A New Age of Naturalists

A new age of naturalists is under way as we explore the distribution and origins of microbial diversity in the natural world

Rachel J. Whitaker

Charles Darwin and other naturalists of his time plied the globe by boat and machete to survey the diversity of plants, animals, and insects, and their distributions around the world. They combined these surveys with their contemporary understanding about the history of the Earth to develop an evolutionary model for the origins of species through natural selection. In *The Origin of Species*, the geographical distribution of plants and animals plays a central role in developing the theory of descent with modification. Darwin and others noticed that organisms from the “same areas of land and water” shared characteristics which he called the “deep organic bond” of inheritance. Darwin argues, based on this bond, that “varieties” of organisms must have descended from a common ancestor that dispersed and later became disconnected and diverged. As a mechanism of divergence he states, “dissimilarities of the inhabitants of different regions may be attributed to modification through variation and natural selection.” To back up this argument, two chapters of *The Origin of the Species* are devoted to developing plausible dispersal mechanisms and changes in landscape that could link populations, that now appear to be disconnected, to a common ancestor.

One hundred and fifty years later, microbial naturalists are uncovering a whole new world of diversity that will lead to a sea change in our understanding of evolution. Using DNA sequencing as a powerful lens through which to resolve relationships between minute cells that look the same even with the most powerful microscopes, we are discovering new microbes each year, even in the most mundane places. Already, the diversity of microbes these tools have uncovered puts the entire menagerie of macroorganisms to shame and has changed the way we understand the evolution of life on Earth. Using DNA sequences to map the relationships between these and other organisms has resulted in a completely new tree of life on which humans, and the vast array of plants, animals, insects, and fungi that captivated Darwin, are just tiny twigs. The new tree shows that we are not at the top of the evolutionary ladder, but are equal, in terms of evolutionary history, to all of the extant microbes on Earth today. Most spectacularly, reordering these relationships revealed three domains of life instead of the five kingdoms most of us grew up studying: Bacteria, Archaea, and Eukarya.

It was the discovery of the Archaea, gaining broad acceptance in the early 1990s, that motivated me to become a microbiologist. I was inspired by the 1997 issue of the journal *Science* on Frontiers in Microbial Biology, in which Norman Pace described the microbial diversity that was being uncovered by the new, high-resolution, molecular lens. A companion article described the extraordinary work of Carl Woese, who had pioneered the field using molecular tools to lay out the framework for the tree of life, identified Archaea, and was continuing to investigate the origins of life and the fundamentals of cellular evolution. How could I resist the chance to study a new domain of life? The challenge for me, and for the next generation of microbial naturalists as the breadth of diversity continues to be uncovered, is to understand how this diversity evolved. Are the evolutionary mechanisms of microbes similar to their distant macrobial cousins? Many of the answers will come, as they did for Darwin and the naturalists of his time, by looking at the natural distribution of microbial diversity and putting it in its appropriate context of space and time.

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Evolutionary biologists studying macroorganisms have been studying the mechanisms through which diversity evolves—the origins of species—for hundreds of years. Conventional evolutionary theory says that new species arise when populations become disconnected so that DNA is unable to move between them. What constitutes a genetic barrier that can disconnect populations? For macroorganisms, the primary mechanism is geographic isolation, such as a mountain range or an ocean, that prevents migration and consequent mixing between populations. These are the barriers that Darwin discusses at length in *The Origin of Species*. Once populations are isolated, they are free to adapt specifically to the local environmental conditions. Natural selection can favor a long, pointed beak in one place, and a blunt, rounded beak in another. These barriers therefore allow “dissimilarities” or diversity to evolve. Of course, evolution does not only occur through natural selection; accidents can cause the extinction of one population and the continuation of another. The key is that, even if random events drive divergence, once isolated, populations are independent and free to follow their own evolutionary course.

Initial surveys of microbial diversity suggested that microbes did not fit this conventional model of evolution. Microbial naturalists were unable to identify mechanisms that would disconnect microbial populations from one another. Microbes seemed too small and too abundant to be restricted by typical geographic barriers. In addition, the lens of DNA sequencing of highly conserved genes uncovered seemingly identical organisms in far-flung corners of the globe, suggesting that geographic isolation did not exist for microorganisms.

Struck by the conundrum of extreme diversity with no evolutionary mechanism to promote it, John Taylor and I set out to ask whether geographic barriers could isolate populations and allow their independent evolution. To test this, we turned to the so-called “extremophiles” which prefer what to humans are extreme conditions, and thus are the least likely to be able to travel the world freely. We chose to examine *Sulfolobus*, from the domain Archaea, which prefer volcanic hot springs where the water is close to the pH of battery acid at a scalding 80°C. We reasoned that with such a limited and discontinuous habitat, if geographic barriers existed for any microorganism, we would find them there. Based upon the increasing resolving power provided by the lens of complete genome sequences, we set out to test whether *Sulfolobus* would be able to disperse across the globe.

This is where the adventuresome part of being a microbial naturalist comes in. While I never sailed a schooner or swung a machete (for this project), I did helicopter into the caldera of a volcano, travel by Soviet-era troop transport over roadless tracts of the Kamchatka Peninsula, and bushwhack into trailless areas of Yellowstone and Lassen National Parks to find hot spring basins untouched by tourists. Back in the lab, these adventures paid off when we found that each population was endemic to each geothermal region. *Sulfolobus* cannot successfully disperse across large distances. Although not visible under a microscope, the sequence differences between *Sulfolobus* from distant locations are in the same range as the differences between Darwin’s finches on the Galapagos islands and the species on the mainland, which are clearly discernable by their physical features.

Biogeographic distributions of microbial species have now been described in many environments that are not considered extreme, such as soil, human intestines, and freshwater lakes. Even environmentally similar locations that are separated by small distances, in some cases, appear to host unique microbial populations. If biogeographic patterns for microbes become the rule, microbial diversity is orders of magnitude greater than our already eye-popping recent estimates. Imagine the vastness of microbial diversity in which unique types are endemic to each centimeter of soil on Earth!

Beyond uncovering diversity of literally astronomical proportions, the biogeographic distribution of microbes opens a new window on the evolutionary processes that shape each one. In line with the theory of descent with modification, each microbial population can adapt to its unique local “island” habitat, allowing microbiologists to link an organism to its environment to uncover essential drivers of natural selection for different traits. Independent populations provide replicates in Nature’s experiment under different conditions. The challenge is to resolve the difference in conditions on a microbial scale. In addition, physically isolated populations are much more likely than globally distributed populations to acquire differences that result from
random chance events. Fragmented microbial populations are more prone to extinctions. This could change how we view microbial diversity. Instead of assuming that all variation is functionally important to a cell’s survival, geographic isolation allows variation to exist for no reason other than historical accident.

Already, what has been found by examining the evolutionary process in island microbial populations has surprised us. The system of isolated volcanic “islands” hosting unique but similar species of *Sulfolobus* allowed us to trace the independent evolutionary history of three populations from a shared common ancestor. Again, using high-resolution genome sequencing, we identified the elements of genomes that had changed since populations diverged.

Our initial analyses suggest that primary differences come not from natural selection by abiotic environmental variables between locations, but from interactions between microbes in each location, specifically between *Sulfolobus* and its viruses and other microbial parasites. Since viruses are not only predators, but also agents of gene transfer, moving DNA between often divergent cells, it appears that interactions between *Sulfolobus* cells and their viruses play a large part in defining the diversity and evolutionary history of *Sulfolobus*. This is something that is likely to distinguish the evolutionary process of microorganisms from their distant microbial cousins.

*Sulfolobus* populations may be disconnected from each other, but each one is tapped into a wealth of genetic potential essentially “housed” in viral storage. The factors that define the spread of genetic material through this viral conduit are still unknown; however, investigating these dynamics uncovered even more surprises. Recently, elegant work by many others in the field identified an adaptive immune system that plays an important role in the interactions between many microorganisms and their parasites in natural environments. Studying the evolution of this newly discovered immune system in *Sulfolobus* provides an even stronger lens into the evolutionary dynamics within microbial populations. Based on the mechanisms that have been established for this adaptive immune system, it appears this system evolves through a process that is more Lamarkian than Darwinian, i.e., adaptive changes within the genome of *Sulfolobus* occur not through random uncorrected mutation but in response to viral infection. The impact of these dynamics is only beginning to be understood. Watching evolutionary dynamics unfold in natural populations like those we have defined for *Sulfolobus* demonstrates the power of looking outside the lab at the evolutionary process happening all around us.

Despite the intrigue, adventure, and potential for discovery, it seems to me that microbial naturalism has not caught on as much as it should have in the 21st century. The study of microbial evolution lags behind that of macroorganisms, and it seems there are relatively few microbiologists choosing microbial naturalism. Historically, this was because we lacked the strong lens we have now with sequencing tools that have both revealed microbial diversity, and allowed us to develop new evolutionary models to understand it. Rather than exploring the distribution of diversity in the natural world, most microbiologists instead chose a very different approach to microbiology, using genetic and molecular biology in laboratory experiments to disassemble the inner workings of a few captive model microbes.

When I entered graduate school, I faced a tough decision between the two historically alienated conceptual frameworks of microbiology. I was tempted by the approaches of genetics and molecular biology, which solve complex puzzles in a way that is satisfying, elegant, and precise. I was most interested in microbial evolution, but letting Nature conduct the experiments and learning to interpret the results seemed so much more complex and diffuse, often needing theoretical modeling and statistics. Ultimately, I was swayed by the compelling questions of microbial evolution. I was fascinated not just by the mechanics of the cellular machinery, but by the prospect of learning how the diversity of machines came to be.

I still perceive the tension between these two approaches to microbiology that has recently been eloquently described by Carl Woese and Nigel Goldenfeld. However, more and more the power of a meaningful synthesis of the two approaches is being demonstrated. After 50 years of molecular biology, we know more about biology than Darwin could have dreamed. Integrating this knowledge to understand the distribution of diversity will be the basis for microbial naturalism, and will allow
microbiologists to develop a deeper understand-
ing of diversity than has ever been known. It is
clear that the new generation of microbial natu-
ralists who set out, as Darwin did, to explore the
distribution of natural diversity now wield not
only the strong lens of genomics but also the
power of molecular biology and genetics, which
will enable them to discover the fundamental
laws of evolution that apply to all of life on
Earth.

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SUGGESTED READING
Woese, C. R., and N. Goldenfeld. 2009. How the microbial world saved evolution from the Scylla of molecular biology and