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ABSTRACT Any two lineages, no matter how distant they are now, began their divergence as one population splitting into two lineages that could coexist indefinitely. The rate of origin of higher-level taxa is therefore the product of the rate of speciation times the probability that two new species coexist long enough to reach a particular level of divergence. Here I have explored these two parameters of disparification in bacteria. Owing to low recombination rates, sexual isolation is not a necessary milestone of bacterial speciation. Rather, irreversible and indefinite divergence begins with ecological diversification, that is, transmission of a bacterial lineage to a new ecological niche, possibly to a new microhabitat but at least to new resources. Several algorithms use sequence data from a taxon of focus to identify phylogenetic groups likely to bear the dynamic properties of species. Identifying these newly divergent lineages allows us to characterize the genetic bases of speciation, as well as the ecological dimensions upon which new species diverge. Speciation appears to be least frequent when a given lineage has few new resources it can adopt, as exemplified by photoautotrophs, C1 heterotrophs, and obligately intracellular pathogens; speciation is likely most rapid for generalist heterotrophs. The genetic basis of ecological divergence may determine whether ecological divergence is irreversible and whether lineages will diverge indefinitely into the future. Long-term coexistence is most likely when newly divergent lineages utilize at least some resources not shared with the other and when the resources themselves will coexist into the remote future.

IN SEARCH OF THE ORIGINS OF MICROBIAL DIVERSITY

In recent decades, microbiologists have discovered an astounding disparity of prokaryotic life. Our field has identified the most anciently divergent prokaryotic lineages, and we have found them to be utterly different in every aspect of their being: their cell architecture, biochemistry, physiology, genome content, and how they make a living. Our understanding of the prokaryotes’ phylogenetic diversity began in large part with Carl Woese’s tree of all life, based on the universal 16S rRNA gene (1). His universal tree yielded the surprising result that the not-yet-named archaea and bacteria, already known to be extremely different in cell structure, represented the deepest divisions of life. Subsequent surveys of 16S diversity, using cultivation-free methods, led to discovery of vast numbers of uncultivated prokaryotic taxa, at all levels from species to phyla, in even the most familiar of habitats (2). While cultivation has yielded discovery of ~30 bacterial phyla, cultivation-free methods focusing on 16S have expanded the number of bacterial phyla to nearly 100 (3). Moreover, we can extrapolate that among rare, presently uncultivable organisms, we will eventually discover ~1,300 phyla (4)! Most recently, single-cell genomic approaches have yielded much greater resolution for prokaryotic phylogeny and have revealed a totally unexpected superphylum at the base of the bacteria tree. This group is
predominated by phyla with limited metabolic capabilities and a shared stubbornness against cultivation (5). These are all exciting forays into estimating the vastness and organization of prokaryotic diversity, but these purely phylogenetic approaches fail to portray the profound distinctness among the most anciently divergent prokaryotes.

What makes the discovery of all these phyla so interesting is their great disparity in cell architectures, physiologies, genomes, and ecologies (6). In some cases, it is clear how ancient architectural differences have generated disparate ways of making a living. For example, the multilayered peptidoglycans of the *Firmicutes*’ cell walls give these organisms constitutive resistance to the osmotic stresses of drought and rewetting found in desert soils (7). Also, the photosynthetic capabilities of the various phototrophic phyla do not emerge from their enzymes alone; their photosynthetic functions depend on complex architectural membrane structures (8). Cells of the *Planctomycetes* phylum (as well as the *Caulobacteriales* of the *Alphaproteobacteria*) sport stalk and holdfast structures that raise the cell above the still water at the surface of the seabed, and so provide access to a greater flow of water and nutrients (9–11). It will be interesting to discover how architecture has led to distinct ways of making a living in other phyla.

The unique capabilities of phyla are based on long-standing, highly conserved differences that are not transferred across taxa (although some transfers were involved early on in the origins of complex traits [12]). That is, the familiar horizontal transfers of simple traits like antibiotic resistance and virulence, which plague public health (13, 14), do not apply to phylum-defining qualities such as the *Firmicutes*’ Gram-positive cell wall. The nontransferable nature of phylum-defining traits suggests that these are complex adaptations based on many genes and proteins (15, 16). Indeed, all *Cyanobacteria* share hundreds of genes that are unique to that phylum (and the derivative eukaryotic chloroplasts) (17), and similar results hold for other phyla (18). It will be fascinating to find out what novel physiologies those 1,300 or so undiscovered phyla might be capable of. Genomics will yield some of the secrets (19), as will studies of habitat differences among phyla (18, 20), but eventual cultivation of these organisms will tell us so much more (21). Fortunately, new high-throughput culture methods promise to make isolates available for many taxa that resist cultivation (22).

Whence did this profound disparity emerge? We cannot yet identify the history of architectural and physiological changes leading to each phylum, but there is one kind of historical moment shared in the emergence of all anciently divergent groups. That is, all lineages, no matter how distant they are now, began their divergence as one population splitting to become two lineages that could coexist indefinitely (23). By addressing the origin of species, population biology can help explain the origins of the deepest divisions of life. My goal here is to explore how one population may split into two lineages that can coexist indefinitely.

As we shall see, this splitting of lineages involves a “transmission” of part of the old lineage into a new ecological niche: the new niche may in some cases be a novel microhabitat with different resources; in other cases, the new microhabitat may hold different physical and chemical conditions; in pathogens and other endosymbionts, the niches may be different hosts or different host tissues; in still other cases, the niches may not be in different places at all but instead represent different sets of resources in the same microhabitat. Whether ecological divergence between lineages brings about their persistence in the same or different habitats, we will refer to the outcome of persistence as “coexistence.” How do these splits happen, and what is necessary for newly divergent lineages to ride the long haul of time to eventually become profoundly different creatures?

**THE DYNAMIC PROPERTIES OF SPECIES**

Let us begin with what speciologists have described as the quintessential attributes of species (24, 25). First, species are understood to be ecologically distinct, since ecological distinctness is required for the groups to coexist into the future. Then there is the property of cohesion within but not between species. That is, lineages within a species are constrained in their divergence, while lineages from different species are not. In the case of the bacteria, we’ll see that cohesion within species involves a force called “periodic selection,” which purges the diversity genome-wide within but not between species-like populations (26). The flip side of cohesion is that upon speciation, two lineages lose their cohesion and can for the first time diverge indefinitely (although we will give a caveat to the meaning of indefinite divergence). After they have become different species, two lineages can no longer fuse back into one, and so species lineages are irreversibly separate (25). Finally, at some point in their divergence, species become recognizable as sequence clusters for most genes in the genome as they accumulate neutral sequence divergence (27).

The species-like property of irreversible separateness, whereby species can coexist indefinitely, is necessary for
two lineages to eventually diverge to the point of becoming two genera, or two families, or even two phyla. However, this property is clearly not sufficient—only the rarest pair of newly divergent species will become two phyla! So, in what sense can we say that two new species have the potential to diverge “indefinitely”?

Two species should at least be ecologically different enough that one will not globally outcompete the other to extinction (28), noting that very small ecological differences can in principle lead to indefinitely long coexistence (29, 30). However, new species are not immune to the vicissitudes of nature that may extinguish them, and so pairs of new species are unlikely to persist indefinitely, regardless of how much they diverge from one another. For example, two new species may fail to coexist into the indefinite future if they have diversified within a single individual host but fail to be transmitted before the host dies. We may think of this as “shortsighted” speciation, analogous to the shortsighted evolution discussed by Levin and Bull, when a pathogen evolves high virulence within a host but fails to be transmitted (31). Here I will attempt to identify the properties of newly divergent species that give them a chance of coexisting into the remote future.

How can bacteriologists study the origins of species? Studies of animal and plant speciation have long focused on the origins of sexual isolation, owing to the influence of Ernst Mayr’s ideas on speciation through much of the 20th century (32). That is, the quintessential element of species’ origins was thought to be the evolution of inability to interbreed and exchange genes. In this view, unless the propensity to interbreed is reduced drastically between two groups, the groups are prevented from diverging, a process known as “Mayr’s brake” on diversification (33). In recent decades, however, zoologists and botanists have accumulated evidence that very little sexual isolation is needed for indefinite divergence, sometimes just the physical distance between two adjacent types of microhabitat (34–36).

In the case of bacteria, a growing literature of experimental evolution experiments indicates the potential for ecological diversification within a single vessel and without any sexual isolation. In some cases, there is some spatial separation of populations (scum on the surface versus the water column) (37, 38), but in other cases, there is no spatial separation whatsoever, and newly evolved populations differ ecologically only in the soluble resources they utilize (39, 40).

Nevertheless, several recent studies have claimed that sexual isolation is a necessary precondition for ecological divergence among bacterial populations (41–43). Their evidence was finding that closely related, ecologically distinct clades had reduced capacity for exchanging genes with one another, while lineages within each clade showed little or no sexual isolation. They concluded that the higher rates of recombination within the clades prevented ecological diversification. However, these studies did not actually attempt to determine whether the more rapidly recombining close relatives within the clades may have diversified ecologically, without the benefit of sexual isolation (44). My colleagues and I recently investigated, in hot spring *Synechococcus*, the possibility of ecological diversification among extremely close relatives that were not sexually isolated, and we found that the highest rates of recombination within clades did not prevent their ecological diversification (44). Where it has been investigated in bacteria, an absence of sexual isolation has not precluded ecological diversification among closest relatives (45). We may conclude that sexual isolation is not a prerequisite for ecological diversification in bacteria, and that sexual isolation is more likely to be the effect than the cause of ecological divergence.

More such studies will be desirable, given the hegemony of Mayrian ideas in speciology, but for theoretical reasons we should not expect to find an important role for sexual isolation in bacterial diversification. That is, recurrent genetic exchange is unlikely to prevent ecological divergence among bacterial populations (46, 47). J. B. S. Haldane showed long ago that when a niche-specifying allele is beneficial for one population but is harmful for another, natural selection can limit the niche-specifying allele to negligible frequency in any population where it is harmful (48). Haldane’s model predicts the frequency of a harmful, foreign allele to be $c_b/s$, where $c_b$ is the rate of recombination between populations and $s$ is the intensity of selection against the foreign allele; because the rate of recombination between bacterial populations is extremely low ($\sim 10^{-6}$ or less per gene per generation) (47), adaptive divergence between bacterial populations will not be stifled by recombination between them.

I will therefore not focus here on the origins of sexual divergence in bacteria. Instead, I will show that we can more usefully focus on the origins of ecological diversities that allow bacterial populations to coexist (46, 49, 50).

**THE FOCAL SPECIES OF BACTERIAL SPECIOLOGY**

Speciologists have traditionally studied the origins of species by focusing on the species taxa recognized by systematists and characterizing their divergence.
If species taxa are newly divergent enough, the differences among new species may be reasonably attributed to the process of species splitting, and not to divergence that happened after the lineages had split (51). This is not a bad approximation for animal and plant species, but it is not so useful for bacterial speciation. A recognized bacterial taxon is typically already extremely diverse in physiology, genome content, and ecology, and contains multiple lineages that each hold the species-like properties we have discussed (52–56).

The problem is that while the classification scheme of bacterial systematics has focused on finding species that are significantly different from one another in DNA sequence identity, genome content, and physiology (57), the classification is not aimed to ensure that each individual species is homogeneous in any characteristic (52, 54, 55, 58). To pick on a particularly notable example, we would not learn much about speciation by studying the divergence between the closely related Escherichia coli and Escherichia fergusonii species taxa, as lineages within E. coli are already hugely diversified. Some populations of E. coli are specialized as pathogens and others as commensals; within one host species, some lineages of E. coli may be specialized to colonize the large intestine and others the urinary tract (59, 60); populations may be specialized to different hosts (61) or even to living outside of any host (62, 63). These environments reflect vast differences in temperature, pH, and extracellular secretions and matrices; moreover, the ecological and physiological adaptations to these environments are based on huge genomic differences (60, 64). Most other named species also contain a high diversity of ecologically, physiologically, and genomically distinct populations (45, 65–75). In addition, genome sequencing is suggesting substantial ecological differences among closely related isolates within a given species taxon (43, 76, 77). Studying the divergence between the species taxa recognized by bacterial taxonomy is clearly not the way to study speciation; we need to zoom in within species taxa to find the origins of the most newly divergent lineages with species-like properties.

My colleagues and I have studied the dynamics of bacterial speciation by focusing on the origins of “ecotypes” (78–80). We define ecotypes as the most newly divergent, ecologically distinct bacterial populations that can coexist indefinitely as a result of their ecological differences. (As noted earlier, by indefinite coexistence we mean only that newly diverged ecotypes will not outcompete one another to extinction.) Ecotypes are defined such that lineages within an ecotype are largely homogeneous; to the extent that there are ecological differences within an ecotype, they are defined to be too small to allow indefinite coexistence (81) (Fig. 1). We will consider ecotypes to be the most newly divergent bacterial species, and we will refer to the origins of ecotypes as speciation, following recent usage by microbial ecologists (43, 45, 55, 78).

I will first discuss progress toward identifying the most newly divergent ecotypes. I will then explain how these efforts have allowed us to characterize the genetic basis of ecotype divergence, to find the ecological dimensions along which ecotypes have diverged, to identify the species-like characteristics held by bacterial ecotypes, and to estimate the tempo of bacterial speciation. In particular, I will address how the lifestyle of a bacterial group and the genetic basis of speciation can affect the dynamic attributes of speciation and the likelihood of long-term coexistence. I will focus on pathogens wherever possible.

FIGURE 1 Ecological divergence between ecotypes and ecological homogeneity within ecotypes. The ecological divergence between ecotypes is sufficient for them to coexist into the indefinite future. Ecotypes are defined so that any ecological differences among lineages within ecotypes are not sufficient to allow them to coexist indefinitely. We may thus refer to ecologically distinct lineages within ecotypes as “ephemeral ecotypes.” The different styles of dashed lines within ecotype 1 refer to different ephemeral ecotypes; note that only one of these lineages persists to the present. The different weights of solid lines represent different ephemeral ecotypes within ecotype 2 (52). Used with permission from Elsevier.

TO IDENTIFY THE MOST NEWLY DIVERGENT ECOTYPES

Bacterial systematists have approached species demarcation under the premise that each species should contain a standard and large level of sequence diversity, for example, a level of ∼1% divergence in the 16S rRNA molecule (82). As we have seen, the universal molecular
criteria for demarcating species have led to species taxa like *E. coli* that are notably diverse in their physiology and ecology. In contrast, several algorithms use sequence data from the taxon of focus to identify the appropriate level of sequence diversity for distinguishing groups likely to have the dynamic properties of species. These include Ecotype Simulation (83), BAPS (84), GMYC (85), AdaptML (45), Minimum Entropy Decomposition (MED) (86), and Accessory Gene Network Analysis (77). For example, Ecotype Simulation identifies sequence clusters that are consistent with ecotypes, assuming that new ecotypes form and cohesive forces purge diversity within ecotypes at rates that the algorithm infers from the sequence data (79).

The algorithms AdaptML and MED require the habitat of isolation as input data, while Ecotype Simulation, BAPS, and GMYC are blind to ecology and demarcate ecotypes based solely on patterns of sequence clustering. Ecology-blind and ecology-informed approaches both have their advantages (87). The ecology-informed AdaptML and MED are useful when the investigators have hypothesized one or more environmental parameters they believe to be important in ecotype divergence. For example, Dana Hunt and colleagues believed that newly divergent ecotypes in marine *Vibrio* would differ in the size of small particles they inhabit, and they were right (45)!

The ecology-informed algorithms not only discover the putative ecotypes, but they also confirm their ecological distinctness by testing for significant differences in habitat preferences. In contrast, the ecology-blind algorithms can identify ecotypes even when the researcher is ignorant of the environmental differences that might be important in ecotype divergence. Moreover, ecology-blind methods can discover ecotypes even when they do not differ in their preferred habitats (83).

So, how successful are these algorithms in finding the most closely related ecotypes? We must require two properties of the “putative ecotypes” that are hypothesized and demarcated by the algorithms. One requirement is that the putative ecotypes must be ecologically distinct from one another; the second requirement is that each putative ecotype must be ecologically homogeneous. By and large, when these algorithms have predicted putative ecotypes, these groups have been confirmed to be ecologically distinct based on their habitat differences (45, 58, 79, 80). For example, putative ecotypes of soil *Bacillus* hypothesized by Ecotype Simulation have been shown to differ in preferences for solar exposure, soil texture, rhizospheres, elevation, and geochemical stressors (78, 79); putative ecotypes of *Synechococcus* from hot spring mats have differed in their temperature and depth associations (58, 80); putative ecotypes of *Legionella* have differed in the amoebae they can infect (that is, they differ in their host ranges) (88); and putative ecotypes within *Vibrio splendidus* differed in the sizes of particles they were attached to and in their seasons of abundance (45). In addition, many ecologists have noted that very closely related sequence clusters (demarcated by intuition rather than by algorithm) were different in their habitat associations (62, 72, 89–91). Over all, putative ecotypes identified as closely related sequence clusters in a great diversity of phyla (*Actinobacteria*, *Cyanobacteria*, *Firmicutes*, *Proteobacteria*, and *Spirochaetes*) have shown ecological distinctness through habitat preferences.

Microbial ecologists have also confirmed the ecological distinctness of putative ecotypes by finding the physiological and genomic differences that underlie their habitat associations. For example, putative ecotypes of *Bacillus subtilis* and *Bacillus simplex* that are associated with more direct solar exposure have been shown to have membranes yielding greater thermal tolerances (78, 92). Putative ecotypes of *Synechococcus* farther from the source of the hot spring (and living in cooler water) were found to be less tolerant of extremely high temperatures (93), and they had genes enabling utilization of ions that are relatively more abundant downstream (94, 95).

Do the putative ecotypes meet the second requirement for ecotypes, that they must be ecologically homogeneous? We should note that attempts to identify putative ecotypes have so far drawn from low-resolution phylogenies, based on only one to three genes. If speciation is occurring rapidly, one to three genes might not provide enough resolution to find the most recently divergent ecotypes as sequence clusters, and so each putative ecotype could actually be an amalgam of many newly divergent ecotypes. When we explore the rates of speciation for various bacterial phyla, we shall see that the ecological homogeneity within putative ecotypes varies across lifestyles. The hypothesized putative ecotypes are in some cases the most newly divergent ecological species; in other cases, they are not, but they at least represent an early phase of ecological diversification.

We'll next consider the genetic and ecological basis of early diversification in bacteria.

**THE GENETIC BASIS OF EARLY DIVERSIFICATION**

Bacteria can evolve to change their ecology through various kinds of genetic changes: mutations in genes they already have, replacement of one allele by another
at a genetic locus through homologous recombination, and acquisition of suites of novel genes either by horizontal genetic transfer (HGT) of chromosomal genes or through infection by a plasmid or a phage (47, 96, 27). As we shall see, the genetic basis of divergence may have implications for the long-term coexistence of ecologically distinct lineages.

Like the animals and plants, bacteria can diversify ecologically through changes in the genes they already have. The capacity for mutation-driven diversification has been demonstrated many times in laboratory evolution experiments, where a single bacterial clone diversifies by mutation into multiple coexisting ecotypes (37, 98–100). In addition, mutations have been shown to be responsible for bacterial diversification in nature, primarily in pathogens. Mutations have resulted in diversification of populations specializing in different mammalian host species in Borrelia (91, 101); mutations have also resulted in diversification of populations specializing in different tissues and microhabitats within hosts by E. coli (102), Pseudomonas aeruginosa (103, 104), and Bartonella bacilliformis (105, 106). Also, mutations in Salmonella enterica have resulted in a “top-down” diversification, in which populations are differentiated by their defenses against amoebic predators (107). Mutation alone can even generate biochemical novelty (e.g., utilization of a new carbohydrate), especially when the gene involved has been duplicated (108).

While bacteria and the higher organisms share mutation as a driver of diversification, bacteria are distinguished by the huge role that HGT plays in their diversification. This is in part because transfer can occur across vast phylogenetic distances in the prokaryotes (109) but also because transfer is usually limited to a small stretch of DNA (47), ranging from several thousand bases in genetic transformation (110) to several hundred thousand in conjugation (111, 112). The short lengths transferred provide that a recipient can acquire a small adaptive set of genes from a donor without also acquiring a huge number of genes (“genetic baggage”) that would be maladaptive for the recipient (110).

A horizontal transfer event can profoundly alter the ecology of a recipient bacterium in a single step and may constitute an instantaneous speciation event. That is, acquisition of DNA by horizontal transfer may cause the recipient lineage to differ markedly from the preexisting lineage in the resources it can utilize or the conditions it can tolerate (113, 114), such that the two lineages can coexist indefinitely.

We should note that horizontal transfer of an adaptation does not necessarily make the donor and recipient lineages more similar ecologically. Instead, a transferred adaptation can allow the recipient to build on its unique, preexisting adaptations to either invade a new niche or improve its performance in its current niche (87). For example, E. coli and Burkholderia cepacia share a niche-transcending adaptation that does not make these lineages more similar ecologically. The shared class 5 fimbriae allow each lineage to better attack the epithelial cells of its respective niche, whether in the small intestine for E. coli or in the lung for B. cepacia (in cystic fibrosis [CF] patients) (115), but these lineages nevertheless still attack different tissues (28, 87). Likewise, when ecologically disparate human pathogens acquire the same antibiotic resistance factors by HGT (116), their ecological niches are not converging beyond their response to natural selection by antibiotics.

We have noted that once two populations have diverged ecologically, sexual isolation is not required for maintaining their ecological divergence (47). However, sexual isolation can impact the creation of ecological divergence by limiting the opportunities for HGT. This is because bacteria are much more likely to acquire genes through HGT from close relatives, both because lower sequence divergence will foster homology-facilitated illegitimate recombination (117) and because closer relatives are more likely to share habitats (109).

What might be the scope of transferable adaptations that can bring about speciation? Transfer of adaptations is limited by the short length of DNA segments that can be transferred by the various mechanisms of recombination. An ecology-changing horizontal transfer can be as small as a single gene, even a small set of nucleotides within a gene. This is the case for homologous recombination of penicillin resistance alleles that have been transferred from one Neisseria species to another and from one strain of Streptococcus pneumoniae to another (118).

Also, an adaptation coded by several genes may be transferred in one event, provided that the genes lie contiguously on a small segment of chromosome. For example, the lac operon, which has been studied so famously in E. coli (119), was acquired by an Escherichia ancestor by HGT, as evidenced by the operon’s unusual codon usage (120). The operon has all the components needed for uptake and metabolism of lactose, as well as regulation of the whole transcription unit.

Jeffrey Lawrence’s “selfish operon” theory explains that many metabolic capabilities have transferred between lineages owing to the operon organization of the genes involved (121). The selfish operon model predicts that natural selection will favor the contiguous
arrangement of a functionally related set of genes as an operon, enabling the gene set to be successfully transferred as a single, functional element across taxa; that is, natural selection acts on the operon itself rather than on the organism (121–123). Operons may thus make possible a wholesale transfer of metabolic capabilities even in the context of very short recombinant segments. Beyond carbohydrate utilization functions, transferable units include the sets of genes coding for synthesis of siderophores (113), synthesis of outer membrane structures (124, 125), heavy-metal resistance (126), and antibiotic resistance (127). Many such transfers have created new populations that are ecologically distinct from their parental populations.

Microbiologists have become accustomed to evolution of adaptations through HGT, to the extent that HGT is seen as a deus ex machina that can solve any ecological challenge. For example, when a pathogen needs a new outer surface structure to evade the immune system, it can just grab it from another species through HGT (128). HGT makes possible the saltational evolution toward “hopeful monsters” that was hypothesized long ago by Richard Goldschmidt for animal evolution (129).

But there are limits to the complexity of adaptations that can be transferred: the genes coding the adaptation must fit on a transferable piece of DNA and the adaptation must be compatible with the physiology and architecture of the recipient (47). For example, the extremely resilient cell wall of the Firmicutes is a remarkable structure that grants constitutive resistance to osmotic stress. Surely, other organisms would do well with the osmotic resistance of the Firmicutes’ cell wall, but this structure has never been transferred across phyla. We can imagine that the complexity of the Firmicutes’ cell wall architecture cannot be coded on a small piece of DNA, nor would it be compatible with the existing architecture of some other phylum. Likewise, the stalked structure of the Planctomycetes phylum is highly adaptive in raising a cell above the still water at the surface of the seabed, and we can imagine that a stalk structure would provide greater nutrients to any other bacteria residing in that habitat. However, this structure has not been transferred either, likely for being too complex to travel across phyla. While HGT is a marvelous device for acquiring simple adaptations, there are clearly limits to what can be transferred (18).

Bacteria can also change their ecology by acquiring a plasmid that comes fully adapted to change the ecological niche of its host. For example, a Rhizobium cell may be adapted to infect and provide nitrogen fixation for a given legume species by virtue of its “symbiosis” plasmid. A Rhizobium lineage may change its host adaptation by gaining a symbiosis plasmid for one plant host and losing the plasmid for another, or it may adapt to life in free soil by losing all its symbiosis plasmids (47, 130). Also, within E. coli there are several pathogenic populations whose virulence capabilities are determined by plasmids (e.g., enteroinvasive, enterotoxigenic, and enteroaggregative E. coli), and one virulence type is determined by a phage (enterohemorrhagic E. coli). These extrachromosomal elements can all move readily from one lineage to another, thereby converting an E. coli lineage to a new ecology (131, 132). Likewise, lineages within Bacillus cereus sensu lato can move from the niche of a gut pathogen of mammals (B. cereus) to that of causing systemic infection in mammals (Bacillus anthracis) to that of an insect pathogen (Bacillus thuringiensis) by acquiring and losing different plasmids. These dynamics each present a paradoxical case where the various niches may persist into the remote future, yet those bacteria inhabiting those niches may never diverge as independent and irreversibly separate lineages. Their divergence is prevented by any given bacterial lineage being thrust from one ecotype into another and then back again through recurrent transfer of plasmids (81).

There is one circumstance where plasmid determination of niches can help precipitate irreversible divergence among bacterial lineages. This is the case when closely related bacterial lineages diversify, perhaps gradually, to specialize on the services of different plasmids. This appears to be happening within agricultural lineages of Rhizobium leguminosarum, where there are five sequence clusters that can each be infected by various symbiosis plasmids that adapt the bacteria to either vetch or clover (133). While these clusters have no known physiological features that would explain their coexistence, they appear to differ in their propensities to be infected by the various symbiosis plasmids (52). Differences in association between plasmid adaptations and bacterial sequence clusters cause the clusters to differ dramatically in the plants they infect. It is not clear why the sequence clusters differ in their frequencies of infection by different plasmids, but this could represent an early stage of irreversible ecological diversification of the Rhizobium sequence clusters.

A later stage of permanent diversification of bacteria through specialization on different plasmids was observed among R. leguminosarum lineages, in this case sampled from nonagricultural species of clover (134). Here, three chromosomally defined sequence clusters were each infected by a different plasmid lineage, and so the Rhizobium lineages largely infected different species.
of clover. This study concluded that plasmids can promote indefinite coexistence of different bacterial lineages. Likely the specialization on different plasmids was fostered by a slow rate of transfer of plasmids, yielding a long residence time of plasmids in any given bacterial lineage; this would give a particular bacterial chromosomal lineage an opportunity to adapt to its plasmid before the plasmid leaves for another lineage.

To what extent can HGT of chromosome-based genes lead to reversible ecological divergence? One possibility is that the transfer of an operon could first create a new population that is ecologically distinct from its parental population; then, if the new population should lose the operon, the divergence between the new and old populations would be reversed. However, there is little evidence that ecological divergence can be reversed in this way, and indeed two factors appear to prevent ecological reversals through acquisition and then loss of chromosome-based genes.

One limiting factor is that the rates of acquisition and loss of chromosome-based functions are not likely to be as high as for plasmid-based genes, except possibly in the case of homologous recombination of niche-determining alleles. For example, penicillin-binding protein alleles can be recurrently gained and lost through homologous recombination among close relatives of *Neisseria*, thus converting lineages from one ecologically defined population to another (135).

Another factor limiting the reversibility of ecological divergence is that horizontally acquired genes can create secondary selection to improve the adaptations they provide. In contrast to plasmids circulating continuously through a given bacterial clade, the adaptations provided by a novel suite of genes from a distant taxon are not fully adapted to a given recipient. As a result, many horizontally acquired genes engender a round of ameliorative evolution that improves the adaptation’s compatibility with its new genetic background (47, 136, 137). Modes of ameliorative evolution include changes in genes whose products interact with the new adaptation (138–140) and in some cases changes in the transferred genes themselves (137). Moreover, the incompatibilities brought by a transferred adaptation can yield a cascade of evolutionary changes (141).

We can expect that the ameliorative evolution following a horizontal transfer complicates the possibility of reversible evolution. Whereas a *Rhizobium* lineage can return to its previous ecological niche by simply losing its symbiosis plasmid, a lineage that has changed its ecology by acquiring a transfer from a distant relative cannot so easily return to its previous niche. The ameliorative changes that have occurred in the rest of the genome might not be easily reversible (142).

In summary, we can hypothesize that the most easily reversible ecological divergences will involve acquisitions and losses of plasmids that circulate through a given taxon, as seen in *R. leguminosarum* (133), *E. coli* (131), and *B. cereus* (143). Perhaps next easiest is acquisition of an adaptation through homologous recombination, as in the case of antibiotic resistance in *Neisseria* (135), although even a single gene can create ameliorative evolution (139, 142). Less reversible will be evolution brought about by a horizontal transfer from a distant relative, given the abundant opportunities for ameliorative evolution to follow the transfer (136). Finally, we may hypothesize that the least reversible evolutionary transitions will be those effected by a series of mutations, since there will be many events that each must be reversed.

### THE ECOLOGICAL DIMENSIONS OF EARLY DIVERSIFICATION

What are the ecological changes that have allowed one population to diverge into two ecologically distinct and coexisting lineages? There are two fundamental ways that populations may distinguish themselves ecologically. First, populations may diverge in their habitat preferences, and may coexist because they are each the superior competitor in different habitats. This is the classic meaning of “ecotype” from the botanical literature, where, for example, closely related populations within a plant species taxon may each be the superior competitor at different elevations (144). Alternatively, populations may coexist in the same microhabitat because they prefer different resources within that habitat. In the animal world, this is commonly seen among closely related species that specialize on different food resources from the same habitat (145). As we shall see, most of the bacterial ecotypes characterized thus far have diverged by habitat type, probably because these differences are easier to document.

We need to take into account that an adaptation yielding an ecological change is not by itself sufficient for ecological diversification. If a new genotype is to found a new population that can coexist indefinitely with the old population, the genetic change must come with a trade-off in fitness. If instead an adaptive mutation were to convey a new resource at no cost in utilizing the old resource, the adaptation would simply be an improvement in the old population. The new mutation would sweep through the old population, and in the end we
would have one improved population, not two different populations (47) (Fig. 2). One possibility is that a trade-off comes intrinsically with the ability to use some new resource. For example, suppose we begin with a cyanobacterial population that is adapted to low levels of light. A new mutant that utilizes high levels of light can coexist as a separate population with the low-light population if the mutation comes at a cost to utilizing low light (30, 80). An intrinsic trade-off can yield coexistence of a new population with the old, even when the populations are living in the same microhabitat (sympatric speciation) or when the populations live in different microhabitats that are in easy dispersal range of the populations (parapatric speciation) (47, 146).

However, trade-offs that yield coexistence need not be intrinsic to the new adaptations. An alternative is possible when nascent ecotypes are geographically isolated (allopatric speciation). In this case, a population may adapt to some new microhabitat with no intrinsic losses in its ability to compete in the old microhabitat; nevertheless, adaptations that are specific to the old microhabitat may be costly to keep in the new habitat and may disappear (147). Even without intrinsic trade-offs, each population eventually evolves to be superior in its own microhabitat and inferior in the other’s (148).

We may divide the ecological dimensions of speciation into changes in tolerances to physical and chemical conditions versus changes in resources consumed. Of course, changes in the conditions tolerated will ultimately result in consumption of resources from microhabitats that differ in their conditions (149).

Physical and Chemical Conditions

In the Cyanobacteria, hot spring Synechococcus and marine Prochlorococcus have both been studied for early diversification in temperature tolerances. For hot spring Synechococcus, temperature has proved a difficult dimension on which to diversify, at least more difficult than diversification by resources. We found two temperature ecotones, one at 61 to 62°C and another at 64 to 65°C, over which evolutionary transitions have been rare (80). That is, comparatively large clades are found nearly entirely on one side of a temperature ecotone or the other, but within each clade, there are many ecotypes that have recently diverged in resource adaptations (e.g., by light and oxygen levels) (150).

However, studies on Prochlorococcus show a contrasting pattern. Here the most newly divergent clades within P. marinus differ in their temperature adaptations, but only the most anciently divergent clades differ in the light levels they are adapted to (151). What is easiest and what is hardest varies with taxa, even among Cyanobacteria!

Diversification in temperature adaptations between newly divergent ecotypes has occurred also in heterotrophic Bacillus. Following the “Evolution Canyon” paradigm (152), organisms were sampled from soil of the north- and south-facing slopes of an east-west desert canyon. The principle of Evolution Canyon is that, at least for very shallow soil samples, the south-facing slope has much more intense solar exposure and higher daytime temperatures (153), indeed even different plant species (78). This approach led to the discovery that very closely related putative ecotypes of Bacillus differed significantly in their associations with solar exposure (78, 79, 92). Moreover, those putative ecotypes associated with greater solar exposure were found to be better adapted to higher temperatures through greater abundance of lipids conferring reduced membrane flexibility (78, 92).

Adaptations to chemical conditions appear to evolve very quickly in Bacillus. Along a geochemical gradient with toxic levels of various metals and metalloids, there was an astounding diversity of tolerance even among extremely close relatives within a single putative ecotype (154). This was an indication that the putative ecotypes demarcated in Bacillus by one or several genes do not provide the resolution to identify the most newly divergent ecotypes.

Resource Differences among Newly Divergent Free-Living Organisms

Beyond light levels, another resource dimension of diversification for newly divergent lineages of Cyanobacteria is the set of mineral nutrients they consume. In hot spring Synechococcus, upstream and downstream populations differ, beyond temperature, in their nutrient utilization capabilities. Phosphate, the easiest phosphorus ion to utilize, is depleted by the upstream populations, leaving the more difficult ions for the downstream populations (94). In addition, the downstream clades showed a genomic capacity for storage of nitrogen, a nutrient largely depleted by the upstream populations (94). Similarly, closely related clades of Prochlorococcus differ in their abilities to take up inorganic minerals (155).

Generalist heterotrophs are likely to change their resource base frequently (156). All that is needed is HGT of a functional set of genes conferring metabolism of a new resource. We have seen that operons make possible a wholesale transfer of metabolic capabilities even in the
FIGURE 2 The consequences of fitness trade-offs in ecological diversification. Improvement of an ecological function by mutation or HGT (indicated by the enlarged triangle) can lead to a periodic selection event (A) or an ecotype formation event (B or C). Each individual is represented by a circle, and each individual’s degree of adaptation to two resources (or conditions) is indicated by the sizes of the square and triangle, respectively. In panel A, adaptation to the triangle resource or condition is increased in one individual (indicated by increased triangle size), and the resultant strain is now able to outcompete the membership of its ecotype by virtue of its more generalist ecology. In panel B, the increase in adaptation to the triangle resource intrinsically decreases the adaptation to the square resource. Thus, increase in one ecological capability comes at the expense of a preexisting capability. In this case, acquisition of the new function leads to a new ecotype, which can coexist with the preexisting ecotype. This has been seen repeatedly in experimental populations of *E. coli* that primarily used glucose for carbon; a mutation to utilize secreted acetate created a new ecotype because the acetate-utilizing bacteria were less able to utilize glucose (39, 47). An alternative possibility is shown in panel C, where there is no intrinsic trade-off to the new adaptation yet a new ecotype can form. Here the new genotype invades a new habitat where the new adaptation is selected for. If the “square” adaptation is not utilized in one habitat and the “triangle” adaptation is not utilized in the other, under the Black Queen hypothesis, the unnecessary adaptations may be lost (147). This will make each ecotype the superior competitor in its own microhabitat. Adapted with permission from reference 47.
context of very small recombination events (120). For example, Bacillus is a generalist heterotroph with a history of rapid change in metabolic capabilities: subspecies taxa within B. subtilis are different in utilization of scores of carbohydrates (81).

Instead of diverging in the sets of resources they can metabolize, generalist heterotrophs may also diverge quantitatively in the extent to which they utilize the same set of resources. For example, within a single putative ecotype of B. subtilis, a genome comparison found only quantitative ecological divergence—all the lineages studied shared the same set of resources but differed in the ability to utilize some of these resources. For example, while all the lineages were able to use maltose, one lineage had additional genes for metabolizing this resource and was superior to other lineages when maltose was the only carbon source (81).

With generalist heterotrophs, ecological divergence can take place within the same microhabitat. What is needed is specialization to different soluble compounds within one habitat. This has been observed repeatedly in experimental microcosms, most often when one ecotype cross-feeds from the exudate of another ecotype within the same vessel (39, 40) but also when the medium contains a rich diversity of organic compounds (38, 157). There are probably many instances of sympatric diversification among closely related heterotrophic ecotypes in nature, but much more attention has been paid to diversification by microhabitat type (43, 158).

More-specialized heterotrophs appear less likely to change resources frequently than generalists (156). Some phylogenetic groups specialize on a particular single-carbon (C1) molecule, and many ecotypes and even taxonomic species in these groups share the same organic resources. For example, different taxonomic species of Methylobacter appear to be limited to consuming methane as an organic carbon source. Nevertheless, speciation is possible in other resource dimensions, as closely related clades within Methylobacter are adapted to different depths (and oxygen levels) in a crater lake (159). In some C1 heterotrophs, speciation is possible by changing specialization from one C1 molecule to another (160).

Resource Differences among Pathogens
Pathogens may diversify by specializing on different host species. For example, in the Mycobacterium tuberculosis complex, which is responsible for tuberculosis, sequence clusters correspond to different clades of hosts (humans, artiodactyls, pinnipeds) (161). Also, within the species Anaplasma phagocytophilum, the pathogen responsible for tick-borne fever, sequence clusters are associated with different mammalian hosts (70). Within Borrelia burgdorferisensu stricto, the North American Lyme disease spirochete, some sequence clusters are associated with different rodent species (101, 162), although it is not yet clear whether the adaptations to specific hosts are genome-wide adaptations or are due primarily to a single outer-surface protein (163). In some cases, host specificity is determined by plasmids, and the bacteria can adapt from one host species to another with acquisition of plasmids, as seen in legume-infecting Rhizobium ecotypes (133) or mammal- versus insect-infecting ecotypes in B. cereus sensu lato (143).

Phylogenetic approaches can address the rate that a pathogen group moves from one host species to another. For example, consider the case of aphids and the obligately endosymbiotic Buchnera lineages that infect them. The aphids and their bacteria have congruent phylogenies, in that a splitting of aphid lineages corresponds to a splitting of their Buchnera endosymbionts, implying that nearly all Buchnera diversification has emerged as cospeciation with aphids (164). A Buchnera lineage rarely ventures into a new host lineage except by passive vertical transmission. This pattern of passive transmission and cospeciation occurs also in some viruses, with hantaviruses rarely transmitting to a new lineage; in contrast, the arenaviruses frequently change host lineages (165).

Can we predict the host species most likely to share pathogens? Humans are a good place to start, as obviously there is a lot of interest in discovering the animal species that are most likely to share their contagions with us. Relatedness is a predictor of the probability of a pathogen spillover, as the primates have given us ~10% of our novel pathogens in the last 3 decades (166). However, the much more distantly related ungulates, rodents, and bats have introduced us to many more diseases in recent decades. There is clearly a role of shared environment (ecological opportunity) here, as rodents infect us through cohabiting our housing and cities with us (167), and our caring for domestic ungulates has introduced us to novel diseases from ancient times to the present (168, 169). Bats have introduced (and reintroduced) us to several diseases in recent decades through sharing their environment with our domestic animals: Middle East respiratory syndrome through camels (170), Nipah through pigs (171), and Hendra through horses (172). Bats have also introduced severe acute respiratory syndrome to us through infecting civets in a bushmeat market (173). In addition, bats have directly and recurrently transmitted Ebola to us (174).
Daniel Streicker has recently investigated the parameters that determine the likelihood of a pathogen moving to another host species and then becoming established there (175). Focusing on lineages of rabies virus that infect different species of bats, he found that the most important determinants were sharing the same geographic range and the relatedness of the bat hosts. Less important was a shared ecology among the bat species, for example, having the same roosting behavior.

The Streicker study suggests that there may be many more host shifts than we appreciate, given the tendency of microbial systematics to lump ecologically distinct lineages into a single taxon. When we see a particular microbe taxon, e.g., rabies virus, infecting a huge diversity of bat species, the established taxonomy does not make clear whether we have a generalist virus that can infect dozens of host species or whether there are many specialist viruses that each primarily infect one host lineage. Streicker’s work shows the latter, and implores us to fine-tune our systematics to accommodate rapid diversification of pathogens (52).

Pathogens may also diversify by the host tissues they infect. For example, Yersinia pseudotuberculosis is a gut pathogen transmitted by the fecal-oral route, but it has the capacity to be lethal when it (rarely) invades the lungs or blood (176). The plague bacterium, Yersinia pestis, which is derived from Y. pseudotuberculosis, inherited its ancestor’s capacity for systemic infection, but it evolved to reside primarily in the hosts’ blood through transmission by fleas (177). Similarly, ecotypes within Streptococcus pyogenes are differentiated to infect the throat, causing strep throat, versus the skin, causing impetigo (178).

Pathogens with a free-living phase have many more opportunities for ecological diversification than obligate pathogens. Living outside of hosts allows diversification of ecotypes by specialization to different environmental carbon sources, for example, as seen in some members of the Enterobacteriaceae such as E. coli (179) or in some Firmicutes such as Listeria (180).

**MODELS OF SPECIATION: SLOWLY SPECIATING, LONG-LIVED TAXA**

As we have discussed, the properties attributed to species include (i) ecological distinctness and irreversible separation of different species, (ii) cohesion within species but not across species, and (iii) distinguishability of species as sequence clusters. Earlier ecotype models assumed that all of these species-like properties would follow once two populations diverged to be ecologically distinct (26). However, it now appears that these properties follow ecological divergence only under certain circumstances (28, 43, 124, 156).

We begin with the stable ecotype model of speciation, where all the species-like properties hold. Here, each ecotype is assumed to be long-lived, and speciation is infrequent (Fig. 3). The long-term coexistence of ecotypes may be fostered by a qualitative ecological divergence, where each ecotype can use at least some resource that is not used by its close relatives (81). Such ecotypes are most likely to persist into the long haul of time because the ecotypes’ unique resources provide a haven protecting them from extinction by competition from one another (52). Nonsharing of resources may be facilitated by HGT, whereby novel resources or capabilities can become available to a recipient ecotype (26), but changes in expression of existing genes can also lead to utilization of novel resources (81). Whether ecotypes will coexist into the indefinite future will depend also on whether their unshared resources will persist into the future.

Newly divergent ecotypes are unlikely to fuse to become a single ecotype once again (28). While fusion may be possible for closely related animal and plant species, owing to their higher rates of recombination (182), the low rates of recombination in bacteria (183) make population fusion unlikely. We have discussed how natural selection against niche-specifying alleles from other populations will hold those alleles to negligible frequencies and thereby prevent species fusion (47, 48). So the only way fusion can occur is if the preferred niches of two ecotypes disappear and there emerges a new niche to which hybrids are adapted. Studies on Campylobacter by Sam Sheppard and colleagues have demonstrated the emergence of a new agricultural niche for hybrids between C. coli and C. jejuni. However, this is not a fusion because the two original species remain adapted to and persist in their respective niches (184).

We might hypothesize that ecotype fusion is most likely among free-living ecotypes that have adapted to infinitesimally different regions of a continuous environmental gradient. However, even a continuous gradient will result in a discontinuity of ecotypes that are adapted to discretely different points on the gradient, as modeled for continuous light variation in a photosynthetic mat (30). If niche-specifying genes from one such ecotype were transferred into another ecotype adapted to a discretely different point on the gradient, it is likely that selection against the maladaptive, foreign genes would prevail and prevent fusion (47). In bacteria, the impetus for irreversible diversification, Fernando
FIGURE 3 Models of bacterial speciation. Ecotypes are represented by different colors, periodic selection events are indicated by asterisks, and extinct lineages are represented by dashed lines. The letters at the top represent the resources that each group of organisms can utilize. In cases where ecotypes utilize the same set of resources but in different proportions, the predominant resource of each ecotype is noted by a capital letter. (A) The stable ecotype model. In the stable ecotype model, each ecotype endures many periodic selection events during its long lifetime. The stable ecotype model generally yields a one-to-one correspondence between ecotypes and sequence clusters because ecotypes are formed at a low rate. The ecotypes are able to coexist indefinitely because each has a resource not shared with the others. (B) The speedy speciation model. This model is much like the stable ecotype model, except that speciation occurs so rapidly that most newly divergent ecotypes cannot be detected as sequence clusters in multilocus analyses. (C) The nano-niche model of bacterial speciation. In the figure, there are three nano-niche ecotypes that use the same set of resources but in different proportions (noted by Abc, aBc, and abC). Each nano-niche ecotype can coexist with the other two because they have partitioned their resources, at least quantitatively. However, because the ecotypes share all their resources, each is vulnerable to a possible speciation-quashing mutation that may arise in the other ecotypes. (D) The species-less model. Here the diversity within an ecotype is limited not by periodic selection but instead by the short time from the ecotype’s invention as a single mutant until its extinction. The origination and extinction of each ecotype $i$ is indicated by $s_i$ and $e_i$, respectively. In the absence of periodic selection, each extant ecotype that has given rise to another ecotype is a paraphyletic group, and each recent ecotype that has not yet given rise to another ecotype is monophyletic (81). (E) Recurrent niche invasion model. Here a lineage may move, frequently and recurrently, from one ecotype to another, usually by acquisition and loss of niche-determining plasmids. Red lines indicate the times in which a lineage is in the plasmid-containing ecotype; blue lines indicate the times when the lineage is in the plasmid-absent ecotype. Periodic selection events within one ecotype extinguish only the lineages of the same ecotype. For example, in the most ancient periodic selection event shown, which is in the plasmid-absent (blue) ecotype, only the lineages missing the plasmid at the time of periodic selection are extinguished, while the plasmid-containing lineages (red) persist. Ecotypes determined by a plasmid are not likely to be discoverable as sequence clusters. Reproduced from reference (81).
Baquero’s *ex unibus plurum*, appears much greater than the opposite impetus for reunification, *ex pluribus unum* (185).

The longevity of ecotypes in the stable ecotype model fosters two species-like properties. First, over the long lifetime of an ecotype, ample opportunity is provided for the ecotype to acquire a unique set of neutral mutations in each gene in the genome. Thus, each ecotype will eventually become distinguishable as a sequence cluster. Second, there may be opportunity for many incidences of cohesion during the long life of an ecotype.

What provides cohesion for a bacterial ecotype? Owing to the rarity of recombination in bacteria, natural selection favoring each adaptive mutation purges the diversity within an ecotype to near zero across the genome, the process of periodic selection (28). Periodic selection purges the diversity within but not between ecotypes, and may occur many times during the long lifetime of ecotype (Fig. 4).

We may test whether the stable ecotype model applies by first using an algorithm to hypothesize ecotypes from sequence diversity, e.g., with Ecotype Simulation. We may then test whether the putative ecotypes are ecologically distinct and finally whether the putative ecotypes are each ecologically homogeneous. If the putative ecotypes are predicted to each contain up to 0.5 to 2.0% average nucleotide divergence (a divergence great enough to detect ecotypes with the resolution of a single gene of 1,000 bp or more), finding ecological homogeneity would indicate a slow rate of speciation (156). This would mean that in the time the average gene has accumulated 0.5% neutral divergence or more, no speciation events have occurred.

So, what bacteria abide by the stable ecotype model? Our surveys of diversity in the photoautotrophic *Synechococcus* of Yellowstone hot springs indicate slow speciation and long-lived species in this group. Each putative ecotype contains ~0.5% sequence diversity genome-wide and appears to be ecologically homogeneous (80, 95). One piece of evidence for ecological homogeneity was that each putative ecotype consisted of several sequence types that maintained the same relative frequencies across a great range of environments. These environments included different hot springs and

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**FIGURE 4** The dynamics of ecotype formation and periodic selection within an ecotype. Circles represent different genotypes, and asterisks represent adaptive mutations. (A) Ecotype formation event. A mutation or a recombination event allows the cell to occupy a new ecological niche, founding a new ecotype. A new ecotype can be formed only if the founding organism has undergone a fitness trade-off, whereby it cannot compete successfully with the parental ecotype in the old. (B) Periodic selection event. A periodic selection mutation improves the fitness of an individual such that the mutant and its descendants outcompete all other cells within the ecotype; these mutations do not affect the diversity within other ecotypes because ecological differences between ecotypes prevent direct competition. Periodic selection leads to the distinctness of ecotypes by purging the divergence within but not between ecotypes. Reproduced with permission from reference 193.
different depths and temperatures within the same hot spring, as well as experimental manipulations of temperature and light. Note that if the different sequence types within a putative ecotype had been specialized to different environments, we would have expected the frequencies of the constituent sequences to vary across environments. Another piece of evidence emerged from comparing multiple genome sequences. Within a given putative ecotype, there was no indication of different histories of positive selection (95), which would have suggested ecological diversity (186). So, in the time it took 0.5% sequence diversity to accumulate, apparently no speciation events had occurred. While it is possible that lineages within a putative ecotype had diverged to accommodate fine differences in conditions (29), there is no evidence that they have done so.

On the other hand, the generalist heterotroph *B. subtilis* and its close relatives in Death Valley soils have shown extremely rapid ecological diversification. We surveyed genome sequence diversity within one putative ecotype and found that every lineage studied had a unique history of positive selection, suggesting that each isolate represented a different ecotype (81). This supported Ford Doolittle’s hypothesis that bacterial diversification is so rapid that any given cell is unlikely to be ecologically identical to any beyond its immediate offspring (187).

The contrast between *Synechococcus* and *Bacillus* suggests a general hypothesis for predicting rates of speciation and the circumstances under which the stable ecotype model applies. Among free-living bacteria, we may predict that generalist heterotrophs like *Bacillus*, with many options for metabolic diversification, will speciate rapidly (156). Alternatively, photoautotrophs and any other ecological guilds that consume few or no organic resources will have limited options for metabolic diversification and will speciate slowly (156).

This hypothesis was supported by a recent metagenomic survey of diversity over time within an acidic lake in Wisconsin, United States (156, 188). Matthew Bendall and coworkers assembled metagenome sequences into clusters with usually up to 2% sequence divergence within them. One such cluster was shown to lose its diversity in a genome-wide sweep over 8 years, providing direct evidence for a periodic selection event in nature. The authors also found evidence that some genome-wide sweeps had taken place in the community before their study began. Each cluster that was swept genome-wide of its diversity was interpreted as being ecologically homogeneous, such that an adaptive mutant could outcompete all the lineages within the cluster. Because these clusters did not diversify in the time that 2% divergence accumulated, we may conclude that diversification within these clusters occurred at a slow rate consistent with the stable ecotype model.

So, what did those clusters abiding by the stable ecotype model have in common? While they were from several phyla, both photosynthetic and heterotrophic, all but one cluster appeared limited in its carbon sources. These clusters included photoautotrophs of the phylum *Chlorobi* as well as heterotrophs limited to consuming single-carbon molecules from various phyla (156). The one possible exception was a genome-wide sweep within a cluster from the *Rickettsia*, a genus of obligately intracellular pathogens. Nevertheless, two aspects of the *Rickettsia* lifestyle may have contributed to slow diversification: its radically reduced metabolism (189) and the reduced opportunity for an obligately intracellular pathogen to infect new host species. The stable ecotype model may be limited to the ecological guilds of photoautotrophs, C1 heterotrophs, and obligately intracellular pathogens.

The metagenomic study also identified various taxa that had less profound sweeps, where only a small chromosomal region was swept of diversity while the rest of the genome remained heterogeneous (188). Bendall and colleagues argued that a genome-wide sweep was prevented in these clusters by high recombination rates; however, they did not supply evidence for a recombination-based explanation. Likewise, other authors finding single-gene sweeps in other systems have drawn on recombination to explain their results (43, 187, 190, 191). However, periodic selection is predicted to cause a genome-wide sweep only *within an ecotype* (192), and in many of these cases, the single-gene sweep was known to have traversed across ecologically distinct populations (43, 187, 190, 191). Moreover, recombination is too rare in bacteria to prevent genome-wide sweeps within an ecotype (183, 193).

A more likely explanation for the single-gene sweeps found in the metagenome study and elsewhere is offered by the “adapt globally, act locally” model (194) and the negative frequency-dependent selection model (195) (Fig. 5). Following these models, the metagenome clusters showing single-gene sweeps may be interpreted to each contain a heterogeneous amalgam of many ecotypes. Assuming that an adaptive mutation driving periodic selection confers a fitness benefit to each of the ecotypes within a cluster, here is the sequence of events that could lead to a single-region sweep. First, an adaptive mutation appears in one ecotype, and the resulting periodic selection drives a genome-wide sweep within
FIGURE 5 Genome-wide and single-chromosomal-region sweeps within a sequence cluster of closely related bacteria. Each row of panels represents a different model of sweep within a metagenome cluster: (A to B) a genome-wide sweep, where the metagenome cluster is populated by only a single ecotype, and where recombination is rare enough to allow a genome-wide sweep within an ecotype \((28)\); (C to D) a narrow sweep (homogenizing only the chromosomal region near the adaptive mutation), where again the metagenome cluster is populated by a single ecotype, but here recombination (indicated by purple arrows) is frequent enough to prevent a genome-wide sweep within an ecotype (model favored by Bendall et al. \((188)\)); and (E to H) a narrow sweep, where the metagenome cluster is populated by many ecotypes (in this case, three), and recombination is rare enough to allow genome-wide sweeps within an ecotype but frequent enough to allow an adaptive mutation to recombine (it need happen only once!) between one ecotype and another \((46)\). In each row, the wide horizontal arrows represent the course of time. In each panel, the rectangle represents one metagenome cluster and each circle represents a single organism. The asterisk represents an adaptive mutation, which allows its carrier to outcompete other organisms in the same ecotype but not organisms from other ecotypes. In panels A to D, the metagenome cluster is ecologically homogeneous, and in panels E to H, the metagenome cluster is ecologically heterogeneous and represents three ecotypes, separated by the vertical dashed lines; the different ecotypes are coded by blue, green, and red. The sequence diversity within an ecotype is represented by different shades of the ecotype color and by different styles of line (dotted, dashed, and solid). In the case of low recombination rates (A to B and E to H), the adaptive mutation causes a genome-wide sweep within the ecotype containing the mutation. In panels E to H, the adaptive mutation is potentially beneficial in different ecotypes and can transfer on a short chromosomal segment to another ecotype, where it precipitates a new genome-wide sweep within its new ecotype. Reproduced with permission from reference \((156)\).
that ecotype; then the small region containing the adaptation transfers into another ecotype and causes a genome-wide sweep within that ecotype; and so on, until the adaptive mutation has swept to fixation in all the constituent ecotypes of the cluster. The result is that the cluster will be swept of diversity in the region containing the adaptive mutation, but the cluster will maintain its genetic heterogeneity elsewhere on the chromosome.

Thus, those clusters from the metagenome survey that showed a single-region chromosomal sweep could be interpreted as containing multiple ecotypes within the cluster’s 2% sequence divergence, owing to rapid speciation (156). The taxa showing single-region sweeps in the metagenome study were largely generalist heterotrophs, like Bacillus, and supported the hypothesis that a highly plastic metabolism yields rapid speciation (52).

To sum up, the data for free-living organisms (plus Rickettsia) largely support the hypothesis that taxa with little opportunity to diversify tend to speciate slowly and abide by the stable ecotype model, whereas those taxa that can use a vast diversity of organic resources tend to speciate rapidly.

Where do pathogens fit in between the extremes of metabolic plasticity from Bacillus to Synechococcus? Those pathogens that diversify primarily by changes in host range or host tissue may be most likely to speciate slowly, especially when there are only a small number of hosts or tissues that a particular pathogenetic lineage can adapt to. That is, transmission to a new host or tissue may be a rare event. We have seen that in several groups of pathogens (including the M. tuberculosis complex of species [161], A. phagocytophilum [20], and B. burgdorferi sensu stricto [162]), each sequence cluster corresponds to a specialist to a different host species; moreover, each cluster appears to be ecologically homogeneous (52). Likewise, the stable ecotype model may apply to ecotypes that have diverged to infect different tissues of the same host species, for example, the case of Y. pseudotuberculosis, focusing on the gut, versus Y. pestis, specialized toward systemic infection and transmission by fleas. Each Yersinia taxon appears homogeneous in its ecology and so may be considered an ecotype specialized to a different tissue, while each is a generalist with respect to host species.

We may hypothesize that the longevity of newly divergent ecotypes that are specialized to different host species may be short, especially if the host species are closely related. This is because two closely related host species would be likely to be extinguished by the same environmental disturbances (196, 197). On the other hand, ecotypes such as Y. pseudotuberculosis and Y. pestis may be destined to coexist into the far future. Because they are both generalists with respect to host species, they are not likely to become extinguished with the extinction of an individual host species. On the other hand, the two tissue sets to which these ecotypes are specialized are likely to persist into the indefinite future.

Some pathogens may speciate at a quick rate, owing to many more opportunities for ecological diversification. One possibility is that those pathogens with a free-living phase can diversify by the resources and physical and chemical conditions available to them outside of their hosts, as seen among ecotypes within Burkholderia seminalis (181). Another possibility for rapid diversification in pathogens is that they can diversify as they escape the immune system, an issue to which we will return.

In sum, at least some bacterial groups abide by the stable ecotype model, where speciation is slow and ecotypes hold all the species-like properties conceived by speciologists. Some of these ecotypes may persist into the indefinite future. On the other hand, it is clear that many bacteria undergo speciation rapidly. As we shall see, there are several models of rapid bacterial speciation, and in each model, ecotypes are missing at least one of the species-like properties.

MODELS OF SPECIATION: RAPIDLY SPECIATING TAXA

The Speedy Speciation Model

Speedy speciation is a model of adaptive radiation, where opportunities for niche diversification are plentiful (Fig. 3). As in the stable ecotype model, ecotypes are cohesive owing to recurrent periodic selection events. In speedy speciation, the rate of speciation is too high to allow the individual ecotypes to be identified as sequence clusters based on one or a few genes—more resolution is needed to distinguish these newly divergent lineages, perhaps even the resolution of the entire genome. Ecotypes abiding by the speedy speciation model may persist into the indefinite future, depending on whether the ecotypes have some unique resources and on whether their resources are likely to persist into the future.

The speedy speciation model appears to apply to some human pathogens, especially in cases where a host individual is usually infected by only a single clone. The infecting clone can then diversify by specializing into all the niches available to it within the host, provided that specialists already adapted to these niches are not likely to follow the original clone into the host. This pattern
appears to hold in *P. aeruginosa* following lung infection of a CF patient. From a single inoculating clone, the descendant bacteria evolve into multiple populations with different nutrient requirements, antibiotic resistance, and virulence (198). This within-host diversification is frequently aided by evolution of higher mutation rates (199) and eventually an attenuation of virulence that could foster coexistence among the populations (200, 201). If instead CF patients were infected by multiple *P. aeruginosa* lineages already specialized to the various lung niches, there would not be the opportunity for *in situ* diversification under the speedy speciation model. It is the shortsightedness of the diversification (in not leading to transmission to other CF patients, *sensu* Levin and Bull) (31) that allows this diversification to occur from scratch in many CF patients.

Similarly, adaptive radiation appears to occur when *M. tuberculosis* infects a human host individual. An individual patient is likely to host an *in situ* diversification of ecologically divergent ecotypes (202), often facilitated by evolution of hypermutation (203). While specialized ecotypes of *P. aeruginosa* and *M. tuberculosis* may be transmitted from one host to another, it is unlikely that a suite of ecotypes will be transmitted, making possible *in situ* adaptive radiation from a single inoculating clone. In this model of within-host diversification, persistence of newly diversified ecotypes is unlikely to extend beyond the life of a single host individual.

Another possibility for rapid speciation in pathogens is diversification in their free-living stage between their times in hosts. The free-living stage may have the same opportunity for diversification as free-living generalist heterotrophs. Pathogens with free-living stages include many members of the *Proteobacteria*, including *E. coli* (62) and *B. seminalis* (181), as well as the *Firmicutes*, including members of the genus *Listeria* (180).

### The Species-less Model

The species-less model differs profoundly from all other models of bacterial speciation, which assume a force of cohesion within species (Fig. 3). The species-less model assumes both rapid speciation and rapid extinction, with a high turnover of species. These species are lacking in cohesion because they do not exist long enough to experience even a single periodic selection event (28, 204).

In the species-less model, ecotypes evolve not by becoming more efficient in utilizing their current ecological niche but instead by invading a new ecological niche. We recently provided evidence that, at least in an experimental microcosm, a lineage of *Bacillus* will split to form new ecotypes at about the same rate that existing ecotypes improve their adaptations (38). This suggests that under the right circumstances, the rate of speciation may be fast enough to make periodic selection unlikely. Little is generally known, however, about the rate of extinction of ecotypes in nature.

However, pathogens may provide a case for both rapid formation and extinction of ecotypes. Mutations that provide an escape from the immune system may each found a new ephemeral ecotype (28, 205, 206), so the species-less model may apply to the diversification of epitope diversity. The species-less model may also apply in cases of bacterial succession, particularly when the descendants of a single colonizing individual at a site must adapt to rapidly changing conditions. For example, successions that occur on mine tailings, with pH and oxidation levels changing rapidly, may yield the high turnover of ecotypes that is compatible with the species-less model (207). Like the case we discussed earlier for *in situ* diversification of *P. aeruginosa* and *M. tuberculosis* within one patient, the *in situ* diversification in a succession is most likely if dispersal to the site brings only a single founding ecotype. Rapid speciation would not occur if dispersal between different mine tailing sites provided all the ecotypes necessary for each successional stage. Provided that each succession engenders a novel diversification of ecotypes, and the products of each succession are not likely to disperse to other similar habitats, the products of diversification will be short-lived. They will not become the stuff on which novel higher taxa are founded.

### The Nano-Niche Model

In the nano-niche model, closely related ecotypes are subtly and only quantitatively different in their ecology (52). “Nano-niche” ecotypes use the same set of resources and conditions, but they coexist much like closely related animal species by using their shared resources and conditions in different proportions (Fig. 3). Not having any unique resources that might constitute a haven from competition from other ecotypes, each ecotype is expected to be ephemeral and vulnerable to extinction from competition with other ecotypes. For a time, the various ephemeral ecotypes may coexist, and each may even have its own private periodic selection events. At some point, however, an extremely competitive adaptive mutant (which we call a speciation-quashing mutation) from one ephemeral ecotype may extinguish not only the other members of its own population but also other closely related ecotypes (193). In the nano-niche model, divergence among very closely related ephemeral ecotypes
is limited by these speciation-quashing mutations. We should not expect any nano-niche ecotypes to coexist long enough to form any higher-level taxa.

Genome comparisons have provided evidence for the nano-niche model in *B. subtilis* (81). Five extremely closely related isolates were found ecologically distinct on the basis of different histories of positive selection, but the genome content differences indicated no unique resources for any of the isolates. The isolates appear to have diverged by specializing quantitatively on resources that they all share (e.g., with one strain being a superior competitor on maltose), supporting the nano-niche model. We suspect that the high-throughput genome sequencing that is now available will provide examples of quantitative divergence, if researchers focus on sequencing sets of extremely closely related isolates.

The nano-niche model may also apply to bacterial ecotypes that adapt over a long course of infection within a host individual, for example, within the gut. Among closely related bacteria, the course of evolutionary adaptation to each human body may lead to each human having its own ecotype, each adapted to the peculiarities of the host’s physiology, diet, and the other bacteria cohabiting the gut. However, the individual hosts might not be different enough to support indefinite coexistence of individual-specific ecotypes. Any speciation-quashing mutation, which makes an individual bacterium superior not just in its own host individual but also in other hosts, would put an end to the speciation among the various nano-niche ecotypes adapting to different host individuals.

**Recurrent Niche Invasion Model**

In the recurrent niche invasion model, plasmids or phages determine the niches of their bacterial hosts (Fig. 3). We have seen that *Rhizobium* lineages may move from one ecological niche to another by simply gaining the symbiosis plasmid adapting the bacteria to one plant host while losing another symbiosis plasmid, and similar dynamics are seen for plasmids providing virulence to lineages of *E. coli* and *B. cereussensu lato*. In this model, ecological divergence is in principle reversible, provided that the host lineages do not have an opportunity to adapt and specialize to a given plasmid that is infecting them. When hosts fail to specialize to their plasmids, host lineages will move back and forth between plasmid-defined populations and will not diverge irreversibly. Nevertheless, the niches made available by the plasmids may persist into the indefinite future.

On the other hand, sometimes different host lineages may specialize to their own plasmids. This may occur if the plasmids are not fully compatible with all their potential hosts and if the plasmids reside in a given lineage long enough for evolution toward compatibility to occur. We have seen various stages of permanent accommodation of host lineages to different niche-defining plasmids (52). As seen in the case where *R. leguminosarum* lineages are completely associated with different symbiosis plasmids (134), a host-plasmid system can leave the recurrent niche invasion model’s realm of reversible divergence and enter the stable eco-type model’s realm of irreversible divergence. Here the plasmids are providing the same dynamics of diversification as any chromosome-based gene that was originally acquired by HGT.

**CONCLUDING REMARKS AND FUTURE DIRECTIONS**

We began by challenging the field of population biology to explain the origins of profoundly disparate creatures within the bacterial world. Each pair of higher-level taxa began their divergence long ago as one ordinary population that split to become two lineages that would coexist to our time. So, we may think of the rate of origin of a higher-level taxon level as a product—the rate of speciation times the probability that two new species coexist long enough to reach a particular level of divergence. Here I have tried to use population biology to give insights into these two parameters of disparification.

I have focused on speciation as a process where one population splits into two ecologically distinct lineages that can coexist indefinitely as a result of their ecological divergence. I have hypothesized that the rate of speciation varies among taxa, depending on the ecological opportunities available for invading a new niche (156). I have suggested three ecological guilds where speciation may be restricted by a small number of niches available to a given lineage: photoautotrophs, C1 heterotrophs, and obligately intracellular pathogens. Generalist heterotrophs, with many more opportunities for resource-based diversification, appear to have much higher rates of speciation. The longitudinal metagenome survey approach launched by Bendall and colleagues (188) holds great promise for testing these hypotheses and exploring other lifestyles that may promote slow or fast speciation.

I have identified several features of nascent species that may promote their coexistence into the remote future. First, ecological diversification should not be easily reversed by losing the adaptation that creates the new niche. Two factors contributing to irreversibility are a slow rate of gain and loss of niche-defining adaptations.
and a fitness cost created by the original adaptation, which causes a secondary round of ameliorative evolution. Also, two nascent species are most likely to coexist into the distant future if they each utilize some resources that are unavailable to the other (47). This is likely to occur when HGT adds a new resource to a recipient’s repertoire, at a trade-off cost of diminishing access to an established resource. Also, we can predict that long-term coexistence of lineages will depend on long-term coexistence of their resources.

We may add to these hypothesized conditions for long-term coexistence through a systematic approach pioneered by Laurent Philippot and Noah Fierer and their respective colleagues (18, 20). They have aimed to discover whether “ecological coherence” may exist for higher-level taxa. By comparing both genomes and habitats of many taxa, they discovered properties that were shared, for example, by all the members of a particular phylum but not by other phyla. For example, the Acidobacteria are found largely in acidic soils, in contrast to other related phyla, and we now know some of the genomic basis of their acid tolerance. This approach holds promise for identifying the traits that enable long-term coexistence of newly divergent lineages. We can hypothesize that those traits distinguishing the genera or families within a given phylum are responsible for granting the long-term coexistence of the higher-order taxa. Thus, we may predict that when new species diverge in these traits, they may have a greater probability than average of coexisting into the remote future.

In sum, bacterial diversification depends on transmission of bacteria into new habitats and resources, whereby one lineage splits into two that can coexist as a result of their ecological differences. Population biologists and historians of life will be challenged to identify those transmissions that yield lineages capable of coexisting for billions of years.

ACKNOWLEDGMENTS
I am grateful for Teresa Coque, Fernando Baquero, Emilio Bouza, and José Antonio Gutiérrez-Fuentes for their invitation to participate in the symposium on transmission in microbiology. This work was supported by grants from Wesleyan University.

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