

## Chapter 5

# Are Species Cohesive?— A View from Bacteriology

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Tom Whittam's research career created a productive synergy between the fields of evolutionary biology and public health. His insights into the evolutionary origin of enteric pathogens allowed him to contribute uniquely to public health efforts to characterize pathogen diversity. From my point of view as a speciologist, his studies of newly divergent lineages of pathogens provided important insights into the role of horizontal transfer in the origin of ecological diversity, the ecological dimensions by which bacterial lineages become irreversibly separate, and the order in which new lineages accumulate adaptations as they establish themselves in novel ecological niches. Tom's work provides a trove of inspiration for those like me who are fascinated by the origins of bacterial diversity.

Are there species in the world of bacteria? To bacterial systematists, there is no doubt that species exist and that it is useful to classify, name, and describe them (17, 73, 78). In bacterial systematics, species are defined as groups of close relatives that are separated from one another by large gaps in phenotypic and molecular characters (78). Charles Darwin would be quite comfortable with this concept of bacterial species, as he had proposed just this rationale for species demarcation in animals and plants—that species are closely related groups that can coexist as phenotypically distinct entities (22, 49, 75). Although bacterial systematists have argued about whether the species we recognize are too widely or too narrowly defined and the appropriateness of different criteria for species demarcation (29, 72, 74), it is clear that the named bacterial species hold Darwin's essential property of being groups of close relatives with gaps between them.

However, to some microbial ecologists (17, 67, 69, 82), as well as most systematists outside of microbiology (21, 23, 51, 53), species have usually been understood to be more than closely related groups separated by gaps. Largely

under the influence of Ernst Mayr (51, 53), Darwin's species concept has been rejected (49). Instead, species of animals and plants have come to be understood as cohesive groups, in the sense that some force of evolution constrains the diversity within species, while different species are not constrained from diverging. In highly sexual species, such as most animals and plants, the force constraining diversity within species is understood to be genetic exchange. In Mayr's biological species concept, speciation requires certain unusual circumstances that allow newly divergent populations to break free of cohesion by recurrent, high-frequency genetic exchange; speciation is therefore understood to be rare (51).

Many concepts of species have been proposed since Mayr's biological species concept, but they generally share certain essential, dynamic properties: that members of a species are subject to cohesion, species are invented only once, and different species are ecologically distinct and irreversibly separate (23). In what we might call Mayrian concepts of species, the essential dynamic properties of sexual animal and plant species have been extended to other groups where genetic exchange is rare or absent, such as the bacteria. The forces of cohesion within a bacterial species are thought to include periodic selection (17), which causes a genome-wide purging of diversity; genetic drift (42); and genetic exchange (27).

As Doolittle (25) has reviewed, some authors have argued that species in the bacterial world *must be* cohesive (14, 85). Doolittle has challenged advocates of dynamically defined, cohesive bacterial species to prove not just that such species do exist on occasion, but that they must generally exist. Here I will take up this challenge, but I will also address the possibility that while many bacteria reside within dynamically defined, cohesive species, others do not.

My own doubt about whether all bacteria belong to Mayrian, cohesive species stems in part from a related debate occurring now within the zoology community (24, 48, 64). James Mallet has challenged Mayr's model that species cohesion usually prevents the splitting of one species into two (48). Mallet explains that ordinary, adjacent populations within ordinary animal species are frequently poised to diverge into separate species. Provided that natural selection favors different adaptations in adjacent environments, populations may split into separate species despite genetic exchange between them. In short, "speciation is easy" (48).

I will explain how Mallet's hypothesis of easy speciation likely applies even more strongly to bacteria than to animals and plants, owing to intrinsic differences in the population dynamics of these major groups. Also, I will address what it means generally for any species to be cohesive. I will suggest that many animal and plant species are cohesive only in a very limited sense—that they are no more cohesive than are distantly related bacteria that occasionally exchange genes, and they are not cohesive in a way that prevents their populations from diverging as they adapt to different environments. Finally, I will make the case that speciationists from zoology and botany can learn a great deal from speciation in bacteria.

## COHESION IN MAYRIAN SPECIES

Under the influence of Mayr, neo-Darwinians have seen species as a unique and special taxonomic level (51, 52). Species are seen as the largest cohesive groups, such that divergence within a species is constrained by an active force, genetic exchange in the case of the highly sexual animals and plants, while divergence between species is not constrained. Thus, in sexual organisms, so long as populations can successfully interbreed and exchange genes, their divergence is understood to be constrained.

Before we discuss the existence and importance of cohesion, it will be important to consider the various ways that species may cohere. One form of cohesion may be termed “speciation-quashing cohesion,” preventing one species from splitting into two (16). In the paradigm of the biological species concept, when nascent species lose their ability to exchange genes, they become free to diverge indefinitely and irreversibly. From the presumed difficulty of populations breaking loose to specieshood, improvements within a species lineage (anagenesis) are understood to occur much more frequently than speciation (cladogenesis).

A second mode of cohesion promotes homogeneity of populations that are ecologically different. In what we might call “niche-transcending cohesion,” ecologically different populations across a range of organisms may share, through genetic exchange, genes that are adaptive across populations (16, 20).

Finally, “intrapopulation cohesion” promotes genetic identity among ecologically homogeneous individuals. Natural selection may promote genetic identity within such a population by favoring an adaptive mutation over other alleles, but within a highly sexual population of an animal or plant species, genetic exchange will purge the genetic diversity of only the locus under selection and linked loci (90). Genetic drift is likely the most important force of cohesion within ecologically homogeneous animal or plant populations.

The Mayrian concept of cohesive species may be extended to asexual and rarely sexual organisms, such as the bacteria (9, 55, 77). In rarely sexual organisms, natural selection provides a powerful constraint, genome-wide, on the accumulation of diversity within an ecologically homogeneous population, in a process known as periodic selection (4, 40, 43). Under rare recombination rates, such as observed in the bacteria (11, 54, 79), not only will natural selection favoring each adaptive mutation within a population cause the adaptive mutation to become fixed at 100%, but the selection will also cause nearly the entire genome to be purged of diversity. Rare recombination can prevent a total purging of diversity, but quantitative modeling has shown that bacterial recombination is insufficient to prevent purging of diversity, even in the most frequently recombining bacteria (10, 12; W. Hanage, personal communication).

We may then extend the Mayrian concept of cohesive species to bacteria by defining a species as an ecologically homogeneous group (called an “ecotype”), whose diversity is constrained recurrently by periodic selection or genetic drift (9, 17). Diversity within an ecotype may accumulate until it is purged by an adaptive mutation (or an adaptive recombination event), whereby one

organism becomes superior in fitness to all others in the ecotype. In this model, intraecotype diversity is ephemeral, awaiting its collapse with the next periodic selection event. Diversity within an ecotype can also be constrained by genetic drift in bacteria with modest effective population sizes, such as pathogens and vertically transmitted mutualists, whose effective population size may approach that of their hosts (17, 42). Thus, bacteria, like the highly sexual animals and plants, are subject to intrapopulation cohesion.

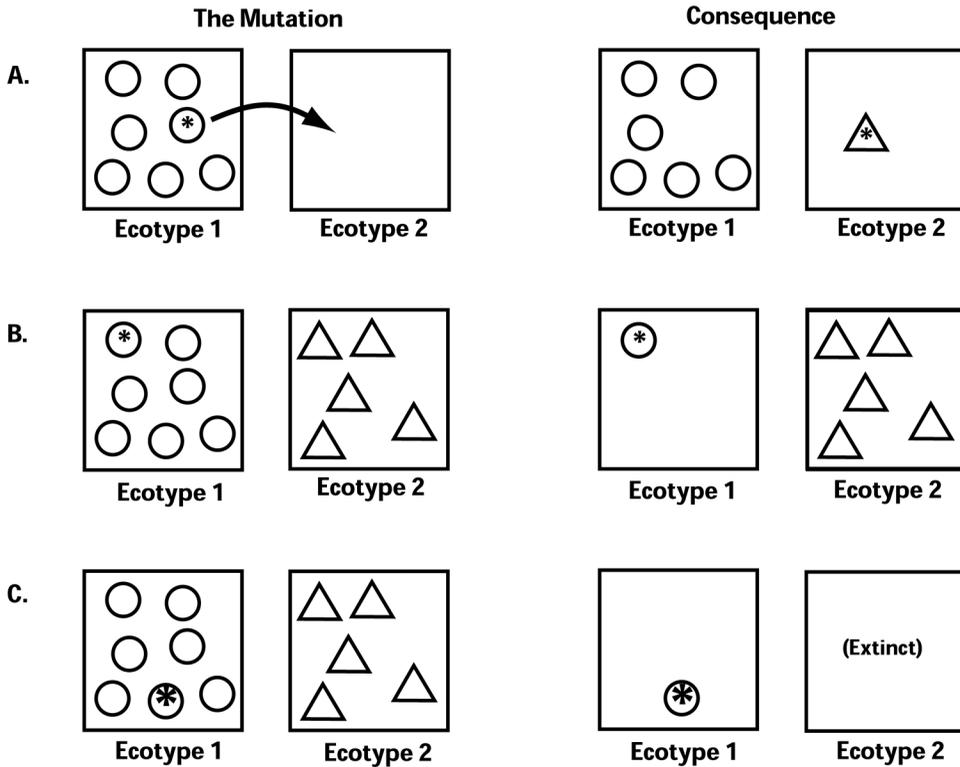
My colleagues and I have previously proposed that speciation within bacteria occurs when a mutation or recombination event places a bacterium into a new ecological niche and creates a new ecotype (9, 17, 82). Then the diversity-purging events in the old ecotype cannot extend to the nascent ecotype, owing to the ecological differences (Fig. 1).

In one model of bacterial species and speciation known as the Stable Ecotype model (17), formation of new ecotypes occurs rarely, and ecotypes are long-lived, such that each ecotype undergoes many periodic selection events during its long lifetime. Each ecotype is constrained in its diversity owing to recurrent periodic selection events and/or to genetic drift; and divergence between different ecotypes is not limited by periodic selection or drift, owing to their ecological distinctness. Provided that an ecotype is founded by only one lineage, these ecotypes appear to hold one essential property of Mayrian species—that periodic selection and/or drift provides cohesion within ecotypes but not between them.

But are bacterial ecotypes irreversibly separate? While the adaptive divergence between ecotypes cannot be reversed by periodic selection, we must consider whether recombination between ecotypes might reverse their adaptive divergence. Clearly in the case of animals and plants, with obligate sexual exchange, populations must be sexually isolated to some extent to successfully diverge as ecologically distinct populations. This sexual isolation may involve genetically based changes in mating behavior, reduced fitness of hybrids, or perhaps most important, ecologically based isolation owing to residence in different microhabitats (21). Consider next whether recombination can likewise prevent adaptive divergence in bacteria, given profound differences in the nature of genetic exchange between bacteria and the highly sexual animals and plants.

Here I will briefly summarize the most important differences in genetic exchange (13). In bacteria, genetic exchange is unidirectional, where a usually small chromosomal segment transfers from a donor to a recipient. Also, bacterial recombination may involve homologous or heterologous transfer of genes. Recombination in bacteria occurs at extremely low rates, even in the most frequently recombining bacteria, ranging from less than the mutation rate to around 50 to 100 times the mutation rate, per gene segment (11, 13, 45, 79). Finally, bacterial recombination can occur across vastly more divergent organisms than is possible in animals and plants (38, 47).

Let us now consider whether recurrent recombination between bacterial ecotypes can hinder their adaptive divergence. I will begin with the potentially harmful effect of recombination of *niche-specifying genes*, those genes that promote adaptation of one population but are harmful when recombined into



**Figure 1.** Three classes of mutation and recombination events that determine ecotype diversity in bacteria. The circles and triangles represent individuals within ecotypes 1 and 2, respectively; the asterisks represent adaptive mutations. (A) Niche-invasion mutations. Here a mutation changes the ecological niche of the cell, such that it can now escape periodic selection events in its former ecotype. This finds a new ecotype. (B) Periodic-selection mutations. These improve the fitness of an individual such that the mutant and its descendants outcompete all other cells within the ecotype; periodic selection events precipitated by these mutations generally do not affect the diversity within other ecotypes, owing to the differences in ecological niche. Periodic selection enhances the distinctness of ecotypes by purging the divergence within but not between ecotypes. (C) Speciation-quashing mutations in the Nano-Niche model. Even if two ecotypes have sustained a history of separate periodic selection events, an extraordinarily adaptive genotype may outcompete another ecotype to extinction. Competitive extinction of another ecotype (ecotype 2) is possible only if all of ecotype 2's resources are also used by ecotype 1. Used with permission from Landes Bioscience (10).

another population (16). In general, populations will successfully diverge in their adaptations in spite of recombination if the effect of selection is greater than that of recombination (9, 33, 48). More specifically, the equilibrium frequency of a recurrently recombined niche-specifying gene from another pop-

ulation is equal to the rate that the gene enters the population by recombination ( $c_b$ ) divided by the selection intensity against the foreign allele ( $s$ ), yielding  $c_b/s$ . Many adjacent animal populations have successfully diverged in adaptations in the face of recurrent recombination, at  $c_b > 1\%$  per generation, presumably because  $s \gg c_b$  (48).

In the case of bacteria, it is straightforward to make the case that recurrent recombination of niche-specifying genes cannot prevent adaptive divergence (9, 16). Recombination among closest relatives within an ecotype ( $c_w$ ) occurs extremely rarely (at about the mutation rate,  $10^{-8}$  per gene segment per generation) (11, 54, 79). Then, even under the most conservative assumption of *no sexual isolation* between populations, recombination of niche-specifying genes is predicted to be inconsequential. That is, if recombination between ecotypes ( $c_b$ ) were every bit as frequent as recombination within ecotypes ( $c_b = c_w = 10^{-8}$ ), the equilibrium frequency of maladaptive niche-specifying genes imported from other populations would be extremely low (at  $10^{-8}/s$ ). No one has quantified the fitness penalty ( $s$ ) for incorporating a single niche-specifying gene from another ecotype, but a reasonable guess would be the selection advantage of a mutation contributing to adaptation in laboratory environments, with  $s$  values ranging from 0.08 to 4.5% (39). It is then reasonable to assume that selection would keep the frequencies of maladaptive niche-specifying genes from other ecotypes extremely low, and that recombination cannot halt or reverse adaptive divergence between young ecotypes. Therefore, the evolution of sexual isolation between ecotypes is not a necessary milestone toward species formation. This is in contrast to the case for the highly sexual animals and plants, where between-population recombination must be reduced at least by residence in different microhabitats, if not through intrinsic sexual isolation (9, 17).

Recently, Sheppard and colleagues have claimed that recombination can prevent or even reverse the divergence among closely related bacterial species (66). They point to the case of two *Campylobacter* species that have recently been given an opportunity to exchange genes more frequently, owing to changes in animal husbandry that have put the species in close proximity. They note a recent history of increased recombination in certain genes used in multilocus sequence analysis, and claim that these two groups are “despeciating.” However, it is important to consider that the genes included in multilocus sequence analysis have been chosen such that recombination between organisms is of little or no fitness or niche-specifying consequence (44). Thus, the genes seen to be recombining successfully between species may be considered to be niche neutral; these are not the niche-specifying genes whose recombination could potentially hinder the adaptive divergence of ecotypes. Recombination of niche-neutral genes may be a hindrance to our taxonomy (by making it difficult for us to assign an organism to its proper ecotype), but it is not a hindrance to the adaptive divergence of ecotypes.

We may conclude, then, that the critical threshold of divergence in ecotype formation is the ecological divergence that allows the new ecotype to escape the periodic selection events of its former ecotype. At this point, the new ecotype is free to accumulate additional niche-specifying genes that further distin-

guish it in ecology from related ecotypes. Furthermore, recombination between ecotypes cannot stall or reverse the adaptive divergence of ecotypes. To the extent that we are able to predict the future of divergence, ecotypes that are outside one another's periodic selection events appear to have the species-like quality of irreversible separateness. Thus, speciation-quashing cohesion postulated by Mayr, where recombination prevents one species from splitting into two irreversibly separate populations, does not appear to apply to bacteria.

We next move to the challenge of identifying ecotypes, or bacterial species that are subject to intrapopulation cohesion provided by periodic selection and/or drift.

### THE FAILURE OF SYSTEMATICS TO DISCOVER ECOLOGICALLY HOMOGENEOUS SPECIES OF BACTERIA

The discovery of dynamically defined, cohesive species would appear to be much easier for the familiar animals and plants than for bacteria. Systematists' demarcation of many animals and plants is informed by a familiarity with the morphological differences underlying their ecological divergence. Our knowledge of differences among animal species and our ability to classify animals based on these differences are likely to predate modern ecology and systematics, and may even be the result of eons of human evolution for ready distinction of organisms with life-or-death consequences for human hunters and gatherers (35, 92). Thus, zoological and botanical taxonomy may have benefited from (but ultimately may be distracted by) what has been called our species' "umwelt," the biological foundation for our species' way of seeing the natural world (92).

Bacteria are, of course, far outside our species' prehistorical umwelt, and the process of demarcating bacterial species has had to be invented wholly by microbiologists. Bacterial species taxonomy is also challenged by the difficulty of anticipating the traits most important for ecological divergence among closest species (17). The problem is that ecological divergence between bacterial species frequently results from acquisition of genes and operons through horizontal genetic transfer (16, 30, 59), and the genes imported by this mechanism and the donor sources of the genes are not known ahead of time. It is therefore difficult to predict the phenotypic characters that should be ecologically relevant in the divergence of new species (17).

It is easy to see the attraction that objective criteria held for midcentury bacterial systematists, beginning with numerical taxonomy (92). Numerical taxonomy attempts to find groups of similar organisms based on overall phenotypic similarity, without requiring knowledge of the most reliable or important traits. Beginning with Sneath's analysis of *Chromobacterium* species, numerical taxonomy was widely used in phenotypic demarcation of bacterial species from the 1950s through the 1970s (until molecular criteria became available). In principle, a numerical taxonomy approach based on a large set of random characters could have yielded the most recent products of speciation, the ecologically homogeneous and cohesive ecotypes.

However, there were some significant obstacles to reaching this goal through numerical taxonomy. First, identification of extremely newly divergent species by phenotypic analysis would be most likely successful if the phenotypic characters responsible for speciation were included in the analysis, but as I have noted, it is difficult to predict the characters underlying the ecological divergence driving speciation. Also, numerical taxonomy might have been handicapped by systematists' pragmatic choice of analyzing presence versus absence of metabolic characters. Each such difference is likely to reflect differences in ecologically significant traits. Thus, clusters of phenotypically similar but nonidentical organisms would likely contain ecologically heterogeneous populations, and defining species as such clusters would lump many long-coexisting, ecologically distinct groups within a single species.

Indeed, the species demarcated by numerical taxonomy are huge by any criterion, including the very characters used in numerical taxonomy (6). From the beginning, numerical taxonomists, as well as less quantitatively oriented taxonomists, could have demarcated smaller, more homogeneous phenotypic clusters. In Kämpfer and colleagues' *Streptomyces* study, for example, a similarity level of 80% for species demarcation was chosen, yet there were apparent clusters at higher levels of similarity (37). It is not clear why such huge phenotypic diversity within species was sought, but in the absence of an instinctive Umwelt for describing bacteria, this level of within-species diversity was established artificially as our Umwelt, and bacterial systematics has enforced this Umwelt for decades (78).

As molecular approaches to species demarcation have become available, molecular criteria for demarcation have been calibrated to yield the species defined by the broad phenotypic clusters previously established (14). For example, whole-genome DNA-DNA hybridization, a measure of gene content shared between organisms, was the first molecular tool for species demarcation. A criterion of 70% annealing in DNA-DNA hybridization was set as the best general criterion to yield the established, recognized species (86). Given the technical and logistical difficulties of performing DNA-DNA hybridization (29), direct calculation of genome content shared has been suggested as a substitute for attaining the DNA-DNA value sought by systematics (31).

As DNA sequencing became available, systematists utilized the sequence of the small ribosomal subunit (16S rRNA) for species demarcation. A level of 3% divergence was originally set as a guide for declaring organisms to be in different species, but more recently a level of 1% divergence has been taken to be a reliable guide for demarcating the species originally classified by phenotypic clusters and then by DNA-DNA hybridization (72). We should note that the 16S levels used for demarcation, if applied to animals, would put all primates in the same species (74). Other molecular guides for species demarcation have been suggested, including the average nucleotide identity of all shared genes in the genome (at a level of 94%) (31).

There is no doubt that as more discerning and convenient molecular tools become available, bacterial systematists will be able to calibrate them to obtain the species fitting our newfound Umwelt for bacterial species, as based origi-

nally on phenotypic clustering. If we knew these species to be ecologically homogeneous, and to be the most recent products of speciation, there would be nothing wrong with this. However, at least some of the recognized species of bacterial systematics are enormously diverse in their ecology. For example, the work of Tom Whittam and his colleagues has characterized the ecological diversity within *Escherichia coli* and within related species. They characterized lineages divergent in their preferred environments and ways of making a living, including commensalism, enteropathogenicity, and uropathogenicity, as well as in their degrees of virulence (50) and persistence outside of hosts (80, 81).

Other recognized pathogenic species have also shown ecological subdivision, with *Legionella pneumophila* containing 11 ecotypes, some of them distinguishable by host range differences and others by gene expression patterns (15). The lung pathogen *Mycobacterium tuberculosis* has several ecotypes distinguished by their associations with host mammalian species (69); similarly, there are lineages associated with different host ranges within the Lyme disease pathogen *Borrelia burgdorferi* (26).

Beyond the pathogens, the recognized marine heterotrophic species *Vibrio splendidus* has been found to include 15 sympatric phylogenetic groups that are distinct in their associations with substrate particle size and season (36). Another free-living heterotroph, *Bacillus simplex*, includes nine ecotypes varying in their associations with solar exposure in semiarid soils (41); a similar result has been obtained for *Bacillus subtilis*, with ecotypes distinguishable by solar exposure and soil texture associations (18). While systematists have long been untroubled by the ecological heterogeneity within the species they recognize, the heterogeneity of genome content, now explicitly seen through genome sequencing (87, 89), is leading to a growing sense among systematists that bacterial species demarcations should be reevaluated (29, 71).

My colleagues and I have previously argued that recognition of cohesive, ecologically homogeneous species by bacterial systematics would benefit microbiologists of many fields, including epidemiology, biotechnology, genomics, population genetics, and ecology; moreover, this could be done without upsetting the current systematics of species (17, 41). We next illustrate a general approach for identifying such cohesive and ecologically homogeneous ecotypes.

## ALGORITHMS FOR DISCOVERY OF BACTERIAL ECOTYPES

Several laboratories have recently developed universal, sequence-based approaches for identifying newly divergent bacterial ecotypes. These methods eschew the universal molecular cutoffs espoused by systematics; instead, they attempt to find appropriate criteria for demarcating ecotypes within a taxon of focus, taking into account the genes being analyzed. These each require a molecular survey of diversity among close relatives, based on molecular markers that are not necessarily responsible for the niche-specifying divergence among ecotypes (and usually are not). Clearly, the ability of any such sequence-based approach will be limited by the rate of speciation and the rate of evolution in the markers; so sequence-based approaches are best suited to finding the long-

standing bacterial species that fit the Stable Ecotype model of bacterial speciation.

The algorithms Ecotype Simulation (ES) (41), BAPS (19), and GMYC (5) are designed to find long-lived, stable ecotypes using only sequence data, usually the nucleotide sequences of several protein-coding loci. Higher resolution of more newly divergent ecotypes could be obtained through analysis of many more genes, in the extreme the entire set of genes shared among genomes. These algorithms search for sequence clusters whose diversity appears to be purged recurrently. Because these algorithms do not utilize any data directly related to ecology (either phenotypes or habitats of isolation), the putative ecotypes hypothesized by these algorithms must be confirmed independently as ecologically distinct. On the plus side, the lack of ecological input allows these algorithms to demarcate ecotypes even when the ecological dimensions of divergence among ecotypes are beyond our imagination (16, 18). In contrast, the algorithm AdaptML requires information about the ecology of the organisms surveyed, in the form of an environmental description of their habitats of isolation (36). The AdaptML algorithm has the advantage that it can simultaneously identify ecotypes and confirm them as ecologically distinct, based on finding statistically significant differences in their habitat associations. The downside for AdaptML is that it can identify only those ecotypes that are divergent in habitat types anticipated by the investigators (16, 18).

Confirmation of ecotypes hypothesized by these algorithms requires two steps. The first and most straightforward part is demonstrating that the hypothesized ecotypes are ecologically distinct. Confirmation of ecological distinctness of hypothesized ecotypes is important because under some models of bacterial diversification, an ecotype may contain multiple, ecologically interchangeable sequence clusters, particularly when geography plays a role in the diversification of populations (17).

One approach to confirming the ecological distinctness of ecotypes is to test putative ecotypes for differences in their associations with contrasting microhabitats (18, 36, 41, 83). Also, ecotypes can be tested for physiological differences that might adapt the ecotypes to their favored microhabitats (2, 18, 68). Full-genome comparisons are expected to play a growing role in finding physiological differences between closely related ecotypes (91), either by identifying ecologically significant biochemical pathways that are not shared across ecotypes (7) or by identifying shared genes whose divergence has been accelerated by natural selection (70). These approaches have confirmed that the ecotypes hypothesized by ES and AdaptML are ecologically distinct (15, 18, 36, 41).

The more difficult, second part of confirming ecotypes is to demonstrate that the putative ecotypes identified by sequence analysis represent the most recent products of speciation. That is, each ecotype must be shown to be homogeneous in its ecology, and thereby potentially cohesive. Alternatively, ecotypes hypothesized by ES or AdaptML might contain within them different, ecologically distinct groups. In this case, the hypothesized ecotypes would not be cohesive because periodic selection and drift would be limited to the sub-

set groups of organisms that represent the true, ecologically homogeneous ecotypes.

There are several models where an ecotype hypothesized by sequence-based approaches belies ecological homogeneity when there is actually ecological diversity hidden within it. One possibility is the Speedy Speciation model, a Mayrian model where each ecotype is cohesive, with recurrent purges of diversity; speciation is simply too rapid for the most recent products of speciation to be detected by the resolution provided by one to several loci (17). Perhaps in these cases, the resolution of whole-genome sequencing might discern the most newly divergent ecotypes.

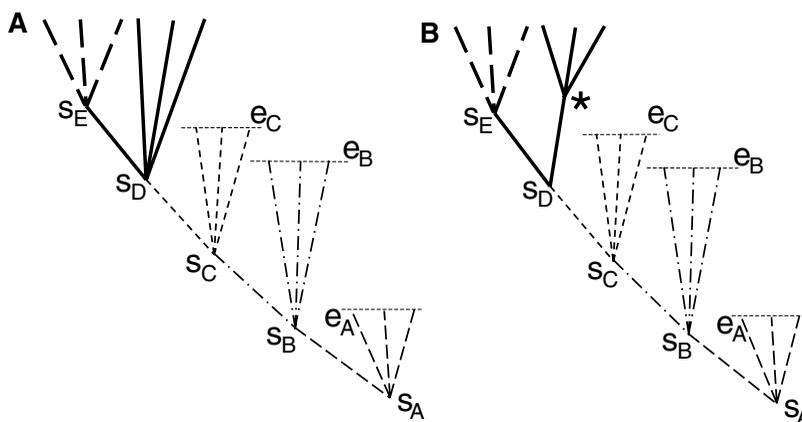
We next consider a diversity of models of speciation that do not follow the Mayrian paradigm; in these cases, bacterial ecotypes do not represent units of intrapopulation cohesion. Under each of these non-Mayrian models, sequence-based approaches are unlikely to identify ecologically homogeneous ecotypes.

### NON-MAYRIAN MODELS OF BACTERIAL SPECIATION

Consider first the Species-less model, where species are formed and extinguished at very high rates, with each nascent species living only a short time before going extinct (17, 84). Each such nascent species is ecologically homogeneous, and so these ecotypes could incur periodic selection events that would purge within-ecotype diversity, as in the cohesive Stable Ecotype model. However, because of their short existence, these species have very limited opportunity for adaptations leading to periodic selection (16). Thus, within-species diversity in the Species-less model would be limited not by recurrent diversity-purging forces, but instead by the limited amount of time from the founding of an ecotype by a single mutant (or recombinant) cell until the ecotype's extinction (Fig. 2). If some species manage to spawn a new species before they go extinct, a large clade of such species could be dominated by cladogenesis events with very few anagenesis events.

The Species-less model may apply when the habitats to which a new species adapts are ephemeral, so that when a habitat disappears, the species specialized to the habitat also disappears. Martin Polz and colleagues have suggested that short-lived ecotypes specialized to ephemeral habitats may constitute a major part of marine bacterial diversity (61). They term these ecotypes "opportunitrophs," where each has colonized and specialized to a particular particle of marine snow. If there is limited opportunity for a specialist of one form of snow to colonize another such particle, there will be parallel niche invasions by many opportunitroph ecotypes, each extremely short-lived.

Mark Achtman has suggested that pathogens may frequently follow the Species-less model when they evolve new immune-escape adaptations (1). An established serotype might have only limited success because most individuals of a host population have developed immunity, but the evolution of a novel serotype not recognized by the hosts' immune system will have a flare of success before it, in turn, becomes widely recognized. Provided that each



**Figure 2.** The Species-less model of bacterial diversification. In the Species-less model, the diversity within an ecotype is not limited by periodic selection but instead by the short time from the ecotype's invention as a single mutant until its extinction. Each ecotype is represented in the figure by a unique line style; the origination and extinction of each ecotype  $i$  are indicated by  $s_i$  and  $e_i$ , respectively. (A) 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. If two closely related ecotypes represent a monophyletic-paraphyletic pair (as in the case of ecotypes D and E, in bold), then we may conclude that a periodic selection event has not occurred in the parental ecotype since the origin of the daughter ecotype. (B) If instead a periodic selection event has occurred in the parental ecotype since the founding of the daughter ecotype, then the parent and daughter ecotypes will be sister monophyletic groups. Observing that pairs of most-closely-related ecotypes usually form monophyletic-paraphyletic pairs would indicate that the origin of new ecotypes is more frequent than periodic selection events in established ecotypes.

immunity-defined ecotype has a short lifetime and can coexist with its parental ecotype, this will constitute a Species-less dynamic.

Consider next how we might test the Species-less model (Fig. 2), where diversity within an ecotype is not recurrently purged by periodic selection. If one lineage within such a species should give rise to a new species, the parental species will appear in a phylogeny as a paraphyletic lineage (i.e., the ecotypes will be nested; Fig. 2A). That is, the most recent common ancestor of the parental ecotype will include among its descendants the more basal parental ecotype as well as the nascent, daughter ecotype. Such a result would indicate that the parental ecotype incurred no periodic selection events after the origination of the daughter ecotype. If it had, both parental and daughter ecotypes would appear as sister, monophyletic clades (Fig. 2B). This analysis requires that we have the molecular resolution to identify the most recent products of speciation, possibly through analyzing the thousands of genes shared across full-genome sequences.

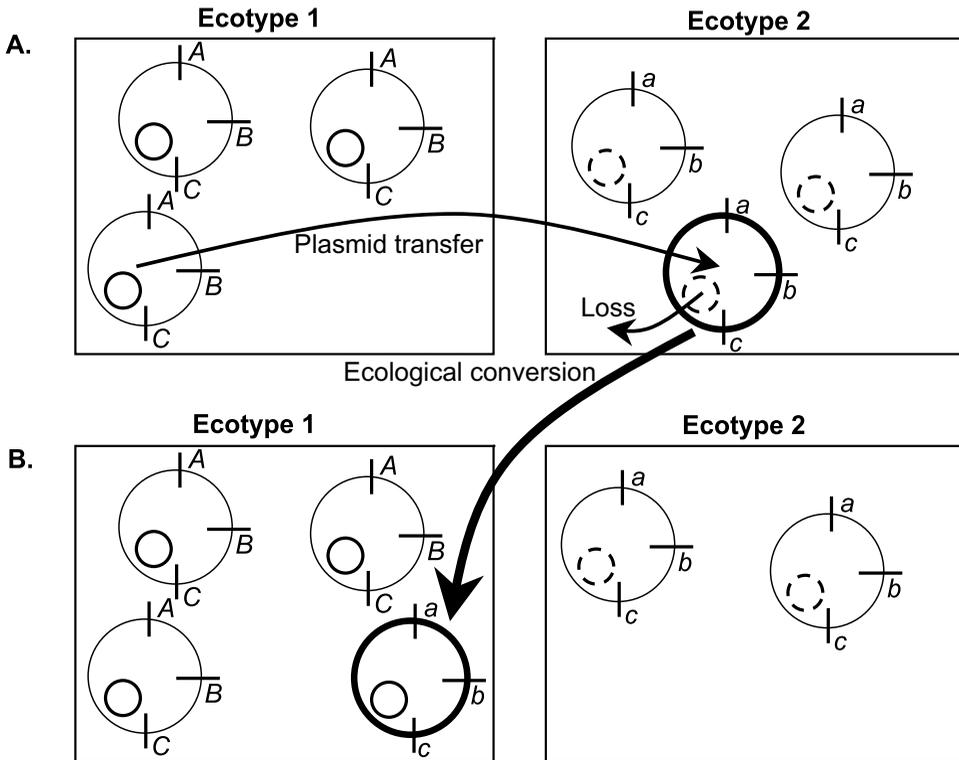
Another non-Mayrian model of diversification is the Nano-Niche model (17). Here ecotypes are formed rapidly and invade extremely similar niches, such that the ecotypes use entirely the same set of resources but in different proportions. While each ecotype may incur its own periodic selection events, the sharing of resources allows for the possibility that there will eventually be a periodic selection event emanating from one ecotype that can completely extinguish its closest relatives (Fig. 1C). The Nano-Niche model invokes cohesion, but it is not the single, ecologically homogeneous species at which cohesion applies; the cohesion applies across multiple, young ecotypes.

Finally, the Recurrent Niche Invasion model takes into account the role of mobile genetic elements, such as plasmids or phage, in determining bacterial niches (17). For example, in the case of *Rhizobium*, a bacterial lineage may acquire a symbiosis plasmid, which will adapt it as an endosymbiotic mutualist to a particular set of legume hosts; then the lineage may lose that plasmid and acquire another symbiosis plasmid, thereby adapting it to another set of legumes (65). In general, a cell is converted from one ecotype to another by acquiring and/or losing a niche-specifying plasmid or phage, and so a lineage moves back and forth between memberships in different, previously existing ecotypes.

If there is a high rate of conversion of lineages from one ecotype to another through plasmid or phage transfer, the molecular approaches to ecotype demarcation we have discussed will fail to identify ecotypes. This is because each time a cell changes from one ecotype to another (by acquisition and loss of a plasmid or phage), this event implicitly transfers the alleles at every chromosomal locus from membership in the cell's current ecotype to membership in its new ecotype, all without any recombination beyond the transfer of the plasmid (Fig. 3). In this way, two ecotypes could coexist for eons and yet never be distinguished as separate sequence clusters for niche-neutral, chromosomal genes. This role of plasmids and phage in instantly and recurrently pulling bacterial lineages from one ecotype to another may be unique to the prokaryotes.

In the Recurrent Niche Invasion model, cohesion may stretch across all the bacteria that can potentially utilize the same set of niche-specifying plasmids (or phage). At any one time, this will include bacteria that are in different plasmid-specified ecotypes. The cohesion will transcend the various plasmid-based ecotypes if each chromosome-based adaptation within each such ecotype is adaptive in the context of all the plasmids utilized by these bacteria.

Alternatively, cohesion could be limited to each plasmid-defined ecotype. This would occur if each ecotype accumulates its own chromosomally based improvements, which are useful only in the context of its own plasmid and are deleterious in the context of other ecotypes' plasmids. In this case, the ecotypes will no longer share plasmids, as each transfer of a plasmid from one ecotype to another will result in a recipient that is not well adapted to bearing the transferred plasmid. Each ecotype will thus incur intrapopulation cohesion and will diverge from other plasmid-based ecotypes irreversibly; that their niche-specifying adaptations were originally all on plasmids will over time become irrelevant. This model predicts that chromosome-based clades will be signifi-



**Figure 3.** Ecological conversion from one plasmid-defined ecotype to another. Each cell is represented by a large circle, and its chromosomal genotype is represented as *ABC* or *abc*. Ecotype 1 is determined by a plasmid represented by a small solid-line circle, and ecotype 2 is determined by a plasmid represented by a small dashed circle. (A) A transfer of an ecotype 1-determining plasmid into a recipient member of ecotype 2 (indicated by the bold circle), along with subsequent loss of the ecotype 2-determining plasmid. (B) The recipient is converted to become a member of ecotype 1, so all of its chromosomal genes are effectively transferred into ecotype 1.

cantly different in their associations with different niche-specifying plasmids, as seen in nonagricultural *Rhizobium* (88).

Whether plasmid- or phage-based ecotypes are in nature cohesive as single ecotypes, or are instead cohesive as pools of related ecotypes, has not been adequately explored. There are known cases where a chromosome-defined clade of strains can hold a diversity of niche-specifying plasmids, e.g., as in the case of the insect-killing plasmids of *Bacillus thuringiensis* (76) or the virulence-conferring phage in *Listeria monocytogenes* (58). This is consistent with cohesion occurring at the level of multiple plasmid-based ecotypes. Another interpretation is that different chromosome-based subclades are adapted preferentially to different niche-specifying plasmids, but they are very recently divergent and are somewhat generalist in their plasmid preferences.

### COHESION THROUGH SHARING OF NICHE-TRANSCENDING GENES

Beyond the harmful effects of recombination on niche-specifying genes, zoologists and botanists have long understood a significant adaptive effect of recombination—this is the recombination of “niche-transcending genes” (16). Occasionally, a mutation that is adaptive in the context of all an animal species’ populations may appear. This niche-transcending gene may adaptively spread across the species range while populations maintain their adaptive divergence in niche-specifying genes. The tension between niche-specifying and niche-transcending adaptations may be illustrated by human populations in the early agricultural era. The rates of recombination between distant human populations in this time would have been low enough to allow populations to maintain their adaptive divergence, in skin color for example, while allowing the sharing of niche-transcending genes that provided widely useful adaptations to agricultural living (34). In many cases, recombination within and between closely related animal and plant species has allowed sharing of niche-transcending genes while allowing each population or species to maintain its niche-specifying adaptations (20, 35, 63).

Likewise, niche-transcending genes appear to have recombined and spread within the bacterial world. The dynamics of their spread is predicted by the Adapt Globally Act Locally model (46). Here a niche-transcending gene initiates a periodic selection event within its original ecotype. The niche-transcending gene then recombines on a small segment into another ecotype, where the recipient will outcompete other members of the recipient’s ecotype and cause a purging of diversity there. The Adapt Globally Act Locally model predicts that ecotypes can maintain their ecological distinctness and their sequence divergence across the genome, while becoming homogenized for the segment of DNA carrying the niche-transcending gene.

Recently, comparative genomics has produced evidence for the spread of niche-transcending genes among ecologically distinct bacteria populations. This is seen, for example, in two *Synechococcus* ecotypes (A and B’) that are adapted to different temperatures and concentrations of mineral nutrients in a Yellowstone hot spring (2, 7), and are consequently found in different microhabitats (85). That they have coexisted for a long time is evidenced by their 78% average nucleotide identity (7). However, the ecotypes share a 35-kb segment containing the nitrogen fixation pathway, which is nearly identical in the two ecotypes. We may conclude that the *nif* pathway has recently recombined across these ecotypes, either directly or by way of other organisms. The two ecotypes, adapted to different thermal and nutrient zones, have thus each become better adapted to their respective stations by sharing these genes.

One could alternatively interpret this as a periodic selection event within a single population (32, 60). For example, one might hypothesize that (i) the A and B’ *Synechococcus* groups are really members of a single, ecologically homogeneous population; (ii) one individual within this population acquired the *nif* region by horizontal genetic transfer; (iii) this organism and its descendants

outcompeted other individuals within the population; and (iv) because of recombination, the only segment to become homogenized was the *nif* region. However, there are several problems with this hypothesis (46). First, the A and B' clades are adapted to 65° and 60°, respectively (2), and accordingly live in different microhabitats (62, 83), so it is difficult to see how an improvement in an A organism would cause that organism to outcompete members of the B' group, or vice versa. Also, if A and B' were a single population, it is difficult to understand why they would appear to us as two long-coexisting lineages with an average nucleotide identity of only 78%. Finally, recombination in bacteria has been shown to be infrequent enough such that selection for an adaptation will purge diversity genome-wide, not just in the selected chromosomal region, as occurs in the highly sexual animals and plants (10; Hanage, personal communication). Nevertheless, this single-population periodic selection explanation has been invoked, mistakenly I believe, to explain a chromosomal region of homogeneity within the ecologically diverse *E. coli* (32) and a similar observation in ecologically distinct groups of *Halorobrum* (60).

Horizontal genetic transfer of niche-transcending genes is understood to be an important part of adaptive evolution in bacteria, as shown by the great number of genomic islands shared across both close and distant relatives (30, 89). The sharing of niche-transcending genes between ecotypes does not appear to reverse their adaptive divergence. Rather, it allows the recipient to build on its unique, preexisting set of adaptations either to invade a new niche or to improve its performance in its current niche (13). For example, Tom Whittam and his colleagues showed that enterotoxigenic *E. coli*, which attack the epithelial cells of the small intestine, have shared the class 5 fimbriae by horizontal genetic transfer with *Burkholderia cepacia* (3). The within-human niche of *B. cepacia* is quite distinct from that of enterotoxigenic *E. coli*, as *B. cepacia* resides in the lungs and attacks the respiratory epithelium. Acquiring the niche-transcending class 5 fimbriae genes has not caused donor and recipient to converge ecologically, and this is likely true for other transfers of niche-transcending genes.

It would be interesting to quantify the relative importance of niche-specifying versus niche-transcending adaptations. In some cases, the set of niche-specifying adaptations separating a set of closely related populations may be severely limited. This is observed, for example, in pitcher plant mosquitoes, where niche-specifying adaptations simply adjust the season of a population's activity, such that all the various populations across a latitudinal range are active in nearly the same range of temperature conditions (but at different times of year) (8). In this case, all adaptations to this shared set of conditions are niche transcending. Perhaps this applies also in bacteria for host specificity within a closely related group of pathogens, if adaptation to a particular host membrane molecule is the extent of niche specificity, and all other adaptations are niche transcending. At the other extreme, nearly every gene in the genome may be niche specifying. This is likely the case for hot spring bacteria populations living on a sharp temperature gradient, where most genes are adjusted to optimize protein activity within an organism's temperature range (56).

### WHITHER COHESION?

Let's now revisit the central Mayrian notion of species and speciation—that divergence within a species is constrained by cohesive forces but divergence between species is not. We will consider each of the three levels of cohesion in turn.

Consider first to what extent individual, ecologically homogeneous populations are subject to intrapopulation cohesion. In the world of animals and plants, each such population is subject to cohesion genome-wide primarily by drift. In the bacterial world, some pathogenic and endosymbiont populations will also be subject to strong drift. However, periodic selection is the most likely force of cohesion for free-living populations, at least those that survive long enough for adaptive mutations to occur. In the Species-less model, with short-lived ecotypes, there may be no opportunities for adaptive improvement and consequent purging of diversity; so these short-lived ecotypes would not be cohesive. It is not clear whether cohesive, long-lived ecotypes are common at all in the bacterial world, or whether the bacterial world is primarily populated by short-lived ecotypes that never have a chance at cohesion.

Second, recombination does not seem likely to be a cohesive force that quashes speciation in either the macroorganisms or bacteria. Most clearly in bacteria, recombination is not sufficient to prevent adaptive divergence in niche-specifying genes, and sexual isolation is not required for bacterial speciation; two nascent ecotypes do not even need the physical separation of residence in different microhabitats. Likewise, animals and plants are able to diverge into ecologically distinct populations and maintain the integrity of their niche specificity, in spite of modest recombination between them. Owing to the obligate nature of recombination in animals and plants, the rate of recombination between populations must be reduced from that within populations, but apparently residence in different microhabitats is sufficient sexual isolation in many cases. Studies of the adaptive divergence between adjacent, interbreeding animal and plant populations indicate that these populations have the dynamic properties of species: each is ecologically distinct and appears to be irreversibly separate from other such populations, at least to the extent that we can predict the future of divergence from past coexistence. Provided that these populations can continue to live in spatially separated and contrasting habitats, it is reasonable to hypothesize their irreversible separateness. Like the case for bacteria, macroorganismal populations appear not to be subject to species-quashing cohesion at the hand of recombination.

There is, however, one circumstance where ecological divergence among long-coexisting, interbreeding populations of animals and plants can cause them to lose their niche specificity through a speciation-quashing event. Futuyma has hypothesized that during bouts of catastrophic global climate change, divergent populations tracking their optimal environments will inevitably find themselves sympatric (28). If in sympatry there is no reproductive isolation between populations, the accumulated genetic differences between them may be reset to zero. In this case, the potential to exchange genes across ecologically divergent

populations may act in the Mayrian sense of speciation-quashing cohesion. I will note, however, that this model does not force cohesion for populations that differ in their optimal microenvironments and are able to find their own particular optimal environments as populations track climate change.

There are also some circumstances in the bacterial world where ecologically distinct populations are prevented from diverging. In the Recurrent Niche Invasion model, where ecotypes are determined only by highly mobile genetic elements such as plasmids or phage, the chromosomal genomes of different ecotypes may never diverge. Also, in the Nano-Niche model, closely related ecotypes, which use the same set of resources but in different proportions, may coexist and experience their separate periodic selection events. However, a speciation-quashing adaptive mutation may allow a highly adaptive mutant to outcompete and extinguish not just other members of its own ecotype but also closely related ecotypes that use the same resources.

Third, cohesion by sharing of niche-transcending genes may be a red herring for species studies. The tradition of species studies in zoology and botany has focused on the origins of a total blockage of interbreeding and recombination among populations. As I have argued, the principal consequence of interbreeding is that it enables ecologically distinct populations to share niche-transcending genes, while interbreeding appears unable to prevent irreversible divergence between populations (in both macroorganisms and bacteria). By focusing on the stoppage of gene flow, the speciology of animals and plants has implicitly emphasized the end of sharing of niche-transcending genes, and has deemphasized the quotidian origins of ecological distinctness among interbreeding populations. In contrast, studies of speciation in bacteria have focused on the origins of niche-specifying adaptations that distinguish newly divergent species, by investigating the ecological dimensions of speciation (18, 36, 69, 83) and the roles of horizontal genetic transfer (7, 57) and homologous recombination (27, 66) in bacterial speciation.

This emphasis on the origins of ecological divergence was forced on bacteriology because bacteria can acquire niche-transcending genes potentially from any organism; so it would be futile to study the end of sharing niche-transcending adaptations in bacteria. It appears that, fortuitously, bacteriology has produced a paradigm of value for species studies in macroorganisms as well as bacteria—that our focus should be on the origins of ecological diversity and not on barriers to recombination.

**Acknowledgments.** This work was supported by NSF FIBR grant EF-0328698 and research funds from Wesleyan University.

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