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 by ecologists and evolutionary biologists. This knowledge gap is rapidly closing with recent technological advances and an increasing appreciation for the role of microbes 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, where the genetic make-up 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 towards a better understanding of microbial adaptation in nature.

Keywords: bacteria, experimental evolution, local adaptation, lateral gene transfer, microbial ecology, time shift experiments.

Introduction

In a 1990 *AREES* review entitled "Experimental Studies of Natural Selection in Bacteria," Daniel Dykhuizen pointed out that:

"*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 (Dykhuizen 1990) highlighted the power behind using simple flasks with broth or agar plates inoculated with a single clone to study the process of evolution in bacterial populations. Indeed, twenty-five years since this review was written the use of 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 been made in the field of microbial ecology, where technology-driven studies continue to reveal novel phylogenetic and functional groups across all possible environments. Although microbial ecologists have historically conducted their research 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 characterizing patterns across natural populations and communities, while our understanding of the process of microbial adaptation has primarily been gained from experimental evolution studies performed under artificial laboratory conditions (Figure 1). One outstanding question in the field is therefore how well predictions generated from in vitro results meaningfully translate to patterns observed in nature, and vice versa. There are a number of reasons to expect this not to be

the case, as natural populations face a suite of selection pressures, are shaped by dispersal and complex spatial structure, and in some cases can utilize foreign DNA to speed up adaptation to a given environment. Indeed, recent studies of experimental evolution that incorporate more realistic ecological conditions often find strikingly dissimilar patterns to those previously uncovered under artificial conditions (e.g. Habets et al 2006, Kerr et al 2002, Morgan et al 2005). A series of experiments run in semi-natural soil microcosms in which the target study organism, *Pseudomonas fluorescens,* was introduced into soil either with or without the natural microbial soil community demonstrate that adaptive diversification of the bacterium is greatly reduced in the presence of the natural community (Gómez & Buckling 2013) and that coevolution between the bacterium and its bacteriophage parasite follows an entirely different trajectory in the presence of the natural community than it does in vitro (Gómez & Buckling 2011).

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, arguing that the process of adaptation follows a continuum across scales and that real insight to the patterns observed in nature will only come through an appreciation of scale and using a combination of approaches. Note that in order to focus our discussion we discuss only adaptation in Prokaryotes (Bacteria and Archaea) and not Eukaryotic microbes or viruses (when not in the context of their bacterial hosts).

Measuring adaptation in natural bacterial populations

The key starting point for the study of ecological and evolutionary processes in the microbial world is a clear understanding of microbial **fitness**. This 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 non-linear and contrasting across species and strains. For example, manipulation of substrate patchiness (i.e. how often nutrients are supplemented into the growth media) across two marine bacteria demonstrated that while

one species out-competed the other under a one-time supplementation, the other species performed best when nutrients were added gradually (Pernthaler et al 2001). Similarly, evidence from *Escherichia coli* cells grown in a microfluidic chamber, allowing researchers to follow the life history of single cells, suggests that while 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). Since the latter will depend on the environment in which the cell is growing, the lifetime reproduction of a given bacterial cell will be highly variable, as exemplified by the common discrepancy between generation times measured in the lab and those measured in natural populations (Jannasch 1969).

Culture-dependent methods

Studying clonal lineages 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 using 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), where the presence of genetic variation in genes of known function offer 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), which could be linked to selection in the local environment. 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 them in microcosms mimicking 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. In this case, microcosms can range from the completely artificial (e.g. broth in shaken flasks, agar plates or even 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)). An approach that is much less commonly taken is the incubation of isolated clones in their original environment. An early but elegant example of this is a study where bacteriophage **transduction** frequencies were measured in a *Pseudomonas aeruginosa* lab strain incubated in a polycarbonate cylinder (sealed with 0.2 μm membranes allowing nutrient diffusion) suspended in a lake (Morrison et al 1978).

Culture-independent methods

Molecular methods based on selective sequencing of marker genes or the non-selective sequencing of meta-genomes 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 insights into community composition and has become the most common approach taken for characterizing microbial diversity (Ward et al 1990), but 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 antibiotic resistance in natural, agricultural, and clinical settings (Allen et al 2010). However, it 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. The current limitation on metagenomic approaches is 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) and specific phenotypes can be discovered through functional metagenomics, where 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). These correlation patterns are important to establish, but causation can be more directly addressed through controlled manipulation of the environment, such as the artificial warming of soil (Rousk et al 2012).

Evolutionary mechanisms underlying adaptation

Natural selection is ultimately reliant on genetic variation, and a wide array of mechanisms are known to be responsible for creating novel genetic variants in bacteria, ranging from simple point mutation to deletions of large chromosomal regions and parasexual processes, where cells actively or passively procure DNA from the environment and incorporate it into their genome. As the manner in which bacteria adapt to their environment is crucially dependent on the rate and type of genetic variation populations are supplied with, 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 type of genetic change where, in its simplest form, an individual nucleotide is replaced by another type of nucleotide. When resulting in an amino acid change, point mutations (or SNPs) can lead to the acquisition of novel traits (e.g. SNPs in the *rpoB* gene conferring resistance against the antibiotic rifampicin (Jin & Gross 1988) or, when occurring in a regulatory gene, mutations can vastly alter patterns of gene expression controlling major phenotype changes such as multicellular development (Yuen-Tsu et al 2010). Mutations generally occur quite rarely; a recent study on *E. coli* found that point mutations occur only around 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 harbour 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 have a particular 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 2001a). For example, a series of elegant experiments on an *E. coli* mutator strain demonstrate 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 2001b).

Genomic changes can be much more substantial than single base pair substitutions. Over the course of 1500 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 overall. Instead, selection seemed to favour 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). Apart from the loss of genes, 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 (IAD) model (Näsvall et al 2012) poses that when a weak, secondary gene activity becomes more important (e.g. after a change in environment), gene duplication is favoured as it results in increased protein production. Having selection for- rather than against- multiple gene copies thus allows different 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).

Genomic change 'from without': incorporating foreign DNA

Although eukaryotes experience hybridization and **lateral genetic transfer (LGT)** (Keeling & Palmer 2008), their level of genetic promiscuity is minute compared to that of

prokaryotes. Horizontal modes of inheritance are so frequent in bacteria that vertical patterns of descent can be to a large degree obscured, complicating the reconstruction of evolutionary histories (Puigbo et al 2013). A wide variety of mechanisms contribute to the lateral transfer of DNA among bacteria, but they all have in common that they result in relatively short fragments of DNA being transferred from one cell to another. Discussions on gene transfer between cells are usually framed around the different mechanisms responsible; the three best-studied mechanisms being **transformation**, **conjugation** and **transduction** (although novel types of LGT are still being discovered; for instance nanotube-based mechanisms [Dubey & Ben-Yehuda 2011]). One crucial distinction between these different mechanisms is whether they themselves are likely to represent a bacterial adaptation or not (Seitz & Blokesch 2013). For mechanisms where infectious elements are involved, such as in conjugation and transduction, this will mostly not be the case. However, for transduction (Johnston et al 2014), where cells actively take up free DNA from the environment, this has been argued to be likely (Vos 2009). One other way to classify bacterial gene transfer is based upon the type of DNA transferred: homologous stretches (novel or identical alleles) or non-homologous 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 nontranformable mutants of the human-associated bacterium, *Helicobacter pylori,* demonstrated more rapid adaptation of competent populations when passaged in a novel laboratory environment (Baltrus et al 2008). In the second case, the uptake of foreign DNA can lead to wholesale changes in phenotype as the transfer of genetically and functionally distinct genes are transferred ‘in one go’ (e.g. (Hehemann et al 2010)). This classification of the uptake of foreign genes is not clear-cut, however, as LGT events can take place due to non-homologous recombination but also through homologous recombination when genes that are not shared are flanked by genes that are shared between donor and recipient (Polz et al 2013).

Horizontal transfer of DNA, homologous or non-homologous, 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 analysed, homologous recombination contributed more to genetic diversity than 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. Indeed, over large evolutionary timescales LGT events can completely transform the genomic make-up, metabolism and ecological life-styles 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), the effect of ecology can override the effect of 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).

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 (non-random) 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, microbial N_e, is more elusive (Lanfear et al 2014). N_e is, by definition, smaller than the actual (census) population size, for instance due to population bottlenecks caused by host-to-host transmission of pathogens or symbionts, or blooms in seasonal environments. Neutral diversity in bacteria ranges over several orders of magnitude, with very low diversity species, such as *Yersina pestis,* likely having relatively small census population sizes, experiencing frequent bottlenecks upon transmission, and having emerged relatively recently (Achtman 2008). For ubiquitous species, N_e estimates range from 10^7 (the gut bacterium *E. coli*; Charlesworth & Eyre-Walker 2006) to 10^{11} (the oceanic

photosynthesizing *Prochloroccus*; Baumdicker et al 2012). Difficulties in reliably estimating N_e aside, it is obvious that this parameter will differ widely for species with distinct ecologies and will have great potential to differentially influence the process of adaptive evolution.

Bacterial adaptation across space

A central question in microbial ecology and evolution has historically been whether adaptation is more often the result of mutational change and subsequent evolution within populations in response to local selection pressures, or of colonization by a particular clonal lineage or species that, by chance, was pre-adapted to the environment. This idea was first put forward by Baas Becking (1934) and his quote "everything is everywhere, but the environment selects" is still frequently cited by microbiologists (De Wit & Bouvier 2006). There is now good evidence that the environment selects, 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 here to mean passive displacement, e.g. the dispersal of cells by splashing raindrops or ocean currents. 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 show evidence of adaptation to local temperature (54°C upstream and 39°C downstream) despite frequent gene flow among them, as demonstrated using genetic markers (Miller et al 2009). In this case, selection acting on one small genomic region (~5 kB), containing genes involved in nitrogen fixation, was found to lead to divergence of this region in both mutations in homologous sequence and gene content across populations.

Bacterial biogeography

Passive dispersal through the atmosphere is likely to be extensive (Smith et al 2013), as is dispersal through ocean currents. However, a model developed for the ubiquitous marine planktonic bacterium *Pelagibacter ubique* shows that currents are not

extensive enough to erase genetic divergence of populations inhabiting different parts of the ocean by mutation (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) but 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 (Bell 2010). 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 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), emphasizing that the spatial scale at which adaptation is occurring is defined both by spatial distance and by the spatial heterogeneity of selection across the landscape.

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, while 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 within soil was found to differ across a 150 meter transect of tropical forest, primarily in response to local pH conditions (Tripathi et al 2014), while the metabolic potential of bacterial isolates from highly contaminated soils was found to vary up to 10,000-fold across samples that were less than one cm apart (Becker et al 2006). Similarly, 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) while 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; (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 hosts/microbes whose fitness depends either directly or indirectly on the presence of a particular bacterial symbiont/eukaryotic host. 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), and this can have important cascading effects to 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 adaptation is by comparing the fitness of individuals from one environment in either their local environment or a foreign environment (reviewed in Kawecki & Ebert 2004). This measurement of "local adaptation" requires some a priori predictions of the particular traits expected to confer adaptation to a local environment as well as the spatial distance at which the selective pressures shaping such traits are expected to differ. If such details are known or can be predicted for a given system, then a reciprocal transplant among environments should reveal the existence of any specific adaptations that have evolved in response to one environment versus the other. If we return 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. Indeed, a reciprocal transplant study comparing local adaptation of grass species (which are not directly affected by nitrogen-fixing bacteria) and 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 as bacteria were transplanted away from their home site at a rate of about six percent per meter; a rate which 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, it is unlikely that such results are generalizable across systems. As mentioned above, the spatial scale of adaptation will be 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 finding differences across soil populations separated only by centimeters (Vos et al 2009), and in the other case finding no signature of local adaptation across leaves from the same horse chestnut tree, but strong phage local adaptation 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 the abiotic conditions of soil or the tree immune defense, that shape the spatial differentiation among populations. Reciprocal transplant experiments of microbial populations and communities across soil types have found similarly mixed results. While there was no evidence for bacterial local adaptation to soil from forest floors dominated by trembling aspen versus white spruce (Hannam et al 2007), there was 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 de-glaciated unvegetated sites along a soil moisture and 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 changes is likely to differ from that of larger organisms. A striking difference can be observed, for example, between the typical rate of adaptation in bacterial populations and that of plant populations. Whereas the time scale of genetic differentiation of plant populations is found to be over 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 will be 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. However, there are also many cases where adaptation to the local biotic environment will be comparable to adaptation to the local abiotic environment; for example when a bacterial pathogen is adapting 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 abiotic environment may occasionally 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 with overlap between the two.

Adaptation in response to the abiotic environment

Just as for eukaryotes, the abiotic environment experienced by bacteria can vary over a wide range of timescales from within a single generation to geological timescales (Figure 3). 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 environment change to select for **phenotypic plasticity** and more long-term change to result in genetic change over time. 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 temperate, UV, and moisture. These within-generation fluctuations have resulted in numerous adaptations which 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 been shown to have circadian programming of gene expression even in cases where cells divide in under 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 Plain grassland sites resulted in functional differences among microbial communities, where increasing variability in moisture variability was associated with increased demand for nitrogen and decreased carbon use efficiency (Tiemann & Billings 2011). Furthermore, experimental evolution of *Escherichia coli* under fluctuating conditions of resource availability was found to result in both increased maximum growth rate and reduction in the lag time of growth upon the arrival of new nutrients relative to the 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 over the course of three growing seasons found evidence for seasonal reoccurrence 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, the changing biotic environment experienced by bacteria can vary across either short or long-term timescales (Figure 3), and these environmental changes can either result in increased **phenotypic plasticity** or in genetic change. Even in response to the same biotic selection pressure, such as bacteriophages, bacteria have been shown to respond via numerous resistance mechanisms including those that are plastic (such as abortive infection whereby a cell commits suicide upon infection in order to prevent phage reproduction or phase variation in expression of surface receptors to which phages bind) and those that are genetic (such as mutations leading to loss or alteration of particular surface receptors) (reviewed in (Labrie et al 2010)). These diverse adaptations are likely to be the result of differing strength as well as continuity of phage-mediated selection in the environment. For example, there is good

evidence that phage prevalence will vary predictably over time; bacteriophage infection of bacterioplankton in the North Sea was found to follow diurnal cycles, with the highest prevalence of infected cells occurring after peaks in bacterial growth and activity and lysis typically occurring overnight (Winter et al 2004). Furthermore, the abundance of phages from the rumen of sheep fed once daily was found to be 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 there is evidence for seasonal variation in the abundance of bacterivorous nematodes in soil after a peak in bacterial diversity and abundance (Papatheodorou et al 2004).

Time shift approach to measuring adaptation

In terms of measuring the rate of adaptation of bacterial populations, a particularly powerful approach is to utilize a "time shift" experiment in which the fitness of individuals from the past, present and future are directly compared (Blanquart & Gandon 2013). This approach can be used to understand the temporal pattern and magnitude of adaptation in response to either biotic or abiotic environmental change. For example, in the laboratory, bacterial populations have been shown to be more resistant to coevolving bacteriophages from 15 bacterial generations earlier (4 days) than to contemporary phages (Buckling & Rainey 2002). Similarly, comparison of bacterial resistance against sympatric phage populations from the horse chestnut phyllosphere demonstrated the evolution of resistance and of phage counter-adaptation across months, such that bacteria were more resistant to phages from a month earlier and less resistance to phages from a month later in the growing season (Koskella 2013). Although there are only few examples of time shift experiments being used to understand the rate of bacterial adaptation to phages in nature, results from experimental microcosm studies suggest that both increased mixing of populations (Brockhurst et al 2003) and increased resource supply (Lopez-Pascua & Buckling 2008) can accelerate the rate of bacterial adaptation to phages, and vice versa. Time shift approaches can also be taken to understand the rate of adaptation to abiotic conditions. For example, adaptation to local water chemistry was examined by comparing growth of bacterial isolates when grown in lake water from three time points, separated by three months and then 22 months (Fox &

Harder 2015), although in this case little evidence for adaptation was uncovered. A similar approach was used to examine the importance of past (two to eight 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 salinity 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 the local environment can often only fully be appreciated when examined in a time-lagged fashion (Koskella 2014).

Population- versus community-level adaptation

Microbes are key to biogeochemical cycling and ecosystem functioning, which is generally achieved at the level of whole communities rather than populations (Torsvik & Øvreås 2002). Community adaptation following exposure to a novel environmental regime can be defined as a shift in community composition leading to increased growth and ecosystem performance. Such adaptation can take place over long time scales (as is the case with global warming) or in much shorter, recurring time scales, as is the case with seasonal changes. For instance, analysis of microbial community composition in soil from an alpine dry meadow uncovered not only strikingly different species composition before and after snowmelt, but also found that microbial communities from pre-snowmelt samples had a higher proportion of respiration at 0 °C relative to 24 °C than did communities sampled post-snowmelt (Lipson et al 2002). With the growing interest in global climate change, an increasing number of studies test how temperature or other environmental variables influence key microbial community functions such as nitrification, productivity and decomposition (Wallenstein & Hall 2012). In contrast to experiments where 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 manipulation of the environment, increasingly utilizing metagenomic sequencing to identify key shifts in phylogenetic community composition.

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). In fact, population genetics and community ecology can both be analyzed within the framework of neutral evolution (Hu et al 2006). Populations consist of alleles that are introduced through mutation or gene flow and, equivalently, communities consist of species that are introduced through speciation and migration. The null hypothesis is that distributions of specific alleles or species are governed by random forces (drift) and selection needs only be invoked when distributions deviate from the theoretical expectation. Interestingly, in microbial ecology 16S rRNA marker gene sequences are typically equated with species, completely removing the boundary between community ecology and population genetics. The fact that LGT results in genes being transferred among different species further blurs distinctions between the two fields. One nice illustration of how changes in species abundances in a community co-occur with changes in gene abundances within species in the community in response to environmental perturbation can be found on a study investigating resistance against Quaternary Ammonium Compounds (QACs), which are biocides that persist in the natural environment (Oh et al 2013). Three bioreactors, one provided with dextrin/peptone (control), one with dextrin/pepton and a QAC and one with a QAC only were inoculated with the same polluted environmental sample. After prolonged time, QAC resistance was quantified, which 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, QAC exposure was found to lead to the disappearance of many taxa and the enrichment of the species *Pseudomonas nitroreducans*. The selective amplification of this particular species was also accompanied by specific point mutations, as well as putative LGT events, in genes implicated in QAC metabolism.

The Black Queen hypothesis

The interplay between genomic evolution within species and community composition turnover has recently been highlighted in the form of the ‘Black Queen

hypothesis' (Morris et al 2012). This hypothesis states that the provision of a suite of extracellular metabolic compounds by other members of the community obviates the need for individual cells to be able to produce these compounds, selecting for the loss of the genes responsible and increasing inter-dependence among species. A functionally diverse community can thus promote 'genome streamlining,' as selection acts to remove genes with redundant function within a community. This process superficially resembles the population-level process of cooperative public good production, where losing the ability to produce a costly public good, whilst still being able to utilize public goods produced by others, allow freeloading cheats an evolutionary advantage over producers (West et al 2006). However, unlike these cells that differ in the ability to produce a single extracellular molecule that serves as a public good, there are likely to be myriad additional differences between **cross-feeding** community members. Different species are likely to be limited by different resources and so are not necessarily direct competitors; the relationship in this case is thus most likely to be that of **commensals**.

Conclusions and future directions

As we've highlighted throughout, 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, and thus the spatial structuring of bacterial populations and communities is likely to differ remarkably across the traits, species and systems being examined. Similarly, although bacterial populations can respond remarkably quickly to local selection, their rate of adaptation may often be more limited by the speed at which the environment changes rather than the adaptive potential of populations, and will again fall across a continuum of rapid to relatively slow population and community-level change. Finally, the many ways in which 'core' genomes can be rapidly populated by different combinations of environment-specific genes result in 'highways of sharing' (Beiko et al 2005) between distinct species inhabiting the same spatio-temporal location or between not-so distinct strains from geographically remote locations. As such, it is often unclear whether a response to selection will occur within a single individual (de novo mutation), within a mobile

genetic element, or across multiple species simultaneously. The challenge to better understand microbial adaptation therefore lies in measuring key parameters governing changes in individual genomes as well as whole communities, over relevant time scales and spatial scales (Figure 3). In light of these complexities in selection across scales, 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, time scale, and genetic complexity) will translate into 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. Evolution experiments 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; Pál et al 2014) could be used to test adaptive benefits of large-scale genomic variations.
3. Experiments can increasingly be designed to combine the reality of the (a)biotic environment with the robustness of experimental evolution.
4. Cultivation-based methods need to catch up with molecular-based methods in order to be able to more fully understand 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 datasets 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 populations and communities will be unable to respond to changing environmental conditions.

Acronyms/definitions list

Clonal interference competition between different beneficial mutations present in different individuals

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

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

Conjugation: a process whereby DNA is transferred between cells that are in physical contact. The transferred DNA is typically in the form of a circular plasmid, which usually carries the genes responsible for the contact and transfer

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

Effective population size: represents the number of individuals that equally contribute to future generations; species with small N_e are more sensitive to random events affecting the reproduction of particular individuals and will experience less efficient selection

Fitness: typically defined as the reproductive success of an individual over its lifetime

Great Plate Count Anomaly: the observation that the vast majority of bacteria in a sample do not grow on any given cultivation medium because the nutrient conditions are not right, cross-feeding is not possible or growth is very slow

Lateral Gene Transfer (LGT): the transfer of genes between individual cells (contrasting with vertical transfer of genes from mother- to daughter cell)

Phenotypic plasticity: the ability of a genotype to produce different phenotypes as a response to environmental conditions

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

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

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

Figures and Legends

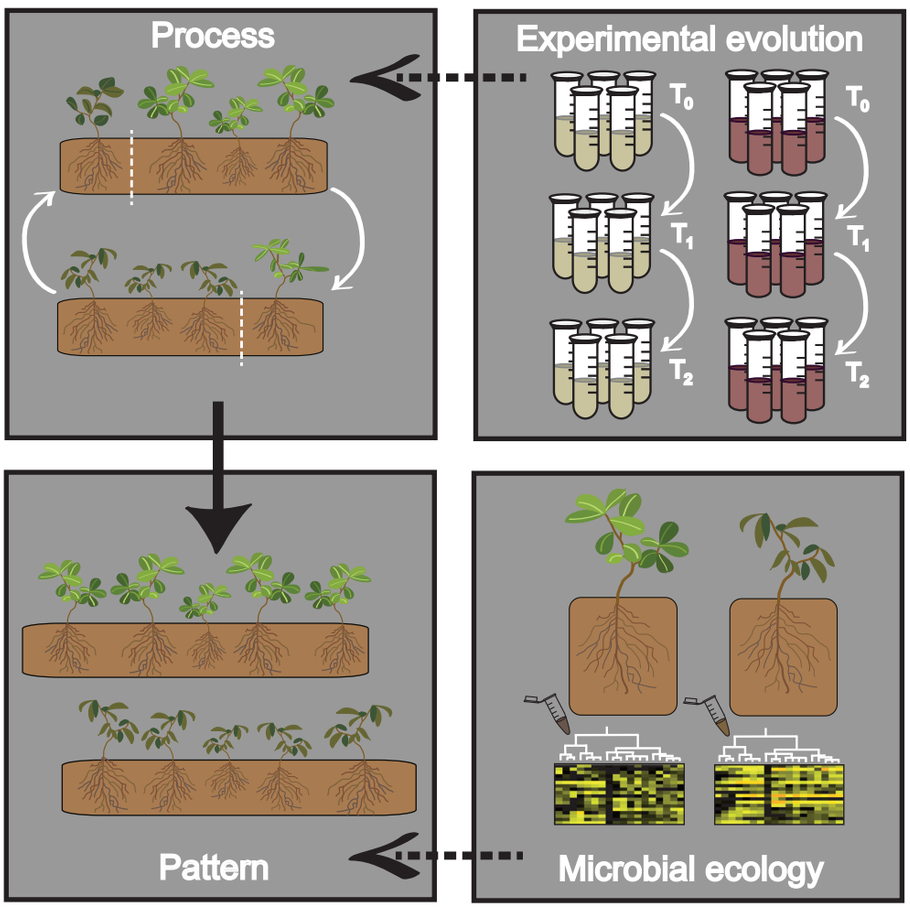

Figure 1. Illustration of the use of experimental evolution and studies manipulating environments (either artificially or in situ) directly to examine the processes underlying microbial adaptation, and microbial ecology approaches to uncover patterns of adaptation in nature.

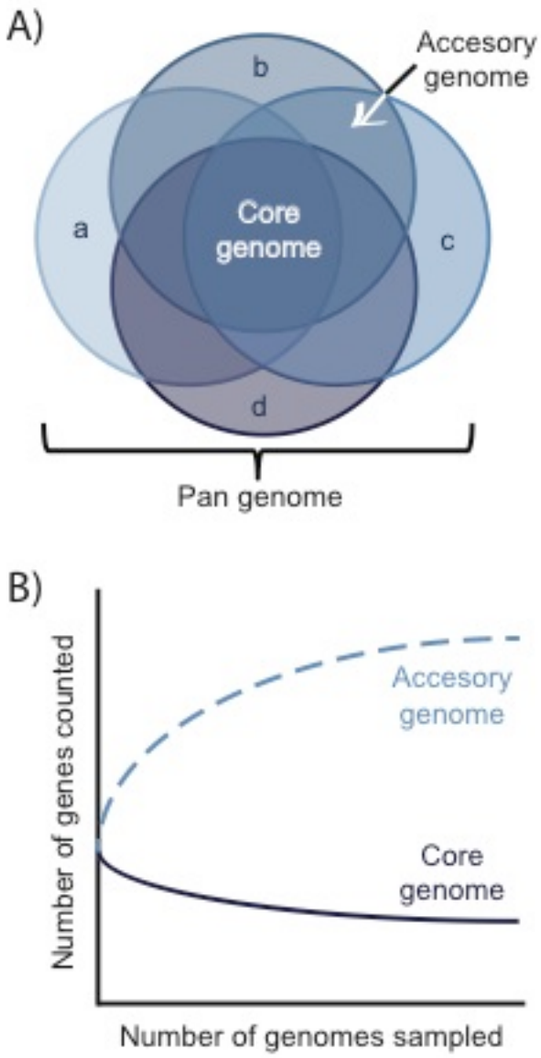

Figure 2. A) A Venn diagram depicting gene content of four bacterial genomes (a-d). The genes that are shared between all genomes are part of the core genome, genes that are present in less than four genomes are part of the accessory genome, the total complement of genes is termed the pan genome. B) when sequencing genomes of new strains, the pan genome will increase due to finding more accessory genes; the size of the core genome will decrease as some genes present in known strains will not be present in new strains.

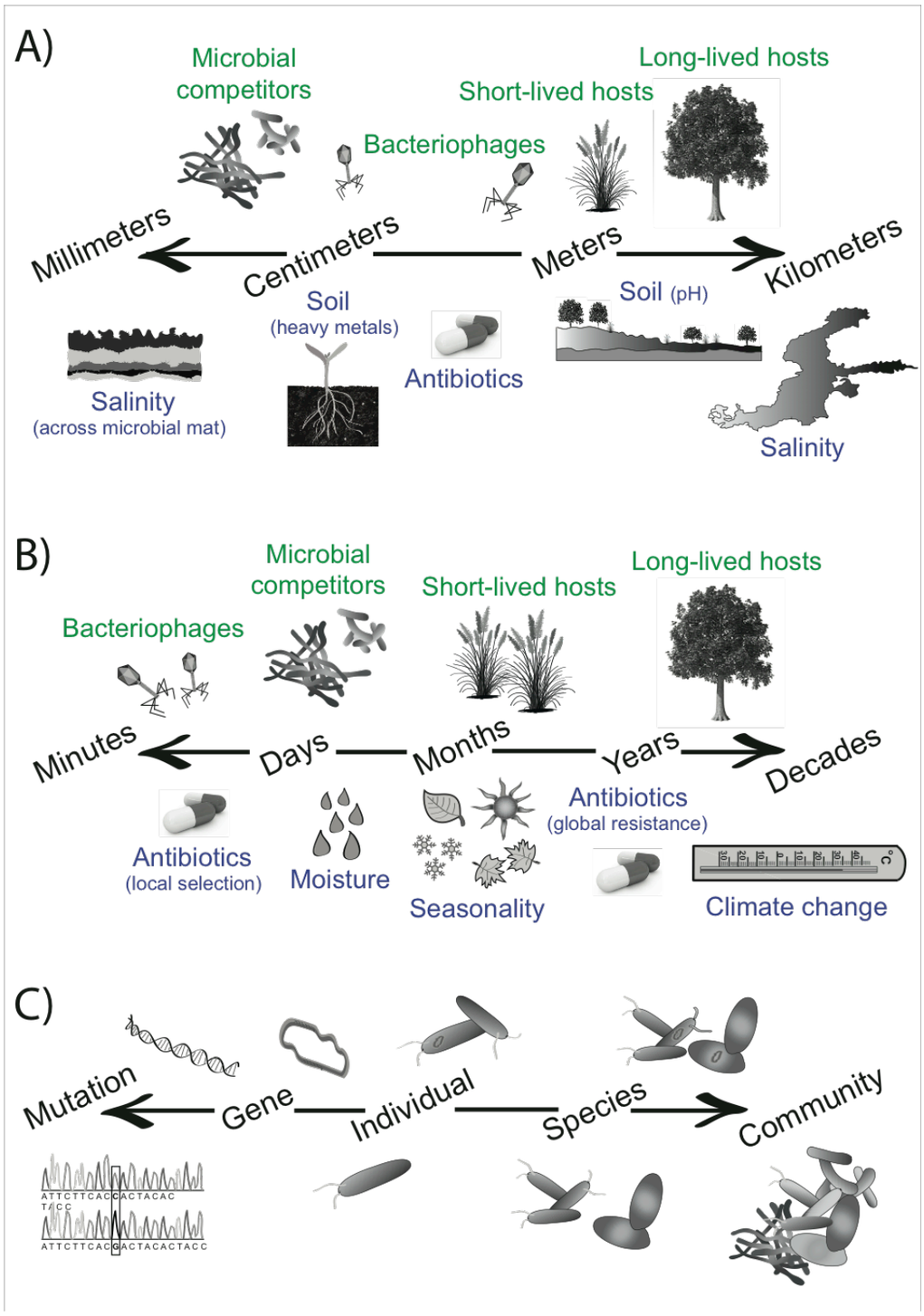

Figure 3. Three panels describing the scales of bacterial adaptation in nature. A) Exploration of the spatial scales, where abiotic selection gradients can range from millimeters (as is the case for salinity across a microbial mat; Kunin et al 2008) to many kilometers (as is the case for salinity across the Baltic sea; Herlemann et al 2011), and similarly biotic selection gradients can occur across very small scales (e.g. for bacteria coevolving with competitor species or bacteriophage viruses) and very large scales (e.g. for bacteria inhabiting long-lived hosts). B) Illustration of the continuum in temporal scales of bacterial adaptation, where the environment can change rapidly (as is the case of coevolving bacteriophages as well as antibiotic concentrations in response to enzymatic

degradation (Wright 2005) or relatively slowly (as is the case for the use of antibiotics over time or climate change). And 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 plasmdis) among bacterial species.

5

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