

# A Macrobiological Perspective on Microbial Species

Species concepts are key to understanding how microbes behave in natural settings, including during infectious diseases

David M. Ward

**A**s a microbial community ecologist, I am interested in the composition, structure, and function of microbial communities, and, in particular, in the fundamental units of which they are comprised. Those studying plant and animal biology—macrobiology—typically have a strong sense of the importance of species to communities. The late Ernst Mayr, a leading biologist of the 20th century, considered species “the basic unit of ecology. . .no ecosystem can be fully understood until it has been dissected into its component species and until the mutual interactions of these species are understood.”

Macrobiologists often disagree over how to group individual organisms into species. Micro-

biologists similarly disagree over whether to use morphological or phenotypic criteria, a polyphasic approach that includes phenotypic criteria and phylogenetic relatedness, phylogenetic clustering alone, ecological, or other criteria to demarcate species. Beyond these disagreements is the idea that horizontal gene transfer (HGT) might be so rampant that microbial populations simply do not fit a traditional species definition.

Debating species concepts is not merely an esoteric exercise. On the contrary, a species concept is central to achieving a predictive understanding of the composition, structure, and function of microbial communities, the population biology of disease outbreaks, and the emergence of new diseases. Indeed, microbiologists can learn by carefully reviewing decades of thought about species in macrobiology. It has not been natural for microbiologists to do so because we tend not to look at the world as macrobiologists do—and that may be reason enough for microbiologists to foster the lateral flow of ideas between these fields.

## Reviewing How Macrobiologists Think about Species

Nearly a decade ago, a group of leading macrobiologists met to review species concepts. Their analysis, summarized in *Species: The Units of Biodiversity*, includes 22 species concepts, some of which are useful for microbiologists to consider. Despite differences of opinion over the many concepts, the evolutionary species concept, stands out in the mind of Richard Mayden of St. Louis University in St. Louis, Mo. The Evolutionary Species Concept describes a species as

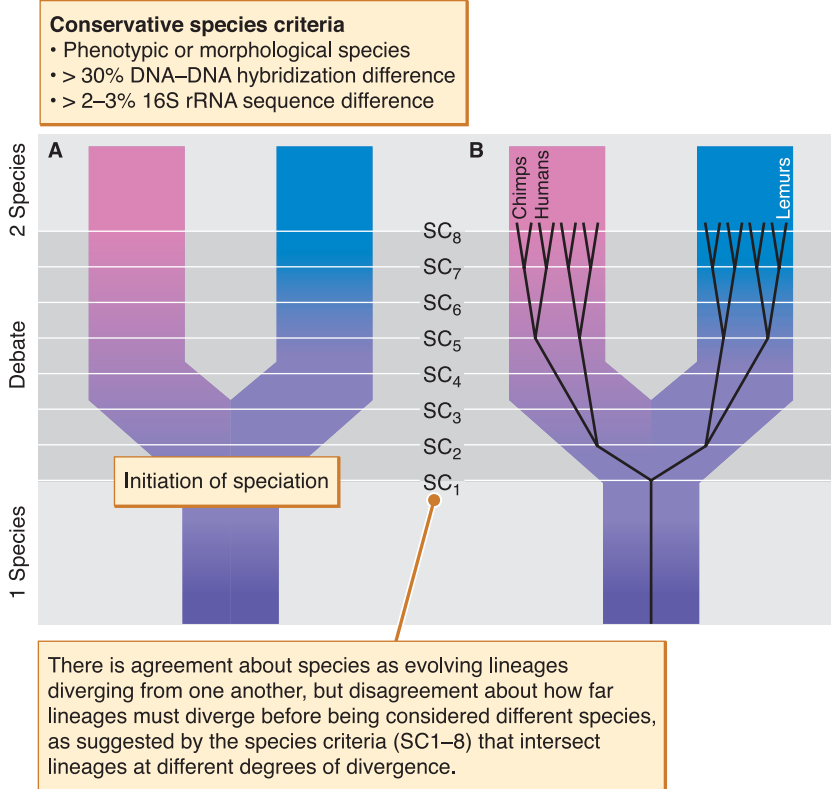
### Summary

- A species concept is central to achieving a predictive understanding of the composition, structure, and function of microbial communities, the population biology of disease outbreaks, and the emergence of new diseases.
- Molecular biology continues to have a big impact on the tradition of assigning microbial species on the basis of phenotypic similarities, and some microbiologists have used molecular cutoffs to demarcate species.
- The terms ecotype and geotype describe populations with unique distributions along ecological or physical gradients, and can be used to identify basic community units that occupy unique ecological niches or are found in distinct locations.
- Horizontal gene transfer is important as an evolutionary process and might be an important way by which microorganisms speciate.

David M. Ward is a Professor in the Land Resources and Environmental Sciences Department at Montana State University, Bozeman.



FIGURE 1



de Quieroz suggestion for separating a concept of species as an evolving lineage segment from criteria used to delineate species (SC1–8). (A) The criterion initiating speciation is far removed from conservative species criteria commonly applied in microbiology. (B) Primates have obviously diverged many times within lineages that have reached such conserved species criteria. (Modified from de Quieroz, 2005.)

“a lineage (an ancestral-descendant sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies.” He calls this concept “primary” because it “is suitable [for]. . . guiding our quest for species as taxa and our search for natural order.”

Separating species concepts from species criteria provides a useful way to accommodate different ideas about species, according to Kevin de Quieroz of the Smithsonian Institution in Washington, D.C. (Fig. 1A). Most of these concepts take into account that species are evolving population lineages. However, disagreements arise over which criteria to use when demarcating species as changing populations diverge—where should we draw the line? As Darwin stated in *The Origin of Species*: “. . .there is no possible test but individual opinion to determine

which. . . shall be considered as species and which as varieties.” Some species criteria are related to the initial division of one population into two (e.g., formation of a subpopulation able to occupy a new niche or physical isolation of a subpopulation; see below), while others come later as populations diverge further (e.g., sexual isolation in animal and plant species).

### Reviewing How Microbiologists Think about Species

Traditionally, microbiologists have relied on phenotypic properties to demarcate species. One exception is the International Committee on Taxonomy of Viruses, whose members agreed to define viral species based on lineages that occupy particular ecological niches.

For prokaryotic microorganisms, molecular biology has had a big impact on the tradition of assigning microbial species on the basis of phenotypic similarities. This development is healthy because it adds phylogenetic relatedness to the concept of species, resulting in a polyphasic approach that combines phylogenetic relationships with phenotypic characteristics.

Even so, molecular sequence data have been used to calibrate the amount of sequence divergence of species that

were named on phenotypic grounds, hence remaining faithful to the notion that phenotypically defined species are true species. This practice, in turn, has led some microbiologists to suggest using molecular cutoffs to demarcate species (Fig 1A). For instance, “gold standards” of >30% variation in DNA-DNA hybridization (and >2 to 3% variation in 16S rRNA sequence) have been proposed as genetic distances needed to ensure that two strains belong to different species, and these standards have been used to conservatively estimate the number of microbial species in nature.

Upon closer examination of diverging lineages, as two populations evolve apart, each may undergo many subsequent divergences in the time it takes for the members of the two initial lineages to reach these “gold standard” molecular cutoffs (Fig. 1B). In other words, such

## For Ward, Mainly Montana along with Occasional Away Games

It took one summer in West Yellowstone 31 years ago to convince Dave Ward that his future was in Montana. A native of Hampton, Va., who grew up in Ohio and was educated there and in Wisconsin, Ward fell in love with Montana in 1975 while a postdoctoral fellow in the Laboratory of Thermal Biology run by his graduate advisor, Thomas Brock.

When Ward later heard that Montana State University (MSU) was looking for a microbial ecologist, he was not to be denied the position. “I’ve tried to make MSU pleased with their decision to hire me from the day I took the job,” he says, looking back to 1977. Now he is a professor in the MSU Department of Land Resources and Environmental Sciences. His primary interest is “to understand the principles governing the composition, structure, and function of microbial communities.” He tends to reject traditional labora-

tory approaches when studying those communities.

“I began to develop serious doubts about how well the naturally occurring microorganisms were being described by traditional methods,” Ward says. “Were laboratory cultivation procedures microbiology’s equivalent to bad traps? I have always suspected lab-oriented approaches of introducing biases, and have thus preferred an in situ approach.” That insistence on doing research “IN SITU,” he says, “is captured on one of my personalized license plates, so I guess this is part of my credo.”

Ward and his collaborators, with National Science Foundation support, are conducting a high-resolution genome-based analysis of whether “species matter in microbial communities—and, if so, how molecular variation groups into clusters that represent species-like units.” He believes that “we will never reach a point at

which we have a predictive knowledge of microbial communities and how to control microbes in natural and applied settings unless we come to know these fundamental ecologically distinct units that (1) occupy unique niches, (2) make unique contributions to community function, and (3) rise and fall as the environment changes,” he says.

Ward, 56, was raised in Worthington, Ohio, and studied microbiology as an undergraduate at nearby Ohio State University. At first, he wanted to become a veterinarian. He chose not to after heeding advice from his grandfather, Park H. Struthers, a naturalist at Syracuse University, who told him “get a B.S. as a base, then decide on a future.” This advice led him to study microbiology as an undergraduate. Soon, “undergraduate research pretty much convinced me to consider microbiology research as a career,” he says. He received a Master of Sci-

conservative species criteria would likely lump otherwise distinct species into one. For instance, these criteria would lump all primates, including humans, into at best two species! Mounting evidence from high-resolution population genetics studies such as multilocus sequence typing indicates that named prokaryotic species contain many closely related but ecologically specialized populations, according to Fred Cohan, an evolutionary biologist of Wesleyan University in Middletown, Conn. He notes that a named bacterial species may be more like a genus than a species. Such thoughts challenge our traditional ideas.

### Patterns of Molecular Diversity

Microbiologists are rapidly accumulating data on molecular diversity in natural microbial com-

munities. A big unanswered question is whether—and, if so, how—molecular diversity relates to species-like populations within microbial communities. Molecular-based analyses often reveal patterns of evolutionary relatedness among variants as well as patterns of distribution of these variants along gradients—in many cases, leading to hypotheses about the causes and significance of the variations within populations.

For instance, many closely related molecular variants appear to be patterned along ecological gradients, suggesting the existence of populations that diverged due to adaptive radiation (Fig. 2). Examples include light adaptation of marine *Prochlorococcus* and marine prokaryotes that contain proteorhodopsin genes and thermal adaptation of hot spring cyanobacteria.

In other cases, genetic diversity, even among closely related variants, is patterned based on



ence degree in 1973 and a doctorate in 1975, both from the University of Wisconsin. After a postdoc at the University of California, Los Angeles, he arrived in Bozeman in 1977.

Ward's interest in molecular biology and microbiology stirred earlier. He recalls that his high school biology teacher shared a 1963 *Life* Magazine article about DNA replication, protein synthesis, and the genetic code. Its contents "must have had something to do with my interest in biology in general and molecular approaches in particular," he says. Recalling its importance, he recently bought a copy of the article and decorated his wall with images from it.

Ward learned to ski in northern Ohio as a youth, and dreamed of skiing in the Rockies—a dream long since fulfilled. "After teaching my kids how to ski, it was a distinct pleasure to learn how to telemark ski from them," he says. "I don't have the legs of youth, but I sure do enjoy carving pow-

der on free heels! It doesn't hurt to live close enough to a resort to take advantage of powder days."

Ward credits his best friend, companion, and wife of 35 years, Nancy, for keeping him in balance throughout his career. He also enjoys genealogy, specifically learning about his family. "Although I never knew my grandfather as a naturalist, I have enjoyed discovering what he did in his career," he says. "I am trying to put together his CV, since one hasn't survived. I take great pride in including in my article a photo of birds he collected in Puerto Rico in the 1920s, which I was able to track down at the University of Nebraska natural history museum." Moreover, he adds, "the pursuit of his collections has made me, the microbiologist without formal education in ecology, evolution, and population genetics, see the obvious from his work about how macrobiologists group individuals into species."

Ward credits his parents with encouraging him toward indepen-

dence, a sense of self-worth, and a solid work ethic. At his father's 80th birthday party some years ago, Ward praised his dad for helping him with his schoolwork while not giving away the answers—"expecting me to figure [them] out on my own." Ward recalls that his father then "claimed he didn't know the answers."

His parents made family outings to Ohio State football games and "through many pleasurable years I had the opportunity to witness Woody Hayes' program, which had a positive influence on everyone in the Columbus area, demonstrating what you can accomplish with the right dedication," he says, adding: "I try to make it a point every fall to give a seminar at a major university where I can take in a college football game—and will consider any reasonable offer."

**Marlene Cimons**

Marlene Cimons is a freelance writer in Bethesda, Md.

coevolution with host populations or on geographic distribution, suggesting the existence of populations that have diverged due to physical isolation (Fig. 3). Examples include microbial symbionts that appear to have coevolved with their animal partners, geographically distinct hot spring *Sulfolobus* and cyanobacterial populations, and geographically distinct populations of soil pseudomonads.

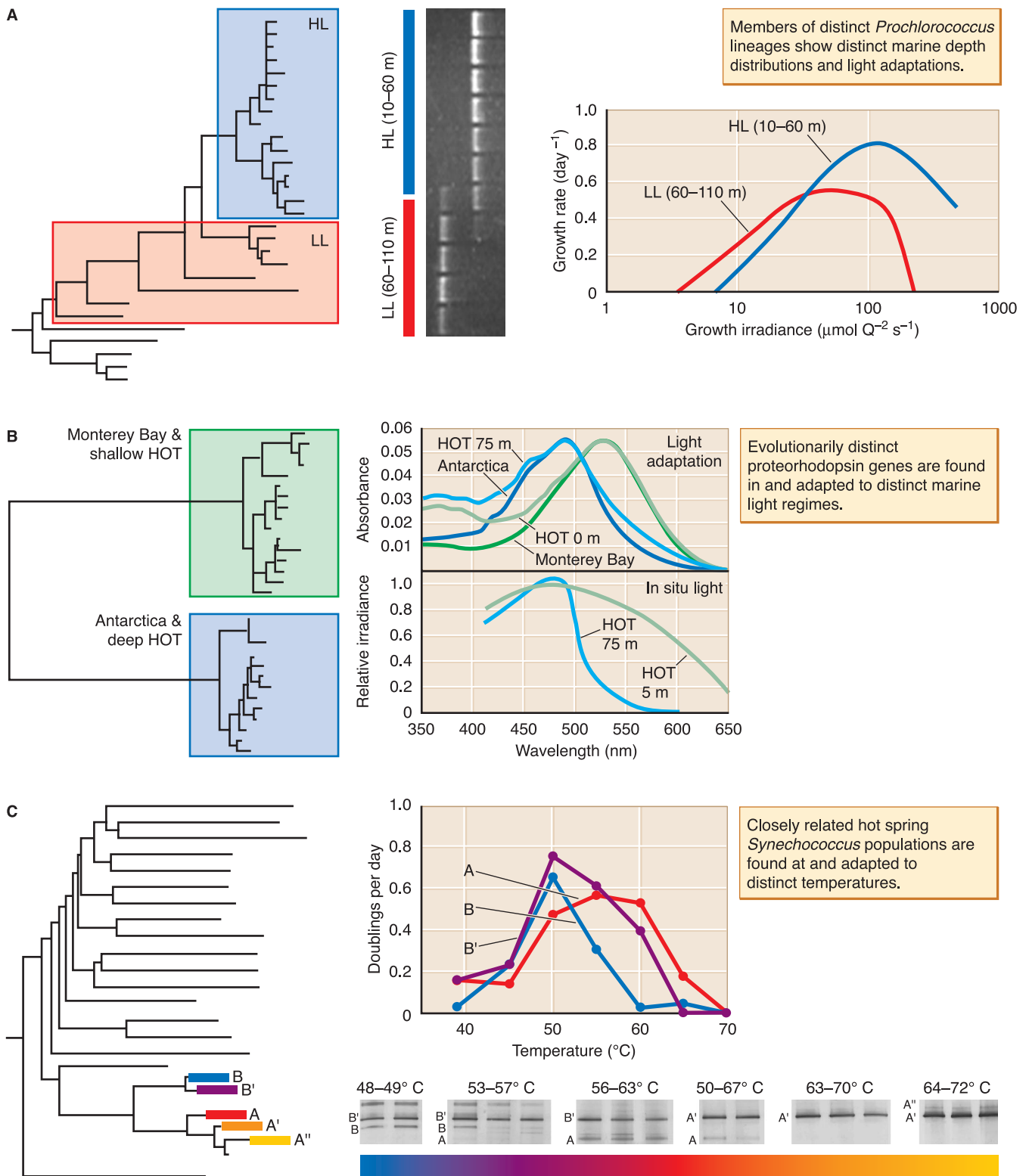
The broader implication of such patterning is that the same forces that generate plant and animal species, including adaptation and physical isolation, are likely also involved in generating microbial species.

Because of the ecological specialization of populations within the hot spring microbial mats we study, we began to use the term "ecotype" to describe molecular populations with unique distributions along ecological gra-

dients. When geographic distribution studies provide appropriate evidence, we term the genetically distinct populations "geotypes." These terms are less controversial than is the word "species," but reflect our goal as ecologists to identify basic community units, the populations that occupy unique niches and vary uniquely in response to changing environmental parameters.

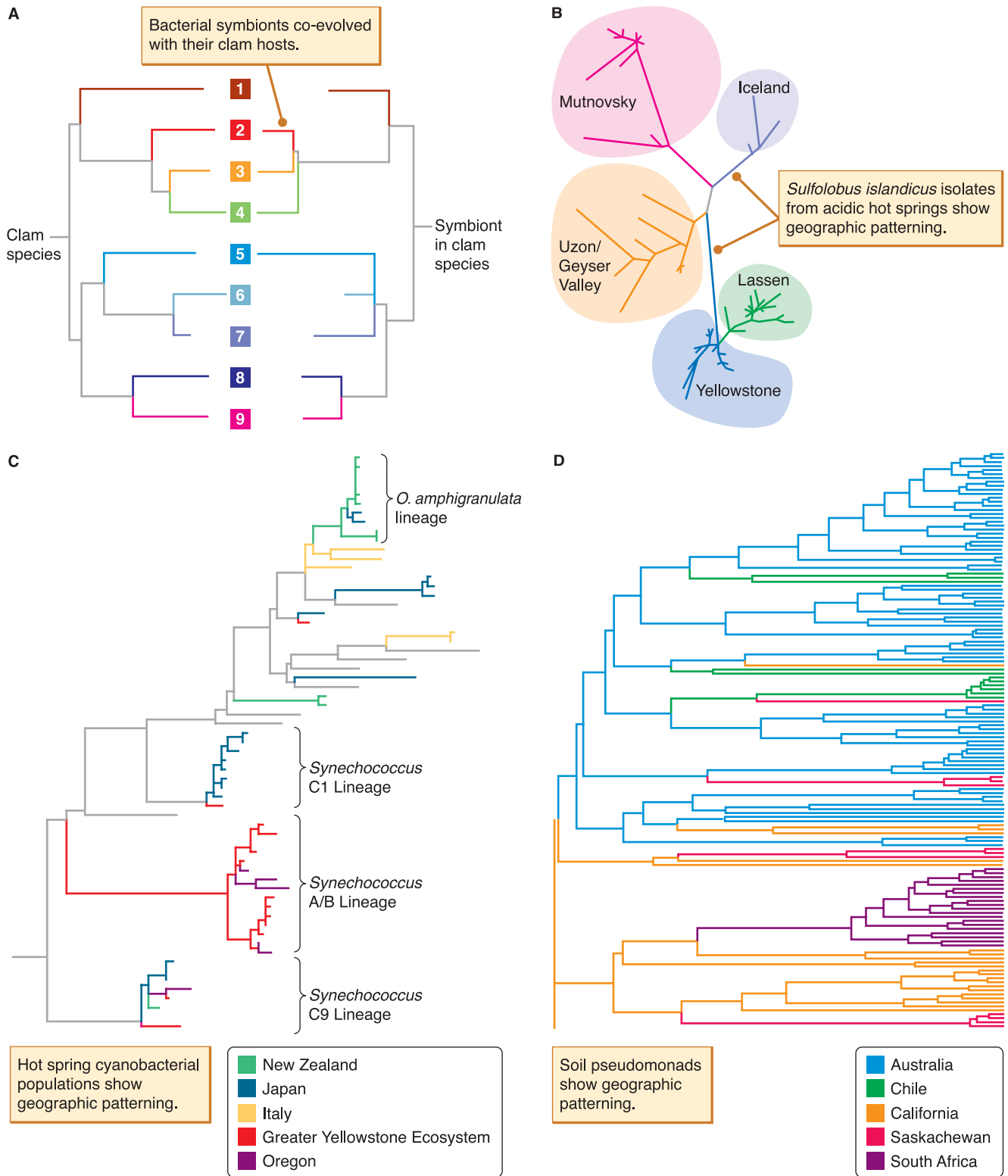
In many cases, ecotype and geotype populations are much more closely related than the 2–3% 16S rRNA sequence divergence criterion for demarcating species allows. Indeed, some distinct ecotypes have identical 16S rRNA sequences, leading to another big unanswered question. How will we know that we have accounted for all the basic units of a community? High-resolution molecular sequence analysis will help us, but a robust evolutionary ecology theory will also be essential.

**FIGURE 2**



Patterns of molecular diversity correlated with ecological gradients. (A) Distinct *Prochlorococcus* 16S rRNA clades associated with different depths in an oceanic water column and distinct light adaptations (West and Scanlan, *Appl. Environ. Microbiol.* 65:2585–2591, 1999. Adapted by permission from Macmillan Publishers Ltd: [Nature] (Moore et al., 393:464–467), copyright 1998). (B) Distinct *Synechococcus* proteorhodopsin clades associated with different depths and spectral adaptations (Reprinted by permission from Macmillan Publishers Ltd: [Nature] (Beja et al. 411:786–789), copyright 2001). (C) Distinct hot spring *Synechococcus* 16S rRNA genotypes associated with different temperatures and thermal adaptations (Ferris and Ward, *Appl. Environ. Microbiol.* 63:1375–1381, 1997 and Allewalt et al., *Appl. Environ. Microbiol.* 72:544–550, 2006).

**FIGURE 3**



Patterns of molecular diversity correlated with physical (including geographic) isolation. (A) Co-evolution of vesicomyid clams and their bacterial endosymbionts (Peek et al., PNAS 95:9962–9966, 1998). (B) *Sulfolobus islandicus* isolates form clades associated with geographically separated acid thermal springs (Adapted with permission from Whitaker et al., Science 301:976–978. Copyright 2003 AAAS). (C) Distinct *Synechococcus* and *Oscillatoria* 16S rRNA clades in geographically separated neutral to alkaline hot springs (Papke et al., Env. Microbiol. 5:650–659, 2003). (D) Distinct REP-PCR clades of *Pseudomonas* isolates from geographically separated soils (Cho and Tiedje, Appl. Environ. Microbiol. 66:5448–5456, 2000).

## Predictions of Evolutionary Ecology Theory

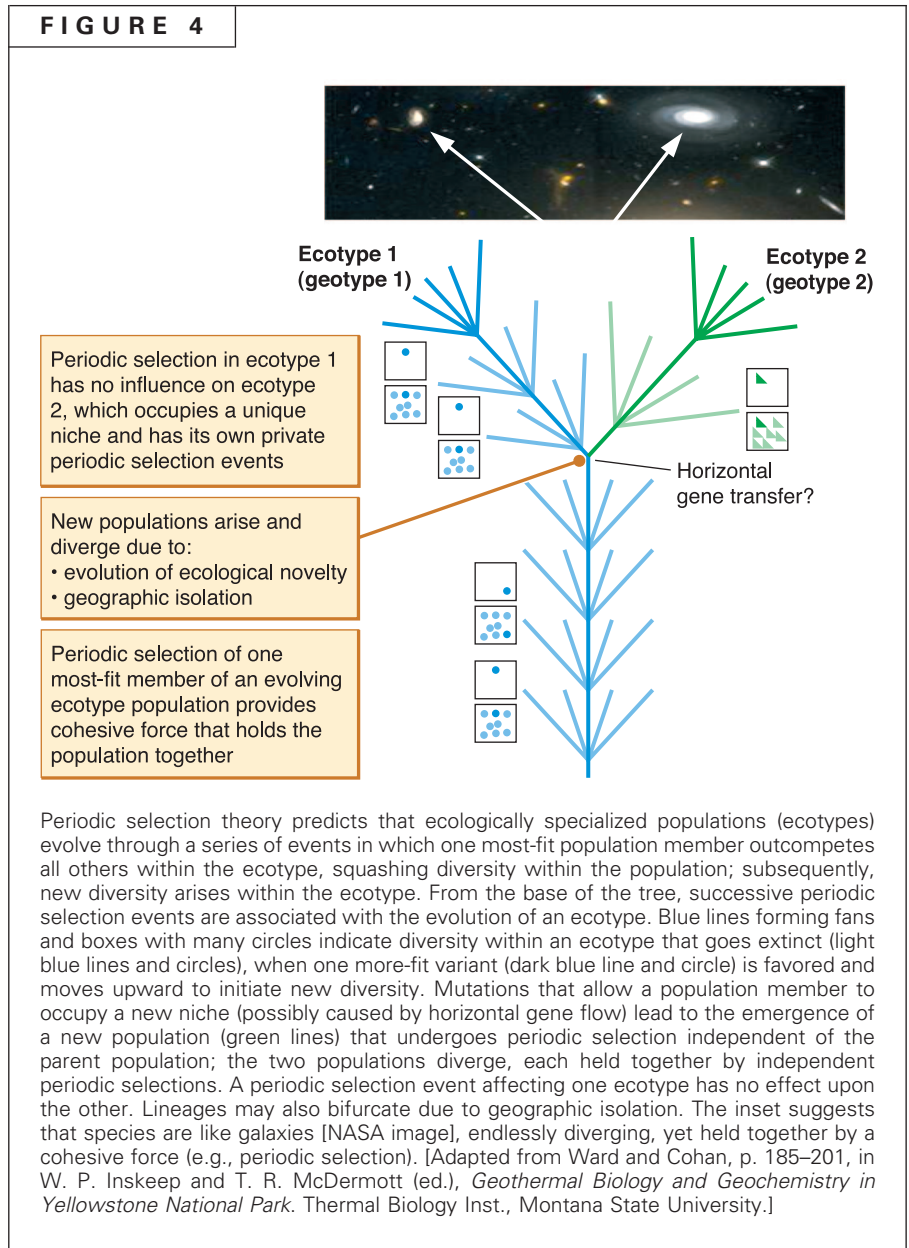
Fred Cohan introduced me to speciation theory (Fig. 4). Like galaxies, species consist of many individuals that are held together by some cohesive force. Yet species, like galaxies, are diverging irreversibly.

Microbiologists think of species as populations of nonidentical individuals (Fig. 5). Thus, they collect many different specimens to take variations into account when demarcating a species. However, I fear that microbiologists who do not typically think of microbial populations in terms of variation, and who describe a species using a fixed set of phenotypic properties (e.g., *E. coli* is a gram-negative, facultative, rod-shaped. . .) may be inadvertently teaching students to accept the fixity instead of the dynamics of species. Perhaps some of us need the same epiphany Charles Darwin described in one of his letters: “. . .I am almost convinced (quite contrary to the opinion I started with) that species are not (it is like confessing a murder) immutable.”

For species that reproduce sexually, sexual isolation (the basis of the Biological Species Concept) is the cohesive force that holds a species together and keeps one species separate from another. But, what cohesive force holds asexual species together? As Mayr suggested, it is “customary to combine into species those asexual individuals and clones that fill the same ecological niche or that play the same role in the ecosystem.”

Like Cohan, Mayr was probably thinking of how natural selection acts upon variation within a population (Fig. 4). From time to time an individual may arise with greater fitness than others. Against the changing pressure of the environment, this superior variant may be selected to the exclusion of others (periodic selection), and perpetuate the population, which eventually again develops diversity. Cohan and others point out that a series of periodic selections can act as a cohesive force holding together a population within a niche, with the survivor of

FIGURE 4



each periodic selection pulling forward its entire genome, including all the genes that define its ability to occupy the niche. Cohan here uses the term ecotype to define “the set of organisms utilizing one ecological niche, such that an adaptive mutant out-competes to extinction members of its own ecotype (and thereby purges genetic diversity within the ecotype, genome-wide); however, an adaptive mutant cannot out-compete members of other ecotypes owing to their ecological distinctness.”

Sometimes, however, a variant arises that can occupy a new niche, leading to a new population



FIGURE 5



Macroecologists view species as populations with variation. Birds collected by author's grandfather, Dr. Parke H. Struthers, Syracuse University.

that is not influenced by periodic selection events affecting its parental population. The reason is simple, the variant and its descendants occupy different niches. Each population undergoes “private” periodic selection events and the two populations further diverge. Given time, a series of periodic selection events and random neutral mutations that accrue within each separate population leads to genetically distinct clades of two distinct ecotypes.

The division of one population into two may also occur as a result of physical isolation, the mere separation of one subset of a population from another, as the two isolated populations will randomly accrue neutral mutations. Given time, this will lead to genetically distinct clades of distinct geotypes.

Jim Staley of the University of Washington in Seattle has suggested a Phylopecies Concept (essentially a Phylogenetic Species Concept) for situations where there is no evidence of ecological or geographic distinction between separate phylogenetic clades. At a recent meeting that he, Brian Spratt, and Matthew Fisher of Imperial College, London, hosted at the Royal Society of London on “Species and Speciation in Micro-Organisms,” Staley commented that we should think of prokaryotic species as “the smallest irreducible phylogenetic clusters.” I like this idea because it recognizes the possibility that the genetic markers we use to measure clusters may not have sufficient variation to discern the clades that represent distinct ecotypes and geotypes

(i.e., use of another marker exhibiting greater variation would “reduce” such clades into several ecotypes or geotypes). Indeed, several cases have been published indicating that higher-resolution molecular markers are needed to reveal ecotype and geotype clades previously undetected using 16S rRNA analysis. Attaining sufficient resolving power was a common plea among speakers at this meeting.

Cohan believes that neutral genetic differences that accumulate in long-diverging populations may provide a way to recognize ecotypes through theory-based evolutionary simulation. While it may seem that gene content differences and adaptive genes should offer the best chance to detect unique ecotypes, it is the neutral variation that can be used to predict the existence of putative ecotype clades. Once ecotypes are identified, gene content differences and adaptive genes can ultimately tell us how the members of ecotype clades differ. The approach is restricted to long-diverging populations because nascent ecotypes (or geotypes) would not exhibit enough neutral genetic difference. Cohan has developed models that not only take into account the evolution of ecotype clades, but also consider that geographic isolation and high rates of genetic exchange are sometimes part of the complex reality of prokaryote speciation. Importantly, he also proposes ways to test these competing hypotheses. For instance, if one ecotype population evolves to form multiple geotypes due to physical isolation, which then later reinhabit the same environment, one would expect  $>1$  phylogenetic clade per ecotype rather than a 1:1 correspondence between clades and ecotypes.

What role does HGT play in speciation? Cohan has argued that HGT is “rare, but promiscuous,” on the basis of empirical evidence that both recombination and mutation occur at low frequency. Thus, while genes can be exchanged between distantly related organisms (even across domains), the frequency of HGT is too low to mix gene pools and thus keep ecotype populations from diverging.

Though rare, HGT is nonetheless enormously important as an evolutionary process. Consider the impact of the endosymbiotic origin of chloroplasts from cyanobacteria on the interdomain evolution of photosynthesis, which led to algae and plants. Heterologous recombination might be the primary way by which microorganisms



speciate. Among microorganisms with high recombination frequencies, homologous recombination may be sufficiently frequent to blur evolutionary and ecological patterning of diversity. Not all plant and animal species exhibit the same evolutionary and ecological strategies—why should all microbial species?

### A Divergence of Fields

Early microbiologists were natural historians. For instance, according to Brock in *Robert Koch, a Life in Medicine and Bacteriology*, Robert Koch, noting that anthrax bacilli grew in the eye fluids of infected rabbits, used the fluids from uninfected rabbits to cultivate the bacilli, then to prove that the isolated bacterium caused disease. Koch's later development of the plate technique for obtaining pure cultures had an important influence on the development of microbiology.

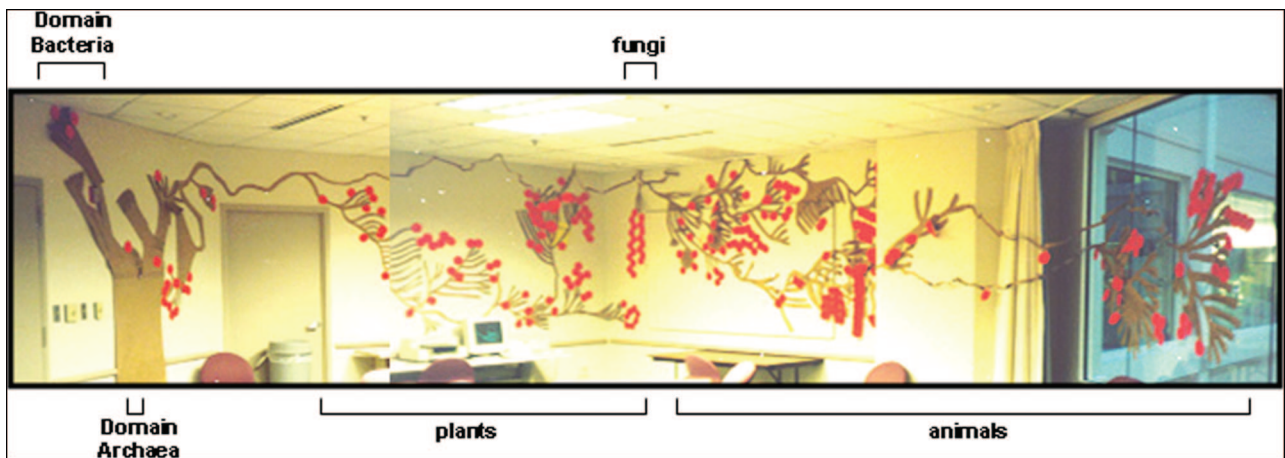
Yet, this approach is microbiology's boon and bane. The tradition of laboratory-based approaches caused many microbiologists to deemphasize field studies. While ecologists observed organisms in situ and developed a principled basis for understanding diversity in terms of evolutionary and ecological forces, most microbiologists focused their energies within labs. Microbial ecologists took a predominantly process-based, as opposed to population-based,

view, doubting that culture methods would ever be able to describe the true diversity and ecology of microorganisms. The reliance on cultivated microorganisms and phenotypic characteristics led to pessimism that an understanding of microbial evolution would ever be reached. Now molecular methods have provided optimism that we can understand microbial evolution and in situ diversity and ecology, but we are poorly prepared to interpret the results. Few microbiology curricula include courses in ecology and evolution, even though they are of fundamental importance to understanding biology.

Is microbiology a separate field, and are microorganisms unique compared to plants and animals? Or, are microbiologists simply trained to see the world differently compared to botanists and zoologists? In any case, there is a considerable rift between microbiology and the rest of biology, as dramatically illustrated by a severe imbalance in funding between macrobial and microbial systematics (Fig. 6).

It is time for macrobiologists and microbiologists to communicate more effectively with one another. Information should flow in both directions, with macrobiologists gaining greater appreciation for microorganisms and microbiologists gaining greater appreciation of the principles of evolution and ecology. Perhaps, by adopting a more natural view in microbiology, we can train better environmental scientists

FIGURE 6



A three-domain funding tree of life I observed on an NSF panel room wall. Domains *Bacteria* and *Archaea* are dwarfed by Domain *Eukarya*, which radiates across parts of three walls. The red dots represent awards from the NSF Systematic Biology Program in the 1990s! (photos courtesy of Mary C. McKittrick)



for the future. Toward this end, my colleagues and I developed a curriculum called Environmental Biology, which might be a useful model for combining the best of both disciplines to provide students with a principled view of all organisms large and small. (<http://landresources.montana.edu/Curriculum/EnvBiologyDescription.htm>).

### Do Species Matter in Microbial Communities?

Species matter in microbial communities. We must discover how molecular variation is organized into the fundamental units that make up microbial communities to understand how community function is organized by composition and structure, to be able to predict the populations that will rise and fall with environmental changes, to understand which populations are associated with disease outbreaks, and to understand the emergence of new species-like populations, including new pathogens.

We also have to learn how special properties of microorganisms, such as the ability to exchange genes, set them apart from plants and animals. Studies in experimental evolution indicate that some microbial populations respond rapidly to changes with the emergence of new ecological strategies. Is the response of a microbial community to perturbations limited to the changing dynamics of existing species, or is the formation of new species a part of that response?

The species debate is a healthy exercise, one that is changing microbiology, allowing us to develop more advanced and natural approaches to systematics, ecology, and both medical and environmental microbiology. Cohan and I are working with others under a NSF Frontiers in Integrative Biology Research award to further investigate the species question. This program includes an annual workshop; additional information about this program and Cohan's course on microbial species and speciation are available on our website (<http://landresources.montana.edu/FIBR/>).

### ACKNOWLEDGMENTS

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