan, F. M. 1995 Recombination and migration rates in natural populations of *Bacillus subtilis* and *Bacillus mojavensis*. *Evolution* **49**, 1081–1094. (doi:10.2307/2410433)

Rossello-Mora, R. & Amann, R. 2001 The species concept for prokaryotes. *FEMS Microbiol. Rev.* **25**, 39–67. (doi:10.1111/j.1574-6976.2001.tb00571.x)

Rydholm, C., Szakacs, G. & Lutzoni, F. 2006 Low genetic variation and no detectable population structure in *Aspergillus fumigatus* compared to closely related *Neosartorya* species. *Eukaryot. Cell* **5**, 650–657. (doi:10.1128/EC.5.4.650-657.2006)

Sano, E., Carlson, S., Wegley, L. & Rohwer, F. 2004 Movement of viruses between biomes. *Appl. Environ. Microbiol.* **70**, 5842–5846. (doi:10.1128/AEM.70.10.5842-5846.2004)

Savolainen, V. *et al*. 2006 Sympatric speciation in palms on an oceanic island. *Nature* **441**, 210–213. (doi:10.1038/nature04566)

Sikorski, J. & Nevo, E. 2005 Adaptation and incipient sympatric speciation of *Bacillus simplex* under microclimatic contrast at "Evolution Canyons" I and II, Israel. *Proc. Natl Acad. Sci. USA* **102**, 15 924–15 929. (doi:10.1073/pnas.0507944102)

Silva, C., Vinuesa, P., Eguiarte, L. E., Souza, V. & Martinez-Romero, E. 2005 Evolutionary genetics and biogeographic structure of *Rhizobium gallicum* sensu lato, a widely distributed bacterial symbiont of diverse legumes. *Mol. Ecol.* **14**, 4033–4050. (doi:10.1111/j.1365-294X.2005.02721.x)

Slatkin, M. 1987 Gene flow and the geographic structure of natural populations. *Science* **236**, 787–792.

Slatkin, M. 1993 Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**, 264–279. (doi:10.2307/2410134)

Sloan, W. T., Lunn, M., Woodcock, S., Head, I. M., Nee, S. & Curtis, T. P. 2006 Quantifying the roles of immigration

and chance in shaping prokaryote community structure. *Environ. Microbiol.* **8**, 732–740. (doi:10.1111/j.1462-2920.2005.00956.x)

Souza, V. *et al.* 2006 An endangered oasis of aquatic microbial biodiversity in the Chihuahuan desert. *Proc. Natl Acad. Sci. USA* **103**, 6565–6570. (doi:10.1073/pnas.0601434103)

Stackebrandt, E. *et al.* 2002 Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int. J. Syst. Evol. Microbiol.* **52**, 1043–1047. (doi:10.1099/ijs.0.02360-0)

Staley, J. & Gosink, J. J. 1999 Poles apart: biodiversity and biogeography of polar sea ice bacteria. *Annu. Rev. Microbiol.* **53**, 189–215. (doi:10.1146/annurev.micro.53.1.189)

Stepkowski, T., Moulin, L., Krzyzanska, A., McInnes, A., Law, I. J. & Howieson, J. 2005 European origin of *Bradyrhizobium* populations infecting lupins and serradella in soils of Western Australia and South Africa. *Appl. Environ. Microbiol.* **71**, 7041–7052. (doi:10.1128/AEM.71.11.7041-7052.2005)

Telford, R. J., Vandvik, V. & Birks, H. J. 2006 Dispersal limitations matter for microbial morphospecies. *Science* **312**, 1015. (doi:10.1126/science.1125669)

Tyson, G. W. *et al.* 2004 Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* **428**, 37–43. (doi:10.1038/nature02340)

Vetsigian, K. & Goldenfeld, N. 2005 Global divergence of microbial genome sequences mediated by propagating fronts. *Proc. Natl Acad. Sci. USA* **102**, 7332–7337. (doi:10.1073/pnas.0502757102)

Via, S. & Lande, R. 1985 Genotype–environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**, 505–522. (doi:10.2307/2408649)

Vos, M. & Velicer, G. J. 2006 Genetic population structure of the soil bacterium *Myxococcus xanthus* at the centimeter scale. *Appl. Environ. Microbiol.* **72**, 3615–3625. (doi:10.1128/AEM.72.5.3615-3625.2006)

Ward, B. B. & O'Mullan, G. D. 2002 Worldwide distribution of *Nitrosococcus oceani*, a marine ammonia-oxidizing gamma-proteobacterium, detected by PCR and sequencing of 16S rRNA and amoA genes. *Appl. Environ. Microbiol.* **68**, 4153–4157. (doi:10.1128/AEM.68.8.4153-4157.2002)

Whitaker, R. J. & Banfield, J. F. 2006 Population genomics in natural microbial communities. *Trends Ecol. Evol.* **21**, 508–516. (doi:10.1016/j.tree.2006.07.001)

Whitaker, R. J., Grogan, D. W. & Taylor, J. W. 2003 Geographic barriers isolated endemic population of hyperthermophilic archaea. *Science* **301**, 976–978. (doi:10.1126/science.1086909)

Whitaker, R. J., Grogan, D. W. & Taylor, J. W. 2005 Recombination shapes the natural population structure of the hyperthermophilic Archaeon *Sulfolobus* '*islandicus*'. *Mol. Biol. Evol.* **22**, 2354–2361. (doi:10.1093/molbev/msi233)

Whitfield, J. 2005 Biogeography. Is everything everywhere? *Science* **310**, 960–961. (doi:10.1126/science.310.5750.960)

Wise, M. G., Shimkets, L. J. & McArthur, J. V. 1995 Genetic structure of a lotic population of *Burkolderia (Pseudomonas) cepacia*. *Appl. Environ. Microbiol.* **61**, 1791–1798.

Wright, S. 1932 The roles of mutation, inbreeding, crossbreeding and selection in evolution. In *Proc. VI Int. Congress on Genetics* (ed. D. F. Jones).

Wright, S. 1943 Isolation by distance. *Genetics* **28**, 114–138.

Wu, X.-L., Friedrich, M. W. & Conrad, R. 2006 Diversity and ubiquity of thermophilic methanogenic archaea in temperate anoxic soils. *Environ. Microbiol.* **8**, 394–404. (doi:10.1111/j.1462-2920.2005.00904.x)

Yannarell, A. C. & Triplett, E. W. 2005 Geographic and environmental sources of variation in lake bacterial community composition. *Appl. Environ. Microbiol.* **71**, 227–239. (doi:10.1128/AEM.71.1.227-239.2005)

Zaballos, M. *et al.* 2006 Comparison of prokaryotic diversity at offshore oceanic locations reveals a different microbiota in the Mediterranean Sea. *FEMS Microbiol. Ecol.* **56**, 389–405. (doi:10.1111/j.1574-6941.2006.00060.x)