Symbiosis—Evolution's Co-Author

Douglas Zook

Abstract Symbiotic integration is a primary contributor to the centerpiece of evolution, genetic novelty. Acquisition of foreign organisms or parts thereof, and potential subsequent assimilation and often internalization of one or several different genomes into another different entity are the foundational expressions upon which natural selection acts, particularly in eukaryotic organisms. Thus, the entire landscape of life—from cells to biomes—is substantially an evolving collection of chimeric communities. Competition may be pronounced and successful in evolution in large part *because* the competing organisms do *not* function as, and indeed are not, individuals. Moreover, growing evidence indicates symbiosis to be on a flexible continuum of physiological expression, often with real plasticity in the organisms' integrating life cycles. Therefore, so-called "mutualism", "parasitism", and "commensalism" as symbiotic reference points and analyses may be outdated and perhaps of dubious use. For example, fundamental ecological principles show us that "parasitism" among two different organisms is often of significant advantage to not only the "parasite" but its "host." Symbiosis system examples are here reviewed and redefined on a more meaningful evolutionary context; namely, symbiosis is the acquisition of one organism(s) by another different organism(s), and through subsequent long-term integration, new structures and metabolism emerge.

Keywords Symbiogenesis · Holobiont · Symbiosis · Margulis · Mutualism · Microbiome

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1 Introduction

This journey reveals how symbiosis permeates the biosphere and its evolutionary history. It emphasizes new perspectives not only about what is in front of us every day and how it got there, but reinforces the revolution in science today—the emerging realization of individuals as ecosystems. Our travels, with stops at dozens of symbiotic examples, many only recently revealed, will thrust through old symbiosis definitions and offer a new workable one. It will dare to step away from the traditional mutualism–parasitism–commensalism gyre into new currents that reflect the fluid reality that is symbiosis. It will culminate with comments from an interview this author conducted a few years ago with longtime friend and sage for so much that we realize today in life science, Lynn Margulis. There is no more profound and revealing place to start than with the pervasive eukaryotes, the algae.

Algae dominate the biosphere. These autotrophic protists, the larger of which are commonly called "seaweeds," significantly impact every biome and nearly every ecosystem on earth. Most are microscopic and are in high densities in the colder regions of the world's oceans, which make up 71 % of the globe's surface. The algae (along with cyanobacteria) are the main fixers of carbon; the primary source of oxygen in the atmosphere; an essential food source for key marine and freshwater food webs; substantial biomineralizers, contributing much of the lithosphere's limestone; principle conduits for critical element flow; emitters of gases that serve as condensation nuclei in cloud formation; and serve as substrates, foundations, and "partners" for biodiverse communities such as mats, crusts, and films. They are the physiological glue of the biosphere, effectively keeping the earth's biosystems productive, efficient, and perpetual. And, their evolution, which extends back to nearly the dawn of eukaryotes two billion years ago, is the result of remarkable symbiotic infection and acquisition events. Indeed, the vast algal groups are among the most prominent evidence for symbiosis strongly sharing the biosphere stage with mutation and recombination as evolution's co-author—with natural selection as the essential and ultimately passive editor.

The first photosynthetic-centered symbiotic event is that which also led to the lineage that emerged as plants—the phagocytosis of a free-living cyanobacterium into a microscopic heterotrophic protist already equipped with other products of symbiosis, mitochondria and the nucleocytoplasm (Archibald 2011). In geologic time that amounts to a flash of lightning, one genome became embedded and functional within another, resulting in a novel now autotrophic organism. Referred to as a "primary symbiosis," this profound acquisition was the biological big bang that still expands outward today, producing phylogenies via little or no gradualism and with mutation as a more secondary influence.

2 Primary, Secondary, and Tertiary Symbiosis

This primary event of autotrophy acquisition resulted in three distinct lineages represented by the Chlorophyta (green algae), Rhodophyta (red algae), and Glaucocystophyta. The latter more obscure algae features a reduced cyanobacterium

known as a "cyanelle" as its evolving photosynthetic organelle. This cyanelle includes the pervasive polymer and cell wall constituent "peptidoglycan," a revealing remnant of its prokaryotic, cyanobacterial acquisition ancestry. While the few representatives of this phylum are extant, this lineage, evolutionarily speaking, was a "dead-end" in that there is no evidence that any new forms branched from it. The same cannot be said of the other two primary symbiosis lineages (Delwiche 1999). At close to 470 mya, green algae from within either the Charophycean class (Lewis and McCourt 2004) or Zygnematales (Wodniok et al. 2011) transitioned from aquatic habitats to the land, eventually leading to the first plants. Thus, all green chlorophyll-containing eukaryotic photosynthesizers, such as plants, are the result of this first cyanobacterial acquisition, likely by a mitochondrion-containing amoeboid-like heterotrophic protist. However, remarkably, this critically significant event—termed a "primary symbiosis"—was only the start of a broad series of secondary symbiosis-generated lineages. Categorized as "secondary" symbiosis, members of what we now recognize as from the primary green and red lineages were phagocytized by another eukaryote. For example, certain chlorophyte algae were engulfed by a heterotrophic protist and emerged as Euglenophyta, while still others became Chlorarachniophyta (Palmer 2003). The latter group is made up of very few species, but has great evidential significance, for these microscopic, colonial forms reveal today a greatly reduced genome, essentially a remnant of the nucleus from the chlorophyte, which it phagocytized. Thus, chlorarachniophytes feature the original "host" heterotrophic eukaryote with its primary symbiosis-derived mitochondria and nucleocytoplasm, as well as a reduced "captured" alga with its now miniscule nuclear expression known as a "nucleomorph." The discovery of the nucleomorph indicated what had been merely suspected previously—that many diverse algal groups are actually well-integrated, multi-genomic consortia (Bhattacharya et al. 2003).

The evidence is further strengthened by the existence of a different nucleomorph that verifies yet another secondary symbiotic event leading to another lineage (Ludwig and Gibbs 1985; Moore and Archibald 2009). This remnant nucleus was that of a species of microscopic red alga, which was engulfed by a heterotrophic protist but not digested. As in the chlorarachniophytes, this red alga counterpart conferred relatively quickly natural selective advantages in the new consortium. This algal lineage, which emerged from the primary symbiont rhodophyte lineage, represents the phylum Cryptophyta. Cryptophytes are mostly freshwater and have two motility organelles ("undulopodia" or what is more traditionally called "eukaryotic flagella"), which it uses in conjunction with specialized ribbon devices known as "ejectisomes." These structures contract and expand and propel the microbe in various directions.

Other secondary symbioses led to other algae of incalculable importance to the biosphere. These include the glass-enclosed (SiO_2 encased) diatoms (Bacillariophyta), the limestone depositing coccolithophores (Haptophyta), and brown algae (Phaeophyta). While there are no remnant nuclei from an acquired symbiont in these and other algal phyla, evidence shows unequivocally secondary symbiosis in action. For example, membrane counting and analysis is a useful indicator. When a heterotrophic protist phagocytizes the alga, the alga becomes

permanently surrounded by that host membrane with its characteristic lipids. Moreover, the plastid enclosed in the red or green algal symbiont also has at least one and often two (or more) membranes, one characteristic of the surrounding cytoplasm of the alga and another of the original cyanobacterial primary symbiosis. These membrane "layers" combined with ultrastructural and gene sequencing comparative data confirm the identity of the integrated genomes within nearly all other algal lineages and show their evolution as derived from secondary level symbiosis (Archibald 2009) (Fig. 1).

Remarkably, one of the most common algae in the oceans and particularly found in symbiosis with larger invertebrate "hosts" such as corals and anemones, dinoflagellates, are sometimes the result not only of the primary and secondary symbioses but a third symbiotic event. For example, species of haptophytes—itself the product of secondary symbiosis—have been phagocytized by yet another likely heterotrophic protist resulting in a tertiary autotrophic dinoflagellate (Inagaki et al. 2000). Such a dinoflagellate can be seen as the sum of up to a dozen genomes or genome remnants without of course counting bacterial gene transfer events over recent or deep time. Indeed, there is growing evidence of many other photoautotrophy-based tertiary symbioses among the protists (Vesteg et al. 2009).

3 Algal Phylogeny: Showcase for Genetic Novelty Through Symbiosis

The algae are deserving of focus from the outset, for there is no more profound example of symbiogenesis—the acquisition-centered impact of symbiosis on evolution. The autotrophic portion of an entire kingdom (or subkingdom) so central to biospheric systems, global biodiversity, and geological substrates is due to the process of symbiosis. In each lineage, genes foreign to an organism were tolerated and eventually incorporated, whole or in part, into the consortium. The heterotrophic protist host would have to undergo unimaginable mutational events to express eventually even a fraction of the consortium's traits. Mutation and recombination influence in the emerging eukaryotic algae without symbiosis is an oxymoron. It is plain to see that there would be no diverse phylogeny of algae as such. In this way, lasting symbiotic mergers through symbiogenesis are not only central to evolution and global ecology but foundational. New species, lineages, varieties can develop within and from the symbiotically constructed lineage, often in turn, leading to new symbiotic mergers. Algal taxonomy reveals life-forms much like bridging silk strands of an orb spider's web, a series of integrated connections that transform the concept of individual to one of a vast symbiotic community, or as the emerging symbiosis-based revolution in science now terms, the "holobiont," as first proposed by Margulis and Fester (1991, p. 2). The term later became more specifically associated with corals (Rowher et al. 2002) and more recently

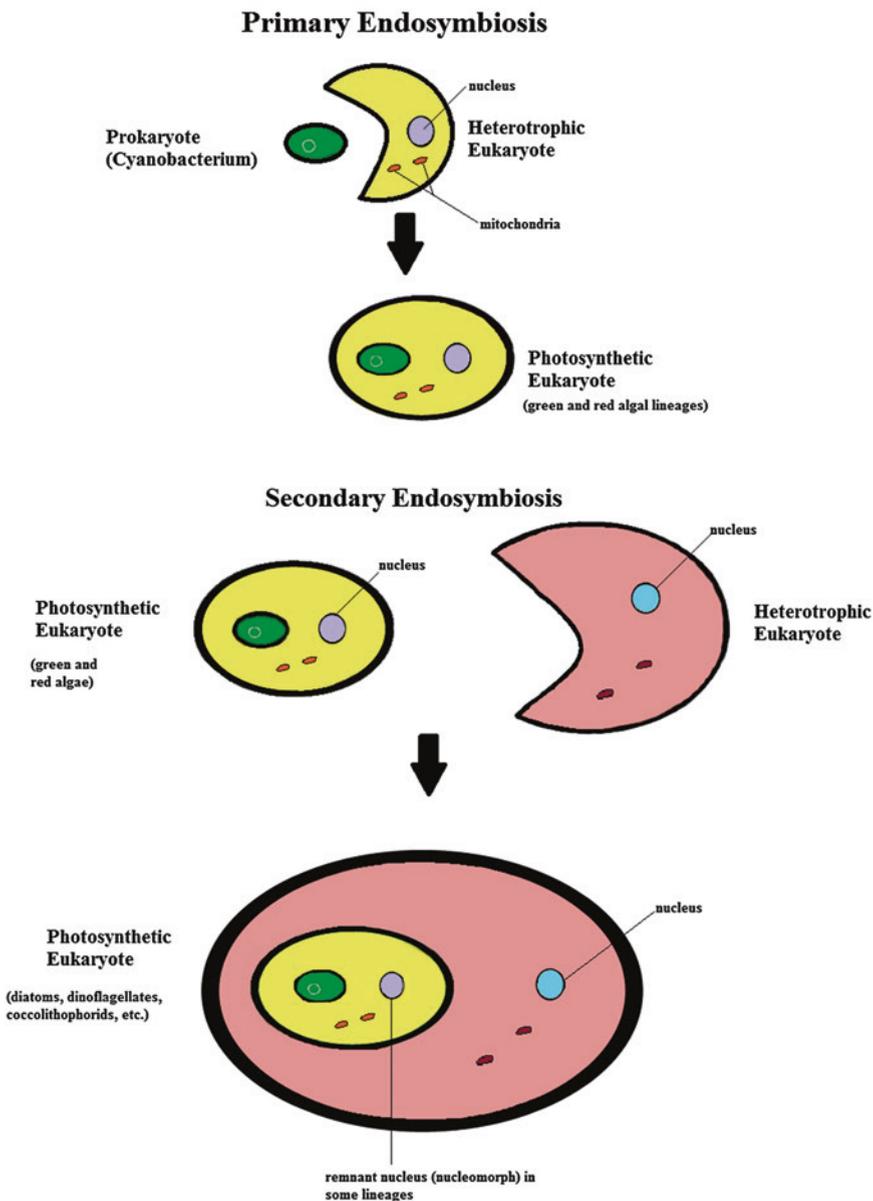# Symbiosis as a Major Speciation Driver

Primary Endosymbiosis

Secondary Endosymbiosis

Fig. 1 Diagram by Olivia Hathaway

transformed to a dynamic concept in understanding the metagenomic unit of selection in evolution (Rosenberg and Zilber-Rosenberg 2011; Gilbert et al. 2012; McFall-Ngai et al. 2013). Indeed, the term "Hologenome" can be used to not only refer to the symbiont genomes but to those identified genes that were horizontally transferred from bacteria for example, as well as possible extra-chromosomal mainstays which may be in evidence with the extraordinary "symbiosis" of the sacoglossan mollusc *Elysia chlorotica* with its *Vaucheria litorea* plastids (Bhattacharya et al. 2013).

4 Outdated Symbiosis Definition

Ironically, symbiosis has often been the outcast of biology, at best seen as an interesting curiosity. Even after the uncovering of prokaryotic DNA (characterized as within a circular chromosome and not containing key histone proteins) in mitochondria in the 1960s (Nass and Nass 1963; Nass 1969), there was antagonism toward any evidence that might suggest the powerful role of symbiosis in evolution. Its original definition, still advocated by many, may not have helped in fostering a clear subdiscipline of symbiosis within Biology. In the late nineteenth century, the German De Bary (1879) labeled it "the living together of unlike organisms" and implied a lasting relationship. But such a definition has proved too all-encompassing. After all, an insect living in the furrow of a particular bark of a tree for a good part of its life cycle could qualify. The tree and the insect are certainly vastly different organisms. They are living together and even in physical closeness for an extended period, with one nestled within the other. If natural selective advantages are considered, we could perhaps find that the bits of waste material from the insect, which get carried down the tracks of the tree bark, end up in the rhizosphere and partly nourish the tree. We can further surmise that the insect in turn gains a secure habitat for an extended time and so on. Of course this is not a symbiosis, but part of the grand expression of fundamental ecology. It is the ubiquitous stuff of ecosystems.

Moreover, extensive research reports and reviews, which clearly state that this is the definition to which their research is tied, would actually have to include systems—pollinators with many angiosperms, epibiont heterotrophic protists on marine macro-algae, and uncountable numbers of other ecological relations—in their data and discussion that are actually outside the purview of symbiosis.

The vagueness of the original definitions also fostered a sense of new categorizations, such as mutualism (that for some could fit the above simplistic insect–tree bark example), parasitism, and commensalism. Much of the symbiosis research over the past one hundred years and right to the present is seemingly intoxicated with having to place symbiosis in one of these boxes. Strangely, it can even guide research, wherein one of the ultimate purposes of many symbiotic studies

is to determine the degree of mutualism or "shared benefit" or whether one form is more parasitic or simply there for the ride without any significant contribution. This has led to subcategories of "cheaters" and "freeloaders," and other terms that seem to not be cognizant that *all* organisms appear to seize on opportunities to enhance their life cycle and balanced or altruistic fits are seldom in play.

Does one really need to use these terms to define or even connote symbiosis? To what degree are these terms actually meaningful and strongly reflective of the biological and evolutionary reality? Do these terms potentially move us away from ecological thinking and replace it with anthropogenic, human chauvinistic thinking? To what degree does such an obsession with these terms skew how we should be investigating and interpreting our findings? In other words, are we subjecting ourselves to research processes that are far less than open ended but rather designed to see how they fit into some prescribed, small set of categories, slots that may reflect more human analysis than nature's reality? I posit that the continued reinforcement of the original definitions and the dogmatic emphasis on the three categories with analysis of the degree of "benefit" or "antagonism" is neither representative nor particularly useful in the now mainstream discipline of symbiosis. The data collected can be outstanding and revelatory but the language and context is often more convenient, habitual, and simply scientifically inappropriate. It is difficult to find a symbiosis research paper that does not become focused on "benefits" and "costs," as well as the mutualism, parasitism, and commensalism.

Moreover, there appears to be little recognition that entire studies and chapters of books within the overarching discipline of ecology discuss "mutualism" and in so doing are referring to both the widespread behavior of pollination and the association of fungi (mycorrhizal) with plant root cells. The latter a symbiosis, the former, in most cases, is not. Mutualisms are very common ecological expressions and for clarity sake alone should not be used to analyze and judge symbiotic systems. To do so only risks greater confusion and again makes symbiosis appear to be synonymous with ecology when it is a central reasonably identifiable discipline within ecology. Further, one can argue with a reasonable degree of validity that most associations of any kind are "mutualistic." Pathogenic organisms that cause death are essential to the continuance of that species (the "victim"). Commensals die and the decaying biochemistry from it becomes part of the ongoing nutrient supply. Parasites ultimately can strengthen the resilience of the species in that natural selection can often favor new varieties more fit for the threatening environmental conditions.

Symbiosis analysis also implies for some a denial of the centrality of competition in ecology and evolution. Rather, the reality can be seen in the context that some competitors are often more fit *because* they have symbiotic "partners" and alliances. Combined with the fact that many eukaryotic organisms (holobionts), as well as bacteria, are naturally selected for efficiency, energy-consuming competition may be less of an evolutionary driver and often more a life strategy that is embedded with frequent caution signs.

5 Symbiosis Redefined More Concretely and as a Better Reality Fit

Those working in symbiosis research know that there are very clear components that make the discipline more concrete. Thus, I found it refreshing when the outstanding symbiosis researcher Angela Douglas in her book *Symbiotic Interactions* (1994, now out of print) indicated some new, clearer criteria, albeit less emphasized in her latest book, *The Symbiotic Habit* (2010). I have adapted some of those ideas into a definition that over the years have helped my students truly identify symbiotic systems less ambiguously, more accurately, and in a more appropriate evolutionary context. Symbiosis is *the acquisition of an organism(s) by another unlike organism(s), and through subsequent long-term integration, new structures and metabolism(s) emerge.*

This definition makes the focal point of symbiosis the specific physical and metabolic outcomes of the symbiosis. For example, the prototype symbiosis can arguably still be seen as the lichen (Sapp 1994). In most lichen symbioses, we have an alga and a fungus, two fundamentally, phylogenetically distinct lifeforms. If I have an alga isolated species with its own morphology and indeed its own genome(s) and I have a compatible fungus separately with its own morphology and genome(s), these organisms are fundamentally its own discreet "selves." But, given genetically programmed signaling and recognition factors, if I axenically bring them physically together in the laboratory for growth on an appropriate nutrient medium and mineral substrate, as was done many years ago by pioneering lichenologist Ahmadjian (1993)—or even in its natural setting, the morphology and indeed the ontogeny change dramatically. Both original forms become substantially unrecognizable as a growing entity. So much so that we are forced to give the new multi-genomic morphotype—this grand "holobiont"—that one can see with the naked eye, a name, the "thallus" (Fig. 2).

Fig. 2 *Cladonia cristatella* ("British soldier") lichen on right and Cladina sp. on left at Parker River Wildlife Sanctuary, Newburyport, MA. Photograph by D. Zook

This construct by both organisms by way of signaling, recognition, environmental conditions, assimilation, and integration is the principal defining characteristic of symbiosis.

Likewise, alga and fungus have vastly different physiological features, such that their metabolic properties are very dissimilar. Fungi cannot photosynthesize. Algae generally do not feed by direct uptake from surface materials. But together in symbiosis, they each bring different degrees of new metabolic expression to such an extent that the fungi have become photosynthesizers through the de facto integration of a "foreign" eukaryote with chloroplasts embedded within the new consortium, resulting in this now lichen holobiont, i.e., the integrated multi-genome entity. Thus, the second defining characteristic is the relatively rapid emergence of new metabolite expressions (e.g., photoautotophy) essential to the holobiont—a physiology(s) and biochemistry that was not there previously in either of the free-living proto-symbionts (Fig. 3).

A third critical component of symbiosis is the process of acquisition. Entire sets of genes with a genome that is "foreign" to one symbiotic partner become sorted, rejected, accepted, and integrated to various degrees in the new multi-genomic holobiont. Acquisition is defined by "coming to control or possess something." Thus, a genome's important functions and components of the partner organism are acquired in that they then belong to a new emerging entity. The acquisition can often best be considered reciprocal in the sense that both once-independent entities are acquiring significant degrees of the gene expression of the other, if not in some cases the whole genome. Often, the acquisition becomes both intimately syntrophic and synergistic.

The proposed symbiosis definition differentiates clearly between relatively short-term, ephemeral, and non-integrated relationships involving different organisms and those that are long term, persistent, and highly integrated. For example, many ecologists consistently cite mutualism for both a coral-dinoflagellate reef-building relationship and a honeybee pollinating a flower. The latter is short term, ephemeral, and relatively unpredictable and is simply one of thousands of

Fig. 3 A new definition of symbiosis as proposed by D. Zook
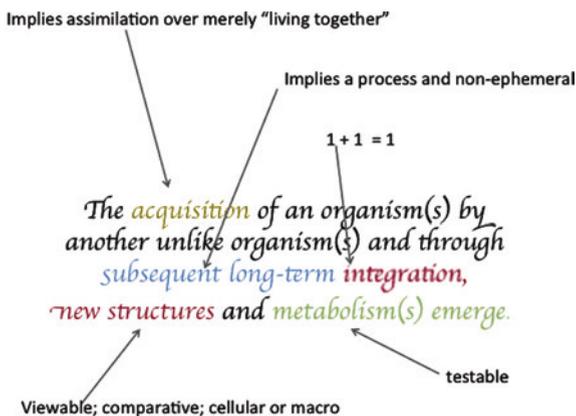
ecological expressions. The former is long term, very persistent, and well integrated, what with specific dinoflagellates changing their morphology and encysting within coral-created membranes in the anthozoan's cells. Within this analysis, the honeybee's relationship to flowers is ecologically mutualistic and not a symbiosis or holobiont with respect to each other, but the bee with its endosymbiotic bacteria is a symbiosis itself (Martinson et al. 2011) and a holobiont. Yet, frequently, we read that both systems represent "mutualists." This only serves to confuse, oversimplify, and relegates symbiosis to a kind of taboo "cooperation" word that should be avoided. The new symbiosis definition does not include ambiguous and confusing "mutualist" and various traditional anthropogenic terms, but rather emphasizes viewable and measurable outcomes that emerge from an acquisition-centered reality. In this context, these novel structures—the thallus of lichens, the rumen or ruminants, the reefs of dinoflagellate–corals, the trophosome of Riftia tube worms, the arbuscles of fungi within root cells, the intercellular Hartig net of ectomycorrhizae, the paunch of termites, the bacteriome of many insects, the light organ of the bobtail squid–Vibrio, the trichome-lined cavity of *Azolla*, the syconium of *Ficus*, the subterranean nests of attine ants, the symbiosome membranes around many intracellular symbionts, and the nodules of Rhizobium with legumes—are all defining central characteristics of symbiosis and reinforce this new definition.

6 Ecosystem Thinking Replaces Compartmentalizing

This labeling of systems as "mutualist" or "parasitic" can also be misleading, given the nature of symbiotic systems. Over both the diurnal and full life cycle of the holobiont, any of the given integrated genomes can, often through disrupted signaling and alternative feedbacks, be more dominant or subservient than the other. Prominent examples reside in the endophyte symbioses, such as those involved in Epichloë (fescue) plants and *Claviceps* fungi (Schardl 2001). In these holobionts, switching via enzyme triggering (Tanaka et al. 2008) to a sexual cycle in the fungus fosters a more pathogenic expression such that hyphal growth becomes so prolific that it chokes out floral development of the grass. This switch may be promoted by the fact that metabolites of the grass, which typically restrict the fungal partner growth, become relegated instead to the energy needs for grass reproductive structure and function. Yet, a different selective advantage expression dominates during asexual fungal periods much of the year in the same holobiont. At these times, the plant is less susceptible to drought conditions and herbivory due to secondary metabolites of the fungus residing intercellularly in the grass leaf and often in the seed (Eaton et al. 2011).

There is also evidence that very limited genetic changes can move a symbiont from necessary holobiont entity to assimilated food source. This is the case in the amoeba *Dictyostelium discoideum* which harbors among many bacteria, two strains of *Pseudomonas fluorescens*. The protective anti-fungal more "mutualistic"

strain is converted by a single point mutation at the activator gene to the edible and thus rapidly ingested strain (Stallforth et al. 2013). Recently, Wooldridge (2010) challenged the long-standing compartmentalizing of hermatypic coral animals with dinoflagellate symbionts by emphasizing a kind of animal winnowing mechanism that results in the most effective photosynthate-transferring varieties, as well as suggesting significant fitness cost for the algal symbionts. Such characteristics among many suggest less of a "mutualism" than a kind of "controlled parasitism," a term originally proposed for lichens many years ago (Ahmadjian and Jacobs 1981) and again which could apply at various parts of the holobiont life cycle to many symbioses.

We are starting to see a refreshing perspective emerge that the guide for symbiosis enquiry and interpretation needs to be on open-minded exploration of physiology, ecology, ontogeny, and cell communication. There is no prescribed screenplay but an ongoing series of images that tell a story within an ecosystem context. For example, the review of microbial symbiont transmission by Bright and Bulgheresi (2010) states at the outset, "The key question is how the symbiont is transferred to the host progeny, *regardless of the type of symbiosis" (italics mine).* They go on to emphasize in their astute and comprehensive analysis "how the conversation between partners… is initiated." That said, they also show a contradiction in that they are focusing on those organisms that maintain "protracted physical contact and involve most of the host population," caveats that are not clear within the de Bary definition to which on the next line they pledge allegiance.

While some symbiosis researchers are facing this compartmentalizing stigma head on by offering such terms as "context-dependent symbioses" (Daskin and Alford 2012), this could be considered a malapropism as it is hard to realize the life history of any holobiont as being independent of context. Arguably, there can be gradations of "context dependency." The "poster child" of symbiosis dating back nearly 150 years has always been the lichen. These algal–fungal and occasionally cyanobacteria-inclusive extracellular consortia have evolved an impressive array of biochemical and physiological features that allow them to secure strongly their niches across nearly all terrestrial biome conditions and even on many aquatic substrates. No feature is more valuable to their fitness than their production of a laboratory full of secondary compounds, commonly labeled as lichen acids. These not only break down substrates to their mineral or particulate constituents and thus enhance element cycling, but they are strongly anti-herbivorous and often antimicrobial. Even those lichens that do not have such acids, such as those that have cyanobacteria as a main phycobiont, may compensate trait loss for such biochemistry through the presence of antibiotic-producing actinobacteria (Zook 1983). Moreover, there is growing evidence that the lichen holobiont may involve and perhaps require a consistent community of bacteria (Cardinale et al. 2006; Grube et al. 2009). Yet, even in this stalwart symbiosis, there is a fluidity and plasticity that defies simple categorization. The algal and fungal symbionts within the holobiont are tantamount to mammalian organs, certainly subject to break down but coordinated through positive and negative feedbacks, often involving pH

changes that allow for functional and even thriving life cycles. At any given time and dependent on environmental conditions such as alternations of wetting and drying, either the algal or the fungal genome can be controlling or dominant. The emerging realization of lichen symbiont fluidity can be seen in many Cladoniaceae lichens which have even been placed outside of key co-speciation possibilities and instead adapting to the environment needs of the holobiont through frequent algal symbiont switching (Piercy-Normore and Depriest 2001). Various fungal symbionts such as *Colletotrichum* spp. function across the spectrum of mutualistic to parasitic and so-called commensal depending on with which plant species they are associating, as well as environmental conditions (Redman et al. 2001).

The emergence of holobiont thinking, recently represented also within the hologenome theory (Rosenberg et al. 2009), emphasizes that the unit of selection is the multiplicity of genomes and genome constituents in what has usually been called an "individual," but in reality is with all eukaryotes a symbiotic community (Gilbert et al. 2012). This outlook reflects a new and necessary ecological and environmental framework, which in turn reveals the fluidity inherent in context-dependent nature. A key extension of this fluidity is that many symbioses now appear to be highly variable, flexible, and adaptable, consistently utilizing associated "foreign" genomes or genomic remnants to fulfill essential metabolic expressions.

7 Symbiogenesis Rooted in Lamarck, Darwin, and Kozo-Polyansky

Acquisition has actually been central to evolution thinking since the early nineteenth century. For example, Lamarck developed the first organized view of evolution, in which he proposed that characteristics can be acquired by organisms and then be inherited into the next generation (1809, reprint 2011). While this idea was subsequently derided, his views became a running thread through Charles Darwin's *Origin of Species* (1859, reprint 2011). A variation of this thinking that Lamarck would not have been able to realize at that time is a reality today in the concept of acquisition-based evolution which we term "symbiogenesis," originally proposed by Russian biologist Kozo-Polyansky (1924), later resurrected by Margulis (1990) and in a new translation and interpretation by Margulis and Fet (2010). Kozo-Polyansky originated the term and summarized its meaning as the origin of evolutionary novelty by the merger of different organisms into one. Ironically, Darwin himself had some sense of this, "We cannot fathom the marvelous complexity of an organic being; but on the hypothesis here advanced this complexity is much increased. Each living creature must be looked at as a microcosm—a little universe, formed of a host and a self-propagating organism, inconceivably minute and as numerous as the stars in heaven." (Darwin 1858, p. 453).

Today, symbiogenesis connotes the emergence of this acquired multi-genomic entity (holobiont) over evolutionary time perpetuated by natural selection.

8 Holobiont Selection Allows for More Rapid Adaptation and Greater Fitness

Two insect symbiotic systems dramatically illustrate this intricate coordination and fluidity of various symbionts as a community, the holobiont.

The tsetse fly, made up of over 30 species in the genus *Glossina,* are large, biting flies which are prolific in North African arid and desert regions. They produce four generations each year and are hematophagous. The tsetse holobiont consists not only of itself and its mitochondria but an interlocking array of microbes. Two are obligate gammaproteobacteria of *Wigglesworthia* spp. A third is *Sodalis glossinidius,* a more recent symbiont as evidenced by its ability to be extracted and cultured (Snyder and Rio 2013). All three are vertically transmitted through the maternal milk glands (Balmond et al. 2013), which carry specific proteins and lipids to the uterus for the viviparous symbiosis-accommodating offspring development (Attardo et al. 2008; Ma and Denlinger 1974).

Wigglesworthia are mostly intracellular, being located in specialized cells known as "bacteriocytes." The collection of bacteriocytes make up a defined region of the insect, the "bacteriome." Such a new structure, a proposed defining characteristic of symbiosis, is commonly found in various insect holobionts (Baumann 2005). Because the fly lacks B vitamins in its blood diet, selection has favored these bacteria which provide not only vitamins but stabilizes the fly immunological development and digestion, and influences the degree of trypanosome infection (Snyder and Rio 2013). Verifications of this symbiont dependency have been shown by providing *Wigglesworthia* cell extracts to aposymbiotic, immune-weak mother flies. Such a treatment restores immune vitality (Weiss et al. 2012). While the role of the *Sodalis* bacterial symbiont, also vertically transmitted via the milk glands, in the holobiont community remains unclear, it is undergoing considerable genome reduction, which indicates likely integration through gene elimination and possible transfers to the other holobiont symbionts. Perhaps indicative of the tight community nature of this holobiont, the demise of *Wigglesworthia* causes a corresponding loss of *Sodalis* (Snyder and Rio 2013).

A fourth (facultative) symbiont in the tsetse fly is a *Wolbachia* species within the bacterial family Rickettsiaceae. *Wolbachia* is the most common bacteria affecting the reproductive system of animals known. It is most commonly found in arthropods and confers dominance of females through various male-reducing and male-eliminating strategies (Werren et al. 2008). In the tsetse fly, it induces cytoplasmic incompatability, which ultimately means that females that are uninfected by *Wolbachia* cannot mate with males which are infected. Because *Wolbachia* can only be transmitted by females, this promotes *Wolbachia* reproduction and viability (Werren 1997).

The salivary gland hypertrophy virus (SGHV) of the Hytrosaviridae family can be considered as another tsetse holobiont genome, albeit a facultative virus. This viral infection of the tsetse fly may confer gonad abnormalities and reduce reproductive success (Sang et al. 1999). Thus, this genome within this holobiont

community mirrors large ecosystems conventionally studied, in that population regulation through disease and death is an ongoing necessity for optimal fitness and viability of the whole.

Because the holobiont approach tends to minimize the necessity for anthropogenic and often misleading terms such as "host," we can of course consider the sixth major symbiont in this holobiont to be the tsetse fly genome itself. For example, Wang and Aksoy (2012) founded that a fly peptidoglycan recognition protein PGRP-LB, similar to that found in Drosophila, prevents immune deficiency signaling stimulation and thus is closely associated with *Wigglesworthia* infection and maintenance. It is produced by adults and also transferred via milk glands to offspring after the latter's initial blood ingestion (Wang and Aksoy 2012).

Findings with the *Planococcus citri* (mealy bug) from the Pseudococcidae family reinforce and even expand the symbiosis-centered holobiont community concept. These cosmopolitan scaly insects are only female in the adult stage and commonly feed on plant sap. Males do not feed and live only until fertilization of the female. Recently, mealybugs have been found to contain not just a bacterial symbiont, but the smallest known bacterial genome at 139 kb, considerably less than both free-living bacteria and other symbiotic bacteria. Husnik et al. (2013) surmised that such gene reduction may be similar to organelle development as in the endosymbiotic origins of mitochondria and plastids in eukaryotes. However, quite rare for prokaryotes, they found that the bacterium *Tremblaya* had acquired a 538 kB genome bacterium, *Moranella*, now completely within its cytoplasm. A considerably larger genome at 538 kb than the near-organelle level of *Tremblaya*, this bacterium was found to code for many of the essential metabolites needed by its bacterium in which it is situated. Moreover, key enzymes and proteins for mealybug function were not merely the result of genes coded within these holobiont bacteria, but were substantially due to lateral transfers of genes from three diverse bacterial lineages over recent evolutionary time. In essence, this tripartite mealybug symbiosis is a holobiont mosaic that may be a model for many holobiont systems across the phyla of life. It is noteworthy that this symbiotic story indicates that pathways other than transfer of symbiont genes to a "host" nucleus, as in the case with many organelles, may be at play among holobionts, given that little evidence was found that the reduction of the genomes among the symbiotic bacteria was due to gene movement to the mealybug nuclei (Lopez-Madrigal et al. 2011).

Studies which reveal the complex holobionts of tsetse flies and mealybugs actually evolved from the many years of research on the aphids–*Buchnera* bacteria symbiosis—except now we know that this is not a pair-wise holobiont, and indeed, it may not be obligate or even appropriate to pigeonhole as a so-called mutualist. Pea aphid holobiont includes associated facultative bacteria not located in the aphid's bacteriocytes. Koga et al. (2003) showed in a landmark study that aposymbiotic aphids infected with only y-proteobacteria secondary symbionts appeared to compensate for much of the *Buchnera* contributions in that the aphid was able to reproduce successfully through several generations. These non-*Buchnera* symbiotic aphids were smaller, and their fecundity was less, but nevertheless, they were fully functional. Interestingly, these substitute secondary symbionts were found to

not only be in the usual intercellular regions but intracellularly within the primary bacteriocytes usually occupied by *Buchnera*. The question arises, however, as to how these secondary symbionts—so effective at compensation in laboratory experiments—confer advantages to the holobiont in nature. The answer may lie in the heat stress to which *Buchnera* is susceptible. The secondary symbionts were found to positively impact aphid reproduction under usually detrimental high heat conditions (Montilor et al. 2002). Generally, when both *Buchnera* and the secondary symbionts exist with the pea aphid, the secondary symbionts convey periodic negative effects.

These data indicate that facultative symbionts, once thought to be unimportant or solely detrimental, can under certain environmental conditions compensate for *Buchnera* weakness or loss. To think that a vertically transmitted obligate symbiosis likely "locked in" for over 100 million years (Moran et al. 1993) evolved a compensation factor involving facultative bacterial genomes on standby reinforces not only the community mosaic of symbiotic holobionts but the fluidity and resilience that argue against static categorization of symbiosis.

Bark beetles are another prime example of holobiont community dynamics. These prolific insects dwell in tree phloem somewhat devoid of nutrients and among regions where there are plant-produced anti-herbivory toxins. They thus depend on an array of microbial symbionts—an "expanded genetic repertoire" as leading insect symbiologist Six (2013) calls them. Several bark beetle species colonizing conifers feature novel symbiotic structures called "mycangia," which house obligate associated fungi that provide nutritional selective advantages (Six 2012). Some beetles carry additional fungi, which tap into sapwood and transport it to the phloem, where it is available for the larvae which gain significant amounts of nitrogen, a particularly limiting nutrient in these substrates (Bleiker and Six 2008). A few associated fungi produce sterols that are necessary for the hormones that stimulate reproductive metabolism. The determining factor in the degree of integration for many of the fungal–beetle associations is often temperature. Sudden or unexpected temperature changes can alter fungal populations, a particular concern with increasing anthropogenic climate change threats. Yeasts are also prevalent among bark beetles as well as other insects, with indications that some may even be involved in converting tree chemical compounds to pheromones, but much of their functional importance remains unclear (Six 2013).

A holobiont community would seemingly not be complete without the implications of bacterial genomes. While gut microbes are in low diversity in bark beetles likely due to the more sugar centered as opposed to cellulose diet within the phloem, the nitrogen-fixing bacterium *Rahnella aquatilis* is consistently found in all stages of the beetle life cycle (Six 2013). Tree defense compounds and toxins may be degraded by bacterial symbionts within some beetle species (Boone et al. 2013).

Tropical rain forest biomes are particularly dominated by symbiotic systems (Zook 2010), with one of the most revealing being the attine ant holobiont with its the cascade of adapting players in a symbiosis that likely dates back 65 million years (Mueller et al. 2001). This extraordinary holobiont features the leaf-cutter ant in association with a fungus from the Lepiotaceae family which it cultivates for food but which is consistently threatened by growth of

the ascomycete (order Hypocreales) fungus, *Escovopsis*. Symbiotic actinobacteria of the genus *Pseudocardia* populate the ant's surface and convey antibiotic protection often targeted to the specific variety of the invading fungus (Poulsen et al. 2010). Black yeast species in turn tend to limit the actinobacteria growth not through resistance to antibiotics but more through outcompeting the bacteria for food (Little and Currie 2008). While the gut microbiota of leaf-cutter ants is still unknown, a wide variety of ant species are known to harbor specific bacterial symbionts which mediate diet and digestion. Bacteria species of Burkholderiales, Pseudomonadales, Rhizobiales, and others are consistently a part of ant holobiont communities. Russell et al. (2009) concluded that bacteria have facilitated convergent evolution of herbivory across many ant groups and suggested that "symbiosis has been a major force in ant evolution."

9 Symbiogenic Foundation of Earth Biomes

Much of the earth's biosphere is a geosymbiotic construct, indeed often microbiogenic. The topography of terrestrial and marine regions on earth results from the remnants of symbiotic processes. The coral-dinoflagellate holobiont builds rocky substrates, the calcium carbonate reef, which then becomes one of the most biodiverse ecosystems on earth. The process evolves around free-living *Symbiodinium* algal varieties encysted within specialized membranes—symbiosomes—of coral polyp cells transferring as much as 95 % of its photosynthate, usually as glycerol (Stat et al. 2006), to its surrounding animal partner, albeit the degree and timing of transfer dependent on the dinoflagellate clade representative and environmental conditions (Cantin et al. 2009). Without this infection and subsequent multigenomic integration, there is not the energy or the metabolites to express a reef. The resulting alkaline excretion allows the polyp cells to return to a more acidic, functioning pH (Goreau et al. 1979), albeit the primary selection for such a hermatypic symbiosis may be that coral larvae have a definitive substrate upon which to affix as well as habitats that support organisms which the coral tentacles can capture as sustenance in their multi-trophic lifestyles.

Oceanic reef regions represent only about 0.1 % of the area of the oceans' surface area with approximately 90 % of that total being in the Indo-Pacific. While oceanic reefs—including many of the result of calcareous sponges likely with symbionts—were more prolific in more ancient eras, these water "oases" were always in relatively small patches given the reality that nutrients in tropical waters, which are basically devoid of upwelling, are in short supply. Reef biomass is highly correlated with the diversity of organisms, which depend on the reef structure, not only as habitat but often as a location for pelagic forms to lay eggs before returning to more open waters. In the Great Barrier Reef off the northern coastline of Australia, 30 species of cetaceans live in or visit; 40 species of seabirds, 5000 species of bivalves, 6 breeding species of sea turtles, and 1500 fish live amidst the coral reef architecture http://www.reef.crc.org.au/discover/plantsanimals/

facts_plantanimal.htm. Examined from a symbiosis perspective, these biodiversity numbers increase exponentially when we realize that most of these megaorganisms are themselves holobionts made up numerous microbial symbionts. Moreover, the reef itself supports varieties of free-living microbes, most of which have yet to be discovered, let alone researched.

Through orogeny and terrestrial subsidence, these reefs become part of the lithospheric crust and pedosphere. The reef can then be seen as a limestone-dominated mountain, mountain ridge, peak, mountain chain, rolling hills, plains, or karstic caves. More than 25 % of the surface area of the People's Republic of China is limestone. This includes massive cave regions as well as extraordinary mountain regions in Guangxi region. Even much of the Gobi desert features remnant limestone fine "sand," the result of biogenic rocks ground down by ancient glacial retreat. Biogenic and microbiogenic limestone geology is prolific around the planet, including in North America where one of the largest limestone quarries exists in the state of Michigan.

But, many of the limestone zones are derived from yet another vast holobiont diversity. The most common eukaryote on earth could be *Emiliania huxleyi* and its varieties. This haptophyte alga produces intricate calcium carbonate "tests" known as coccoliths, as it floats within the photic zones in mostly northern temperate seas (Shutler et al. 2010; Holligan et al. 1983). As these massive blooms of algae die, most of the limestone tests gradually reach the benthic regions and accumulate tens of meters thick over tens of thousands of years. Much of the uppermost lithospheric crust of Europe—not merely well-known outcroppings such as in Dover, UK—is remnant coccoliths as well as some foraminifera tests (Huxley 1868). As with all haptophytes, the coccolithophorids are the result of a secondary symbiosis involving a heterotrophic protist phagocytizing a microbial red alga (Archibald 2009), which in turn had of course internalized a free-living cyanobacterium originally.

Thus, limestone-based geology common around the globe and critical to global ecology is a crucial extension and visible reminder of the dominance of symbiogenesis not only in macroevolution but in the emergence and maintenance of the biosphere. The origins and life cycles of karst-depositing hermatypic corals and hapotophytic algae have an impact far beyond its own singular body or colonial structure. The boundaries of holobionts are therefore fluid as well, for they involve the expressions of readily viewable geology, geomorphology, biogeography, and ecosystem dynamics. Symbiosis, as manifested through holobiont communities, is a central component of global ecology.

10 Anthropogenic Threats to Holobiont Global Ecology

If, as the evidence shows, the very foundations of how biomes and its ecosystems emerged and are maintained are substantially symbiosis-reliant, then, one can imagine identifying many symbionts as "keystone" species, i.e., usually

inconspicuous, smaller organisms that have a disproportionately significant impact on the greater biodiversity (Zook 2002). An example is the *Ficus* (fig tree) symbiosis with highly specific fig wasps of the superfamily Chalcidoidea. The flower of this prolific tropical rain forest tree is an enclosed receptacle with often hundreds of florets inside. This evolving fruit is called a "syconium" and can only be entered by specific pollinating female wasps through an ostiole. Using its ovipositor, the wasp lays its eggs deep within the stamens, and offspring later fly out carrying fig pollen. *Ficus* trees are critically important to the biodiversity of the rain forests in that a single tree can mast (produce fruits) up to four times each year, providing abundant food for organisms from throughout the phyla (Janzen 1979). The fig wasp is clearly a keystone species. Indeed, conservation policy directed at preserving fig trees will "automatically" help to conserve a wide range of other species, nearly all of which are likely holobiont symbiotic communities themselves. The *Symbiodinium* spp of hermatypic corals are another classic example of keystone species, and how identifying and conserving such symbionts may be essential in the process of not only understanding the symbiotic system and its environment but establishing policies and initiating actions to maintain biodiversity.

While the demise of coral-dinoflagellate reefs due to bleaching out of the dinoflagellate algae within the coral cells is the most prominent example of anthropogenic climate change effects on symbiotic systems, emerging research indicates other potentially problematic holobiont changes with significant ecosystem implications. Kiers et al. (2010) in a review paper pointed out that in the last forty years, fertilizer use by humans has increased 700 %, which in turn resurrects the long prevalent concern that such excess over an extended period can translate to demise for some mycorrhizal–plant symbioses, as well as *Rhizobium*–Fabaceae nitrogen fixers. Nutrient-rich sites commonly show replacement of strong mycorrhizal strains with weaker, less advantageous (to the plant) strains (Johnson 1993). Wang and Qui (2006) pointed out that some plants in Brassicaceae that typically thrive in high nutrient soils have lost their ability to form symbioses with mycorrhizae. Kiers et al. (2010) warned of a worrisome picture for the near future with symbiotic systems. They emphasize the likelihood of partner switching as "mutualistic" relationships are threatened and even indicate the actual replacement of a symbiont by antagonistic species. However, while the warnings ring true, the overall analysis cites symbiotic and other ecological systems with minimal consideration of bacteria impacts, now well recognized as critically important in holobiont metabolism, viability, and ontogeny.

Problems in this analysis are compounded by the traditional ecology usage of "mutualists." Mycorrhizae fungi with specific plants are lumped into the same group as bee generalists in ephemeral relationships as pollinators. Indeed, the entire article avoids the terms "symbiosis" or "holobiont." This is all the more confounding when in the same paper, the authors readily admit to fluidity in "mutualisms" (which presumably include some symbioses) pointing out how at ecological and evolutionary timescales the partners shift on a bidirectional continuum from beneficial to antagonistic. Key questions of environmental impacts on partnered

organisms are on target, but lost in the questionable uniting of ubiquitous ecological relationships with actual symbioses, as discussed earlier in this chapter.

There are some growing indications that lethal diseases affecting both bats and amphibians worldwide may be related to climate-related temperature changes affecting microbial populations associated with the animals (Daskin and Alford 2012). In bats, there are grounds to speculate that the lethal affect of the fungus *Pseudogymnoascus destructans (*formerly *Geomyces destructans)*, known as white nose syndrome (WNS), may have become pronounced due in part to changes in the bat microbiota. If any of the six species of bats extensively affected are shown to be a holobiont with interacting multiple genomes such as most mammals, some climate change, or environmental effects that helped to foster the fungi could be ameliorated by the fluidity inherent in many bacterial-influenced symbioses. Studies such as that of Daniel et al. (2013) have identified key members of the gut microbiota in the shortnosed fruit bat (*Cynopterus brachyotis*), albeit the authors characterize their work as a search for pathogens. A good start in Chiroptera-microbiota into enquiry of the microbiota of Chiroptera is represented by Phillips et al. (2012) who used comparative metagenomic analysis to not only identify the likely endemic gut microbiota but to indicate how such populations vary dependent on geography, stage of the bat life cycle, and diet.

The destructive agent for amphibians worldwide appears to be fungus *Batrachochytrium dendrobatitdis* (*Bd*) (Kilpatrick et al. 2010). The prevalence and severity of the disease with amphibians have been at higher elevations in the tropics. It is possible that the effects of possible symbiotic bacteria in the animals may have reduced impact on immunity against the fungi in the new temperature regimes influenced by current climate (Daskin and Alford 2012). This view is credible in light of recent work (Myers et al. 2012) that shows antimicrobial peptides (AMP) of the frog *Rana muscosa* secreted onto its skin may work synergistically with metabolites from endemic frog bacteria to confer resistance to the lethal chytridiomycosis. More specifically, *Plethodon cinereus* and skin bacterium *Pseudomonas fluorescens* may be a holobiont in that the bacterium limits the amount of AMP necessary from the frog.

Such findings further promote the concept of bioaugmentation in the face of environmental degradation and climate change. For example, probiotics using anti-Bd bacteria on amphibian skin in vitro reduced the harmful infection (Harris et al. 2009). Administration of specific bacteria to augment immunity in the amphibians could be a necessary conservation measure. Such human intervention is not without risks, for probiotic use could reach other organisms in and beyond the food web or certainly beyond the holobiont. Myers et al. (2012) suggested using an ecological ethics framework such as that of Minteer and Collins (2008) to consider and balance such risks and promote appropriate decision-making that is more conservation helpful than harmful.

Amphibian dependency on its microbiota is perhaps not so surprising given the historic findings of Kerney and colleagues (Kerney 2011; Kerney et al. 2011) and Graham et al. (2013). The eggs deposited as gelatinous masses in shallow waters by North American spotted salamander *Ambystoma maculatum* are later

penetrated by a green alga, *Oophila amblystomatis*. Since first discovered decades ago (Gilbert 1942; Hammon 1962; Goff and Stein 1978), it was presumed that this association was an epibiotic ecological association in that perhaps oxygen emitted from the algae through photosynthesis provided an appropriate environment for egg development in an ecosystem context. However, the recent work shows a deeper story that fits in well with the growing holobiont perspectives. The algae actually enter the developing embryo capsules near the blastopore and settle within the cells and tissues of the salamander embryo. Moreover, while oxygen can be a selective advantage for the animal in the holobiont, the alga actually translocates photosynthate to the salamander embryo as well as inhibiting invasive bacterial growth. Comparative studies with non-infected spotted salamanders confirm that the infecting algal symbiont is essential for optimal growth and viability of the salamander. These discoveries open the door for important follow-up enquiries such as whether the algae foster antibiotic production through associated bacteria; how the holobiont, in particular the chlorophyte alga, populations are regulated; and, of course, the obvious developmental biology and immunology questions of how this infection evolved and emerged as obligate. Moreover, this work is especially noteworthy in modern science, for they represent first definitive evidence of algae in symbiosis with a vertebrate; the latter previously considered a completely foreign domain for photosynthesizers.

Much like the bacteria and algae, with respect to the amphibian sustainability, certain mycorrhizal fungi could be a partial solution to both human-caused and natural environmental threats of a quite different nature: The human-created toxic waste sites scattered around the world. Mining for metals and minerals may allow for a supply of consumables deemed important in our societies, but the extraction process results in massive tons of hazardous waste products. For example, in Poland, as in many countries of the world, metals such as zinc, lead, and silver have been extracted for industrial purposes. Entire natural areas have been transformed and degraded. In some cases, excavation and removal of minerals and the corresponding waste has gone on since the twelfth century, but more intensively since the industrial revolution start in the mid-nineteenth century.

Case in point is the once active Trzebionka Mining Works within a major karst belt in southern Poland. Each year over many decades around two million tons of ore had been extracted. The ground-down waste rock and soils from the process were deposited as a 60 m-high heap covering about 64 ha (158 acres). Now, with the site essentially abandoned, there is little effort to water down the dry barren hazardous waste hill, albeit doing so would only be a very short-term measure. Therefore, some of the waste area is completely devoid of nearly all plant life including what was once there as part of a temperate zone forest biome. The dominant elements in these tailings (ore mining waste) are not organic matter but tons of crushed rock resulting in essentially zinc, cadmium, and lead "sand," all at levels far beyond what is tolerable for most life. These toxic-laced particles, usually about 0.3 mm in diameter, easily blow off from the tailing heap surface in even light winds. Rain and melting snows on the tailings tend to run off into nearby greener zones and can potentially percolate to regions where water is used for

gardens, farms, or drinking. The area is surrounded by fragmented forest zones, a highway, and some farming and village communities. Phytostabilization of the tailing heap is the only viable practical way to ensure reduction of contamination into neighboring villages and ecosystems. Until recently, even this possibility was far-fetched as it was unimaginable that any plant with its roots could grow and take hold on such a low nutrient and toxic substrate. However, within the emerging subdiscipline of "applied symbiosis," the possibility of remediation is now realized through utilizing selected plants that show some evidence of tolerating extremely harsh soil conditions in association with mycorrhizae (Turnau et al. 2012) (Fig. 4).

Mycorrhizae in association with these "extreme" plants not only can act as root extensions and reach limited phosphorus and water, but its mycelium (extensive hyphal network in the soil) can accumulate and store massive amounts of toxic metals. For example, one arbuscular mycorrhizae type can accumulate 10–20 times more cadmium than the plant roots to which it is associated. Identifying and collecting those plants that grow sporadically at the site, its perimeter, or nearby downslopes have resulted in identifying a growing inventory of those plant–mycorrhizal holobionts which may have the best chance at populating the tailings and then continuing to grow and reproduce into distance future generations. Thus, the field of phytoremediation in once-mined regions where toxic metal waste remains situated substantially depends on the capabilities of the mycorrhizal symbiont in symbiotic association with specific plants (Turnau et al. 2012).

All the examples posed and the many not mentioned usually involve a holobiont community interacting with another holobiont community. Nowhere is this more evident than with the spruce beetle and its microbiota involved in mycorrhiza-supported spruce tree substrates. In Alaska and the adjacent Yukon region in the 1990s, consistently warmer than normal temperatures during summers promoted an extra beetle reproductive cycle, such that eggs were annually doubled in what would usually be over a two-year span (Raffa et al. 2013). With some beetle outbreaks, it is not only the increased reproduction as a result of increased temperature, but also the spread into new regions. For example, the mountain pine beetle expansion in western Canada has expanded over the past 40 years into more

Fig. 4 Abandoned heavy metal mining site in southern Poland where bioremediation via the use of specific mycorrhizal plants is being investigated. Photograph by D. Zook

northerly latitudes and higher altitudes with a 1 °C increase. Because bark beetle bacterial symbionts are known to detoxify tree defense chemicals (Adams et al. 2013; Boone et al. 2013), evolving research is focusing on some manipulation of the bacterial community to alleviate the growing invasive strength of the beetles, especially with increasingly alarming data on anthropogenic climate change.

11 Symbiosis as an Ancient Strategy in Evolution

While symbiosis is front and center in the emerging crises involving anthropogenic-caused climate change and related issues, the evidence indicates that as a prevalent system in the biosphere, symbiosis is both ancient and resilient.

For example, it is likely that there were major selection pressures for the endosymbiotic evolution of the eukaryotic cell two billion years ago. We now know that mitochondria resulted from a free-living facultatively aerobic bacteria being assimilated into a chimeric archaea–eubacterium "host." It is likely that this critically important symbiogensis occurred in part due to the environmental pressures of relatively toxic oxygen levels emerging in a substantially anaerobic world. As has so often been expressed, the serial endosymbiotic theory (SET) for the origin of eukaryotic cells resurrected, restructured, and promulgated by the late Lynn Margulis (Sagan 1967) shows clearly the powerful role acquisition-oriented behavior exemplified by symbiogenesis plays in shaping evolution. For nearly a half century, the energy transforming centers of eukaryotes, mitochondria, and plastids have been the *sine qua non* of symbiosis significance in evolution. Yet, it has always appeared as a kind of strange omission or bias that this endosymbiotic basis of so many life-forms and their metabolism—foreign, greatly reduced, but assimilated genomes resulting from symbiotic acquisition—was and remains relegated in textbooks from high school and upward to a page or two or a special sidebar box. With the holobiont-centered revolution in science real and prominent today, this is finally likely to join the newer prolific discoveries as an exemplar of the new evolution paradigm.

The deep time symbiogenesis story is not only about the essential eukaryotic cell components, for there is significant micro- and plant–fossil evidence that symbiosis was an entrenched lifestyle for a variety of organisms through ancient time. One can even think of the dominant microbiogenic features dating back to nearly 3.5 billion years ago (Schopf and Kudryavtsev 2012) and forward through the Paleozoic, the stromatolites, as a kind of ubiquitous precursor to symbiosis on a grand scale. After all, these lithified structures due to binding and trapping of sediment in usually shallow salty water were the creations of a community of bacteria led by specific polysaccharide-excreting cyanobacteria. Moreover, we can be assured that this prokaryotic layering through photoautrophic growth and post-metabolic mineral deposition consistently included substantial gene transfers, such that any given individual bacterium in the community was likely housing genes from neighbors and the past. These stromatolitic structures when still living

entities feature a blue-green color on the rock surface indicating their continued colonization by cyanobacteria and continued growth. Much like the latter biogenic geomorphology represented by limestone generated from secondary symbiont coccolithophorid algal and coral-dinoflagellate holobionts as discussed earlier, stromatolite communities were a dominant biospheric feature with great global ecological importance. These prokaryotic communities became greatly reduced by the Cambrian Period (541–489 mya) as ocean regions became less shallow and less salty and the emergence of a wide variety of algal and cyanobacterial-feeding animals appeared (Schopf 1999).

Dating back to at last 600 million years are the oldest unicellular ancestors of Animalia, the choanoflagellates. Pre-dating sponges, an extant choanoflagellate protist *Salpingoeca rosetta,* has been found to respond to sulfonolipid signaling from associated bacteria that initiates colony formation (Alegado et al. 2012). This is the seed of a fascinating possibility—that multi-cellularity may have arisen through a choanoflagellate–bacterial symbiosis (McFall-Ngai et al. 2013). Sponges actually have choanocytes or "collared cells," much like the choanoflagellate protists. Moreover, nearly all marine sponges are considered now to be symbiotic with wide varieties of bacteria and algae prevalent (Thacker and Freeman 2012). It is striking that these earliest animal forms that remain a highly successful phylum today may be among the most dense and diverse holobiont communities.

Evidence indicates that well before bryophytic and vascular plants, fungi and photoautotrophs were evolving as likely symbioses. The primary terrestrial lifeform most widely associated with symbiosis, lichens, appears now to have had its origins more than 600 million years ago, with the report by Yuan et al. (2005) of hyphae and coccoid cyanobacteria or algae in likely biogenic phosphorite-rich sedimentary rock at Weng'an S. China. In a landmark study, Lutzoni et al. (2001) examined the small and large subunits of nuclear rRNA genes for 52 species from 24 orders of ascomycete fungi that associate as lichens in order to infer the occasions of lichenization and losses of lichenization, as well as to get indications of lichens in more accurate phylogenic placement. The work not only showed lichen symbiosis as more ancient that originally surmised being Late pre-Cambrian in origin, well before the first plants, but that major ascomycete fungal lineages are actually derived from lichen-forming ancestors.

Moreover, electron micrograph examination of fossilized lichens from the lower Devonian (approximately 400–385 mya) indicates, similar to extant lichens, actinobacteria in the medulla layer beneath the photobiont as well bacterial colonies on its surface (Honegger et al. 2013). Reports from the same specialists (Honegger et al. 2009) clearly show well-stratified lichens featuring both cyanobacteria and algae in approximately 415 my strata, while other findings at the Rhynie chert reveal a likely ancient lichen, *Winfrenatia reticulate,* with what are probable filamentous and coccoid cyanobacteria (Karatygin et al. 2009).

One current symbiosis stands out as both very unique and yet with likely deep linkages to ancient terrestrial ecosystems. *Geosiphon pyriforme–Nostoc punctiforme* is one of the few known symbioses involving a fungus and a cyanobacterium. This holobiont grows on soil surfaces and features unicellular bladders about

2 mm long and 0.5 mm in diameter, which house the recognized *Nostoc* filaments. The cyanobacteria are in symbiosomes derived from the fungal plasma membrane. Hyphae are prolific between the symbiosomes (symbiosis-created membranes), and the bladders are substantially chitinous. The *Nostoc* grows and divides within the bladders and produces the non-photosynthesizing specialized spheres (heterocysts) on the filament for nitrogen fixation. It is also photosynthetic in both its sessile colony and its motile hormogonia stages. In fact, there is some evidence it has a higher photosynthetic capacity when associated with the fungus than when isolated (Bilger et al. 2004). The fungus appears to be a likely ancestor of arbuscular mycorrhizal (AM) fungi. Schüssler et al. (2001) showed through SSU rRNA sequencing that both AM fungi and the *Geosiphon* holobiont are a monophyletic group so distinctly separated from other fungi that it constitutes its own new phylum Glomeromycota. This *Geosiphon* symbiosis can be seen as a modern-day remnant of ancient forms that led to mycorrhizal fungi, which in turn later associated with eukaryotic algae en route to initial land plant formation or as an extant more direct AM precursor from which its variations developed into fungal–plant associations. New findings through phylogenetic analysis reveal that six species of liverworts from the earliest diverging clade of land plants, two hornworts and a fern among others associate with Endogone-like fungi (Mucuromycotina) and pre-date the Glomeromycota ancestry back to the mid-Ordovician (475 my) (Bidartondo et al. 2011).

Whether the new endogonaceae family of fungal mycorrhizal data supersede by age or given that the fungi of both the *Geosiphon* and those involved in AM fungi are so similar in features and of the same clade—in either case it is likely that all plant-based terrestrial and even estuarial biomes are and have been foundationally dependent on symbiogenesis at all stages of their evolutionary history.

The initial hypothesis to explain the emergence of plants from a charophycean algal lineage via early mycorrhizal fungi during the Late Ordovician or Early Devonian dates back several decades (Pirozynski and Malloch 1975) has gained further acceptance in more recent years (Turmel et al. 2007; Selosse and Le Tacon 1998). TEM evidence from the fossilized axial prevascular plant *Aglaophyton major* recovered from Early Devonian (419–400 my) strata of the famed Rhynie chert in Aberdeenshire, Scotland, repeatedly shows mycorrhizae fungal infection (Taylor et al. 1995, 2005). Remarkably, other TEM fossil evidence from the same plant and region shows extensive filamentous cyanobacteria colonizing the intercellular spaces of the outer cortex as well as penetrating parenchyma cells within the plant root zone of arbuscular mycorrhizal infection. Often the filaments are seen coiled within the plant cells. Electron micrographs also indicate that entry into the plant is commonly through stomata (Krings et al. 2007a, b). Surface plant openings are often a means of entry in today's plant–cyanobacterial symbioses. For example, in the extant ancient plants *Gunnera* and a variety of cycads, cyanobacterial symbionts enter via surface openings, spread intercellularly, and some become embedded intracellular deeper into the plant structure. Named after a Swedish botanist of the eighteenth century, the herbaceous flowering plant *Gunnera* often features very large leaves of up to 2 m long, and its symbiosis with the cyanobacteria *Nostoc punctiforme* is characterized by prominent glands at the

base of its long petioles through which the cyanobacterial symbiont enters and colonizes. The *Nostoc* fixes nitrogen and is vertically transmitted (directly transferred via in the holobiont germ line rather than horizontally, i.e., being acquired each generation from the environment). This unique plant has been dated back to nearly 100 million years through its distinctive fossilized pollen (Jarzen 1980). These findings in specimens from the lower Ordovician through the Cretaceous lend further credence to the view that symbiosis, even apart from eukaryotic cell origins, is ancient and likely had high selective advantages for organisms, including for transitions to very new environments, adaptation to climate changes, and procuring better access to sustaining resources. Moreover, if the unit of selection is the holobiont as is now being widely considered, natural selection would favor those forms that were able to adapt most quickly, that is, without the extremely slow and usually lethal process of point mutation change.

This speed of symbiogenesis is most readily revealing in the pioneering work of Kwang Jeon. Jeon discovered that one of the amoeba cultures he had been growing in his laboratory become infected with colonies of a still unidentified *Legionella*-like bacterium that could not be separately cultured. These gram-negative rods had the effect of killing off most of the amoebae. However, several amoebae appeared to tolerate the bacterium (Jeon and Lorch 1967). Their numbers peaked regularly at 42,000 per amoeba cell, each sequestered as groups within amoeba-generated membranes or "symbiosomes." Within 18 months or approximately 200 amoeba cell generations, the two genomes became obligately dependent on each other. Indeed, the new symbiosis based on the bacterial infection could no longer coexist with the original amoeba and became restricted to narrow temperature regimes and conditions (Jeon 1995). The emerging amoeba–bacteria holobiont was essentially a new species in the geological time equivalency of a blink of an eye, became the centerpiece of important evolutionary and symbiosis investigations, and continued to thrive through thousands of generations for years after. While the laboratory and its nutrient-filled petri dishes represent an artificially created environment, rather than in nature per se, this series of longitudinal studies extending from 1965 to the present day are nevertheless suggestive of how quickly acquisition of genomes can occur, be viable, and result in potentially new taxa. Increased rates of evolution are also indicated in metagenomic enquiries, including with lichens, wherein Lutzoni and Pagel (1997) showed much higher rates of nucleotide substitutions in nuclear ribosomal DNA in the symbiotic lichenized state and with liverworts associated with fungi than with non-symbiotic associated fungi.

12 The Human Microbiome: A Centerpiece of Symbiogenesis

As 2013 closed out, there were about 1,200 refereed, published articles in journals that appear when the keywords "human microbiome" are inserted. The majority of the titles are mainly in the past six years but date back about ten years. Prior

to that time, there were perhaps a half dozen. Nothing has spurred the renewed recognition of the centrality of symbiosis and bacterial gene movement in our biosphere than this "new" discovery of a biome literally under and including our nose. We as humans and all the mammalian kin and indeed all those that emerged from blastula developmental architecture have joined the rain forests and coral-dinoflagellate reefs as key centers of biodiversity. Due to our proclivity to know as much about ourselves as we can—some would say due to our egocentricity and correspondingly minimal humility in the face of nature—we have poured time, monies, and resounding inquisitiveness into finding out who is inhabiting us and why. Only of course to find out that the us is not *Homo sapiens*, the individual member of a species, but rather *Homo sapiens* the mobile ecosystem comprised of millions of life-forms, indeed more microbes in and on one human body than that human being's total number of cells or to realize that the microbiota of one human body has nearly 100 times more genes that its associated animal "self" (Nelson et al. 2010). While a first reaction might well have been there is more of them than me, we know that we are on the verge of discovering that each one of us was never "I" but always "we" (Gilbert et al. 2012). Could there be alien microbial life in the solar system has now been replaced with what is the function and meaning of the "alien" life in us, the human holobiont community?

The human microbiome, inclusive of interacting bacteria and the less studied viral populations, can be functionally envisioned as a classic wheel model in that the hub of governance and stability is the intestinal organs—the six meter coiled small intestine and the slightly shorter but much wider large intestine. This extraordinary gut system is akin to the hermatypic corals' calcium carbonate reef, as its folds and crevices maximize volume, and house a remarkable diversity of microbial life. Indeed, extensive genomic studies by Eckburg et al. (2005) led them to conclude, "Bacterial diversity within the human colon and feces is greater than previously described, and most of it is novel." The spokes of this hub are the specific array of often bidirectional and biochemical signals to and from the gut microbiota to and from the respective organ systems; namely, immune, circulatory, digestive, reproductive, neuroendocrine, musculoskeletal, and so on, while the wheel rim are these systems to which the spokes are spatially, chemically attached. One could say the outer tire represents the direct contact of this mammalian holobiont with the greater surrounding ecosystems through which the "wheel" traverses. But, what are the evidences for such a scenario and to what degree are such interactions "symbiotic"?

Work in the field of gnotobiology (artificially raised "germ-free" animals) allows one to see whether there are functional deficiencies or defects as compared to those populations raised in a normal microbe-colonized environment. In such studies, gut microbiota were found to be essential for intestinal immune maturation, warding off infections by inducing increased "T" cells (called such for they mature in the thymus). Moreover, the bacterial inducers must be the "correct" recognized ones (Chung et al. 2012). This has implications for medical treatments as well as suggesting that interactions between the human cell and bacterial genomes are likely well-coordinated and not happenstance. In a remarkably

thorough review citing scores of studies, Nicholson et al. (2012) emphasized how the human cells and bacteria are involved in an ongoing "cross talk" through signaling pathways within the immune system and beyond. They point out, "These immune-mediated signaling processes, together with direct chemical interactions between the microbe and the host, act upon multiple organs such as the gut, liver, muscle, and brain…. Multiple bacterial genomes can sequentially modulate metabolic reactions resulting in a combined metabolic process by the microbiome and host genome."

The growing evidence of widespread microbiota controls on the human immune system mirrors the findings with other animal holobionts which often incorporate and maintain a bacterium that confers protection against common infectious agents. For example, *Drosophila neotestacea* is susceptible to infections which lead to sterility by various nematodes. However, those Drosophila that included *Spiroplasma* bacteria as part of its holobiont community were more tolerant of such nematodes and did not become sterile. The bacteria were found to inhibit the actual size and therefore potential reproductive output of the adult female worms (Jaenike et al. 2010). In one of many examples involving *Streptomyces* bacteria, *Philanthus* (a beewolf wasp) larval nests can be overcome by infecting bacteria and fungi, not unlike the threats to the subterranean nests of the leaf-cutter ant. However, most such beewolf wasps are now able to be more protective of offspring through the development of glands at the base of the mother's antenna which house the antibiotic-producing *Streptomyces* spp. The mother wasp actually actively secretes the liquid containing the actinobacteria onto the developing offspring as they spin their cocoons (Seipke et al. 2011). In another example, this one involving vertebrates, the colorful bird revered in Egyptian history and other venues, known as the European Hoopoe (*Upupa epops*) accesses secretions from its uropygial gland through preening. These secretions contain specific volatile chemicals produced by bacterial symbionts, such as *Enterococcus*, which reduce potentially deleterious high numbers of diverse bacteria in this avian holobiont (Martin-Vivaldi et al. 2010).

The human microbiome also shows some affinity with other animals when evaluating the digestive tract. Bacteria as well as some protists and fungi in many vertebrates, birds, reptiles, and amphibians convert food materials to absorbable nutrients and ferment carbohydrates into short-chain fatty acids which foster energy and ultimately allows for more efficient absorption of salts and water. In some herbivores, the larger gut capacity through the presence of a foregut allows for additional fermentation by a microbial community that synthesizes proteins and B vitamins (Stevens and Hume 1998). In the human digestive tract, while the diversity of microbes changes radically with different food intake, Wu et al. (2011) founded specific characteristic bacteria or "enterotypes" associated with long-term diets that dominated the gut microbiome and were not easily altered. *Bacteroides* spp. predominated in diets with high animal fat and protein, while *Prevotella* spp. was the enterotype for high carbohydrates diets. This reinforces the view that food intake is a significant contributor to the human microbiome and once acclimated are not in the short-term susceptible to major change. Pepper and Rosenfeld

(2012) emphasized the need more than ever to see animal bodies as ecosystems and suggest that the consistently more static enterotypes combined with often shifting larger microbial populations reported in the human microbiome may be an example of "multi-stability," in that the bacteria–human holobiont has evolved to stay relatively stable under wide-ranging conditions. This can be compared to migrating species or seasonal eutrophication in larger ecosystems wherein the stability of the latter is not fundamentally altered. The diet-microbiome linkage has ramifications for sickness and obesity study (Ley et al. 2006) in that the pathway of chosen external foods to core gut bacteria to then degree of body size and eventually "good health" may be significantly intertwined. Moreover, the linkage of the mammalian microbiome to global ecology resonates profoundly with research reports from Dominguez-Bello and her team (Clemente et al. 2015). Their extended microbiome studies on the isolated Yanomami indigenous peoples of Venezuela show levels of microbial diversity far in excess to what has been measured in the microbiome of modern western civilization cultures. The results imply that modern day eating habits and related behaviors may strongly limit microbiome potential and ultimately human health. This research opens an exciting and potentially a revealing pathway to understanding the evolution of the microbiome within the mammalian holobiont.

At first, suggestions that the brain may be subject to microbiome influence seems far-fetched, even science fiction, until we simply realize that the brain like all other body organs depends on intake of nutrients conveyed by the bloodstream from the intestines. And, if nutrient supplies, catalysts, processes, degraders, recyclers, and signalers are substantially microbial, the connection becomes profoundly logical. Indeed, Nicholson et al. (2012) pointed out and McFall-Ngai et al. (2013) reinforced that as much as one-third of the metabolites that are distributed through our blood circulatory system to our body organs are of gut microbial origin.

Neuroscience, microbiology, and ecology have begun a prolonged and essential meeting at the human microbiome. The growing number of research papers on this aspect is a testament to this. Particularly noteworthy are the detailed studies such as by Heijtz and his team in Stockholm, Sweden (2010). They found in repeated testing with mice that germ-free mice and normal microbiota (specific-pathogen-free) mice differed significantly in motor control and anxiety behaviors. However, if germ-free mice were exposed to normal gut microbiota very early in life, they display behaviors and motor control similar to the mice with normal microbiota. Human microbes particularly target, they discovered, two key synaptic proteins, PSD-95 and synaptophysin. Intriguing and profound linkages usually involving complex chemical signaling of the gut to the brain and vice versa are being consistently reported (Wang 2002; Forsythe et al. 2010).

The unfolding of the human microbiome energized by the Human Genome Project certainly puts ecology front and center as the science of what we formerly would call the individual. Still more revelatory is that under our symbiotic definitions, including the new one proposed in this chapter, symbiosis can be seen as both prevalent and governing in the functioning of all megafauna and megaflora.

13 Summary

The impacts of symbiosis and symbiogenesis on evolution, ecology, and earth science include the following:

1. *Establishes essential novelty upon which natural selection "acts" through the acquisition of nonself genomes which have a vastly different phylogeny. The emerging holobiont is then further acted upon by natural selection, resulting in a new organism and often the start of expansive lineages. The primary unit of selection is the multi-genomic holobiont.*
2. *Reveals that the integration of genomes from vastly different lineages often fosters new geodynamic substrates—reefs, calcium carbonate/marble deposits, caves, and Ficus-enriched forest canopies—that become physical substrates and habitats for the emergence of novel "communities" and expanding lineages.*
3. *Biome and ecosystem foundations extend deep into the fossil record. Symbiosis was likely ubiquitous in the biosphere from the late Proterozoic through the Phanerozoic to the present. Symbionts can thus often be seen as foundational and serve as "keystone" expressions for both the specific holobiont within the larger ecosystem in a macroevolution perspective and for holistic systems development from an earth history and homeostasis view.*
4. *Renders the concept "individuals" among eukaryotes as mythical. The "self" is incomplete and non-functional without the integration of foreign genomes and frequent gene transfers from "foreign" bacteria and viruses. The reality in the biosphere is that all eukaryotes are actually metagenomic entities functioning as an integrated community, the holobiont. Prokaryotes are often significant symbionts in and on eukaryotic holobionts, albeit the prokaryotic cell itself is a holobiont more from consistent gene transfers than whole genome assimilation.*

14 Epilogue: The Insightful Proponent of Symbiogenesis and the Concept of the Holobiont, Lynn Margulis

The distinguished researcher Margaret McFall-Ngai and her colleagues conducted revealing and often elegant work with the dynamic *Euprymna–Vibrio* bioluminescence research over many years and thereby helped pave the way for the new symbiosis-centered paradigm for life on earth. Her review of this new perspective published with many accomplished symbiosis research colleagues (2013) as well as the brilliant treatise of Gilbert et al. (2012) are already seen as historic contributory bridges to the holobiont perspective and symbiogenesis. In the former paper, McFall-Ngai et al. remarked, "For much of her professional career, Lynn Margulis (1938–2011), a controversial visionary in biology, predicted that we would come to recognize the impact of the microbial world on the form and function of the entire biosphere, from its molecular structure to its ecosystems. The weight of evidence supporting this view has finally reached a tipping point…."

In this context, I share a recorded, previously unreleased interview excerpt that I conducted with the late Lynn Margulis, who was a friend and frequent mentor for over three decades and whose course she passed on to me when she left Boston University and which I taught with the appropriate major updates for twenty-five years. Designed for those just beginning to explore the importance of symbiosis, Lynn informally reviews in this excerpt a few Darwinian basics and discusses some key differences of symbiogenesis and neo-Darwinism. Rather than excerpting words from her extensive publications, which are deserving of the reader's more prolonged attention and study, I share here this brief portion of the interview, focusing particularly on the centrality of symbiogenesis in evolution. (Margulis 2009, interview by Douglas Zook, video recorded by Michael Lee and video/audio edited by Divya Madhavan):

What do virtually all eukaryotes, even diatoms, do that no prokaryotes ever do? Eukaryotes can take up new genomes which may ultimately be symbionts essentially a genome at a swallow. And, that is the crucial point. Eukaryotes have steroid-containing membranes. They open the membranes and they take things in, and they can of course digest those organisms, but they do not have to… If the digester is resisting and under conditions where the digester and the potentially digested then live together for an extended period, you tend to have these associations. So with eukaryosis, there is this ability to open membranes, close them with a foreign genome enclosed, and both survive! That is something you don't see in bacteria. Now, we know that bacteria have invented just about all the main metabolic processes for life….nitrogen fixation, methanogenesis, sulphide reduction, sulfur oxidation, and of course chemo- and photoautrophy. We could go on and on… But the getting together is pretty weird in bacteria. While they form tight communities, their relations are substantially external. They are practitioners of syntrophy, where one produces one product and the other uses it. Are you not amazed with fertilization in eukaryotes?! In fertilization, you open a membrane and something comes in exclusively and closes it again. That's what is going on in symbiogenesis… We have phagocytosis, exocytosis, endocytosis…. We have all these fancy words, but we don't have the intellectual understanding yet that these are all words for basically the same kind of common central process in evolution. And its prevalence in evolution shows us that symbiogenesis becomes the rule of speciation, innovation, higher taxa formation, once you have a eukaryotic world which is always superimposed on a prokaryotic world. The prokaryotic world of course remains and thrives, but members can also be assimilated into the eukaryotic structure.

Ernst Mayr said it well when he pointed out that when you are concerned with evolution, you cannot simply be an evolutionist. It is a multi-component theme. There are many processes involved. What are they? Darwinian evolution has these main components. The tendency of all populations of organisms is to grow exponentially, beyond what the resources available can support. An example is the fungi *Alternaria fusarium* which make 100-150,000 spores per minute for six months. Of course they are growing on a tree. Humans have the potential to have 20-21 children per couple. The bacteria that we can see and count…a single bacterium doubles to two, four, eight, sixteen, thirty two, and so on. A single bacterium can generate the weight of the earth unchecked in less than a week. The potential to grow is everywhere, and that potential is never remotely close to being reached…. And it can be studied in orchids with their tiny seeds, plants that grow vegetatively like the philodendrons here where we sit…every organism can theoretically have a number associated with it, which we call its biotic potential, that is the number of organisms produced per unit time or translated to the number of organisms produced per generation. This is characteristic of all life, always. The fact that the biotic potential is not reached…that we don't have a bacterial planet that is only saturated with bacteria, that is what we call natural selection. Natural selection is the elimination of organisms, the what is left over – because they always have "checks," as Darwin would call it. Checks

are, among many, lack of food, lack of water, lack of space, disease. Those are among the agents of selection. We have wonderful examples of protective coloration where the animal is in a proper environment and it is completely hidden. Those organisms will not be selected against relative to that same animal just a few meters away that is exposed. Natural selection is the fact of biotic potential, which is measurable and is not reached.

So natural selection within evolution maintains what is already there with respect to that environment….It is all about the ones who have made it through to reproductive age and had offspring which then had offspring. And, of course the vast majority of all species to have been recorded on the earth are extinct and the vast majority of all offspring do not move on to produce more offspring indefinitely. In the human species, it acts mostly at the level of two billion sperm per ejaculation and often not even one gets through to fertilize! So, there is a huge example… So with every organism you can show that there is the potential to grow new offspring, and it is not reached.

Now what is the essential difference between the symbiogenic view of evolution and the standard neo-Darwinian view of evolution? Darwin was quite different than neo-Darwinian, indeed he was more Lamarckian in many ways. Well. you and I were taught that the source of all variation, the differences from parents, are the accumulation of random mutations. I remember being told that there was direct evidence that all offspring are not exactly like their parents, and there are lots of reasons for that. And, as Darwin said, we are only interested in the variation that is important to us, and by that he meant the inherited variation. So we are looking at inherited variation – color of our eyes, your blood type, skin, hair qualities and so on with respect to people. There's this variation in traits that are of real interest to evolutionary processes because they have 100 % heritability potential for example. These high heritability traits can be measured. From generation to generation the probability of laying 12 eggs during a week in a season or something like that. This can be inherited. Now here are variations from parent to offspring whether they are non-sexually produced from one parent or whether there are two parents, the source of the inherited variation as told to me and in every book is random mutations. And, when there is enough random mutations accumulated, you have new species. So the main unit of variability is said to be mutational changes in base pairs of DNA, and there's of course recombination and immigration and emigration in natural populations. These are listed as the sources of inherited variation. This is where I part company, not with Darwinism but with neo-Darwinism.

Take a Drosophila and induce random mutations. You will get a sick or dead Drosophila. You don't get a new species. It is nearly always deleterious. I have looked for years for examples of how mutations produce a new species in any literature. Even the best examples from neo-Darwinists involve the acquisition of mycoplasms or other bacteria. The main way that inherited variation is positive, that is it gives you new changes that Lamarck did not understand, is not of inherited characteristics but of entire genomes, bacterial genomes or fungal genomes. There's lots of different examples of course. The random mutations hone, modify, modulate and yes this is important. But when you acquire and integrate a whole genome, you gain the key component in evolution - variability - which often results in speciation. For example, you get a slug that gropes around eating in translucent environments and it is taking in chloroplasts and that animal turns green relative to its non-chloroplast relatives, and in one step, much like punctuated equilibrium, you get a new species. My favorite example is actually the Convoluta symbiogensis examples. Convoluta convoluta is a little flatworm, and it eats and digests all sorts of algae on the western European shorelines but does not retain them. But *Convoluta roscofensis* is a new species from that non-symbiotic lineage. It is green because it took in but did not digest certain Platymonas algae. Every member of the population is green and has phototactic responses. They are all photosynthetic except the eggs, which hatch out, feed and digests other microbes and eventually assimilates the alga it is programmed to recognize. *Convoluta paradoxa* on the other hand is brown, solitary, grows in a different way, is found in a different habitat and has different symbiotic algae, diatoms. There, through these three we can see genome acquisition, variation and hence speciation through symbiogenesis (Fig. 5).

Fig. 5 Author of this
chapter, Biologist Douglas
Zook with Lynn Margulis at
Boston University, 2009 in
an image from previously
unreleased video interview.
Photograph by Michael Lee

Glossary

Following are selected terminology defined by the author that may be of use to
some readers.

Arbuscular Branching tree like hyphae of mycorrhizal fungi within, but not entirely enclosed,
plant root cells

Actinobacteria Filamentous bacteria commonly found in soils and featuring an array of antibi-
otic chemistry

Archaeans Microscopic organisms that thrive in "extreme" temperature or saline condi-
tions. They have many biochemical and genetic features that are closer to eukaryotes than
prokaryotes

Ascomycete Small craterlike features on the surface of fungi and lichens, from which spores are
emitted

Bacteriocytes Specialized intracellular regions of many insects that house symbiotic bacteria
which are transmitted via the insect egg and often grouping during the life cycle to form
functional organs known as bacteriomes

Bioaugmentation Any intervention by humans that seeks to promote the viability and fitness of
a holobiont (organism) living in non-anthropogenic nature

Chimera In the context of a holobiont, it is a collection of different genomes interacting as one
entity

Coccoliths The plates of calcium carbonate (limestone) surrounding holobionts known as coc-
colithophores. These algae in the group haptophta build these structures as part of their outer
covering

Endemic A species that is characteristic of a biogeographical region over a significant period of
geologic time

Extant In the context of biology and evolution, organisms or conditions from more ancient geo-
logical time that have persisted to the present

Endophytes Bacteria or fungi that live symbiotically in between or within plant cells

Epibiotic An organism lives on the surface of another different organism. It may or may not be symbiotic

Facultative An organism that functions with clear options such as being to live in either aerobic or anaerobic conditions

Gnotobiology The study of organisms living in an artificially created environment, namely in conditions where no other living organisms are present

Heamatophagous The ability of certain animals to penetrate body parts of other organisms and feed on blood

Hermatypic Coral–dino holobionts that build exoskeletons known commonly as reefs, as opposed to many corals which do not extrude limestone and thus known as a hermatypic

Holobiont Any living entity (all eukaryotes and rarely some prokaryotes) made of two or more different symbionts—minimally a so-called host species and different symbiont species

Horizontal transmission The passing of a symbiont to following generations through one symbiont acquiring the other symbiont from the environment

Karst Geological formations usually created by the dissolution of carbonate rocks such as limestone

Lithosphere The outermost section of the solid earth, frequently referred to as "crust" but encompassing as well somewhat deeper layers, such as the upper region of the mantle. Much of the lithosphere can be considered part of the region where life can be found, known as the biosphere

Metagenomic The collection of genomes from different organisms as collected directly from the natural environment as opposed to laboratory cultures

Microbiogenic Geological structures and features which are the result of living microbial processes and depositions

Nucleomorph A genetic fraction or remnant of a previously complete nucleus from an alga and now embedded in a new alga with its own nucleus

Pedosphere The outermost layer of the solid earth composed of the soil and rock eroding regions

Peptidoglycan A chemical compound made up of sugars and amino acids that forms a mesh-like cell layer known as the bacterial cell wall. It is the defining characteristic of eubacteria, for it is not found in the microbial domain, Archaea

Phagocytosis The process whereby a cell, usually a eukaryotic one, or an organism envelopes and then internalizes materials or other organisms from the surrounding environs

Rhizosphere The soil regions among the roots of plants, including the organisms and all their interactions

Rumen The specialized first section of the alimentary canal of many hooved animals, wherein fermenting, cellulose-producing microbes are housed

Stromatolites Lithified structures built by the trapping, binding, processing, and then deposition of sediment by cyanobacteria. They are prominent in the fossil record and serve as evidence that our oxygenated atmosphere was substantially the result of cyanobacterial metabolism

Syconium The section of the Ficus (fig) tree that becomes a fruit, but initially is a completely enclosed structure with numerous internal flowers. Only its holobiont specific partners, certain fig wasps can gain entry and promote the necessary pollination

Symbiosome A specialized membrane usually substantially formed by the "host" member of a holobiont which completely encloses the entering or captured symbiont

Syntrophy One species lives off the products of another organism

Thallus The living structure built by the algal–fungal lichen symbiosis. It bears little or no resemblance to the morphology of either the fungus or the alga. Some lichens have a cyano-bacterial holobiont partner which also contributes to its development

Trophosome A specialized symbiosis-created food-processing organ which houses sulfur oxi-dizing and other bacteria, in deep sea vent tube worms

Vertical transmission The persistence from generation to generation of a symbiont(s) through direct transfer via the "host," often through incorporation within or attachment to an egg

Viviparous Animals which produce live young emerging from the body as opposed to the depo-sition externally of eggs

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