PERSPECTIVES

ESSAY

Are biologists in 'future shock'? Symbiosis integrates biology across domains

Margaret McFall-Ngai

Abstract | The study of symbiosis is quintessential systems biology. It integrates not only all levels of biological analysis — from molecular to ecological — but also the study of the interplay between organisms in the three domains of life. The development of this field is still in its early stages, but so far, the findings promise to revolutionize the way we view the biotic world. This Essay outlines some of the challenges facing the field and the implications of its development for all of biology.

At this juncture, biologists cannot be blamed for finding themselves in a kind of 'future shock', the psychological state that was described by Alvin Toffler[1] in 1970 as "too much change in too short a time." Sequencing projects are producing information at prodigious rates and this information is dramatically altering our perceptions of the microbial world. Our view of animal and plant symbioses with microorganisms has been particularly susceptible to change (FIG. 1). Historically, biologists have mainly concentrated on the study of the major macrobiotic groups (animals, plants and fungi) as individuals, characterizing their form and function (or phenotype) as a derivative of their own genotype alone. The exception to this trend lies in the focus of microbiologists on microorganisms as agents that induce pathogenesis. How will new perspectives on microorganisms as evolutionary partners change the way that we think about biological systems? First, let us consider why we, as biologists, think the way we do about these systems.

The history of biology and symbiosis

On 17 September 1683, Antonie van Leeuwenhoek's famous letter to the Royal Society about his observations on the microorganisms associated with his own teeth was published. In viewing these microbial communities, van Leeuwenhoek not only made the first recorded observations of living bacteria, but was also the first individual to observe a microbial symbiosis. It would be nearly 300 years before the concepts and molecular sequencing methods to define the diversity and reproducibility of these assemblages would become available, largely through the pioneering efforts of Carl Woese[2]. However, it is only within the past decade that these methods have been refined to the point at which characterizing the constituents of complex microbial communities can be accomplished in a time-effective and cost-efficient manner. Thus, the type of complex microbial communities living on or in animals (and plants) that van Leeuwenhoek observed and reported to the Royal Society in the seventeenth century have, until recently, been largely unstudied (TIMELINE).

In the intervening years between van Leeuwenhoek and Woese, microbiologists focused most of their efforts on a small subset of bacteria — those that could be cultured in a laboratory setting. Beginning in the mid-to-late 1800s, individuals such as Pasteur, Koch and Winogradsky began to 'tame' microorganisms, isolating them from their natural habitats and growing them under controlled conditions. Microbiologists applied this newfound facility to pathogenic and environmental microbiology, viewing bacteria either as the cause of disease or as agents responsible for recycling (as decomposers or crucial drivers of geochemical cycles, for example). With these emphases in microbiology firmly established, other branches of biology either developed directly as outgrowths of these specific areas or developed without consideration of the microbial world. For example, the field of host responses to the presence of microorganisms has largely been developed within the context of pathogenic microorganisms as the discipline of immunology and, more recently, cellular microbiology.

An obvious example of ignoring microorganisms can be found in the field of mammalian digestive physiology. Despite the fact that it has long been known that microorganisms are present in abundance in the gut, until recently they have all been considered 'commensals', that is, 'eating at the same table' but having no effect on the host. In terms of animal nutrition, almost all of the introductory biology or basic physiology textbooks that are currently on the market only mention microorganisms in the context of situations in which they provide unusual capabilities (for example, in the ruminants). Yet recent studies of gut microorganisms have demonstrated that these microbial communities are highly complex and are likely to have a profound effect on the digestive physiology of all mammalian species[3,4]. This ignorance is not a minor oversight; in effect, there is a whole

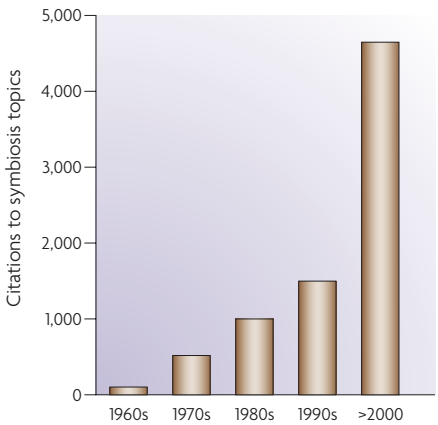
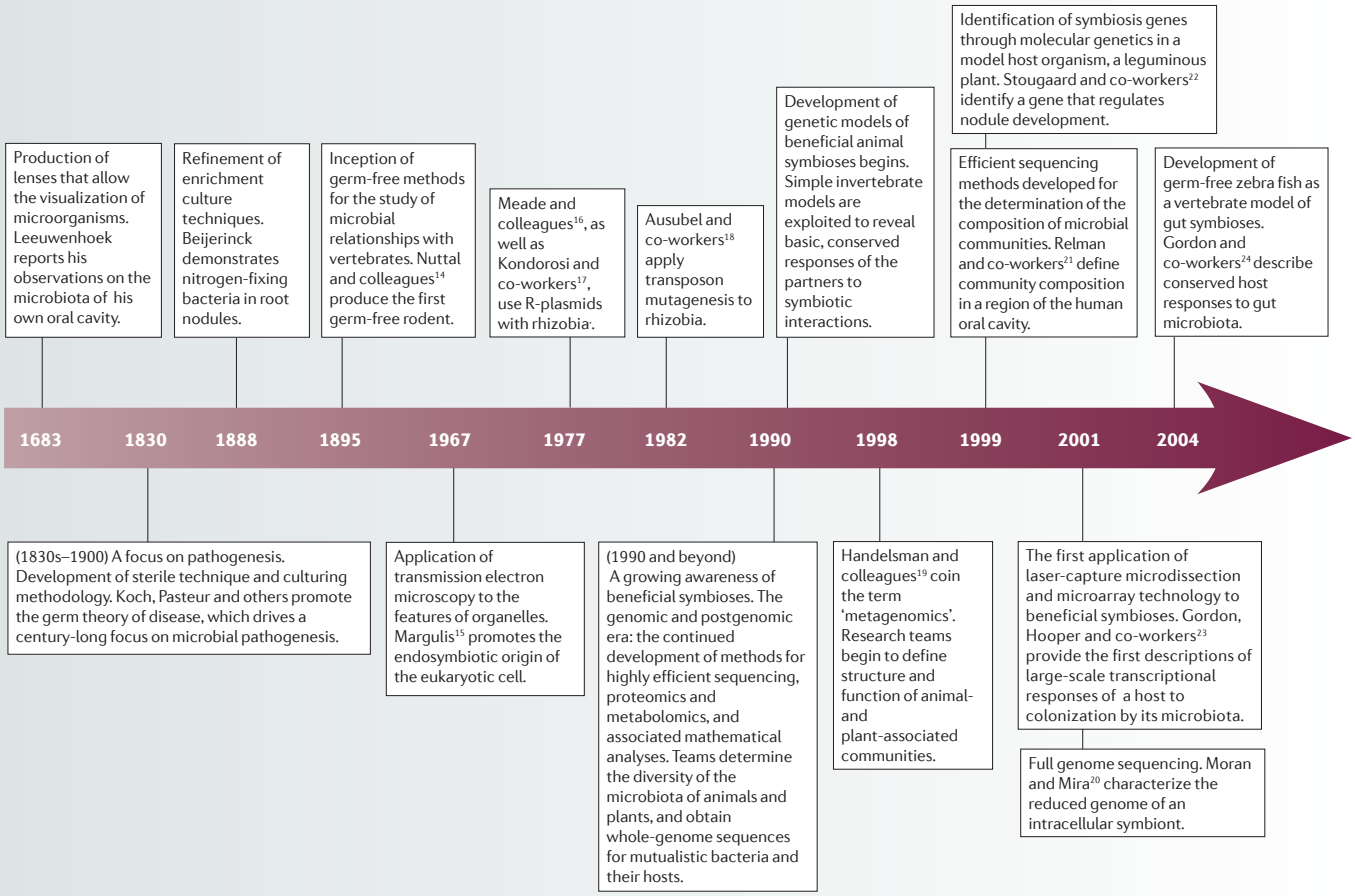


Figure 1 | **Growth of the symbiosis field.** This figure illustrates the rapid growth in the field of symbiosis over the past 40 years, as measured by citations to papers mentioning symbiosis in the title. Data derived from Pubmed.

Timeline | **Milestones in symbiosis**



'organ' — the gut microbiome — that is yet to be described. There are therefore two major components of the human body — the brain and the microbiome — that remain largely mysterious, with the essence of their functional complexity being poorly understood compared with that of other organs, such as the heart or kidney.

If beneficial symbioses have not yet been recognized as central in biology, where do they fit into the historical development of our understanding of animal and plant associations with microorganisms? Although 'symbiosis' encompasses mutualistic, commensal and pathogenic relationships, as a term it has principally been associated with mutualistic associations. The study of symbioses has focused mostly on obvious or dramatic, ecologically or economically compelling examples (see other articles in this Focus issue on Symbiosis), including the associations that are present in the cow rumen and termite hindgut, nitrogen-fixing root nodules, mycorrhizal–root systems, insect bacteriomes, fish and squid light organs, marine–invertebrate chemoautotrophic tissues and zooxanthellae-hosting cnidarian epithelia. These symbioses are fascinating in their own right, but because they are often simple, involving just a few species, they can also take on a new role: that of model systems[5]. As such, they promise to provide insights into evolutionarily conserved features of both host and symbiont biology.

The good news and the bad news

We now have the technology to begin to understand what van Leeuwenhoek saw under his microscope. The good news is that advances in molecular technology have been the key to opening the door to the detailed analysis of symbiotic partnerships. The bad news is that these advances have also revealed the scale of the task ahead. Obtaining an accurate picture of the composition of symbiotic microbial communities presents an unusually difficult challenge. At this point, we are still in the exploratory phase and are developing approaches to ask questions such as: what are the patterns of the associations of microorganisms with animals and plants; how profoundly have these alliances affected the partners' evolution; and how do symbioses affect other aspects of the partners' biology?

Most initial studies were driven by biomedicine and began by exploring these questions in depth in humans and model mammalian species. Like the oceanographic expedition of the HMS Challenger of 1869, which was the first attempt to sample and enumerate the fauna of the deep sea, we have encountered greater complexity than was expected. In the examination of both deep-sea and human microbial communities, the initial studies provided some useful points of reference and revealed the scale of the challenge. To this day, biologists continue to refine our understanding of deep-sea habitats, developing the ever-more sophisticated technologies that are required for the task. It is likely that characterizing the human microbiota will demand a similar commitment but, with a concerted effort, such studies should proceed at great pace. The successes of our first forays have laid the groundwork for the

design of more sophisticated and ambitious studies, such as the Human Microbiome Project (see Further information). However, the sequencing efforts have revealed that the scale of microbial diversity is greater than we could have imagined, and have demonstrated that the lines between species and strains of bacteria can be difficult to define. The development of appropriate methods to allow these problems to be tackled is one of the most vibrant areas of microbiology[6].

In addition to the difficulties in knowing who the 'players' are in a symbiosis, the basic nature of host-associated microbial communities presents some specific challenges. In contrast to habitats such as the deep sea, where emigration and immigration are rare events, the microbial communities that are associated with the mucosal surfaces of animals can often be affected by interactions with other microorganisms coming in from the environment. For example, the community of gut microorganisms would be expected to interact with non-partner phylotypes that enter the gut on food. These visitors will have varying residence times and different effects on the activities of the system, and will also vary in importance, depending on the host species involved. In essence, they introduce a prominent stochastic component and render the determination of the co-evolved, core members of the community difficult. Acknowledgement of the need to differentiate between the co-evolved partners and the 'tourist' microorganisms is crucial for the development of well-framed research questions. Biologists cannot just go out, choose an animal and describe everything in its gut without considering the internal and external forces shaping its microbial community. Thus, studies of these complex systems are fraught with pitfalls, and huge resources might be used with little to show for the effort if great care is not taken in experimental design.

The impact of our increased awareness

Our ability to characterize the microbial communities that are associated with animals and plants is likely to have a wide-ranging influence on biology. For example, recent studies of the human metabolome have indicated that much of the metabolic signature that is detected in bodily fluids (blood, sweat and urine) is actually derived from the associated microbial communities, particularly those that are present in the gut[3,4,7]. For example, in humans, as nutrients are brought to cells and tissues by the circulatory system, so are the metabolic products from our microbial symbionts. Some of these molecules could be the result of secondary metabolism, such as the breakdown products of drugs and antibiotics, and recent studies have suggested that the differences in activities of the microbiota between individuals could explain the highly individualized responses of humans to pharmaceuticals[3].

> "We now have the technology to begin to understand what van Leeuwenhoek saw under his microscope. The good news is that advances in molecular technology have been the key to opening the door to the detailed analysis of symbiotic partnerships. The bad news is that these advances have also revealed the scale of the task ahead."

Looking at the bigger picture, the physiology of each and every cell that is served by the circulatory system has been shaped through the co-evolution of the host and its microbial symbionts. How has this exchange of metabolic products exerted evolutionary selection pressure on the biology of mammals, other vertebrates and invertebrates? Is it possible that animal–microorganism alliances have been the basis of some of the major milestones in animal evolution? Animal biologists have long recognized that symbiosis was important for the evolution of certain groups, such as the ruminants, corals and blood-sucking and phloem-feeding insects, but perhaps we have missed some profound examples because of our ignorance of the microbiota. For example, is it possible that endothermy arose in birds and mammals as a mechanism by which to manage the metabolism of their large symbiont populations? Similarly, is it possible that the patterns of form and function of animal symbioses (for example, the number of partners or whether the habitat is intracellular or extracellular) have had profound influences on the evolution of the immune system, the principal response element to the microbial world?

One of the biggest challenges ahead will be to shift our thinking about pathogenesis. First, microbial symbionts seem to have been recruited from bacterial genera and species that are pre-adapted to living in, or on, animal tissues as a general ecological niche. Although it is not clear for any genus whether the initial association evolved as a beneficial or a pathogenic one, it is interesting to note that almost all bacterial pathogens of humans have congeners that are part of the normal microbiota[8]. Second, the same genetic determinants, such as colonization genes or type III secretion systems[9,10], and effector molecules, including toxins, are found in both beneficial and pathogenic associations. The important difference between the biological outcomes of the expression of these elements seems to be the level of expression and where the genes are expressed in the host[11]. Finally, the conversation between the partners in a beneficial relationship is complex and multi-layered; interestingly, unlike the situation for pathogens, the acquisition of one or a few genes (for example, a pathogenesis island or even a single toxin gene) does not generally allow a non-beneficial microorganism to become a successful symbiont. Clearly, a better understanding of how, and in what context, colonization factors initially evolved and how they have been recruited to bring about different fitness outcomes is of fundamental importance.

Incorporating an appreciation of the impact of the alliances between animals and microorganisms into our thinking might also provide insights into certain environmental problems and conservation issues. For example, when an animal or plant becomes extinct, it is likely that some subset of the microbial world — the co-evolved partners of that multicellular organism — will also become extinct[12]. Do captive-breeding programmes provide the correct environment for the normal transfer of the native microbiota between generations? Likewise, invasive species are likely to bring new microorganisms into the habitat they are colonizing, which could have trickle-down effects on the native microbiota. Is the ability of a species to be a successful invader dependent on its ability to bring along its native microbiota, and if so, might that be a target by which to control the process? How will global warming influence the alliances of animals, particularly ectotherms, with their partners? The possibilities are endless, but such considerations might be crucial to our understanding of how animals and plants relate to their environment.

What's next for symbiosis?

Now that the importance of microbial symbioses is increasingly being recognized as a major theme in biology, how do we reshape our view of life sciences to incorporate these ideas? Perhaps the largest obstacle to the integration of microbial symbiosis stems from the culture gaps among the different disciplines of biology, such as microbiology, biomedicine, and animal and plant biology: the infrastructure as it stands would not foster this process. Academic departments, federal agencies and scientific societies are structured in such a way as to frustrate opportunities for productive interactions. Another impediment is the difference in the trajectory of some of these areas. Since the milestone of Woese's discoveries, the scope of microbiology has broadened. There is a strong balance and dynamic interplay between the microbiologists who study different areas of the hierarchy of life, and between those who work at a molecular level with those who work at an ecological level. Spanning these levels might be more comfortable for microbiologists than other life scientists, as the hierarchy of life collapses in a microorganism: their ecology is, in essence, their biochemistry and molecular biology. The emergent properties across the hierarchy, so striking to biologists who study multicellular organisms, are blurred for a microbiologist and this renders the pull between reductionistic and synthetic approaches less pronounced. By contrast, animal and plant biologists often find themselves in departments where cell and molecular biology are administratively separated from departments of ecology and evolutionary biology. Although all of these impediments exist, for the impact of symbiosis on biology to be realized, other disciplines must be incorporated, such as the example of digestive physiology mentioned above.

In summary, we truly are in a golden age of microbiology[13], and the importance of symbiosis has now been established. To achieve the full potential of this field, however, a vast array of technical and cultural hurdles must be overcome. Luckily, such challenges in science are what render research such an exciting arena.

Margaret McFall-Ngai is at the Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, Wisconsin 53706, USA.
e-mail: mjmcfallngai@wisc.edu

doi:10.1038/nrmicro1982

1. Toffler, A. *Future Shock* (Bantam Books, New York, 1970).
2. Woese, C. R. & Fox, G. E. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc. Natl Acad. Sci. USA* **74**, 5088–5090 (1977).
3. Nicholson, J. K., Holmes, E. & Wilson, I. D. Gut microorganisms, mammalian metabolism and personalized health care. *Nature Rev. Microbiol.* **3**, 431–438 (2005).
4. Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R. & Gordon, J. I. Worlds within worlds: evolution of the vertebrate gut microbiota. *Nature Rev. Microbiol.* **6**, 776–788 (2008).
5. Ruby, E. G. Symbiotic conversations are revealed under genetic interrogation. *Nature Rev. Microbiol.* **6**, 752–762 (2008).
6. Medini, D. *et al.* Microbiology in the post-genomic era. *Nature Rev. Microbiol.* **6**, 419–430 (2008).
7. Li, M. *et al*. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc. Natl Acad. Sci. USA* **105**, 2117–2122 (2008).
8. Dethlefsen, L., McFall-Ngai, M. & Relman, D. A. An ecological and evolutionary perspective on human–microbe mutualism and disease. *Nature* **449**, 811–818 (2007).
9. Silver, A. C. *et al.* Interaction between innate immune cells and a bacterial type III secretion system in mutualistic and pathogenic associations. *Proc. Natl Acad. Sci. USA* **104**, 9481–9486 (2007).
10. Dale, C. *et al.* Type III secretion systems and the evolution of mutualistic endosymbiosis. *Proc. Natl Acad. Sci. USA* **99**, 12397–12402 (2002).
11. McFall-Ngai, M. J. & Gordon, J. I. in *Evolution of Microbial Virulence* (eds Seifert, H. & DiRita, V. J.) 147–166 (ASM, Washington DC, 2006).
12. Staley, J. T. Biodiversity: are microbial species threatened? *Curr. Opin. Biotechnol.* **8**, 340–345 (1997).
13. Maloy, S. & Schaechter, M. The era of microbiology: a Golden Phoenix. *Intl. Microbiol.* **9**, 1–7 (2006).
14. Heinecke, H. The origins of the gnotobiotic technic—Küster/Nuttall/Schottelius/ Thierfelder. *Z. Versuchstierkd.* **33**, 19–22 (1990).
15. Sagan, L. On the origin of mitosing cells. *J. Theor. Biol.* **14**, 255–274 (1967).
16. Meade, H. M., Long, S. R., Ruvkun, G. B., Brown, S. E. & Ausubel, F. M. Physical and genetic characterization of symbiotic and auxotrophic mutants of *Rhizobium meliloti* induced by transposon Tn*5* mutagenesis. *J. Bacteriol.* **149**, 114–122 (1982).
17. Kondorosi, A., Kiss, G. B., Forrai, T., Vineze, E. & Banfalvi, Z. Circular linkage map of *Rhizobium meliloti* chromosome. *Nature* **268**, 525–527 (1977).
18. Hirsch, A. M., Long, S. R., Bang, M., Haskins, N. & Ausubel, F. M. Structural studies of alfalfa roots infected with nodulation mutants of *Rhizobium meliloti*. *J. Bacteriol.* **151**, 411–419 (1982).
19. Handelsman, J., Rondon, M. R., Brady, S. F., Clardy, J. & Goodman, R. M. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chem. Biol.* **5**, R245–R249 (1998).
20. Moran, N. A. & Mira, A. The process of genome shrinkage in the obligate symbiont *Buchnera aphidicola*. *Genome Biol*. **2**, RESEARCH0054 (2001).
21. Kroes, I., Lepp, P. W. & Relman, D. A. Bacterial diversity within the human subgingival crevice. *Proc. Natl Acad. Sci. USA* **7**, 14547–14552 (1999).
22. Schauser, L., Roussis, A., Stiller, J. & Stougaard, J. A plant regulator controlling development of symbiotic root nodules. *Nature* **402**, 191–195 (1999).
23. Hooper, L. V., Wong, M. H., Thelin, A., Hansson, L., Falk, P. G. & Gordon, J, I. Molecular analysis of commensal host–microbial relationships in the intestine. *Science* **291**, 881–884 (2001).
24. Rawls, J. F., Samuel, B. S. & Gordon, J. I. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc. Natl Acad. Sci. USA* **101**, 4596–4601 (2004).

Acknowledgements
The author thanks her students and colleagues for helpful discussions on the issues touched on in this Essay.

FURTHER INFORMATION
Margaret McFall-Ngai's homepage: http://www.medmicro.wisc.edu/labs/mcfall-ngai/index.html
Human Microbiome Project: http://nihroadmap.nih.gov/hmp/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF