Diversity, Ecology, and Evolution of Microorganisms Inhabiting Hot Spring Microbial Mats

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Contents:

Introduction

My students and I have studied hot spring microbial mat communities in Yellowstone National Park since 1977 (**Fig. 1**). We study them as models of microbial community ecology addressing primarily questions about the composition, structure and function of the community. Microscopically, it appears that that community structure is simple: a single, sausage-shaped cyanobacterium (*Synechococcus* sp. revealed by the red autofluorescence of the chlorophyll *a* they contain) appears to build the community together with filamentous bacteria resembling green nonsulfur bacteria (*Chloroflexus* sp.). This simple picture of community structure was reinforced by the fact that readily cultivated *Synechococcus* and *Chloroflexus* strains appeared to have limited genetic diversity, as expected of single species. Since I was a graduate student I have been concerned that the simple morphology of microorganisms might mask an underlying greater diversity. Furthermore, I doubted that the extremely selective nature of laboratory cultivation techniques would make it useful for describing the composition of microbial communities in an unbiased way. In 1984 I took a sabbatical leave with Norman Pace to learn molecular methods for cultivation-independent analysis of diversity within a microbial community. As a result, we have been able to develop approaches to demonstrate that this community is not as simple as it first appears.

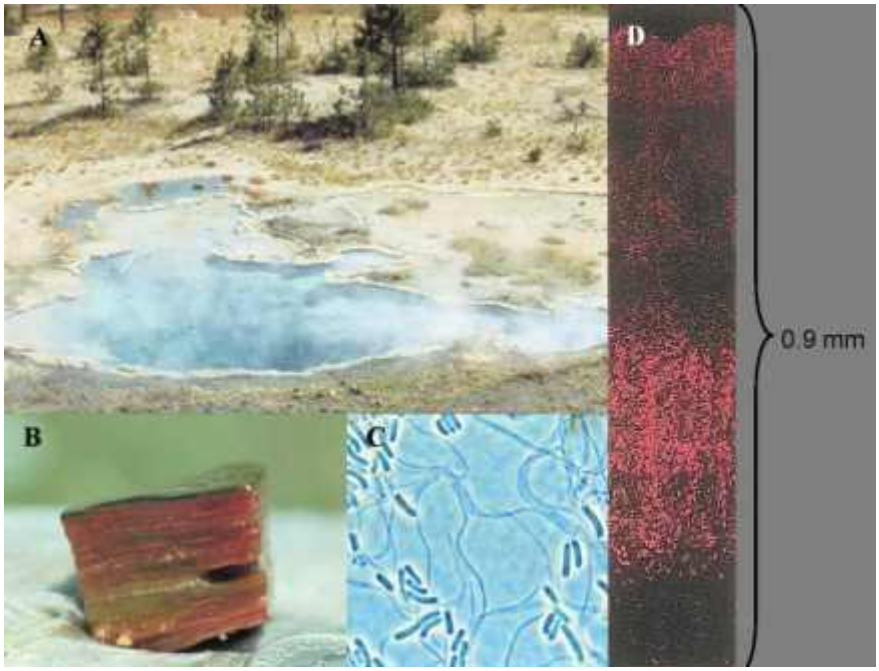

Figure 1. Green hot spring microbial mats occur below ca. 74°C (a). The top green layer (b) is comprised of filamentous green nonsulfur-like bacteria and unicellular cyanobacteria, Synechococcus (c) that form distinct layers of different autofluorescence intensity in the top 1 mm (d).

Molecular analysis of community composition and structure

In the mid-1980s we began to develop and use methods for examining the genetic variation exhibited by the gene encoding 16S ribosomal RNA molecules, using the strategy depicted in **Figure. 2**. DNA is extracted from mat samples, then PCR amplified to produce a mixture of 16S rRNA sequences from community members. These are separated by cloning or gel methods called *d*enaturing *g*radient *g*el *e*lectrophoresis (DGGE) to allow purification of individual 16S rRNA sequences. Purified 16S rRNAs are then sequenced and compared to a large and growing database on the evolution of life based on sequence variation in this molecule http://rdp.cme.msu.edu/index.jsp.

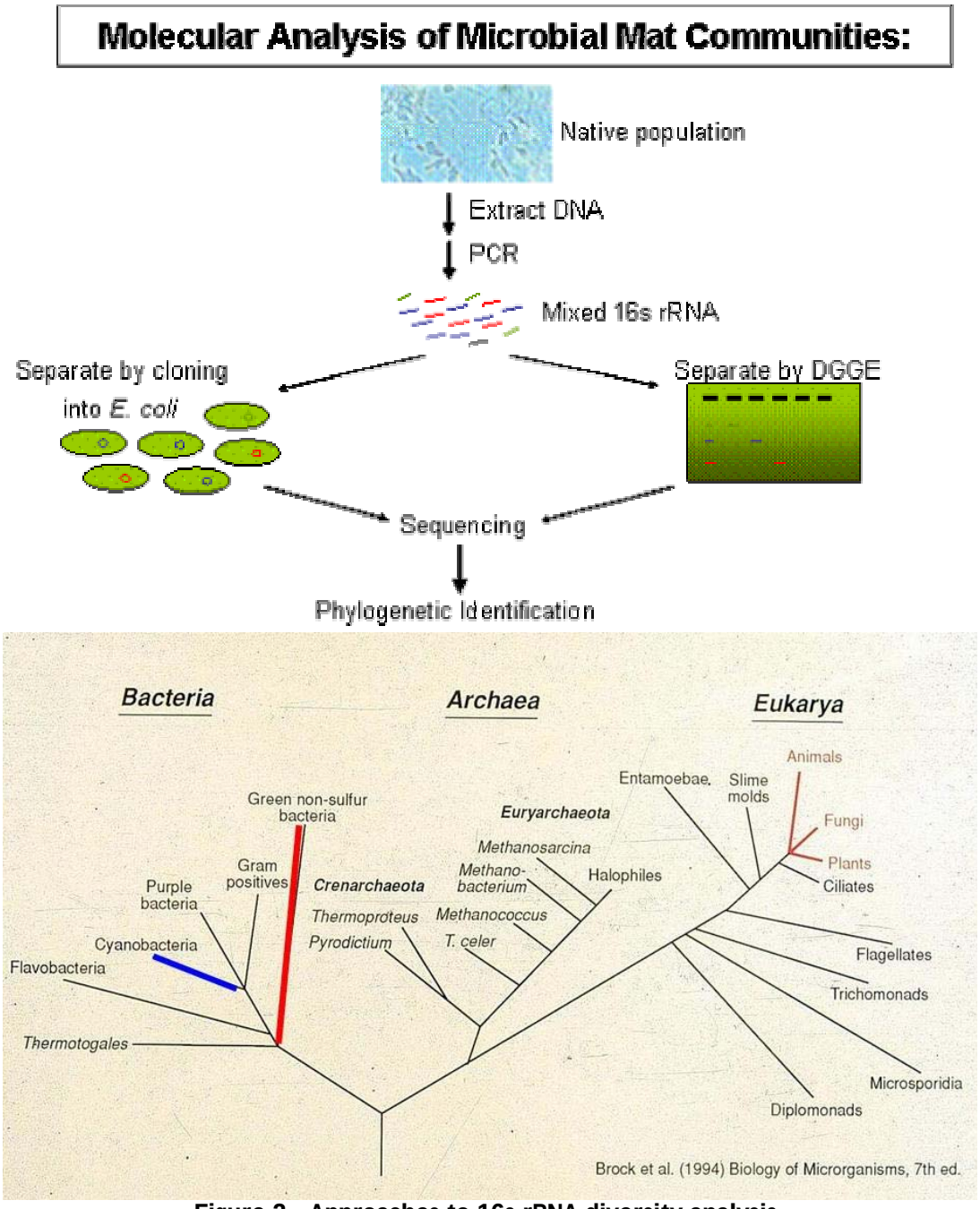

Figure 2. Approaches to 16s rRNA diversity analysis.

As expected, our community contained 16S rRNA sequences related to cyanobacteria and green nonsulfur bacteria, as shown by the blue- and red- highlighted Kingdom-level lineages in the 3-domain tree. The three red-highlighted lineages in Domain Eukarya are the traditional Animal, Plant and Fungal Kingdoms. Since line length separating organisms in the tree are proportional to genetic difference between the organisms, it is easy to note that microorganisms (all other lines) contain much more genetic diversity as assayed by 16S rRNA sequence variation.

Evidence of diversification through adaptive radiation

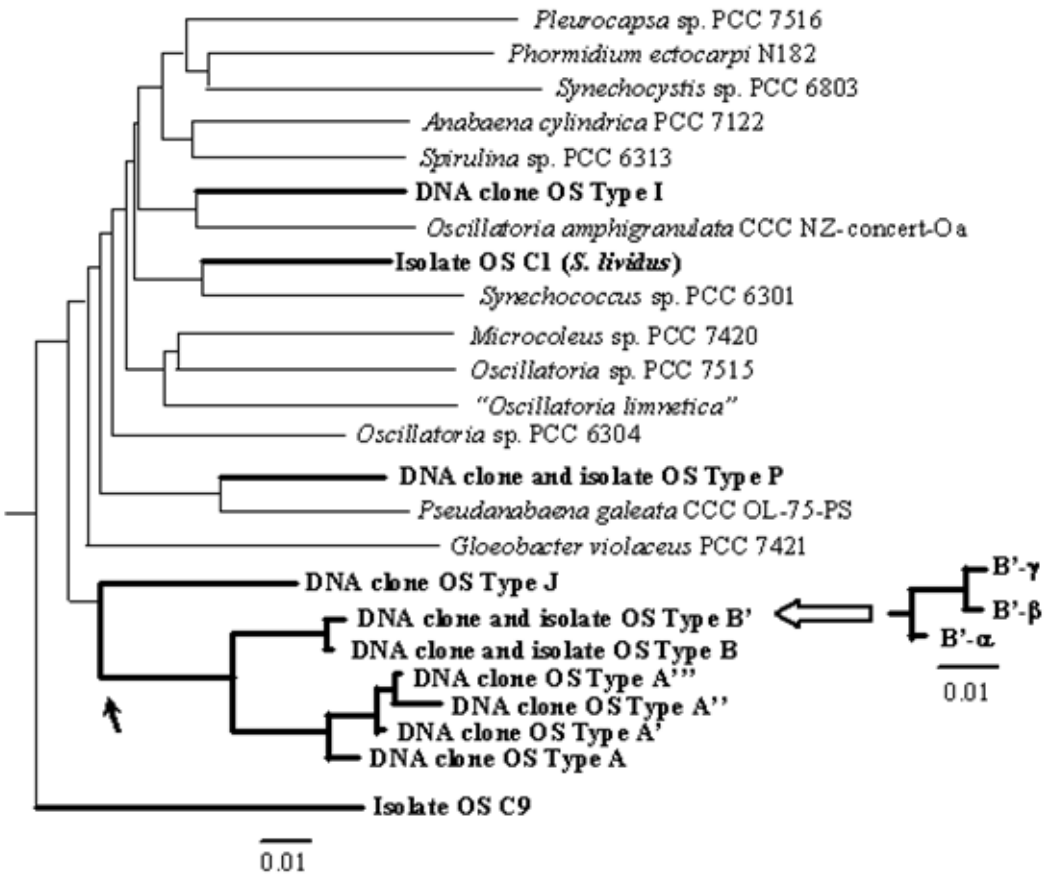

Figure 3. Cyanobacterial 16s rRNA phylogeny. Mat sequences are bold-highlighted. Inset shows ITS variation within 16s rRNA genotype B'

Fig. 3 shows a tree of diversity in cyanobacterial 16S rRNA sequences, with thin lines providing a background of diversity within this kingdom. The thick lines indicate the diverse cyanobacterial 16S rRNA sequences we detected in a hot spring microbial mat. Clearly, the mat contains more cyanobacterial diversity than meets the eye. Again, line lengths separating sequences (in this case horizontal component only) equate to genetic differences. The readily cultivated sequence (Isolate OS C1, *S. lividus*) is unrelated to the predominant ones, which comprise a set of closely related sequences we call A/B types. The difference between type C1 and the A/B types is very large, certainly representing different species, but more likely representing differences on the same scale as the difference between flowering plants and ferns! But, what about the closely related sequences of the A/B group? By studying the distribution of these genetic variants along ecological gradients, we learned that even the most closely related sequences appear to correspond to ecologically distinct cyanobacterial populations. **Fig. 4** shows different A/B genotypes at different temperatures and depths. Note the progression of genotypes from B to B' to A to B' to A'' from low to high temperature and the subsurface position of genotype A corresponding to pigment-rich *Synechococcus* 400-700 µm below the mat surface. We are currently examining pure cultures of A/B lineage *Synechococcus* to evaluate whether, as predicted from distribution studies, these are temperature- and light-adapted ecological populations. Together with evidence from other laboratories, it seems clear that, like plants and animals (e.g., **Fig. 5**), prokaryote diversity is acted upon by natural selection to yield ecologically specialized populations. We term these populations *ecotypes*, but they can be taken as species if an ecological concept of species is applied. [Ward, 1998; Ward et al., 1998, 2002]

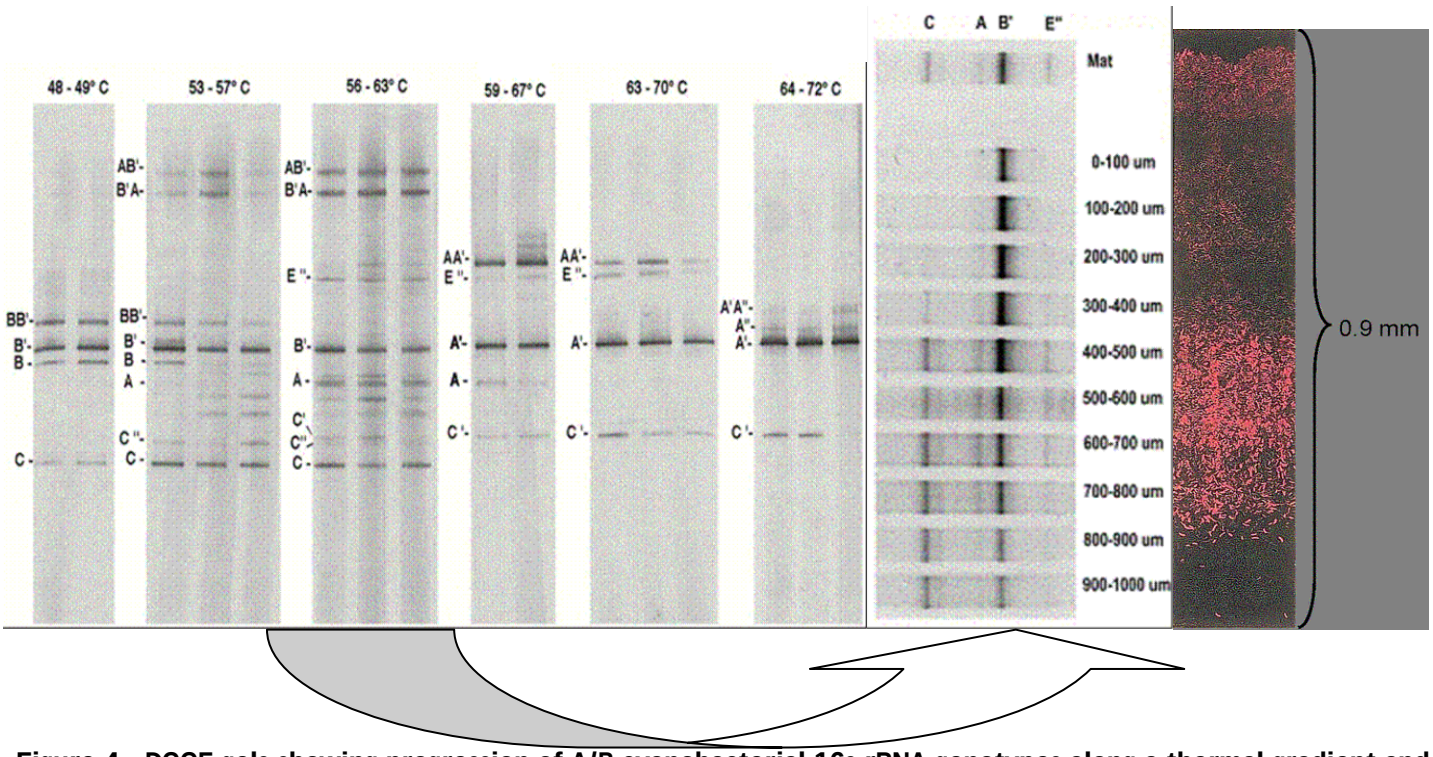

Figure 4. DGGE gels showing progression of A/B cyanobacterial 16s rRNA genotypes along a thermal gradient and along a vertical gradient at a 60°C site.

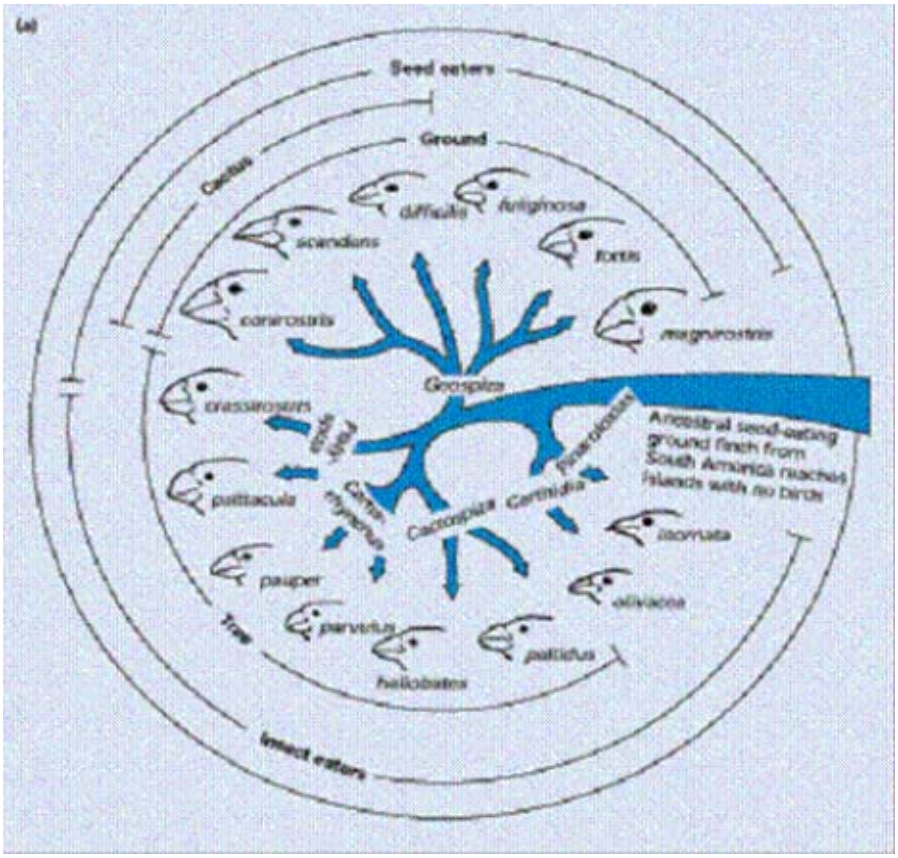

Figure 5. Galápagos finch diversity arose from an ancestral mating pair via adaptive radiation to fill unoccupied niche space. [Begon et al., 1990]

Have we detected all the ecotypes?

We have determined that the 16S rRNA gene may be too conserved to enable us to detect all ecologically distinct populations within microbial communities. At a 68°C site, we also see distinct differently pigmented *Synechococcus* populations near the mat surface and at depth. However, we can detect no difference in depth in 16S rRNA sequence. We developed an approach to PCR amplify the faster-evolving internal transcribed spacer (ITS) region adjacent to the 16S rRNA gene and used it to show that genotypes found at the mat surface (●) are genetically distinct from those in the mat subsurface (■) (**Fig. 6**). [Ferris et al., 2003]

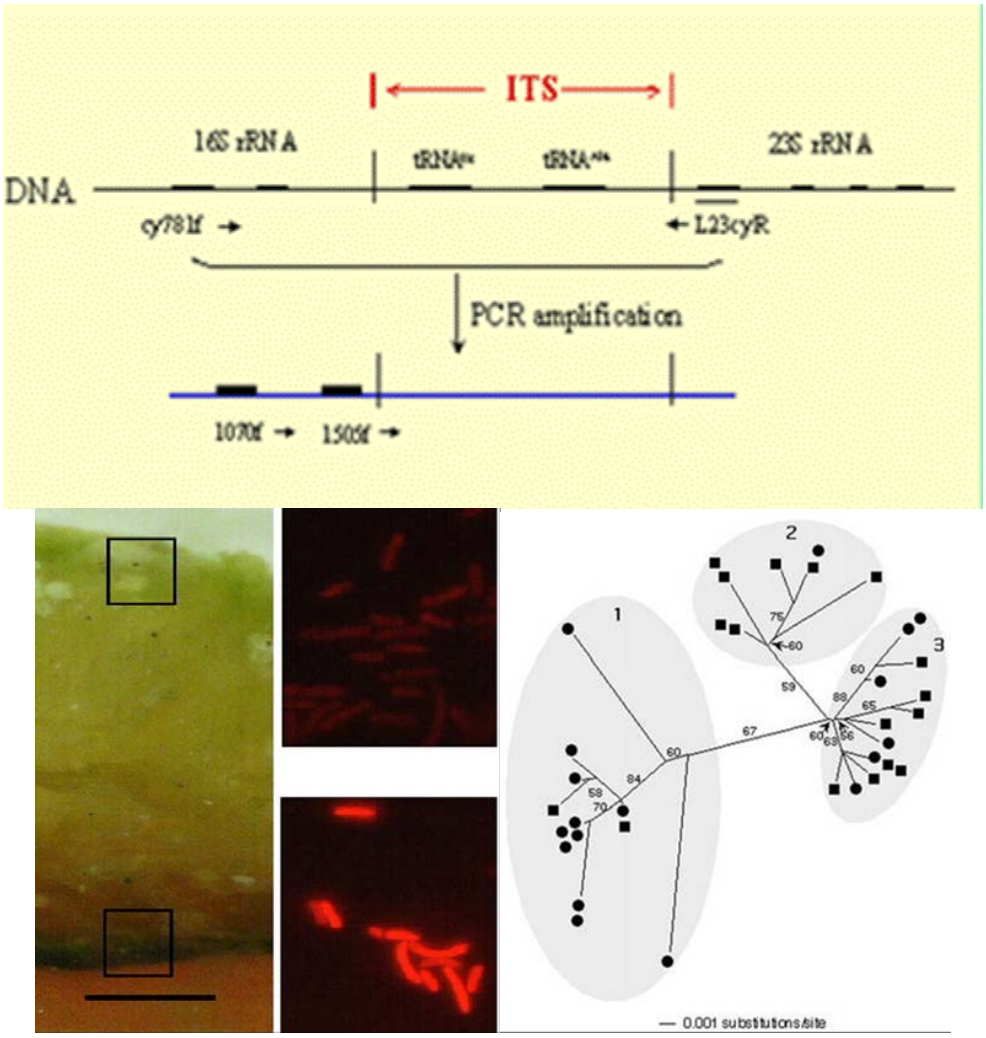

Figure 6. *Top*: **PCR approach to amplification of 16s rRNA gene and adjacent internal transcribed spacer.** *Lower left*: **Vertical section of 68°C Mushroom Spring mat with autofluorescence microscopy images of surface and deeper layers.** *Lower right*: **Unrooted ITS phylogeny for cyanobacterial sequences of a single 16s rRNA type detected in the top layers (circles) or deeper layers (squares).**

Evidence of diversification through geographic isolation

Since bacterial diversity seems to arise through adaptive radiation, it seemed of interest to examine whether the other major driver of speciation in plants and animals, geographic isolation, was important to prokaryote diversification. Considering hot springs to be like islands, we sampled springs in Japan, New Zealand and Italy in addition to North America. We retrieved sequence data for both the 16S rRNA and ITS loci of indigenous cyanobacteria (**Fig. 6**) directly from the mats we sampled. We found unique cyanobacterial 16S rRNA genotypes in each location, as shown in **Fig. 7**. The A/B type *Synechococcus* sequences appear endemic to North America (red and pink for OR). Japan (blue) was dominated by C1-lineage *Synechococcus*, which were also found in North America. New Zealand (green) was dominated by C9-type *Synechococcus* and *Oscillatoria amphigranulata* sequences. In many cases we observed distinct clades for different geographical regions. The geographic distribution pattern could not be explained by different chemical conditions, suggesting that geographic isolation is involved in diversification of hot spring cyanobacteria. We even found evidence of geographic clades at more local spatial scale (e.g., within major thermal basins of Yellowstone Park. Together with similar results from other laboratories, it appears clear that, like plants and animals (**Fig. 8**), geographic isolation also acts upon prokaryote variation to cause diversification. [Papke et al., 2003]

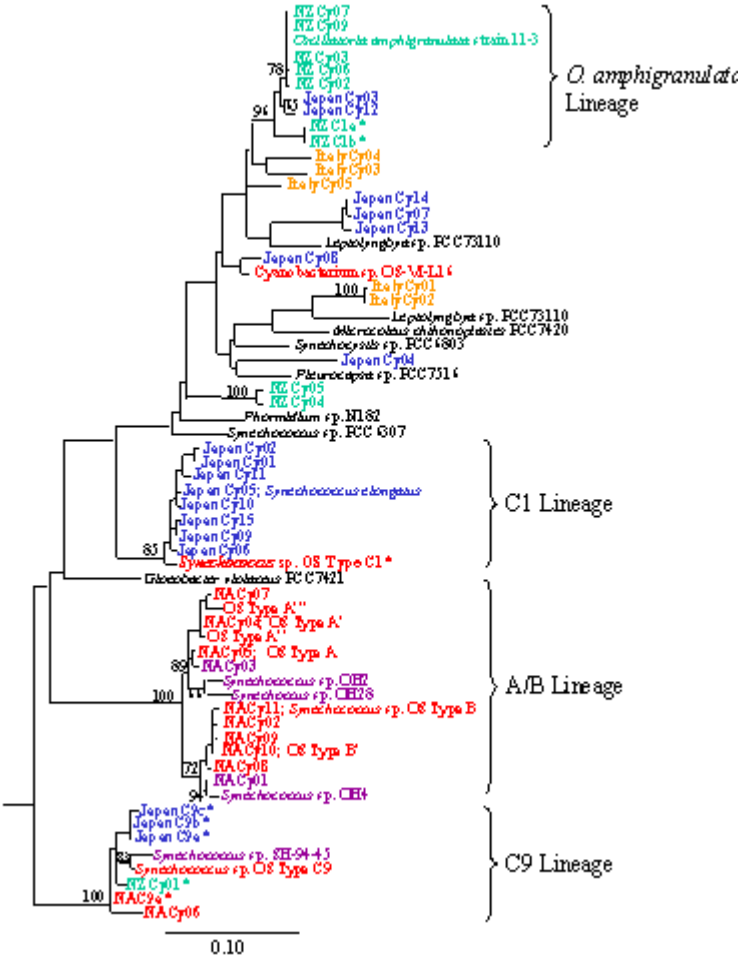

Figure 7. Cyanobacterial 16s rRNA diversity detected in globally separated hot spring mats of North America (OR), Japan, and New Zealand. A/B, C1, and C9 lineages are *Synechococcus* spp.

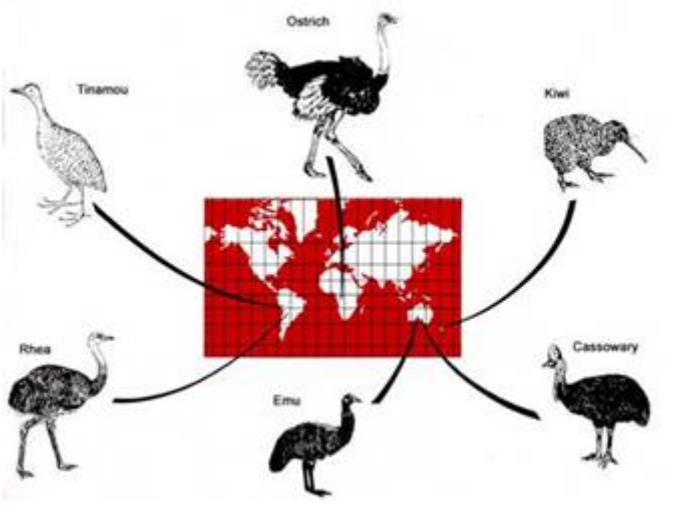

Fig. 8. Distinct flightless birds on different continents exemplify diversification through geographic isolation. [Begon et al., 1990]

Current Research

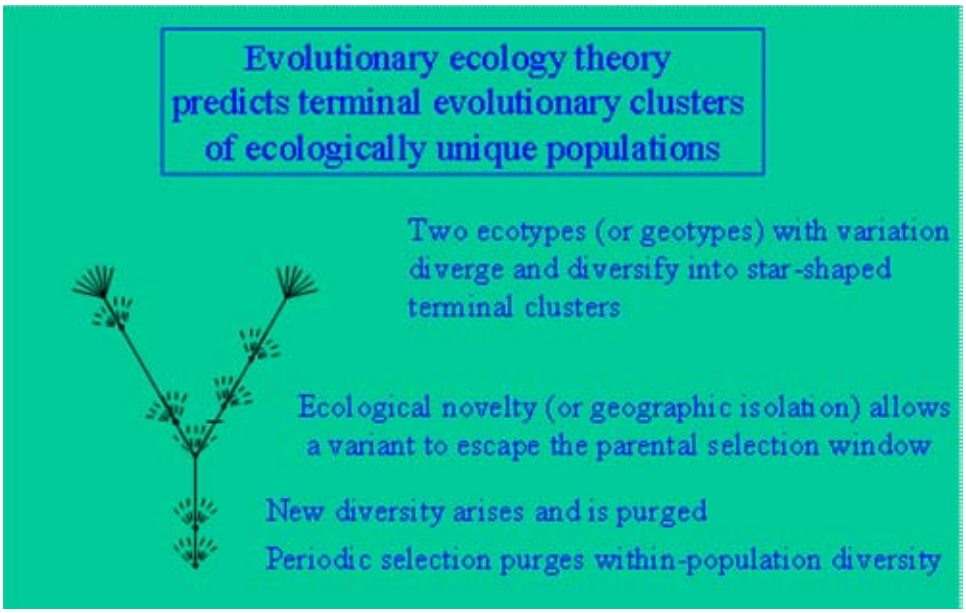

Figure 9. Periodic Selection Theory.

After observing these patterns of diversity and its distribution and their correspondence to ideas about plant and animal speciation, I took a sabbatical leave in 2000 to the lab of Fred Cohan to learn about evolutionary theory. The empirical results we observed in our studies match well with periodic selection theory [Cohan, 2002] (**Fig. 9**). The idea is that populations, which have variation, evolve through a succession of periodic sweeps of diversity. One most-fit variant out-competing all others and carrying forward the genetic know how to occupy the population's niche. However, whenever a variant arises which has a novel ecology (occupies a different niche than the parental population) it is no longer selected in the same way (i.e., survives periodic sweeps of the parental population). It is then free to diverge from the parental population, giving rise to a new population which undergoes its own private periodic sweeps. The eventual result is two ecologically distinct populations. Geographic isolation can have the same effect as ecological adaptation in driving populations apart. Fred has shown that a high-resolution molecular technique for analyzing population genetics has potential to detect these terminal ecotypical clusters. The method, called Multi-Locus Sequence Typing (MLST), involves PCR amplification of 7 rapidly evolving protein encoding genes and sorting variants into clonal complexes. Fred has developed an evolutionary simulation that suggests that MLST clonal complexes equate to putative ecotypes. In a project sponsored by the NSF Frontiers in Integrative Biological Research (FIBR) and NASA Exobiology Programs, Fred and I plan to develop a cultivation-independent MLST approach to study, at very high resolution, *Synechococcus* ecotypes within the mat. As a part of the FIBR project, we will collaborate with John Heidelberg of the Institute for Genomic Research (TIGR) to obtain genomic sequences of two *Synechococcus* isolates that are genetically relevant to the mats we study. John will also conduct direct environmental genomic analysis of predominant mat populations, as a theory-independent means of investigating how genomic diversity may be organized into populations. Genomic sequence data will permit us, in collaboration with Devaki Bhaya and Arthur Grossman (Carnegie Institution/Stanford), to develop microarrays that will be used to investigate gene expression in situ within the mats. Ultimately, we hope to compare the distributions of allelic variants of highly expressed genes, with alleles that mark MLST clonal complexes (putative ecotypes).

Publications

*****=recommended publications

Ward Lab publications:

Reviews and book chapters:

*Ward, D.M., R. Thane Papke, U. Nübel and M.C. McKitrick. 2002. Natural history of microorganisms inhabiting hot spring microbial mat communities: clues to the origin of microbial diversity and implications for micro- and macro-biology, pp. 25-48 in *Biodiversity of Microbial Life: Foundation of Earth's Biosphere* (J.T. Staley and A.-L. Reysenbach, eds.). John Wiley and Sons, NY.

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*Begon, M, J.L. Harper and C.R. Townsend. 1990. Ecology: individuals, populations and communities. 2nd ed. Blackwell Sci. Publ., Cambridge, MA. [This is an excellent introduction to how diversity is linked to evolution and ecology. I highly recommend it for a more natural view than microbiologists usually acquire through their own texts.]

*****Cohan, F. M. 2002. What are bacterial species? Annu. Rev. Microbiol. 56:457-487.

Funding:

- Past:
  The Ward lab appreciates the long-term support provided by the NSF Ecology Program and the NASA Exobiology and Astrobiology Programs. We also appreciate the support of the Montana State University Thermal Biology Institute.
- Current:
  - "Do species matter in microbial communities?" (with Co-Investigators F. Cohan, J. Heidelberg, D. Bhaya and A. Grossman), NSF Frontiers in Integrative Biology Program (FIBR), 1/03 through 9/1/08, $4,999,690.
  - "Molecular and geochemical analysis of hot spring cyanobacterial and Chloroflexus mats as stromatolite analogs" (with Co-Investigators F. Cohan, J. Eisen, J. Heidelberg, M. Madigan, and S. Schouten), NASA Exobiology Program, 5/03 through 5/07, $588,056.