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PREFACE

The year 2009 marked both the 200th anniversary of Darwin's birth and the 150th anniversary of the publication of his landmark book, *On the Origin of Species*. In August 2009, to celebrate these milestones, the American Academy of Microbiology convened a colloquium in the Galapagos Islands, where Darwin made some of his most crucial observations, to consider a new question: what would Darwin have made of the microbial world? The ability to sail to remote sites like the Galapagos, and access to specimens collected by himself and other avid naturalists, gave Darwin the information he needed to develop a conceptual framework for understanding life's visible diversity. Today, new discoveries and technical capabilities in microbiology are providing information that for the first time makes it possible to develop a conceptual framework for deepening our understanding of the diversity of the microbial world.

Darwin focused his attention on visible life forms, which actually make up only a small fraction of the living world—the invisible world of microorganisms was as yet largely unexplored in his time. Yet Darwin's theory has proven remarkably robust; despite some fundamental differences between microorganisms and the rest of the living world, the two lynchpins of Darwin's theory—descent with modification and natural selection—have proven as powerful in explaining microbial evolution as they have in explaining macrobial evolution. Since Darwin, the advent of Mendelian Genetics and the Modern Synthesis have provided a wealth of new tools to evolutionists; these tools are also of fundamental importance in the modern study of microbiology.

The scientists gathered at the colloquium considered two fundamental questions:

- Is the balance of evolutionary mechanisms, for example natural selection or drift, or individual and group selection, consistent among microbes and similar between microbes and macrobes?
- How are the mode and tempo of microbial evolution influenced by Earth's diversity of environments, and the changing global environment, and how are microbes themselves driving these changes?

The colloquium provided an opportunity for individuals with expertise in evolutionary biology, genetic engineering, mycology, virology, microbial ecology, and other fields to discuss these issues and review the areas in which research is needed to fill gaps in our understanding.
INTRODUCTION

Microbial evolution had been underway for at least 2 billion years before the emergence of the first eukaryotic cells, and multicellular organisms arose even later. Even now, most of the biodiversity of life on Earth is microbial. Many of the genes, molecular machines, regulatory, metabolic, and synthetic pathways found in all living organisms today evolved first in microorganisms. Understanding the early evolution of microorganisms will shed light on the origin of the machinery of life common to all organisms. The evolutionary process is ongoing in microorganisms, as it is in all life forms, and depends on genetic variation. Microorganisms have several ways to generate far more dramatic and rapid genetic variation than plants and animals, including genome reduction and horizontal gene transfer.

Determining how the earliest life forms evolved is complicated by the fact that many of the traits that microbiologists study have evolved over vast time scales and the fossil record for microbes is poor and usually lacks the relevant details. Thus, unraveling the earliest events in microbial evolution is very challenging. Microbiologists may compare the presence and absence of particular pathways, genes, and functions, and can compare the sequences of highly conserved genes, but without detailed understanding of the time scales over which these differences evolved—and without living representatives of the intermediate forms—these comparisons are inherently uncertain, and that uncertainty grows as we look farther and farther back in time. On the other hand, the opportunity to observe and study evolution in action—including experiments to test the effects of particular genetic and ecological processes on the rates and outcomes of evolution—provides an important complement to the comparative method, one that is especially powerful for studying microbial evolution.

It is becoming clear that the microbial world must be taken into account when studying the evolution of all other life forms. All plants and animals evolved in environments filled with billions of interdependent microorganisms and all macroorganisms have a co-evolving community of microorganisms living in and on their bodies. From the microorganisms that allow termites to digest wood to those that produce essential vitamins in the human gut, the importance of microbial communities in the health and development of all other organisms raises a rich set of questions for evolutionary biologists. In turn, selective pressures facing plants and animals—their physical environment, diet and interactions with other species—feed back to affect the evolution of their microbial partners. One need only consider the impact of antibiotics, which have both saved millions of lives and driven the spread of antibiotic resistance mechanisms throughout the microbial world, to begin to appreciate how important it is to understand the interplay between macroorganismal and microbial evolution. On a very practical level, understanding the mechanisms of microbial evolution will further our ability to develop more effective antibiotics and vaccines, predict disease outbreaks and changes in virulence, and harness microorganisms’ potential for rapid evolutionary change to create new products and processes.

Again and again, discoveries made in microbiology have proven to have universal biological significance, and microbes have served as model systems for many of the most important discoveries in science. The field of molecular biology continues to rely on microbial model systems, and microbial “tools”—for example, the use of
Microbial Evolution

plasmids and phages to clone genes from one organism to another—have found
many practical applications in the biotechnology sector. Just as microbes’ capacity
to transfer genes became an important tool for the biotechnology revolution, micro-
bacterial evolution can also be harnessed to generate new biological entities capable of
generating fuels from sunlight, producing biomaterials, remediating pollution, and
recycling wastes.

But only with a more detailed and systematic understanding of microbial evolution can
the manipulation of microbial communities be applied to complex problems. Whether
the goal is to use microbial communities to produce desired materials, remedy
environmental damage, or mitigate climate change, a comprehensive and predictive
understanding of how microbes evolve is critical to ensure success and safety.

The study of microbial evolution is also important because of the intimate and
complex relationship between microbes and the environment. Microorganisms
play a crucial role in the cycling of elements at a global scale, thereby profoundly
and directly affecting the environments of our planet, in which all of life evolves.
While microorganisms affect the environment, the environment in turn also gener-
ates evolutionary pressures on the microorganisms themselves. Understanding this
complex system, with multiple, interacting feedback loops, is as critically important
as it is stunningly difficult. Interactions among the atmosphere, the ocean, and
ecosystems with both complex microbial communities and changing sets of macro-
organisms, are as yet poorly understood.

Today, almost every ecosystem on the planet bears the imprint of human influence,
and these impacts extend to the microbial members of those ecosystems. In the
face of profound and ongoing changes to the planet, we have distressingly limited
abilities to predict the response of microorganisms even in the most common-
place, and seemingly simple, systems. What will be the impact of all anthropogenic
changes on the microbial communities that are major drivers of global biogeochem-
ic cycles? Are these cycles resilient to change? If so, to what extent does microbial
evolution play a role in this resilience? Greater understanding of microbial evolution
will be required to answer these questions with any confidence.
EXTRAORDINARY GENETIC DIVERSITY AND VARIABILITY

Microorganisms differ from macroorganisms in a few fundamental ways and some of these differences have profound effects on the mechanisms and tempo of microbial evolution. Much of the early study of evolution depended on comparing physical characteristics (or phenotypes) — beak shape, the shape and relationship of bones, seed and leaf shapes and sizes, etc. Studying the evolution of microorganisms, however, was comparatively difficult. There were a limited number of phenotypes — like shape and nutrient requirements — that could be easily studied. With the discovery of the structure of DNA, it became clear that an organism’s characteristics were related to underlying gene sequences (the organism’s genotype). This launched the challenge of unraveling the genotype-phenotype connection, with implications not only for the study of evolution, but also development, physiology, disease risk and biodiversity. Advances in molecular biology opened the door to studying evolution in microbes in new and powerful ways. Microorganisms employ an impressive array of mechanisms to generate genetic variation and this makes the study of their evolution complex and its implications profound — both for understanding microorganisms themselves and evolution in general.

Horizontal gene transfer

Ironically, while sexual reproduction superficially appears to provide greater opportunity for genetic variation than reproduction by binary fission, in fact, microorganisms display much greater genomic plasticity than organisms reproducing sexually. Sexual reproduction allows genetic mixing between just two individuals of the same species. In contrast, bacteria and archaea engage in horizontal gene transfer; they can share genetic material with distantly related organisms and not only pass that material on to their offspring, but also put it to use immediately. Lateral gene transfer between microorganisms generates variation in a way that is generally unavailable to macroorganisms. Freedom from the requirement to reproduce sexually is one explanation for the vast diversity of Bacteria, Archaea, phages and viruses as compared to that of eukaryotes. Lateral gene transfer can blur the boundaries between species, potentially a problem for those particular aspects of evolutionary theory that rely heavily on the concept of species. The prevalence of horizontal gene transfer also requires some refinement of the concept that evolution results in a tree-like pattern of inheritance. Relationships among the earliest microbial organisms may have looked more like a web than a tree, with the more familiar tree of life emerging later, although that issue remains unsettled. On a conceptual level, it is interesting to consider the relative evolutionary advantages of the genome plasticity enjoyed by microorganisms versus the more stable genomes that characterize eukaryotes. At a more detailed level, it is clear that horizontal gene transfer is widespread and frequent, but it is not random. The rules governing “who
shares what with whom, and when?" have yet to be worked out and the prevalence of lateral gene transfer in the environment is as yet unknown.

The importance of viruses as agents of evolution

Phages and viruses are responsible to a large extent for horizontal gene transfer, and the interactions among phage, Bacteria, and Archaea are a critically important, but relatively poorly understood, component of microbial evolution. A number of studies have revealed that in marine systems at least, viruses that infect bacteria, known as bacteriophage or simply phage, are remarkably numerous (see Suttle 2005 for review). Marine phages play important roles in the survival and evolution of marine microbes, from the level of the individual cell on up to the population and ecosystem levels. They are major agents of mortality, making them an important selective force and driver of local variation. By some estimates, phages kill up to one-third of marine bacteria every day, and it is possible that in some marine ecosystems they are the keystone predator, helping maintain population balances and regulating ecosystem structure by keeping fast-growing populations like Vibrios or Emiliania huxleyi under control. The ability of phage to ‘kill the winner’ in an evolutionary standoff can drive unexpected changes in the structure of microbial populations.

Phages have also driven the evolution of systems that enable bacteria to resist infection and there is evidence that phages play a role in modifying the photosynthetic ability of marine organisms as they transfer genes that are part of the photosynthetic apparatus. These genes allow the host to continue photosynthesis during phage infection, and suggest a potential role for phages in the evolution and transfer of photosynthetic genes.

Metagenomic data from the oceans show that phage genome integration into bacterial genomes is common and can drive variation within populations. It is important to note that evolutionary pressure cuts both ways in host-phage interactions. As obligate pathogens, phages themselves are under continuous selective pressure to be able to infect a host, and are forced to evolve continuously. To date, most of our understanding of the role of phage in microbial communities has been gained through the study of marine systems. Whether phages are more or less prevalent or important in other environments will require considerable study.

‘Pan-Genomes’

Taxonomists have yet to agree on the precise definition of a microbial “species” and current conventions for cataloging microbes remain inadequate. When multiple strains of some microbial species are sequenced, it turns out they share only about 60% of their genes, and the other 40% differ from strain to strain. The shared genes are known as the ‘core genome’ – the set of genes that are essential for that species survival. The rest of the genes are called the ‘dispensable genome’. Together, all of these genes make up what is called the species ‘pan-genome’ – the complete collection of genes found in that species. For some species, it appears that every additional strain sequenced contains additional novel genes, so the pan-genome may never be completely characterized. Other microbial species have a much larger core genome and many fewer genes in the dispensable genome.
Generally, the difference seems to correlate with the kind of environment in which the species live; species that live in highly variable environments are likely to have much larger dispensable genomes. Those that live in isolated and static environments, or that are part of a long-evolved partnership with a host (like many of the bacteria that live in insect guts) will have a far smaller dispensable genome. The evolutionary implications of all this genetic variability are profound. The capacity of a species to maintain a diverse pool of genetic resources in its various strains has clear evolutionary advantages. Maintaining a wide variety of genes in related strains allows the species to retain access to those genes without requiring each individual organism to carry an enormous genome. How is that diversity maintained? How variable is the dispensable genome over time and under different kinds of stress? Can parts of the dispensable genome become extinct, and what does that mean for the species’ long-term evolutionary success? To date, there have only been a handful of projects to sequence full genomes of many individuals from a variety of bacterial species; understanding the pattern and significance of pan-genomes will require many more such projects.

VAST TIME SCALE

Some of the apparent discrepancies between the evolution of microscopic and macroscopic life is due to differences in the temporal scales over which we compare evolutionary changes. In terms of the history of life on Earth, the adaptations discussed by Darwin arose in animals relatively recently. The various beak morphologies of Galapagos finches, for instance, evolved in response to the available food sources on the islands over the course of mere thousands of years.

In contrast, microorganisms have been evolving for billions of years, and many of the traits microbiologists study have evolved over vast time scales. Much of what we know about the earliest microbial evolution is based on a single gene – the SSU (short sub-unit) rRNA gene. Initially chosen by Carl Woese in the 1970s for his pioneering work using phylogenetics to understand the relatedness of bacterial species, the SSU rRNA gene has a number of characteristics that make it ideal for examining deep evolutionary history: it is found in every organism, it is highly conserved (the sequence is 50% shared between organisms as different as bacteria and mammals), and it does not undergo lateral transfer (so its history is reflective of the organism’s history, a statement that would not be true for a gene that had moved between species).

Woese’s phylogenetic trees of cloned rRNA genes from microbes then available in laboratory culture revealed, among other things, a previously unknown domain of life—the archaea—whose rRNA sequences were as different from bacterial rRNA as they were from eukaryotic rRNA. SSU RNA has been an invaluable tool in building a universal tree of life, a tree that extends back to the earliest life forms, reflecting the evolutionary connections among all life forms. However SSU rRNA can only reveal so much. Indeed, when one is using individual genes to infer the earliest events in evolution, one must accept that some information is irrevocably lost; some of the bases in the gene will have mutated more than once, or mutated back to their original sequence, changes that are phylogenetically invisible. Therefore it would be highly useful to construct similar trees using other highly conserved genes, but at present few genes have been sequenced in a wide enough variety of organisms to allow in-depth analysis. Phylogenetics has enormous potential as a tool for unraveling the ancient relatedness of life if such data sets could be assembled.
MULTIPLE LEVELS OF SELECTION?

Darwin recognized that there is some relation between “all organic beings”, an idea that allows for the traits of one species to impact the success of another, Symbiosis, which involves direct genome-genome interactions, is clearly both an important driver of the evolution of countless microorganisms and a potential constraint. More recently, the “super organism concept”—the recognition that all organisms are actually a composite of their own cells and their co-evolved microbiota, and that evolution and selection may act on this composite rather than on the individual — has raised interesting conceptual questions. Studying microbial evolution at levels ranging from “selfish genes” to complex, interconnected species and niches will initially drive the development of models that describe evolution in the most basic ecosystems, but eventually what is learned about the drivers of evolution in simple microbial communities will have implications for evolutionary theory more broadly.

ADAPTATION VS EVOLUTION

A complex issue in the study of microbial evolution is unraveling the process of evolution from that of adaptation. In many cases, microbes have the capacity to adapt to various environmental changes by changing gene expression or community composition as opposed to having to evolve entirely new capabilities.

From experience with certain types of environmental changes, we have gained a general idea of how certain microbes will respond. In the case of oil spills, for example, we know to expect a reduction in diversity and a local increase in the abundance of organisms that mediate hydrocarbon metabolism. Over longer terms horizontal gene transfer can result in the distribution of genes that facilitate hydrocarbon breakdown and the creation and assembly of pathways that allow microbes to utilize hydrocarbons.

In the oceans, research has revealed clear evidence of physiological adaptation at the microbial community level, but we have yet to find evidence of innovation in an existing population as a result of changing conditions. Apparently, changing conditions generally enrich for new populations that are suited to the new niche, rather than for improvements or innovation in the extant populations. Research has revealed evidence of gene flow, but there is less evidence of evolutionary adaptation in response to specific pressures. In complex environmental communities – marine or terrestrial – searching for evidence of evolutionary change has rarely been attempted and is currently intractable. Communities of deeply buried, isolated sediments and other extreme environments may be more manageable for examining ecology and evolution in concert.

There are many examples in which microbes are undeniably evolving in the face of challenges created by humans. Xenobiotics, for example, are substances that come from biological sources, but make their way into environments where they are not normally present. For example, high concentrations of antibiotics are not normally found in humans; this is a recent, pharmacological innovation and has had unforeseen consequences for human health and the evolution of infectious agents. Human hormones and many pharmaceutical compounds that enter the water supply are also xenobiotics. Microbes have already evolved new catabolic pathways for the
degradation of xenobiotics and synthesized compounds like herbicides or industrial pollutants. These pathways seem to be assembled by horizontal gene transfer of different genes from different organisms onto mobile elements such as plasmids, which are then shared and distributed among organisms in the environment. Today, we can find similar plasmids in widely distributed locations, indicating that these capabilities have evolved rapidly and the innovations have been geographically distributed very quickly.

The degree to which microbes will evolve new capabilities versus adapting to new conditions using the capabilities already in place remains an open question. But whether they adjust by evolution or adaptation, microbes will continue to affect global environmental conditions in ways that may or may not be favorable from a human point of view.
WHY UNDERSTANDING HOW MICROBES EVOLVE IS IMPORTANT

EXPERIMENTAL TRACTABILITY

Again and again, discoveries made in microbiology have proven to have universal biological significance and microbes have served as model systems for some of the most important discoveries in biology. Many discoveries that were made by investigating microbes have expanded our understanding of evolution in general. A partial list includes:

- Microbes were the first organisms in which scientists showed that DNA is the hereditary material. (Avery, et al 1944, Hershey and Chase, 1952),
- Microbes represented the bulk of the data that led to the three domain tree of life (Woese. 1990),
- Scientists used microbes to demonstrate that evolution proceeds as a result of selection upon pre-existing mutants that arise spontaneously and at random (Luria and Delbruck, 1943), and
- Observational studies and experiments have demonstrated the importance of host-pathogen arms races as a driver of evolution.

Just as microbes served as highly flexible model systems for molecular biology experimentation, microbes and microbial consortia can also be used for experiments on evolution. Microbes in experimental systems and in real-world situations like infectious disease offer the opportunity to test and observe microbial evolution in action.

With larger organisms, until the advent of molecular biology, biologists were often forced to make inferences about evolution from observation. Genetic study has vastly enriched our understanding of the mechanisms of evolution, but the ability to carry out evolutionary experiments is still limited in long-lived organisms with large and complex genomes. By contrast, experimental evolution with microorganisms offers a rich alternative by providing a testable system based on hypothesis, experiment, and outcome. Control over selective pressures represents another advantage. Moreover, in microbiology it is possible to save and see the “mistakes” or evolutionary dead ends in an experiment; we don’t necessarily “lose the losers” in an evolutionary microbiology experiment.

MICROBIAL EVOLUTION CAN BE USED TO SOLVE PROBLEMS

Microbial evolution can be harnessed as a tool to generate new biological entities capable of generating fuels from sunlight, producing bio-based materials, remediating pollution, and recycling wastes. These approaches show great promise for contributing to a transition to sustainable human societies. ‘Directed’ evolution—forward
engineering—allows us to circumvent our profound ignorance of how a DNA sequence encodes function and represents a highly effective approach to generating useful new biological molecules and metabolic pathways and to modifying whole organisms for human needs. Although humans have been ‘breeding’ microbes for many years, with the genetic tools now available we can exert more control over the process, for example, by controlling mutation rates and directing mutations to specific genes. We can also extract detailed information on the changes that occur during evolution.

Only with a more detailed and systematic understanding of microbial evolution can the manipulation of microbial communities be applied to more complex problems. Whether the goal is to use microbial communities to produce desired materials, remedy environmental damage, or mitigate climate change, a comprehensive and predictive understanding of how microbial communities respond to change and stress is critical. There are many fundamental questions to which we have dangerously incomplete answers. For example, we need to know whether microbes increase their rate of genetic change in response to stress. Do they mutate more often or more quickly? Do they exchange plasmids more often? Are they more susceptible to horizontal gene transfer via phages or other mechanisms? If the answer to those questions is yes, that suggests that microbes may be able to evolve more quickly when environmental conditions are in flux. Understanding such responses to stress will be helpful in efforts to drive microbial evolution to meet practical goals.

Even in the absence of perfect understanding of the ‘rules’ of microbial evolution, it has proven possible to modify some microbial systems to achieve a favorable outcome. For example:

- The ability of microbial communities to remediate oil spills can be enhanced by providing nutrients and co-metabolites like acetate that help microbes break down chlorinated solvents.

- Land use practices like the timing and amount of fertilization or cultivation of riparian strips can be adjusted to minimize denitrification and leaching, thereby avoiding adverse consequences of nutrient export into coastal and freshwater systems.

Many capabilities seem to be latent in microbial communities, and it seems that every new estimate of microbial diversity soon proves too low. This diversity is amplified by the microbial capacity for gene transfer both within and between species, an ability that allows future populations to share genes, mobilize a direct response to selective pressures and rapidly adapt to both natural and human caused global environmental shifts. Due to their large population sizes and large ranges, microbes represent an enormous natural reservoir of genotypic diversity that is available to undergo natural selection.

MICROBIAL EVOLUTION AND CLIMATE CHANGE ARE INTERTWINED

We are currently struggling to understand how human actions affect global processes, but it is worth remembering that from their beginning, microorganisms have had a profound effect on conditions on Earth. Through the evolution
of oxygenic photosynthesis and continuous cycling of carbon, nitrogen and other elements, microbes created and continue to sustain the conditions we enjoy today. This is true at all scales and in all environments, on land, in the ocean, and under the Earth’s surface. Microbes directly modulate the amount of bioavailable nitrogen, carbon, phosphorous, sulfur, and many important metals. The ability to transform nitrogen gas from the atmosphere into a form that organisms use to make critical biomolecules like DNA and proteins (the process of nitrogen fixation), evolved in microbes very early in the history of life and microbial nitrogen fixation continues to serve as a fundamental link in the nitrogen cycle. Microbial chemical cycling also plays a role in the strength of the planet’s greenhouse effect. Denitrifiers and nitrifiers can generate nitric oxide and nitrous oxide, for example, which are powerful greenhouse gases that have 280-320 times more potential for warming than carbon dioxide. Also, phytoplankton produce dimethylsulfide and dimethylsulfoniopropionate, and the cycling of these compounds produces sulfur gasses that impact cloud formation, and, hence, the water cycle and the global albedo (reflectivity). Archaeal methane production is the dominant natural source of methane, a gas that is over 20 times more powerful a greenhouse gas than carbon dioxide.

Human activities are changing the environment in which microbes evolve, creating innumerable new evolutionary pressures. The feedback loop between microbes and the environment now includes another driver. How microbes will react to this new set of variables is critically important, but difficult to predict. The time scale of human-produced changes affects whether they will evoke evolutionary changes or simply adaptation, for example, in microbial community population structure. Over the long term, there is no doubt that if microbial ecosystems are disturbed, their “evolutionary trajectories” will be affected in ways that we cannot predict.

Many of the human-induced changes to the microbial biosphere are felt in the oceans, which represent the largest biome on Earth. Ocean ecosystems are on the receiving end of a great deal of runoff and of other less direct human influences. Chemical runoff from agriculture and industry, including pesticides, herbicides, and metals, stress ocean ecosystems and may increase the availability of metal co-factors like tungsten and molybdenum, which are required for particular proteins and may have been limiting certain biogeochemical processes over time. Increases in temperature, dissolved carbon dioxide, and changes in the water cycle can also be expected in the oceans as the climate continues to change.

Any of these human-caused impacts could have effects on microbial evolution; in combination, the accumulation of stressors may dramatically change the selective pressures faced by microbes in the sea and on land. What will be the impact of all these changes on the microbial communities that are major drivers of global biogeochemical cycles? Are these cycles resilient to change? If so, to what extent does evolution play a role in this resilience? Resilience to change depends on the magnitude of the environmental perturbation, the temporal and spatial extent of the perturbation, and the time required for recovery. After small perturbations, communities recover quickly and internal changes in the community are sufficient for recovery. For more severe perturbations, recovery likely requires dispersal from undisturbed ecosystems, and in the case of severe, widespread, prolonged perturbation, recovery is likely to involve evolutionary change.
RESEARCH NEEDS

What is needed to improve our understanding of microbial evolution enough to permit prediction of the consequences of climate change, antibiotic use, crop fertilization, or other human perturbations of microbial communities? The challenge is great, but the following areas have been identified as critical to improving our ability to answer these important questions.

EVOLUTIONARY MECHANISMS AT WORK IN MICROBIAL POPULATIONS

Fundamental understanding of evolution in microorganisms lags behind that of multicellular organisms. The tools are becoming available to study the vast expanse of time in Earth’s history when evolution was entirely microbial and that saw the evolution of the fundamental pathways shared by all life forms. More effort and resources should be directed to fundamental microbiology research to help us understand the mechanisms through which microbes evolve, especially in response to different environmental changes and challenges. Directing funding solely to applied problems does not fill in these basic gaps in our knowledge in an effective manner. Basic research on microbial evolution, however, has the potential to contribute across sectors and address applied problems in many fields, thereby leading to new approaches to treating disease, raising agricultural productivity, monitoring and addressing climate change, and producing clean energy.

Only by understanding evolutionary mechanisms can we hope to understand and predict how the evolutionary trajectory of genes, populations, or communities will respond to environmental changes like the impact of introduced organisms (including genetically modified organisms, organisms used in biofuels, and exotic species) in the environment. Also, since all animals and plants have an associated microbial community, we need research that will enable us to understand how those organisms’ microbiomes affect their ability to invade new territories or resist invaders, and the impact of extinctions on microbial diversity.

In particular, we need to understand processes that drive evolutionary diversification. Generating sequence data sets from a wide diversity of organisms for many highly conserved genes beyond rRNA will reveal a great deal about early evolutionary events. Distinguishing evolution from adaptation can be challenging at the microbial level. Microbial communities can respond to a change in the environment in different ways, including community level changes (e.g., ecological replacement, in which one organism replaces another), physiological adaptations (phenotypic change in response to the environment), and evolution through natural selection, but the relative importance of these different responses in various environments or under various circumstances is not known.
When making his case for how natural selection shapes evolution, Darwin pointed to the large phenotypic changes that could be achieved in relatively few generations of artificial selection. The same strategy applied to microbes, experimental evolution, has allowed us to observe how rapidly microbes and their biological components (proteins, DNA, RNA) adapt in the face of defined selection pressures and, with the tools now available to sequence genes and even whole genomes, even delineate the precise mechanisms by which adaptation occurs. These studies will continue to yield insights into evolution at a molecular level, and may also provide insight into how microbes have responded in the past and will respond in the future to specific changes in their natural environments. For example, experimental evolution studies have already predicted specific mutations conferring antibiotic resistance. Experimental evolution helps us to understand mechanisms of evolution and to measure and constrain evolutionarily relevant parameters.

At the same time, microbial ecologists increasingly can investigate the population structure of real microbial communities in the field. But they traditionally have not addressed the question of evolution in the complex communities they study. Conversely, evolutionary microbiologists in the laboratory have focused on the mechanisms of evolution in experimental microcosms, but haven’t expanded their findings to complex natural systems. Both communities will gain greatly by collaborating. Microbiologists need to bring these two paradigms together by linking comparative studies of microbial communities in the environment with experimental work in the lab. We need to study systems from the “top down”, by observation, and “bottom up”, through experimentation. Some of the priorities for these collaborative studies include:

- Linking population structure with concepts of microbial fitness based on what has been learned in experimental systems,
- Seeking environmental confirmation of the lessons about fitness that microbial evolutionists are learning in the lab,
- Taking biome mapping to the next level and interpreting the population structures that we see based on what has been learned in past decades in experimental evolution.

Most of what we know about microbial evolution has come from the study of pure cultures of organisms like *E. coli* and *Salmonella*. It is now time to tackle questions of evolution in mixed communities that better reflect the environments in which microorganisms actually evolve. Researchers need to employ tractable, simple systems with small groups of microorganisms in which the pattern and consequences of genetic and phenotypic changes can be manipulated and tracked. Some possibilities include communities from simple extreme environments, like acid mine drainage, or invertebrate gut communities.
The living cells harbored in deeply buried marine sediments (>1 meter to several kilometers in depth), often referred to as the “deep biosphere”, represent another possible natural microcosm of both population-level adaptation and cellular evolution. Studies of sediment cores below one meter have detected population-level enrichment of a very specific population of microbes that are not observed in any other habitat – it is a distinct biome.

The deep biosphere may also contain a sort of “microbial Galapagos” – evolutionary islands where isolation and selective pressures have resulted in speciation. If ocean water can be considered a gel to microorganisms, the deep sediments can be considered a solid. Because these sediments are impermeable, the microbial habitat is marked by increasing isolation and individual cells in the deep biosphere represent time capsules of evolution. Some of these microbes have been effectively isolated for tens of millions of years.

**LINKING MICROBES TO CLIMATE**

Global climate change will have profound impacts on all ecosystems, but we have little understanding of what those impacts will be, especially at the microbial level. The effect of temperature changes on microbial systems is poorly understood, so we cannot predict, for example, the impacts of warming in northern latitudes on biogeochemical cycles or the effect of warmer oceans on primary productivity. Global climate change will also alter the ranges and geographical distribution of insects and other disease vectors and, consequently, the distribution patterns of diseases, with unknown impacts on agriculture, human populations, and ecosystems.

Other research priorities with respect to microbes and climate include:

- Determining how decomposers will respond to the increases in productivity and carbon dioxide consumption that are anticipated as average temperatures climb,
- Exploring the effects of falling pH on productivity in the oceans,
- Distinguishing between cause and effect with respect to microbes and climate to determine whether microbes are causing certain changes or “buffering” the system from faster change, and,
- Exploring the genomics and population structure of microbial systems to determine whether or not there are genetic “backup systems”. In other words, if one species that performs some key function is lost, are there other microbes to perform that function in its stead?

**UNDERTAKING LARGE ENVIRONMENTAL STUDIES**

To understand how microbes will evolve in response to human impacts like carbon emissions and nutrient run-off, microbiology needs a baseline of information about current genetic diversity and the natural history of populations and communities in different habitats. Large-scale space and time surveys of both living and non-living factors in a range of different communities are needed to fill these gaps in our knowledge base.
PREDICTING PHENOTYPE FROM GENOTYPE

Darwin was able to observe that different beak shapes among the Galapagos finches had adaptive significance—a critical piece of evidence linking fitness and natural selection to evolution over time. With the advent of molecular biology, linking characteristics like beak shape (phenotype) to genetic variation (genotype) became feasible, which fueled rapid progress in understanding the mechanisms of evolution. The genotype-phenotype connection is by no means solved for macroorganisms, but the challenge is vastly greater in microorganisms. The function of most microbial genes is unknown—even in well-characterized organisms—and metagenomic studies reveal more and more genetic sequences that encode unknown proteins of unknown function. Thus much of the incredible wealth of genomic sequence data available today is untapped. When a protein’s function is unknown, it is difficult to discern the adaptive or ecological significance of variations in that protein within a population.

Solving the genotype-phenotype question is critical. We need to become better able to make predictions about the structure and function of proteins from DNA sequence data. Progress in this area will not only improve our understanding of microbial evolution. Historically, study of molecular pathways in model organisms like E. coli and yeast has contributed enormously to our understanding of plant, animal, and human physiology and disease. Because so many key metabolic and regulatory pathways are evolutionarily conserved across life’s kingdoms, the same will be true of tackling the genotype-phenotype challenge at the microbial level.

Bioinformatics is a crucial tool for identifying patterns of genetic variation and recognizing genes that are under positive selection. Computational biology can vastly expand our ability to detect these genes and infer their biochemical, physiological, and evolutionary significance.

DETERMINING THE RATES AND CONSTRAINTS ON MICROBIAL EVOLUTION

How quickly can microbes evolve? And what constrains that rate? Researchers need to determine the temporal scales over which different kinds of evolutionary change occur in microbial populations and identify the factors that affect the rate of evolution. We also need tools, methods and conceptual frameworks to measure such evolutionary parameters as:

- mutation rate,
- recombination rate,
- environmental growth rates, and
- effective population sizes.
UNDERSTANDING THE EXTENT AND SIGNIFICANCE OF HORIZONTAL GENE TRANSFER

Horizontal gene transfer is a—perhaps the—major source of genetic variation in microorganisms but our understanding of the ‘rules’ governing which genes are shared among which organisms, and under what conditions, is rudimentary. We need to be able to quantify the relative contribution of horizontal gene transfer from related and unrelated organisms in a given strain and to determine the relative contributions of different methods of gene exchange (transduction, transformation, conjugation) to its genome. Research is needed to explore how the importance of these mechanisms changes between organisms and environments and what implications these mechanisms have for the rates and types of evolutionary changes that occur in genomes and populations. Are there underlying principles governing the structure of pan-genomes, and do these affect organisms’ evolutionary history and potential?

EDUCATION AND TRAINING

Deeper understanding of microbial evolution will have implications across biology because so many of life’s most basic systems and processes evolved first in microbes. Therefore, educational outreach is absolutely critical. Microbiologists have an obligation not only to train the next generation of students to be multidisciplinary but also to recognize, communicate, and incorporate into education the connections between microbiology and the rest of biology and other sciences. The general public and policy makers also need to be informed of work in microbial evolution, and especially of the many potential practical outcomes.

Students and researchers need access to more opportunities for interdisciplinary training and research, for example, integrating evolutionary microbiology and microbial physiology to facilitate a better understanding of the adaptive significance of genomic variations between different organisms.

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CONCLUSION

Evolution has a long history, but it is also an ongoing process. The Earth today is increasingly dominated by human activities, a reality that has profound consequences for global climate, ocean and land management, and other ecological factors that may, in turn, lead to important evolutionary shifts in the microbial world. Human activities are radically changing the Earth’s ecosystems, driving evolution with new selective pressures. What will happen? Can we promote desirable outcomes, and curtail or prevent undesirable outcomes?

Only by grasping the realities of microbial evolution past and present – the drivers, the limitations, and the mechanisms – can we make accurate predictions about future impacts of this global experiment on oceans and terrestrial systems, biogeochemical cycles of the elements, and climate. Participants in the colloquium agreed that research on the areas of basic and applied research described above can contribute to a new synthesis in evolutionary science – one in which microorganisms are fully integrated, and their roles in evolutionary history, the evolution of other life forms, and the state of the Earth’s oceans and atmosphere are fully a part of ongoing discourse on evolution and its mechanisms.