Adaptation in Natural Microbial Populations

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Abstract

Although their diversity greatly exceeds that of plants and animals, microbial organisms have historically received less attention in ecology and evolutionary biology research. This knowledge gap is rapidly closing, owing to recent technological advances and an increasing appreciation for the role microbes play in shaping ecosystems and human health. In this review, we examine when and how the process and patterns of bacterial adaptation might fundamentally differ from those of macrobes, highlight methods used to measure adaptation in natural microbial populations, and discuss the importance of examining bacterial adaptation across multiple scales. We emphasize the need to consider the scales of adaptation as continua, in which the genetic makeup of bacteria blur boundaries between populations, species, and communities and with them concepts of ecological and evolutionary time. Finally, we examine current directions of the field as we move beyond the stamp-collecting phase and toward a better understanding of microbial adaptation in nature.

INTRODUCTION

> A statement of the importance of studying microorganisms to increase understanding of the evolutionary process is required because of the near total exclusion of microbiology from the neo-Darwinian synthesis. This exclusion was not intentional but occurred in part because bacterial species and their phylogenetic relationships were nearly impossible to define until recently. Consequently, microbiology has remained the least evolution-oriented of the biological disciplines.
>
> —Dykhuizen (1990)

In his 1990 review, Daniel Dykhuizen highlighted the power of using simple flasks with broth or agar plates inoculated with a single clone to study the process of evolution in bacterial populations (Dykhuizen 1990). Indeed, 25 years since his review was written, experimental evolution in the laboratory has reshaped much of our understanding of how microbial species respond to selection (Buckling et al. 2009). An equally impressive wave of scientific discovery has since arrived in microbial ecology, in which technology-driven studies continue to reveal novel phylogenetic and functional groups found across highly diverse environments. Although microbial ecologists have historically worked largely in isolation from their macrobial counterparts, and despite differences in methodology, tradition, and the types of organisms under study, the two fields are now converging, allowing for a deeper understanding of the evolution and ecology of microbial life around us (Prosser et al. 2007).

Much of our current knowledge of microbial adaptation in nature comes from observational or comparative studies that characterize patterns within and among natural populations and communities, whereas our understanding of the process of microbial adaptation has primarily been gained from experimental evolution studies performed under artificial laboratory conditions (**Figure 1**). Therefore, one outstanding question in the field is how well predictions generated from in vitro results meaningfully translate to patterns observed in nature—and vice versa—despite the increased ecological complexity of these environments. Indeed, experimental evolution studies incorporating more realistic ecological conditions have repeatedly demonstrated strikingly dissimilar patterns to those previously uncovered under more artificial conditions. A set of experiments conducted under seminatural conditions in which the target study organism, *Pseudomonas fluorescens*, was introduced into soil microcosms either with or without the natural microbial community highlights this point well. First, coevolution between the bacterium and its bacteriophage parasite was found to follow an entirely different trajectory in the presence of the natural community than it did in vitro, likely owing to the increased realization of costs associated with phage resistance in the presence of competitor species (Gómez & Buckling 2011). Second, adaptive diversification of the bacterium was greatly reduced in the presence of the natural community, most likely because there were less available niches to fill and therefore weaker selection for expanded resource use (Gómez & Buckling 2013).

In this review we first discuss the evolutionary mechanisms underlying adaptation of bacterial populations and introduce the numerous approaches used to measure the process and patterns of adaptation in nature. We then highlight studies that characterize change over time, divergence among populations over space, and factors structuring both population and communities. We argue that the process of adaptation follows a continuum across scales and that real insight into the patterns observed in nature only comes through an appreciation of scale and use of a combination of approaches. We focus our discussion on prokaryotes (Bacteria and Archaea) and exclude eukaryotic microbes or viruses (when not in the context of their bacterial hosts).

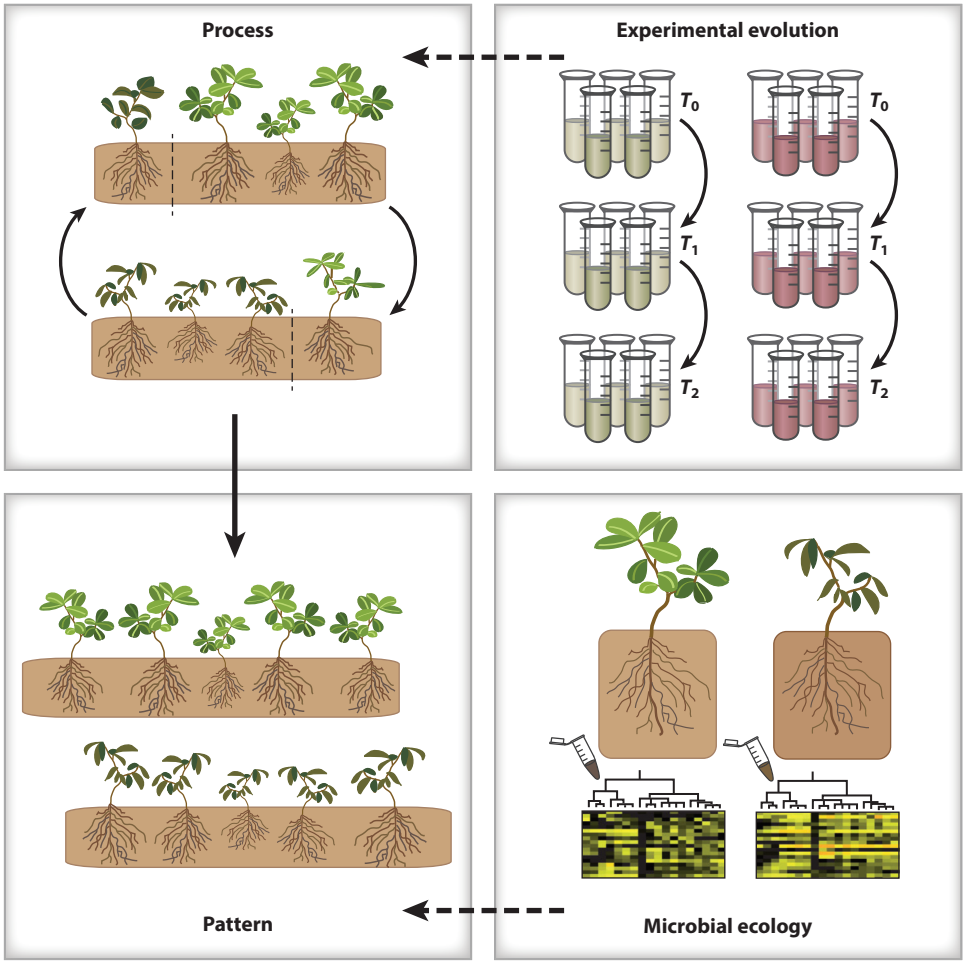

Figure 1

Illustration of approaches used to understand the patterns and underlying processes of microbial adaptation in nature (*dashed arrows*). The process of microbial adaptation can be directly tested using experimental evolution approaches (*top right* panel), in which the abiotic or biotic environment is manipulated and the response of initially identical bacterial populations/communities is measured over time (T) in response to each treatment. The process of adaptation can also be inferred through reciprocal transplants. For example, in the case of soil-associated microbes, plant traits could be compared when grown in the presence of sympatric versus allopatric microbial communities to test for microbe-mediated versus environment-mediated effects (*top left* panel). An alternative approach is to test hypotheses on the outcome of adaptive processes using observations of natural patterns, for example by comparing the composition of plant-associated microbial communities across space or host species (*bottom left* panel). One powerful tool to study microbial ecology, and quantify patterns of adaptation across time or space, is the use of next generation sequencing to compare microbial populations and communities directly, taking into account phylogenetic relatedness among samples (*bottom right* panel). In this way the similarities among whole microbial communities can be compared across biotic or abiotic environments and patterns of adaptation can be uncovered at the genomic level.

MEASURING ADAPTATION IN NATURAL BACTERIAL POPULATIONS

Fitness: the reproductive success of an individual during its lifetime or the relative contribution of an individual's progeny to the next generation

Transduction: the accidental transfer of DNA from one bacterial cell into another bacterial cell by infective bacteriophage

The key starting point for the study of ecological and evolutionary processes in the microbial world is a clear understanding of microbial fitness. This understanding is no small feat, however, as bacterial lifespan and reproduction can be influenced by temperature, nutrient availability, and stress levels (e.g., the presence of antibiotics or bacteriophage predators), and the response is often nonlinear as well as contrasting across species and strains. For example, manipulation of temporal substrate patchiness (i.e., how often nutrients are supplemented into the growth media) across two marine bacteria demonstrated that although one species out-competed the other under a one-time supplementation, the other species performed best when nutrients were added hourly (Pernthaler et al. 2001). Evidence from *Escherichia coli* cells grown in a microfluidic chamber, allowing researchers to follow the life history of individual cells, suggested that although bacterial reproductive rate remains constant throughout the lifetime of a cell, cell death is typically the result of aging due to the accumulation of cell damage (Wang et al. 2010). Because the latter depends on the environment in which the cell is growing, the lifetime reproduction of genetically identical bacterial cells could be highly variable across conditions, as exemplified by the common discrepancy between generation times measured in the laboratory setting and those measured in natural populations (Jannasch 1969).

Culture-Dependent Methods

Studying strains isolated from natural populations under controlled laboratory environments usually reveals a wide diversity of phenotypes with potential adaptive significance. However, these studies are typically limited by the small minority of species that can be cultivated with current techniques and can be biased by the experimental conditions under which they are assayed as well by the subset of traits being measured. Whole-genome sequencing of isolates allows a reverse ecology approach to understanding adaptation (Shapiro & Polz 2014), in which the presence of genetic variation in genes of known function offers clues to ecological differentiation. For example, two oceanic *Vibrio cyclitrophicus* populations in a very early stage of ecological specialization were found to differ in genes controlling biofilm formation and host colonization (Shapiro et al. 2012); this difference could be linked specifically to habitat, thereby representing likely signatures of selection. One key drawback of this approach is that the vast majority of bacterial genes are of unknown function. As such, purely bioinformatical approaches remain limited, necessitating much more laborious subsequent genetic manipulation of niche-associated genes to link sequence to function. An alternative approach is to differentially mark distinct clonal lineages isolated from nature and directly compete these in microcosms that mimic natural conditions. In this way researchers can elucidate fitness trade-offs, examine differences in growth among environments, and compare competitive ability across biotic and abiotic environments. Experimental microcosms can range from the completely artificial (e.g., broth in shaken flasks, agar plates, or microfluidic devices) to almost natural conditions (e.g., containers with unsterilized water or soil samples) (Vos et al. 2013), and manipulation can range from simple (e.g., incubation under different temperature regimes) to complex (e.g., manipulation of community composition) (Celiker & Gore 2014, Lawrence et al. 2012). A much less common approach is the incubation of isolated clonal lineages in their original environment. An early but elegant example is a study in which bacteriophage transduction frequencies were measured in a *Pseudomonas aeruginosa* laboratory strain incubated in a polycarbonate cylinder (sealed with 0.2 μm membranes to allow nutrient diffusion) that was suspended in a lake (Morrison et al. 1978).

Culture-Independent Methods

Molecular methods based on selective sequencing of marker genes or the nonselective sequencing of metagenomes have become a standard tool in microbial ecology, as they circumvent the Great Plate Count Anomaly. Sequencing of the phylogenetic marker gene 16S rRNA gives broad insights into community composition and has become the most common approach for characterizing microbial diversity (Ward et al. 1990). However, it is also possible to sequence genes known to be involved in specific ecological functions. Perhaps the most widespread use of this approach has been to examine the prevalence and spread of genes conferring antibiotic resistance in natural, agricultural, and clinical settings (Allen et al. 2010). However, this approach can also be used to explore microbial adaptation to specific environmental conditions. For example, sequencing of a key functional gene involved in ammonia oxidation in Archaea, *amoA*, from soil samples spanning a range of spatial scales revealed that specific lineages were associated with particular soil pH ranges and not with any other physicochemical characteristic (Gubry-Rangin et al. 2011), shedding light on nitrogen cycling and soil ecosystem function. Metagenomic approaches are currently limited by the huge diversity of species and genes, the latter of which are mainly comprised of unknowns and present a formidable computational, statistical, and biological challenge (Marx 2013). However, in the case of genes of known function, the presence of particular sequences can be correlated with particular environmental characteristics (e.g., Hemme et al. 2010). Genes responsible for phenotypes of interest can be discovered through functional metagenomics, in which random sequence fragments isolated from a community sample are cloned into a vector, allowing host bacteria to be screened for specific traits encoded by these sequences (e.g., Culligan et al. 2012). Correlations between genotypes and environments are important to establish, but causality can be more directly addressed through controlled manipulation of the environment, such as the artificial warming of soil (Rousk et al. 2012).

Great Plate Count Anomaly: the observation that the vast majority of bacterial species cannot currently be cultivated as the appropriate growth conditions are not known

EVOLUTIONARY MECHANISMS UNDERLYING ADAPTATION

Evolution by natural selection is ultimately reliant on genetic variation, and a wide array of mechanisms can create novel genetic variants in bacterial populations. These mechanisms range from simple point mutations to large deletions of chromosomal regions and parasexual processes, in which cells actively or passively procure DNA from the environment and incorporate it into their genome. As the speed with which bacteria adapt to their environment is crucially dependent on the rate and type of genetic variation supplied to populations, this section summarizes the range of important variation-generating mechanisms.

Genomic Change from Within: Mutation, Deletion, Duplication, and Transposition

Mutation is arguably the best-known form of genetic change; in its simplest form, an individual nucleotide is replaced by another type of nucleotide. When coding for an amino acid change, point mutations [or single nucleotide polymorphisms (SNPs)] can lead to the acquisition of novel traits (e.g., SNPs in the *rpoB* gene confer resistance against the antibiotic rifampicin; see Jin & Gross 1988). When they occur in a regulatory gene, mutations can vastly alter patterns of gene expression and control major phenotypic changes such as multicellular development (Yuen-Tsu et al. 2010). Mutations are generally quite rare; a recent study on *E. coli* found that point mutations occurred approximately only once in a thousand generations per genome (Lee et al. 2012), and the available estimates for other bacterial species are on the same order of magnitude (Sung et al.

2012). However, some populations of bacteria have been found to harbor appreciable numbers of strains with an elevated mutation rate caused by defective methyl-directed mismatch repair (so-called mutators; e.g., Oliver et al. 2000). Mutators can realize a fitness advantage when adapting to a novel environment, as they are able to supply new beneficial mutations at a faster rate than wild-type strains. However, when the environment becomes more stable, or once beneficial mutations have been fixed, mutators tend to become disadvantaged because the majority of mutations are deleterious (Giraud et al. 2001b). For example, a series of elegant experiments on an *E. coli* mutator strain demonstrated a more rapid initial adaptation to the mouse gut environment than its wild-type ancestor with normal mutation rate but a disappearance of this advantage over time as adaptive mutations were acquired by both strains (Giraud et al. 2001a).

Genomic changes can be much more substantial than single base pair substitutions. Over the course of 1,500 generations of laboratory evolution in *Methylobacterium extorquens*, 80% of replicate populations were found to have lost the same large region of their genome (Lee & Marx 2012). This parallel loss was not observed in the absence of selection, indicating that although the deletions must be random, their fixation in the population was not. Importantly, genome reduction was not found to be beneficial per se: Longer deletions did not generate higher fitness. Instead, selection seemed to favor the loss of particular genes that did not contribute to fitness under the specific lab conditions, and fitness was found to be lower than the ancestral population when measured under alternative laboratory conditions (Lee & Marx 2012). Gene duplications resulting from replication and repair errors can also contribute to the flexibility of microbial genomes, as a gene copy can be selected to perform a novel function while the function of the original copy is not affected. The Innovation-Amplification-Divergence model (Näsvall et al. 2012) posits that when a weak, secondary gene function becomes more important (e.g., after a change in environment), gene duplication is favored because it results in increased production of gene products. Selection for—rather than against—multiple gene copies thus allows copies to accumulate different beneficial mutations and eventually diverge in function. Evolution experiments have demonstrated that such specialization of duplicated genes indeed readily occurs (Näsvall et al. 2012).

Lateral gene transfer (LGT): the transfer of genes between contemporary cells (in contrast with vertical transfer of genes from mother to daughter cells)

Transformation: the uptake of free DNA from the environment followed by recombination

Conjugation: transfer of DNA in the form of a (circular) plasmid between cells that are in physical contact

Clonal interference: competition between different beneficial mutations present in otherwise clonal individuals

Genomic Change from Without: Incorporating Foreign DNA

Although eukaryotes experience hybridization and lateral gene transfer (LGT) (Keeling & Palmer 2008), their level of genetic promiscuity is minute compared with that of prokaryotes. Horizontal modes of inheritance are so frequent in bacteria that vertical patterns of descent can be largely obscured, complicating the reconstruction of evolutionary histories (Puigbo et al. 2013). A wide variety of mechanisms are capable of moving relatively short fragments of DNA between cells. The three best-studied mechanisms are transformation, conjugation, and transduction [although novel types of LGT are still being discovered; e.g., nanotube-based mechanisms (Dubey & Ben-Yehuda 2011)]. One crucial distinction among mechanisms is whether they themselves are likely to represent a bacterial adaptation or not (Seitz & Blokesch 2013). For mechanisms involving infectious elements, such as in conjugation and transduction, this is mostly not the case. However, transduction (Johnston et al. 2014), in which cells actively take up free DNA from the environment, has been argued to be adaptive (Vos 2009). Another way to classify bacterial gene transfer is on the basis of the type of DNA transferred: homologous stretches (novel or identical alleles) or nonhomologous stretches (novel genes). In the first case, bacterial recombination resembles gene conversion in eukaryotes and results in the creation of novel combinations of mutations. By combining different beneficial mutations in one genome, this process can alleviate clonal interference. Indeed, experimental evolution of either naturally transformable or nontransformable mutants of the human-associated bacterium *Helicobacter pylori* demonstrated more rapid adaptation

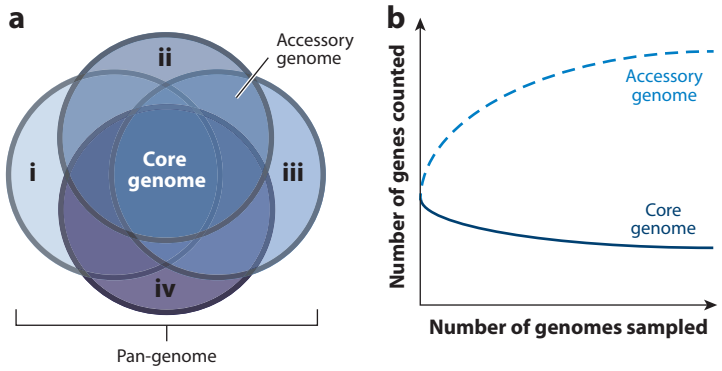

Figure 2

Illustration of the fluidity of prokaryote genomes. (*a*) A Venn diagram depicting gene content of four bacterial genomes (*i–iv*). The genes that are shared between all genomes are part of the core genome, genes that are present in fewer than four genomes are part of the accessory genome, and the total complement of genes is termed the pan-genome. (*b*) As the genomes of new strains are sequenced, the discovery of more accessory genes will increase pan-genome size and the size of the core genome will decrease as some genes present in known strains will not be present in new strains.

of competent populations when evolved in a novel laboratory environment (Baltrus et al. 2008). In the second case, in which genes that are genetically and functionally distinct from those already present in the genome are transferred, wholesale changes in phenotype can occur (e.g., Hehemann et al. 2010). Classification of uptake by type of foreign genes is not clear-cut, however, as LGT events can occur not only through nonhomologous recombination but also through homologous recombination in which genes that are not shared between donor and recipient are flanked by genes that are shared (Polz et al. 2013).

Horizontal transfer of homologous or nonhomologous DNA, through active uptake or through infective intermediaries, can play a profound role in the evolution of natural populations of bacteria. A meta-analysis uncovered that in over half of all prokaryote species analyzed, homologous recombination contributed more to genetic diversity than to point mutation (Vos & Didelot 2008). Likewise, it has been shown that new gene copies arise more often through LGT than through duplication (Treangen & Rocha 2011), and population genomic studies have revealed that isolates with nearly identical nucleotide composition in the genes they share can differ by many hundreds of accessory genes (e.g., Nowell et al. 2014), indicating that LGT might be more important than mutation (**Figure 2**). Indeed, over large evolutionary timescales LGT events can completely transform the genomic makeup, metabolism, and ecological lifestyles of bacterial lineages (e.g., Nelson-Sathi et al. 2012). Although phylogenetic distance is thought to form a significant barrier to the success of gene transfer (e.g., Popa et al. 2011), ecology can override phylogeny in determining patterns of gene flow. For example, in a bioinformatics study on bacteria inhabiting the human body, it could be demonstrated that shared body site or oxygen tolerance was the best predictor of gene transfer rate (Smillie et al. 2011).

Competence: the physiological state in which bacteria pick up free DNA from the environment, of which some fragments can be incorporated in the genome

The Efficacy of Selection

Random genetic changes, be they individual point mutations or the uptake of large genomic islands, are the raw ingredients for evolution, with the fate of any genetic change determined by the balance between (nonrandom) natural selection and (random) genetic drift (Nielsen et al. 2013). The balance between these two forces is determined by (*a*) the selection coefficient acting on a novel

change and (*b*) the effective population size (N_e). The first parameter is relatively straightforward, but the second, N_e, is more elusive (Lanfear et al. 2014). By definition, N_e is smaller than the actual (census) population size. This size difference is due to, for instance, population bottlenecks caused by host-to-host transmission of pathogens and symbionts or by blooms in seasonal environments. Neutral diversity in bacteria ranges over several orders of magnitude, and very low diversity species, such as *Yersinia pestis*, likely have relatively small census population sizes, experience frequent bottlenecks upon transmission, and have emerged relatively recently (Achtman 2008). For more ubiquitous species, N_e estimates range from 10^7 (the gut bacterium *E. coli*; see Charlesworth & Eyre-Walker 2006) to 10^{11} (the oceanic photosynthesizing *Prochlorococcus*; see Baumdicker et al. 2012). Difficulties in reliably estimating N_e aside, it is obvious that this parameter differs widely for species with distinct ecologies and has great potential to differentially influence the process of adaptive evolution.

Effective population size (N_e): the number of individuals that equally contribute to future generations

BACTERIAL ADAPTATION ACROSS SPACE

Historically, a central question in microbial ecology and evolution research has been whether adaptation to the local environment is more often the result of mutational change or of colonization by a particular preadapted clonal lineage or species. The idea that everything is everywhere, but the environment selects, first put forward by Baas Becking (1934), is still frequently cited by microbiologists (De Wit & Bouvier 2006). There is now good evidence not only that the environment selects for success of particular immigrating species but also that microbial species are dispersal limited (e.g., Bell 2010, Finkel et al. 2012, Östman et al. 2010, Telford et al. 2006). Note that, although there are adaptations to increase the probability and distance of dispersal, such as the formation of raised structures containing spores, we use the term dispersal in this review to mean passive displacement (e.g., the dispersal of cells by splashing raindrops or ocean currents). Furthermore, there is clear evidence that bacterial populations can rapidly adapt to local environmental conditions upon arrival, further differentiating populations across space. Work on the cyanobacterium *Mastigocladus laminosus* from thermal springs and streams in Yellowstone Park nicely demonstrates the interplay between the local environment and dispersal in shaping bacterial adaptation. Populations sampled along a 1-km temperature gradient showed evidence of adaptation to local temperature (54°C upstream and 39°C downstream) despite frequent gene flow, as demonstrated using genetic markers (Miller et al. 2009). Sequence data suggested that selection acting on one small (~5 kB) genomic region containing genes involved in nitrogen fixation led to divergence in both homologous sequence and gene content across populations.

Bacterial Biogeography

Passive dispersal of bacteria through the atmosphere is likely to be extensive (Smith et al. 2013), as is dispersal via ocean currents. However, a model developed for the ubiquitous marine planktonic bacterium *Pelagibacter ubique* showed that currents are not extensive enough to erase genetic divergence of populations that inhabit different parts of the ocean (Hellweger et al. 2014). Similarly, a study measuring bacterial colonization across initially identical sterile microcosms along a 497-m woodland transect found evidence of dispersal limitation over short timescales (a few days) (Bell 2010). This study also demonstrated that such limitation was not important in shaping community composition over longer timescales (more than a week), at which point the local environment became the more important explanatory variable. In addition, research characterizing the distance-decay relationship among bacterial colonists of the leaf surfaces of salt-excreting *Tamarix* trees

along a 500-km transect found not only a strong signature of geographic distance in shaping community composition but also evidence that salinity and humidity were important environmental factors in explaining community dissimilarity (Finkel et al. 2012). This evidence highlights how the scale at which adaptation occurs can be defined both by spatial distance and by the spatial heterogeneity of selection across the landscape (Hanson et al. 2012).

Quorum sensing: the regulation of gene expression in response to fluctuations in population density

Relative to larger eukaryotes, we might expect the diversity of habitats and particular niches within habitats to be even more pronounced for bacterial colonists given their small size. For example, although genetic differentiation among plants adapted to differing abiotic conditions such as levels of toxins, fertilizers, herbicides, or light availability is typically found to be on the order of meters to kilometers (Linhart & Grant 1996), bacterial adaptation to the abiotic environment can occur across much smaller spatial scales. Bacterial community composition of soils from tropical forest was found to differ across a 150-m transect, primarily in response to local pH conditions (Tripathi et al. 2014), whereas the ability to metabolize glucose was found to vary up to 10,000-fold across microbial communities that were less than 1 cm apart (Becker et al. 2006). For plants adapting to local biotic conditions, such as competitors, herbivory, and pollination, the scale of genetic differentiation is typically found to be meters (Linhart & Grant 1996), whereas the scale of biotic adaptation for bacterial populations can be dramatically smaller. For instance, different quorum sensing types of *Bacillus subtilis* can be found only millimeters apart (although the adaptive significance of their distributions is not yet well understood; see Stefanic et al. 2012). In many cases we may expect the spatial scale of bacterial adaptation to be influenced by the spatial structure of eukaryotic populations and/or communities—and vice versa. This is especially true for those microbes whose fitness depends either directly or indirectly on the presence of a particular eukaryotic host. By altering the local environmental conditions, for example, the presence of particular host species can increase or decrease the relative fitnesses of different microbial species. Similarly, the distribution of microbial populations and communities across space can influence the relative fitness of eukaryotes. For example, many bacteria are able to tolerate heavy metals in the environment by either sequestering the metals or through enzymatic detoxification (Mejáre & Bülow 2001), both of which alter the local concentrations of metal and can therefore have important cascading effects on the spatial structuring of eukaryotic populations and communities across a landscape.

Bacterial Local Adaptation

Perhaps the most common and straightforward approach to understanding the spatial scale of microbial adaptation is a comparison of the fitness of clonal isolates from the same environment in either their local environment or a foreign environment (reviewed in Kawecki & Ebert 2004). This measurement of local adaptation requires a priori prediction regarding the spatial distance at which the selective pressures shaping fitness might differ. With this information, a reciprocal transplant among sites can be performed to test for adaptations that have evolved in response to one environment versus the other. Returning to plant–microbe interactions, we might predict that the environment to which plants are locally adapted is, at least in part, due to microbial community composition and vice versa. Indeed, a reciprocal transplant study comparing local adaptation of grass species (which are not directly affected by nitrogen-fixing bacteria) with that of legumes (which are directly affected by nitrogen-fixing bacteria) found that grass species were primarily locally adapted to climatic conditions, whereas legumes performed much better when grown in their local soil (Macel et al. 2007). Similarly, a study of bacterial local adaptation to soil from across an old-growth forest demonstrated a decrease in fitness at an approximate rate of 6% per meter

as bacteria were transplanted away from their home site; this rate is similar in scale to plant local adaptation (Belotte et al. 2003).

Although local adaptation experiments are extremely useful in characterizing the strength and spatial scale of adaptation for particular systems, such results are likely not generalizable across microbial systems. As mentioned above, the spatial scale of adaptation is affected by the rate of dispersal and the spatial heterogeneity of the environment, both of which are likely to differ even for the same bacterial species found in two regions or the same pairwise interaction occurring in different environments. For example, in two studies examining phage local adaptation to their bacterial hosts, the spatial scale of adaptation was strikingly different: In one case, differences were found across soil populations separated only by centimeters (Vos et al. 2009), whereas in the other case, no difference was found across leaves from the same horse chestnut tree but strong phage local adaptation was observed to bacteria from the same versus neighboring trees (Koskella et al. 2011). Such differences across systems could be due to the presence of other selection pressures, such as soil composition or tree defenses that shape the spatial differentiation among populations, or could reflect differences in dispersal and/or adaptive potential. Reciprocal transplant experiments of microbial populations and communities across soil types have found similarly mixed results. Although one study found no evidence for bacterial local adaptation to soil from forest floors dominated by trembling aspen versus white spruce (Hannam et al. 2007), other studies have found evidence for bacterial community composition shifts during reciprocal transplants among high-altitude meadow and forest soils (Bottomley et al. 2006) as well as among three deglaciated unvegetated sites along a soil moisture and soil temperature gradient (Zumsteg et al. 2013). Together, the data from bacterial local adaptation studies as well as those characterizing spatial structure using genetic markers suggest that population differentiation can occur across a range of scales, from surprisingly small to surprisingly large (**Figure 3**).

BACTERIAL ADAPTATION ACROSS TIME

Given their relatively short generation times, large population sizes, and flexible genomes, the temporal scale over which a bacterial population can respond to environmental change is likely to differ from that of larger organisms. Whereas the timescale of genetic differentiation of plant populations is measured in years (Linhart & Grant 1996), there is evidence for divergence between natural bacterial populations (Lieberman et al. 2011) and communities (Diaz-Ravina & Baath 1996, Koskella 2014) in well under a year, with divergence among replicate experimental populations occurring within only days (Buckling & Rainey 2002, Lenski & Travisano 1994). Just as for larger eukaryotes, the rate of evolutionary change in bacterial populations is dictated in part by the speed at which the local environment changes. In the case of coevolving bacteriophages (Buckling & Rainey 2002, Koskella 2013) or interacting bacterial species (Hillesland & Stahl 2010), the process of bacterial adaptation may be continual, as interacting species respond to one another in an ongoing coevolutionary race without ever reaching a stable optimum. However, there are also many cases in which adaptation to the local biotic environment is comparable with adaptation to the local abiotic environment—for example, adaptation of a bacterial pathogen to its long-lived host (Toft & Andersson 2010). Similarly, as many bacterial species modify their local environment—for example, by removing antibiotics (Wright 2005), sequestering iron (Wandersman & Delepelaire 2004), or reducing nitrates (López-Gutiérrez et al. 2004)—the local abiotic environment may change more rapidly than the biotic environment. As such, rather than thinking about the temporal scale of adaptation as different depending on biotic versus abiotic environments, as we might usefully do for eukaryotes, it is perhaps more helpful to think about the temporal scale as a continuum.

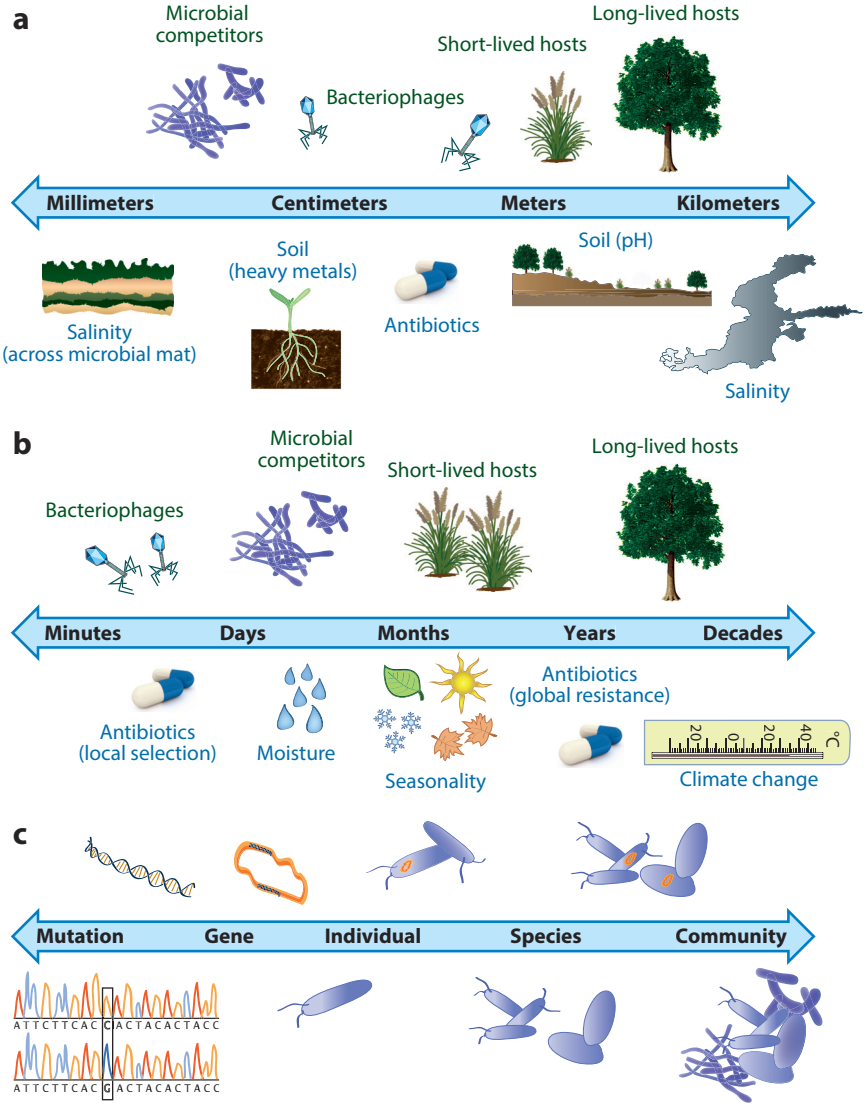

Figure 3

Three panels describing the scales of bacterial adaptation in nature. (*a*) Exploration of the spatial scales of adaptation, in which abiotic selection gradients can range from millimeters (as is the case for salinity across a microbial mat; Kunin et al. 2008) to many kilometers (as is the case for salinity across the Baltic Sea; Herlemann et al. 2011). Similarly, biotic selection gradients can range from very small scales (e.g., for bacteria coevolving with competitor species or bacteriophage viruses; Vos et al. 2009) to very large scales (e.g., for bacteria inhabiting long-lived hosts; Koskella et al. 2011). (*b*) Illustration of the continuum in temporal scales for bacterial adaptation, in which the environment can change rapidly (e.g., as antibiotic concentrations decline with enzymatic degradation; Wright 2005) or relatively slowly (e.g., following cultural shifts in human use of antibiotics over time or climate change; Wallenstein & Hall 2012). (*c*) Depiction of the levels at which selection can act to shape bacterial adaptation, from single mutations to whole communities, especially in light of the mobility of genes (e.g., via plasmids) among bacterial species.

Adaptation in Response to the Abiotic Environment

Phenotypic plasticity: production of different phenotypes by a single genotype as a response to environmental conditions

Abortive infection: process whereby a bacterial cell commits suicide upon infection in order to prevent phage reproduction

Just as for eukaryotes, the abiotic environment experienced by bacteria can vary over a wide range of timescales (**Figure 3**), from within a single generation to epochs. The stability of the environment relative to the generation time of a bacterium is an important factor in shaping evolutionary predictions, as we might expect more rapid environmental change to select for phenotypic plasticity and more long-term change to result in genetic change. Many terrestrial bacterial cells, for example, must cope with drastically changing environmental conditions over the course of their life span as a result of diurnal changes in temperature, UV radiation, and moisture. These rapid fluctuations have resulted in numerous adaptations that can be considered plastic, including light-dependent gene regulation (El-Shehawy et al. 2003), daily shifts in activity levels of aerobic versus anaerobic bacteria as a result of changing levels of plant-released oxygen (Nikolausz et al. 2008), and altered growth and reproduction in response to fluctuating substrate availability over the course of the day (Pernthaler & Pernthaler 2005). Cyanobacteria, for example, have circadian programming of gene expression even when cells divide in less than 24 hours, and this pattern is maintained under conditions of constant illumination (Johnson et al. 1998). Of course not all fluctuations are so regular or predictable: Pulses in resource or moisture levels over time are likely a commonality across niches in the microbial world. Experimental manipulation of fluctuations in soil moisture availability in Great Plains grassland sites resulted in functional differences among microbial communities, in which increasing moisture variability was associated with increased demand for nitrogen and decreased efficiency of carbon usage (Tiemann & Billings 2011). Furthermore, *E. coli* lineages experiencing fluctuating resource availability evolved greater maximum growth rate and shorter lag time for growth upon the arrival of new nutrients relative to their ancestor (Vasi et al. 1994). Bacterial response to environmental changes occurring over longer timescales, from seasonality to climate change, has also led to specific microbial adaptations. A study of pseudomonads isolated from sugar beet leaves during the course of three growing seasons found evidence for seasonal recurrence of particular genotypes, such that certain groups of pseudomonads performed well at one point in the season but were replaced by others as conditions shifted (Ellis et al. 1999).

Adaptation in Response to the Biotic Environment

Just as with the abiotic environment, change in the biotic environment experienced by bacteria can vary in timescale (**Figure 3**) and can result in either increased phenotypic plasticity or genetic change. Even in response to the same biotic selection pressure, such as bacteriophages, bacteria can respond via numerous resistance mechanisms that are either plastic (such as abortive infection or phase variation in expression of surface receptors to which phages bind) or genetic (such as mutations leading to loss or alteration of particular surface receptors) (reviewed in Labrie et al. 2010). Different responses may result from differences in the strength and continuity of phage-mediated selection, especially as phage prevalence can vary predictably over time. Phage infection of bacterioplankton in the North Sea has been found to follow diurnal cycles, with the highest prevalence of infected cells occurring after peaks in bacterial growth and cell lysis typically occurring overnight (Winter et al. 2004), and the abundance of phages from the rumen of sheep fed once daily was found to peak between 8 and 10 hours after feeding (Swain et al. 1996). Temporal change in other biotic selection pressures, such as predator-mediated selection, is also likely, and evidence for seasonal variation in the abundance of bacterivorous nematodes in soil after a peak in bacterial diversity and abundance has been documented (Papatheodorou et al. 2004).

Time Shift Approach to Measuring Adaptation

A particularly powerful approach for measuring the rate of adaptation of bacterial populations to particular environments is the use of time shift experiments, in which the fitness of individuals from the past, present, and future are directly compared in a common garden (Blanquart & Gandon 2013). This approach can measure both the temporal pattern and magnitude of adaptation in response to either biotic or abiotic environmental change. For example, in the laboratory, bacterial populations from 15 bacterial generations (4 days) in the future were found to be more resistant to contemporary phages than bacteria from the contemporary time point (Buckling & Rainey 2002), suggesting they had evolved resistance over the course of the experiment. Similarly, examination of horse chestnut phyllosphere bacteria resistance to sympatric phage populations demonstrated bacterial adaptation and phage counteradaptation across the growing season, such that bacteria were more resistant to phages from a month earlier and less resistant to phages from a month later (Koskella 2013). Although few time shift experiments have been used to understand the rate of bacterial adaptation to phages in nature, experimental microcosm results suggest that both increased mixing of populations (Brockhurst et al. 2003) and increased resource supply (Lopez-Pascua & Buckling 2008) can accelerate bacterial adaptation to phages, and vice versa.

Time shift approaches can also be used to understand the rate of adaptation to abiotic conditions. For example, little evidence for adaptation to local water chemistry was uncovered when comparing growth of bacterial isolates in lake water from across three time points, separated by 3 and 22 months (Fox & Harder 2015). A similar approach was used to examine the importance of past (2 to 8 days earlier) versus contemporary environmental conditions on the structure of bacterial communities from rock pools in Sweden (Andersson et al. 2014). In this case, spatial differences in bacterial community composition were better explained by salinities at the earliest time points than those at the contemporary time point or in the more recent past. Together, these time shift experiments demonstrate that bacterial adaptation to a local environment might only be fully appreciated through incorporation of a time axis (Koskella 2014).

POPULATION- VERSUS COMMUNITY-LEVEL ADAPTATION

Microbes are key to biogeochemical cycling and ecosystem functioning, processes that are generally performed at the level of whole communities rather than populations (Torsvik & Øvreås 2002). Community adaptation can be defined as a shift in community composition following exposure to a novel environmental regime that leads to increased productivity and ecosystem performance. Such adaptation can take place over long timescales (as is the case with global warming) or in much shorter, recurring timescales (as is the case with seasonal changes). For instance, analysis of microbial community composition in soil from an alpine dry meadow found not only strikingly different species composition before and after snowmelt but also sharp metabolic shifts. Microbial communities from pre-snowmelt samples had higher levels of respiration at 0°C relative to 24°C than did communities sampled post-snowmelt (Lipson et al. 2002). With growing interest in global climate change, an increasing number of studies are testing how temperature and other environmental variables influence key microbial community functions such as nitrification, productivity, and decomposition (Wallenstein & Hall 2012). In contrast to experiments in which an ancestral clone placed in a novel environment is tracked over evolutionary time, community-level studies usually measure the rate of change of a focal ecosystem function due to differential species growth or death following an environmental manipulation. These studies increasingly use metagenomic sequencing to correlate functional changes with shifts in phylogenetic

community composition and/or gene presence, as well as metatranscriptomics to explore how gene expression at the community level might change across environments.

Cross-feeding (syntrophy): one species feeding on the metabolic products of another species

Commensalism: a species interaction whereby fitness is positively affected in one partner and not affected in another partner

Genes Versus Species

The distinction between the change in frequency of alleles in a bacterial population (one species) and the change in frequency of species in a microbial community is not clear-cut (**Figure 3**), and both population genetics and community ecology can be analyzed within the framework of neutral evolution (Hu et al. 2006). Populations consist of alleles that are introduced through mutation or gene flow; equivalently, communities consist of species that are introduced through speciation or migration. The null hypothesis is that distributions of specific alleles or species are governed by random forces (drift) and selection needs be invoked only when distributions deviate from the theoretical expectation. Interestingly, in microbial ecology, 16S rRNA marker gene sequences are typically equated with species, thereby removing the boundary between community ecology and population genetics. Gene transfer among species by LGT further blurs distinctions between the two fields.

One recent study illustrates how changes in species abundances in a community co-occur with changes in gene abundances within species in response to environmental perturbation by investigating resistance against quaternary ammonium compounds (QACs), biocides that persist in the natural environment (Oh et al. 2013). Three bioreactors, one provided with dextrin/peptone (control), one with dextrin/peptone and a QAC, and one with only a QAC, were inoculated with the same polluted environmental sample. After prolonged time, QAC resistance was quantified and unsurprisingly was highest in the QAC-only reactor and lowest in the control. When changes in community composition were assessed through metagenomics and amplicon sequencing, it was found that QAC exposure led to the disappearance of many taxa and the enrichment of *Pseudomonas nitroreducens*. The selective amplification of this species was accompanied by specific point mutations in genes implicated in QAC metabolism as well as by putative LGT events.

The Black Queen Hypothesis

The interplay between genomic evolution within species and species turnover within communities has recently been highlighted in the form of the Black Queen hypothesis (Morris et al. 2012). This hypothesis states that provision of a suite of extracellular metabolic compounds by other members of the community obviates the need for individual cells to produce these compounds, selecting for the loss of the genes responsible and increasing interdependence among species. A functionally diverse community can thus promote the streamlining of genomes, as selection removes genes with redundant function within a community. This process superficially resembles the population-level process of cooperative public good production, in which losing the ability to produce a costly public good, while still being able to use public goods produced by others, allows freeloading cheats an evolutionary advantage over producers (West et al. 2006). However, unlike cells that differ in the ability to produce a single extracellular molecule that serves as a public good but that are otherwise identical, there are likely to be myriad differences among cross-feeding community members. Different species are typically limited by different resources and so need not compete directly; the relationship in this case resembles that of commensalism.

CONCLUSIONS

As we've highlighted throughout this review, the sheer number and diversity of individuals and species in natural microbial communities greatly facilitates their rapid adaptation to changing

environments. Bacteria can respond to selection pressures that are heterogeneous across very small to very large geographic distances; thus, the spatial structuring of bacterial populations and communities is likely to differ remarkably across the traits, species, and systems being examined. Similarly, given the rapid rate at which bacterial populations can respond to local selection, their rate of adaptation may often be more limited by the speed at which the environment changes, rather than by the adaptive potential of populations. As such, the rate of evolution will differ among systems and environments, and again should fall across a continuum of rapid to relatively slow population- and community-level change. Finally, the many ways in which genomes can be populated by different combinations of environment-specific genes result in so-called highways of sharing (Beiko et al. 2005) between distinct species inhabiting the same spatiotemporal location or between not-so-distinct strains from geographically remote locations. As such, it is often unclear whether a response to selection occurs as a result of de novo mutation within a population, movement of mobile genetic elements within/among populations, or movement across populations of multiple species simultaneously. The challenge to better understand microbial adaptation therefore lies in measuring key parameters that govern changes in individual genomes, as well as whole communities, across relevant temporal and spatial scales (**Figure 3**). In light of these complexities, it remains unclear how much of our understanding of in vitro microbial adaptation (from studies that are typically limited in their degree of spatial structure, timescale, and genetic complexity) translates into meaningful predictions in nature. However, as we continue to build more realistic ecology into experimental evolution studies and to take advantage of experimental manipulations in natural settings, we are gaining a clearer picture of the fundamental forces governing microbial adaptation.

FUTURE ISSUES

1. Experimental evolution studies can be extended to communities (e.g., Celiker & Gore 2014) to incorporate species sorting and LGT in addition to mutation.
2. Synthetic biology methods capable of radically altering genomes (on a scale not attainable using artificial selection; see Pál et al. 2014) could be used to test adaptive benefits of large-scale genomic variations.
3. Experiments can increasingly be designed to combine complexities of real abiotic and biotic environments with the robust methods of experimental evolution.
4. Cultivation-based methods must catch up with molecular-based methods in order to more fully elucidate microbial function.
5. The current microbial ecology and evolution framework could be more explicitly applied to understanding the assembly, stability, and contribution of microbiomes to plant, animal, and human health.
6. Long-term data sets could be further leveraged to understand how human activity (such as the use of antibiotics in agriculture) can alter microbial evolution, in turn affecting ecosystem function and human health.
7. Experimental evolution and natural studies can be combined to identify the limits to microbial adaptation, beyond which microbial communities and populations are unable to respond to changing environmental conditions.

DISCLOSURE STATEMENT

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LITERATURE CITED

Achtman M. 2008. Evolution, population structure, and phylogeography of genetically monomorphic bacterial pathogens. *Annu. Rev. Microbiol.* 62:53–70

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* 8:251–59

Andersson MGI, Berga M, Lindström ES, Langenheder S. 2014. The spatial structure of bacterial communities is influenced by historical environmental conditions. *Ecology* 95:1134–40

Baas Becking LGM. 1934. *Geobiologie of Inleiding Tot de Milieukunde.* Den Haag, Neth.: WP Van Stockum & Zoon

Baltrus DA, Guillemin K, Phillips PC. 2008. Natural transformation increases the rate of adaptation in the human pathogen *Helicobacter pylori*. *Evolution* 62:39–49

Baumdicker F, Hess WR, Pfaffelhuber P. 2012. The infinitely many genes model for the distributed genome of bacteria. *Genome Biol. Evol.* 4:443–56

Becker JM, Parkin T, Nakatsu CH, Wilbur JD, Konopka A. 2006. Bacterial activity, community structure, and centimeter-scale spatial heterogeneity in contaminated soil. *Microb. Ecol.* 51:220–31

Beiko RG, Harlow TJ, Ragan MA. 2005. Highways of gene sharing in prokaryotes. *PNAS* 102:14332–37

Bell T. 2010. Experimental tests of the bacterial distance-decay relationship. *ISME J.* 4:1357–65

Belotte D, Curien JB, Maclean RC, Bell G. 2003. An experimental test of local adaptation in soil bacteria. *Evolution* 57:27–36

Blanquart F, Gandon S. 2013. Time-shift experiments and patterns of adaptation across time and space. *Ecol. Lett.* 16:31–38

Bottomley PJ, Yarwood RR, Kageyama SA, Waterstripe KE, Williams MA, et al. 2006. Responses of soil bacterial and fungal communities to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon. *Plant Soil* 289:35–45

Brockhurst MA, Morgan AD, Rainey PB, Buckling A. 2003. Population mixing accelerates coevolution. *Ecol. Lett.* 6:975–79

Buckling A, Maclean RC, Brockhurst MA, Colegrave N. 2009. The *Beagle* in a bottle. *Nature* 457:824–29

Buckling A, Rainey PB. 2002. Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. R. Soc. B* 269:931–36

Celiker H, Gore J. 2014. Clustering in community structure across replicate ecosystems following a long-term bacterial evolution experiment. *Nat. Commun.* 5:4643

Charlesworth J, Eyre-Walker A. 2006. The rate of adaptive evolution in enteric bacteria. *Mol. Biol. Evol.* 23:1348–56

Culligan EP, Sleator RD, Marchesi JR, Hill C. 2012. Functional metagenomics reveals novel salt tolerance loci from the human gut microbiome. *ISME J.* 6:1916–25

De Wit R, Bouvier T. 2006. 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? *Environ. Microbiol.* 8:755–58

Diaz-Ravina M, Baath E. 1996. Development of metal tolerance in soil bacterial communities exposed to experimentally increased metal levels. *Appl. Environ. Microbiol.* 62:2970–77

Dubey GP, Ben-Yehuda S. 2011. Intercellular nanotubes mediate bacterial communication. *Cell* 144:590–600

Baltrus et al. 2008. One of the few experimental studies that have demonstrated the adaptive benefits of bacterial recombination.

Bell 2010. A rare example of a study that combined traditional microcosm experiments with field experiments.

Dykhuizen DE. 1990. Experimental studies of natural selection in bacteria. *Annu. Rev. Ecol. Syst.* 21:373–98
El-Shehawy R, Lugomela C, Ernst A, Bergman B. 2003. Diurnal expression of *hetR* and diazocyte development in the filamentous non-heterocystous cyanobacterium *Trichodesmium erythraeum*. *Microbiology* 149:1139–46
Ellis RJ, Thompson IP, Bailey MJ. 1999. Temporal fluctuations in the pseudomonad population associated with sugar beet leaves. *FEMS Microbiol. Ecol.* 28:345–56
Finkel OM, Burch AY, Elad T, Huse SM, Lindow SE, et al. 2012. Distance-decay relationships partially determine diversity patterns of phyllosphere bacteria on *Tamrix* trees across the Sonoran Desert. *Appl. Environ. Microbiol.* 78:6187–93
Fox JW, Harder LD. 2015. Using a "time machine" to test for local adaptation of aquatic microbes to temporal and spatial environmental variation. *Evolution* 69:136–45
Giraud A, Matic I, Tenaillon O, Clara A, Radman M, et al. 2001a. Costs and benefits of high mutation rates: adaptive evolution of bacteria in the mouse gut. *Science* 291:2606–8
Giraud A, Radman M, Matic I, Taddei F. 2001b. The rise and fall of mutator bacteria. *Curr. Opin. Microbiol.* 4:582–85
Gómez P, Buckling A. 2011. Bacteria-phage antagonistic coevolution in soil. *Science* 332:106–9
Gómez P, Buckling A. 2013. Real-time microbial adaptive diversification in soil. *Ecol. Lett.* 16:650–55
Gubry-Rangin C, Hai B, Quince C, Engel M, Thomson BC, et al. 2011. Niche specialization of terrestrial archaeal ammonia oxidizers. *PNAS* 108:21206–11
Hannam KD, Quideau SA, Kishchuk BE. 2007. The microbial communities of aspen and spruce forest floors are resistant to changes in litter inputs and microclimate. *Appl. Soil Ecol.* 35:635–47
Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JB. 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.* 10:497–506
Hehemann J-H, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. 2010. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* 464:908–12
Hellweger FL, van Sebille E, Fredrick ND. 2014. Biogeographic patterns in ocean microbes emerge in a neutral agent-based model. *Science* 345:1346–49
Hemme CL, Deng Y, Gentry TJ, Fields MW, Wu L, et al. 2010. Metagenomic insights into evolution of a heavy metal-contaminated groundwater microbial community. *ISME J.* 4:660–72
Herlemann DPR, Labrenz M, Jurgens K, Bertilsson S, Waniek JJ, Andersson AF. 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J.* 5:1571–79
Hillesland KL, Stahl DA. 2010. Rapid evolution of stability and productivity at the origin of a microbial mutualism. *PNAS* 107:2124–29
Hu XS, He F, Hubbell SP. 2006. Neutral theory in macroecology and population genetics. *Oikos* 113:548–56
Jannasch HW. 1969. Estimations of bacterial growth rates in natural waters. *J. Bacteriol.* 99:156–60
Jin DJ, Gross CA. 1988. Mapping and sequencing of mutations in the *Escherichia coli rpoB* gene that lead to rifampicin resistance. *J. Mol. Biol.* 202:45–58
Johnson CH, Golden SS, Kondo T. 1998. Adaptive significance of circadian programs in cyanobacteria. *Trends Microbiol.* 6:407–10
Johnston C, Martin B, Fichant G, Polard P, Claverys J-P. 2014. Bacterial transformation: distribution, shared mechanisms and divergent control. *Nat. Rev. Microbiol.* 12:181–96
Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. *Ecol. Lett.* 7:1225–41
Keeling PJ, Palmer JD. 2008. Horizontal gene transfer in eukaryotic evolution. *Nat. Rev. Genet.* 9:605–18
Koskella B. 2013. Phage-mediated selection on microbiota of a long-lived host. *Curr. Biol.* 23:1256–60
Koskella B. 2014. Bacteria-phage interactions across time and space: merging local adaptation and time-shift experiments to understand phage evolution. *Am. Nat.* 184:S9–21
Koskella B, Thompson JN, Preston GM, Buckling A. 2011. Local biotic environment shapes the spatial scale of bacteriophage adaptation to bacteria. *Am. Nat.* 177:440–51
Kunin V, Raes J, Harris JK, Spear JR, Walker JJ, et al. 2008. Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. *Mol. Syst. Biol.* 4:198
Labrie SJ, Samson JE, Moineau S. 2010. Bacteriophage resistance mechanisms. *Nat. Rev. Microbiol.* 8:317–27

Hanson et al. 2012. This article is an authoritative overview of microbial biogeography.

Hu et al. 2006. An exploration of the similarities between the neutral emergence of genetic diversity and species communities.

Lanfear R, Kokko H, Eyre-Walker A. 2014. Population size and the rate of evolution. *Trends Ecol. Evol.* 29:33–41

Lawrence D, Fiegna F, Behrends V, Bundy JG, Phillimore AB, et al. 2012. Species interactions alter evolutionary responses to a novel environment. *PLOS Biol.* 10:e1001330

Lee H, Popodi E, Tang H, Foster PL. 2012. Rate and molecular spectrum of spontaneous mutations in the bacterium *Escherichia coli* as determined by whole-genome sequencing. *PNAS* 109:E2774–E83

Lee M-C, Marx CJ. 2012. Repeated, selection-driven genome reduction of accessory genes in experimental populations. *PLOS Genet.* 8:e1002651

Lenski RE, Travisano M. 1994. Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. *PNAS* 91:6808–14

Lieberman TD, Michel J-B, Aingaran M, Potter-Bynoe G, Roux D, et al. 2011. Parallel bacterial evolution within multiple patients identifies candidate pathogenicity genes. *Nat. Genet.* 43:1275–80

Linhart YB, Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Ecol. Syst.* 27:237–77

Lipson D, Schadt C, Schmidt S. 2002. Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. *Microb. Ecol.* 43:307–14

López-Gutiérrez JC, Henry S, Hallet S, Martin-Laurent F, Catroux G, Philippot L. 2004. Quantification of a novel group of nitrate-reducing bacteria in the environment by real-time PCR. *J. Microbiol. Methods* 57:399–407

Lopez-Pascua L, Buckling A. 2008. Increasing productivity accelerates host–parasite coevolution. *J. Evol. Biol.* 21:853–60

Macel M, Lawson CS, Mortimer SR, Smilauerova M, Bischoff A, et al. 2007. Climate versus soil factors in local adaptation of two common plant species. *Ecology* 88:424–33

Marx CJ. 2013. Can you sequence ecology? Metagenomics of adaptive diversification. *PLOS Biol.* 11:e1001487

Mejáre M, Bülow L. 2001. Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends Biotechnol.* 19:67–73

Miller SR, Williams C, Strong AL, Carvey D. 2009. Ecological specialization in a spatially structured population of the thermophilic cyanobacterium *Mastigocladus laminosus*. *Appl. Environ. Microbiol.* 75:729–34

Morris et al. 2012. The presentation of an exciting new hypothesis marrying community ecology and genome evolution.

Morris JJ, Lenski RE, Zinser ER. 2012. The Black Queen hypothesis: evolution of dependencies through adaptive gene loss. *mBio* 3:e00036–12

Morrison W, Miller RV, Sayler G. 1978. Frequency of F116-mediated transduction of *Pseudomonas aeruginosa* in a freshwater environment. *Appl. Environ. Microbiol.* 36:724–30

Näsvall J, Sun L, Roth JR, Andersson DI. 2012. Real-time evolution of new genes by innovation, amplification, and divergence. *Science* 338:384–87

Nelson-Sathi S, Dagan T, Landan G, Janssen A, Steel M, et al. 2012. Acquisition of 1,000 eubacterial genes physiologically transformed a methanogen at the origin of Haloarchaea. *PNAS* 109:20537–42

Nielsen KM, Bøhn T, Townsend JP. 2013. Detecting rare gene transfer events in bacterial populations. *Front. Microbiol.* 4:415

Nikolausz M, Kappelmeyer U, Székely A, Rusznyák A, Márialigeti K, Kästner M. 2008. Diurnal redox fluctuation and microbial activity in the rhizosphere of wetland plants. *Eur. J. Soil Biol.* 44:324–33

Nowell RW, Green S, Laue BE, Sharp PM. 2014. The extent of genome flux and its role in the differentiation of bacterial lineages. *Genome Biol. Evol.* 6:1514–29

Oh S, Tandukar M, Pavlostathis SG, Chain PSG, Konstantinidis KT. 2013. Microbial community adaptation to quaternary ammonium biocides as revealed by metagenomics. *Environ. Microbiol.* 15:2850–64

Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. 2000. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 288:1251–53

Östman Ö, Drakare S, Kritzberg ES, Langenheder S, Logue JB, Lindström ES. 2010. Regional invariance among microbial communities. *Ecol. Lett.* 13:118–27

Pál C, Papp B, Pósfai G. 2014. The dawn of evolutionary genome engineering. *Nat. Rev. Genet.* 15(7):504–12

Papatheodorou E, Argyropoulou M, Stamou G. 2004. The effects of large-and small-scale differences in soil temperature and moisture on bacterial functional diversity and the community of bacterivorous nematodes. *Appl. Soil Ecol.* 25:37–49

Pernthaler A, Pernthaler J. 2005. Diurnal variation of cell proliferation in three bacterial taxa from coastal North Sea waters. *Appl. Environ. Microbiol.* 71:4638–44

Pernthaler A, Pernthaler J, Eilers H, Amann R. 2001. Growth patterns of two marine isolates: adaptations to substrate patchiness? *Appl. Environ. Microbiol.* 67:4077–83

Polz MF, Alm EJ, Hanage WP. 2013. Horizontal gene transfer and the evolution of bacterial and archaeal population structure. *Trends Genet.* 29(3):170–75

Popa O, Hazkani-Covo E, Landan G, Martin W, Dagan T. 2011. Directed networks reveal genomic barriers and DNA repair bypasses to lateral gene transfer among prokaryotes. *Genome Res.* 21:599–609

Prosser JI, Bohannan BJ, Curtis TP, Ellis RJ, Firestone MK, et al. 2007. The role of ecological theory in microbial ecology. *Nat. Rev. Microbiol.* 5:384–92

Puigbo P, Wolf Y, Koonin E. 2013. Seeing the tree of life behind the phylogenetic forest. *BMC Biol.* 11:46

Rousk J, Frey SD, Bååth E. 2012. Temperature adaptation of bacterial communities in experimentally warmed forest soils. *Glob. Change Biol.* 18:3252–58

Seitz P, Blokesch M. 2013. Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. *FEMS Microbiol. Rev.* 37:336–63

Shapiro BJ, Friedman J, Cordero OX, Preheim SP, Timberlake SC, et al. 2012. Population genomics of early events in the ecological differentiation of bacteria. *Science* 336:48–51

Shapiro BJ, Polz MF. 2014. Ordering microbial diversity into ecologically and genetically cohesive units. *Trends Microbiol.* 22:235–47

Smillie CS, Smith MB, Friedman J, Cordero OX, David LA, Alm EJ. 2011. Ecology drives a global network of gene exchange connecting the human microbiome. *Nature* 480:241–44

Smith DJ, Timonen HJ, Jaffe DA, Griffin DW, Birmele MN, et al. 2013. Intercontinental dispersal of bacteria and archaea by transpacific winds. *Appl. Environ. Microbiol.* 79:1134–39

Stefanic P, Decorosi F, Viti C, Petito J, Cohan FM, Mandic-Mulec I. 2012. The quorum sensing diversity within and between ecotypes of *Bacillus subtilis*. *Environ. Microbiol.* 14:1378–89

Sung W, Ackerman MS, Miller SF, Doak TG, Lynch M. 2012. Drift-barrier hypothesis and mutation-rate evolution. *PNAS* 109:18488–92

Swain RA, Nolan JV, Klieve AV. 1996. Natural variability and diurnal fluctuations within the bacteriophage population of the rumen. *Appl. Environ. Microbiol.* 62:994–97

Telford RJ, Vandvik V, Birks HJB. 2006. Dispersal limitations matter for microbial morphospecies. *Science* 312:1015

Tiemann LK, Billings SA. 2011. Changes in variability of soil moisture alter microbial community C and N resource use. *Soil Biol. Biochem.* 43:1837–47

Toft C, Andersson SG. 2010. Evolutionary microbial genomics: insights into bacterial host adaptation. *Nat. Rev. Genet.* 11:465–75

Torsvik V, Øvreås L. 2002. Microbial diversity and function in soil: from genes to ecosystems. *Curr. Opin. Microbiol.* 5:240–45

Treangen TJ, Rocha EP. 2011. Horizontal transfer, not duplication, drives the expansion of protein families in prokaryotes. *PLOS Genet.* 7:e1001284

Tripathi B, Lee-Cruz L, Kim M, Singh D, Go R, et al. 2014. Spatial scaling effects on soil bacterial communities in Malaysian tropical forests. *Microb. Ecol.* 68:247–58

Vasi F, Travisano M, Lenski RE. 1994. Long-term experimental evolution in *Escherichia coli* II. Changes in life-history traits during adaptation to a seasonal environment. *Am. Nat.* 144:432–56

Vos M. 2009. Why do bacteria engage in homologous recombination? *Trends Microbiol.* 17:226–32

Vos M, Birkett PJ, Birch E, Griffiths RI, Buckling A. 2009. Local adaptation of bacteriophages to their bacterial hosts in soil. *Science* 325:833

Vos M, Didelot X. 2008. A comparison of homologous recombination rates in bacteria and archaea. *ISME J.* 3:199–208

Vos M, Wolf AB, Jennings SJ, Kowalchuk GA. 2013. Micro-scale determinants of bacterial diversity in soil. *FEMS Microbiol. Rev.* 37:936–54

Wallenstein MD, Hall EK. 2012. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry* 109:35–47

Shapiro et al. 2012. One of the few comprehensive studies on the early stages of ecological differentiation of bacterial populations.

Wandersman C, Delepelaire P. 2004. Bacterial iron sources: from siderophores to hemophores. *Annu. Rev. Microbiol.* 58:611–47

Wang P, Robert L, Pelletier J, Dang WL, Taddei F, et al. 2010. Robust growth of *Escherichia coli*. *Curr. Biol.* 20:1099–103

Ward DM, Weller R, Bateson MM. 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature* 345:63–65

West SA, Griffin AS, Gardner A, Diggle SP. 2006. Social evolution theory for microorganisms. *Nat. Rev. Microbiol.* 4:597–607

Winter C, Herndl GJ, Weinbauer MG. 2004. Diel cycles in viral infection of bacterioplankton in the North Sea. *Aquat. Microb. Ecol.* 35:207–16

Wright GD. 2005. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Adv. Drug Deliv. Rev.* 57:1451–70

Yuen-Tsu NY, Yuan X, Velicer GJ. 2010. Adaptive evolution of an sRNA that controls *Myxococcus* development. *Science* 328:993

Zumsteg A, Bååth E, Stierli B, Zeyer J, Frey B. 2013. Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield. *Soil Biol. Biochem.* 61:121–32

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