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Endosymbioses Have Shaped the Evolution of Biological Diversity and Complexity Time and Time Again

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Abstract

Life on Earth comprises prokaryotes and a broad assemblage of endosymbioses. The pages of *Molecular Biology and Evolution* and *Genome Biology and Evolution* have provided an essential window into how these endosymbiotic interactions have evolved and shaped biological diversity. Here, we provide a current perspective on this knowledge by drawing on decades of revelatory research published in *Molecular Biology and Evolution* and *Genome Biology and Evolution*, and insights from the field at large. The accumulated work illustrates how endosymbioses provide hosts with novel phenotypes that allow them to transition between adaptive landscapes to access environmental resources. Such endosymbiotic relationships have shaped and reshaped life on Earth. The early serial establishment of mitochondria and chloroplasts through endosymbioses permitted massive upscaling of cellular energetics, multicellularity, and terrestrial planetary greening. These endosymbioses are also the foundation upon which all later ones are built, including everything from land–plant endosymbioses with fungi and bacteria to nutritional endosymbioses found in invertebrate animals. Common evolutionary mechanisms have shaped this broad range of interactions. Endosymbionts generally experience adaptive and stochastic genome streamlining, the extent of which depends on several key factors (e.g. mode of transmission). Hosts, in contrast, adapt complex mechanisms of resource exchange, cellular integration and regulation, and genetic support mechanisms to prop up degraded symbionts. However, there are significant differences between endosymbiotic interactions not only in how partners have evolved with each other but also in the scope of their influence on biological diversity. These differences are important considerations for predicting how endosymbioses will persist and adapt to a changing planet.

Key words: endosymbiosis, prokaryotes, eukaryotes, biodiversity, genome evolution, coevolution.

Significance

Understanding how endosymbiotic interactions have coevolved and shaped life on Earth is critical to developing comprehensive theories and predictions in biology. In this perspective, we compare and contrast how major endosymbioses—starting with eukaryogenesis—have influenced biodiversity and the mechanisms that underlie their evolution. We reflect on how significant differences between categories of endosymbioses should influence our theories and predictions about their persistence in nature.

This Perspective is part of a series of articles celebrating 40 years since *Molecular Biology and Evolution* was founded. It is accompanied by virtual issues on this topic published by *Genome Biology and Evolution* and *Molecular Biology and Evolution*, which can be found at our 40th anniversary website.

Introduction

The evolution of all life—from prokaryotes to complex multicellular eukaryotes—has been shaped by symbiotic interactions with the immense microbial diversity that exists on the Earth (McFall-Ngai et al. 2013). Such interactions generally range from antagonistic to beneficial, placing distinctive evolutionary pressures on the interacting partners (Lynch and Hsiao 2019; Drew et al. 2021). In recent years, beneficial symbioses—including facultative and obligate interactions—have become much better understood as important drivers of biological complexity and diversity (Archibald 2014; Douglas 2014; McFall-Ngai 2015; Chomicki et al. 2019; Perreau and Moran 2022). These interactions are diverse in terms of the phylogenetic array of hosts and microbes involved, the specific services that each provides, and the evolutionary mechanisms employed to sustain them. While such beneficial symbioses are diffuse among biological life, endosymbiotic interactions—microbes living inside the cells of a host—are among some of the most ancient and complex biological interactions known (Archibald 2015a). They generally arise when unexploited resources are available but out of reach for potential hosts (Moran 2007). By bridging distant peaks between adaptive landscapes, endosymbionts provide novel phenotypes to their hosts that unlock environmental resources (Fig. 1; Lynch and Hsiao 2019). As a result, endosymbionts are often removed from the open environment and become wholly dependent on their hosts (Bennett and Moran 2015; Drew et al. 2021). Thus, endosymbioses are paragons of coevolution. They entail the complex evolution of integrated genomes, host tissues and organs, novel host cells and cell structures, and mechanisms of resource exchange and communication between the domains of life (Keeling 2013; Martin et al. 2015; Wilson and Duncan 2015).

Over evolutionary time, endosymbioses have become ecologically pervasive, playing integral roles in shaping—and reshaping again and again—Earth's biological diversity. One could summarize life on our planet as comprising just the prokaryotes and a broad union of organisms derived from endosymbiotic interactions (i.e. anything with mitochondria, plastids, and beyond; Yutin et al. 2008; Martin et al. 2015; Archibald 2015a; McCutcheon et al. 2019). As such, there is an intrinsic and even urgent need to understand the biology and ecology of these interactions. This knowledge is key to discerning the main origins and drivers of biological diversity, as well as to clarifying even our most basic biological and evolutionary theories. Pursuing such grand research goals requires the application of evolutionary principles. This framework elevates questions of "*how* endosymbioses function" to "*why* they function," "*where* they came from," and "*why* they even exist and persist in nature." It further provides a predictive
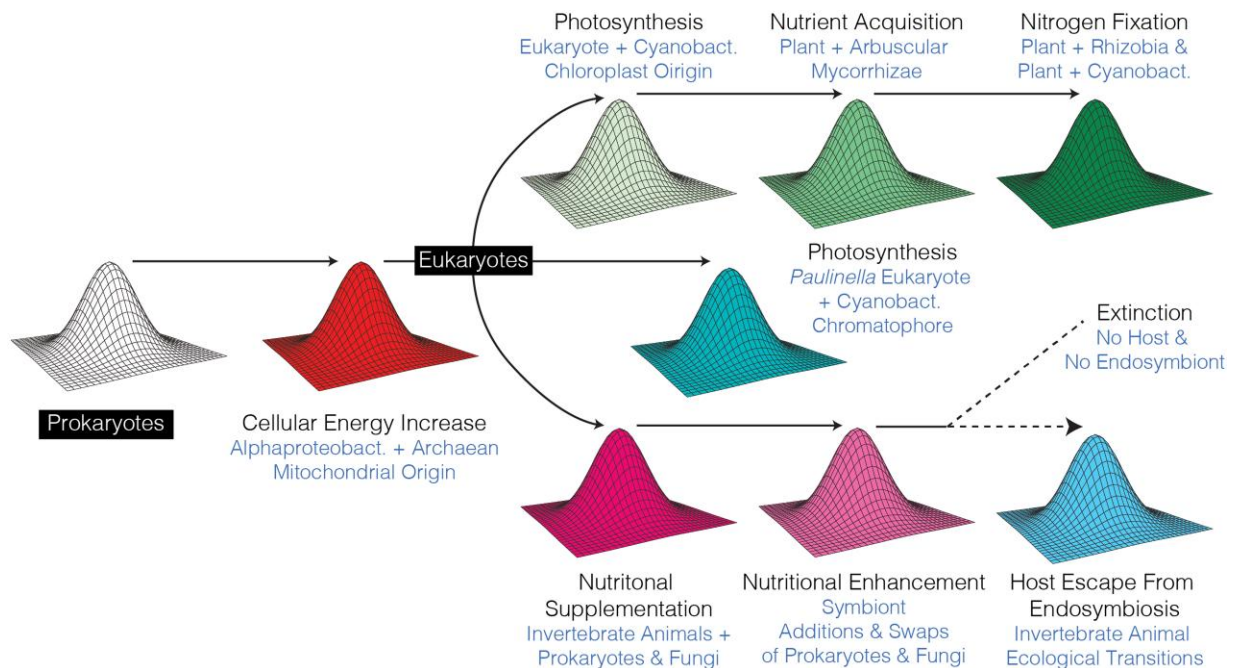
FIG. 1.—Simplified summary of the major endosymbiotic events that have led to significant leaps in the biological diversity and complexity of life. Endosymbionts provide novel phenotypes to their hosts permitting them to leap between adaptive landscapes with new trait axes and peaks. Except for the establishment of mitochondria during eukaryogenesis, all other endosymbioses are built on the more ancient ones that preceded them. Arrows track the evolutionary progression of these interactions. Peaks and labels illustrate some of the major endosymbiotic events.

framework to project our understanding well past our contemporary moment in the evolution of life on Earth.

Building an evolutionary framework into endosymbiosis research necessitates developing a baseline understanding of the diversity, origins, and evolutionary processes underlying these interactions. Over the past 40 years, researchers publishing in the journals, *Molecular Biology and Evolution* (*MBE*), and later *Genome Biology and Evolution* (*GBE*), have tackled these questions and greatly expanded our knowledge of endosymbioses. What we have learned is profound and voluminous. We take readers through this literature, tackling two basic questions: *How have endosymbioses shaped life*? And *how do endosymbioses evolve?*

A Primer on How Endosymbioses Shaped Biological Diversity

With ever more sophisticated molecular tools and technologies, researchers publishing in *MBE* and *GBE* have traced the evolution of endosymbioses up and down the tree of life. Early phylogenetic approaches permitted the identification of endosymbiotic partners for many systems and the development of hypotheses about the origin and evolutionary processes shaping these interactions (Moran 1996; Peek et al. 1998; Spaulding and von Dohlen 1998; Pisani et al. 2007). But the greatest accelerant of our understanding of endosymbioses—particularly since few endosymbiotic microbes can be cultured—is the advent of next-generation molecular sequencing (McFall-Ngai 2015). These technologies cheaply expanded the ability to collect complete molecular information for all symbiotic partners (genomes, transcriptomes, proteomes, epigenomes, etc.) across populations, species, and groups (Brown et al. 2015; Chong et al. 2019; Shinzato et al. 2021; Sun et al. 2021; Gould et al. 2022). Research employing these approaches has yielded novel and refined theories of how endosymbioses function and evolve (e.g. McCutcheon and Moran 2010; Sloan et al. 2014; Shapiro et al. 2016; Yang et al. 2020; Ip et al. 2021). Along the way, we have learned that endosymbionts appear to come from almost everywhere and do almost everything. They provide an array of metabolic and physiological services to their hosts, including the exponential upscaling of cellular energy and the bridging of nutritional deficits on land and in the sea (Lane and Martin 2010; Hansen and Moran 2014; Sogin et al. 2021). From eukaryogenesis to the ability of insects to feed on plants, endosymbiosis has been a perpetual driver of biological complexity and diversity (Fig. 1; Archibald 2014; Mills et al. 2022).

The Original Endosymbioses

The first known endosymbiosis of major biological significance occurred ~1.8 billion years ago when Asgard archaean formed an obligate endosymbiotic relationship with an alphaproteobacterium (Sagan 1967; Fitzpatrick et al. 2006; Pisani et al. 2007; Yutin et al. 2008; Williams et al. 2013; Raval et al. 2023). This relationship gave rise to the mitochondria (also mitosomes and hydrogenosomes) and eukaryotes writ large. The precise origins and steps in the coevolutionary integration of the endosymbiotic interaction have been long debated (Thiergart et al. 2012; Williams and Embley 2014; Geiger et al. 2023). (Note: 1.8 billion years of Earth's history is an immense amount of time and space for evolution to scramble its tracks—a common theme in endosymbiosis research.) Nevertheless, recent theory suggests that mitochondria arose in anaerobic conditions through the dependence of methanogenic archaea on H_2 provided by an alphaproteobacterial ancestor (Martin and Müller 1998; Mills et al. 2022). The permanent establishment of this alphaproteobacterium into the mitochondria greatly expanded the cellular energy budgets of single-celled and multicellular eukaryotes, further assuming roles in cell cycle regulation, signaling, apoptosis, etc. (Gray et al. 1999; McBride et al. 2006; Roger et al. 2017). The benefit of abundant and localized energy vis-à-vis the mitochondria was a necessary preadaptation for establishing *all* other endosymbioses that have followed. The mitochondria, by transferring genes to the nuclear genome, also provided genetic toolkits for integrating and sustaining later endosymbioses in more complex hosts (e.g. nutritional endosymbioses in some insects; reviewed by Mao et al. 2018).

Relatively soon after eukaryogenesis (~1.5 billion years ago), an ancestor to the Archaeplastida (algae and plants) established another significant endosymbiosis with a cyanobacterium (Yoon et al. 2004; Rogozin et al. 2009; Keeling 2013). This relationship led to the primary establishment and evolution of chloroplasts, eukaryotic photosynthesis, massive increases in global primary productivity, and the literal greening of Earth (Moreira et al. 2000; McFadden 2001). Mitochondria were a necessary partner for this event. It provided the fundamental bioenergetic framework to leverage solar energy and the protection of chloroplasts during cellular stress conditions and in the darkness of night (Hoefnagel et al. 1998; Lane and Martin 2010; Mills et al. 2022). In return, the chloroplast endosymbiont provided a ready food source and metabolic support to its mitochondrial partner (e.g. sugars and oxygen; Raghavendra and Padmasree 2003; Oikawa et al. 2021). As a result, some mitochondrial and chloroplast metabolic activities are linked and coregulated by their hosts (Zhang and Glaser 2002; Zhao et al. 2020; He et al. 2023). Some of their essential functions are even supported by the same dual-targeted genes (e.g. essential t-RNA synthesis; Peeters and Small 2001; Carrie et al. 2009; Yogev and Pines 2011). Thus, mitochondria and chloroplasts are, perhaps, the first dual endosymbiosis—a relatively common feature of later endosymbioses of plants and invertebrate animals.

The ability to photosynthesize is such an important adaptive eukaryotic trait that ancestrally nonphototrophic

eukaryotes (ancestors to euglenids, apicomplexans, cryptomonads, etc.) have repeatedly stolen the ability (Yoon et al. 2005; Baurain et al. 2010; Dagan et al. 2013; Keeling 2013). Over evolutionary time, and through the process of secondary endosymbiosis, nonphotosynthetic eukaryotic hosts have intracellularly acquired archaeplastid endosymbionts many times and sometimes repeatedly (e.g. tertiary endosymbiosis; Douglas 1998; Keeling 2013). However, the arrangement for the interned photosynthetic eukaryote is less than ideal. The secondary host essentially dissolves the archaeplastid and absorbs necessary genes into its genome to sustain their stolen plastid (Archibald 2015b; Ponce-Toledo et al. 2019). Endosymbioses derived from secondary, tertiary, etc. origins have led to key biological diversity with Earth-changing outcomes (Dorrell and Howe 2015). For example, *Symbiodinium* dinoflagellates, the product of a red algal secondary endosymbiosis, are themselves endosymbionts of a wide range of marine invertebrates (jellyfish, anemones, nudibranchs, etc.; LaJeunesse et al. 2018; Liu et al. 2018). Notably, *Symbiodinium* in corals enabled the massive diversification of marine reef systems, which are among the most diverse, important, and threatened ecosystems on the planet (Plaisance et al. 2011; Levin et al. 2016).

The Later Endosymbioses That Shaped Plant and Animal Diversity

Beyond the serial establishment of mitochondria powerhouses and chloroplast primary productivity, disparate eukaryotic lineages have continued acquiring additional endosymbionts. These "later" endosymbioses provided key adaptive advantages to their hosts that generally include novel metabolisms and enhanced access to environmental resources (Moran 2007; Archibald 2014). They have occurred in everything from single-celled eukaryotes (e.g. *Paulinella*; Marin et al. 2005; Nowack et al. 2008, 2011) to more biologically complex plants and invertebrate animals (e.g. Fabaceae leguminous plants, some marine deep-sea vent invertebrate animals, and hemipteran plant-feeding insects; Sloan et al. 2014; Manzano-Marín et al. 2015; Warshan et al. 2018; Ip et al. 2021). The importance of these endosymbioses in shaping biodiversity is now well in view.

The diversity and success of land plants are attributable, in part, to endosymbiotic interactions with arbuscular mycorrhizae (AM; Parniske 2008; Bonfante and Genre 2010) and nitrogen-fixing cyanobacteria and rhizobia bacteria (Kiers et al. 2003; Warshan et al. 2018). Up to 80% of land plant species engage in endosymbiosis with the ancient Glomeromycota AM (Bennett and Groten 2022). By extending plants' abilities to scavenge essential nutrients from soils (e.g. nitrogen, phosphorous, and minerals; Parniske 2008), endosymbiosis with AM provided a key adaptive advantage for plants in the terrestrial environment. This relationship was also likely an important factor

in the early success of land plants as they established on Earth's barren landscapes >450 million years ago (Delaux and Schornack 2021). In contrast, endosymbioses between land plants and bacteria are comparatively restricted, possibly because plant cell structures and physiology limit intracellular invasion by microbes (Geurts et al. 2016; Delaux and Schornack 2021). Nevertheless, endosymbiotic bacteria in plants have contributed to the ecological success and diversification of several important plant groups. Nitrogen-fixing cyanobacterial endosymbioses have independently evolved in a range of hosts that include *Gunnera*, some liverworts, cycads, and ferns (Rikkinen 2017; Warshan et al. 2018; Delaux and Schornack 2021). Similarly, root-nodulating rhizobial endosymbioses have led to the diversification of the ecologically and agriculturally important group of plant orders that includes Fabales, Cucurbitales, and Rosales (Markmann and Parniske 2009).

Invertebrate animals have been particularly successful at establishing additional endosymbioses. In an evolutionary framework, these exceedingly diverse interactions permitted hosts to thrive in totally unsuitable environments, often leading to global-scale adaptive species radiations (Moran 2007). For example, a wide diversity of marine invertebrates (e.g. some clam, mussel, and snail species) ally with chemosynthetic bacterial endosymbionts for CO_2 fixation into consumable biomass and sugars (Ozawa et al. 2017; Sogin et al. 2020; de Oliveira et al. 2022). These endosymbioses permit their hosts to dominate some of the most extreme and energy-limited environments on Earth, including oceanic sediments and deep-sea thermal vents (Sogin et al. 2021). But this diversity of endosymbioses in invertebrate animals is just the tip of the iceberg.

Far more diverse groups of invertebrate animals, including some nematodes and insects, owe their origins to endosymbioses (Jiggins et al. 2002; Brown et al. 2015; Chong et al. 2019). In particular, insect endosymbioses have received intense attention in recent years. Insects have been dubbed a "fairly land" of endosymbiosis and for good reason (Buchner 1965). These endosymbiotic interactions are responsible for at least 20% of insect species diversity (>1 million species) and underlie their terrestrial dominance (Douglas 2011). Origination events are generally ancient (e.g. tens to hundreds of millions of years old; Bennett and Moran 2013; Patiño-Navarrete et al. 2013) and are too numerous to summarize (e.g. at least ~50 independent origins in the order Hemiptera, alone; Bennett and Moran 2015; Sudakaran et al. 2017). The principal role of insect endosymbionts is to provide nutrition lacking in host diets (Hansen and Moran 2014). For example, plant sap-feeding insects in the hemipteran order (cicadas, leafhoppers, aphids, whiteflies, etc.) have acquired a diverse array of bacteria to provide essential amino acids lacking in their plant phloem and xylem diets (Sloan and Moran 2013; Santos-Garcia et al. 2014; Mao et al. 2017; Garber et al.

2021). Similar nutritional interactions are known from a broad diversity of insect groups, including ants, cockroaches, and tsetse flies to name just a few (Williams and Wernegreen 2012; Medina Munoz et al. 2017; Kinjo et al. 2018). Insects often take these interactions much further by acquiring multiple endosymbiotic bacteria and fungi that have completely different origins and that make distinct contributions to their endosymbioses (McCutcheon and Moran 2010; Weglarz et al. 2018). They are also actively evolving ever-novel endosymbioses with environmental bacteria (Oakeson et al. 2014), leading to the routine replacement of ancient endosymbionts with younger ones (Sudakaran et al. 2017).

Evolving Biologically Complex Endosymbioses

A major area of interest in evolutionary biology—and Society for Molecular Biology and Evolution (SMBE) journals—is the evolutionary processes that shape the establishment, integration, and long-term persistence of endosymbioses. We have previously described this process as an ever-deepening and spiraling "rabbit hole" (Bennett and Moran 2015). In this framework, endosymbioses are shaped by at least four key factors: (i) the metabolic purpose of the endosymbiosis, (ii) the number of partner endosymbionts involved, (iii) the host's abilities to transmit and support their endosymbionts across generations, and (iv) the age of the endosymbiosis. The first two factors dictate the minimum genetic repertoire endosymbionts must retain to fulfill their host-dependent functions (e.g. oxidative phosphorylation pathways in mitochondria and nutritional pathways in invertebrate animal endosymbionts; Gray et al. 1999; McCutcheon and Moran 2010). Many symbiotic systems further depend on multiple collaborative endosymbionts to provide single metabolisms (e.g. chloroplast–mitochondrial interactions and dual nutritional endosymbioses in insects; Douglas 2016; Gossett et al. 2023; He et al. 2023). The third factor dictates how strongly host selection and drift are in shaping endosymbiont evolution and the extent to which hosts can support their endosymbionts across generations (Jiggins et al. 2002; Salem et al. 2015; Leftwich et al. 2020; Perreau and Moran 2022; Romero Picazo et al. 2022). The modality in which endosymbionts are acquired (i.e. environmental vs. vertical transmission) generally plays a large role in how these processes unfold over evolutionary time. Finally, as endosymbiotic relationships age, the effects of living in an endosymbiosis become more pronounced on the decreasing cellular and metabolic integrity of endosymbionts (McCutcheon et al. 2019). Hosts, in turn, must continually adapt to sustain their endosymbionts (Mao et al. 2018; Perreau and Moran 2022).

Endosymbiont Evolution: Streamlined for Dysfunction

Perhaps one of the most generalizable outcomes of endosymbiosis is that the genomes and related cellular functionality of endosymbionts are streamlined to fit their ecological and symbiotic requirements. In many cases, this process leads to the extreme reduction of microbial genomes to a mere fraction of those found in their free-living relatives (Khachane et al. 2007; McCutcheon and Moran 2012). However, the obvious effects of genome streamlining are less pronounced in endosymbionts that are horizontally acquired, particularly those with life phases in the open environment (Bright and Bulgheresi 2010; Fisher et al. 2017). For example, while rhizobia bacteria and AM contain genes necessary for their endosymbiotic interactions, they also tend to have large versatile genomes required to cope with complex soil environments (>7 and >150 Mb, respectively; Young et al. 2006; Tisserant et al. 2013).

For vertically transmitted endosymbionts, the evolutionary procession shaping their reduced genomes has been relatively well characterized (Khachane et al. 2007; Wernegreen 2015). Early in the establishment of an endosymbiotic interaction, endosymbiont genomes become adaptively streamlined to lose redundancy with those of their hosts and other endosymbiont partners (Gray et al. 2001; Dale et al. 2003). Genes encoding redundant biosynthetic activities are purged via relaxed selection and eventual excision (Bennett and Moran 2015; Wertheim et al. 2015). This process occurs even in systems with multiple obligate endosymbionts that evolve to perfectly complement each other to meet the needs of their hosts (McCutcheon and Moran 2010; Monnin et al. 2020). However, early on in their establishment when endosymbiont population size and selection are reduced, their genomes can expand with noncoding content that rapidly obliterates redundant and nonessential genes (Koga and Moran 2014; Oakeson et al. 2014). As this early upheaval settles, endosymbiont genomes shrink toward essential integrated metabolisms and functions.

A distinct mode of adaptive genome streamlining occurred in mitochondria, plastids, and the chromatophore of *Paulinella*. The ancestors of these endosymbionts translocated sizable portions of their genomes to their hosts—a process known as endosymbiotic gene transfer (EGT; Dagan et al. 2013; Ku et al. 2015). The advantages of EGT are thought to lie in host control of gene expression, aiding endosymbiont escape from genetic drift, and economized energetics of protein production and transport (Kelly 2021). The EGT evolutionary process, as well as extreme genome reduction, also occurs in the genomes of primary archaeplastid algal hosts unfortunate enough to become enveloped in secondary endosymbioses with other single-celled eukaryotes (Keeling 2010; Uthanumallian et al. 2022). A significant evolutionary contrast can be made with endosymbionts that are established later in multicellular plant and animal hosts. They generally exhibit little evolutionarily significant EGT. These endosymbionts are rarely in direct contact with germline nuclei and have

limited opportunity for heritable EGT to occur (Nikoh et al. 2010).

Finally, as vertically transmitted endosymbioses mature—particularly those found in invertebrate animals—endosymbionts become locked into the endosymbiotic relationship. They experience drastically reduced effective population sizes and strong intergenerational genetic bottlenecks (Moran 1996; Woolfit and Bromham 2003; Vogel and Moran 2013; Hendry et al. 2016). Along with the reduction in their DNA repair mechanisms, drift and the inability to fix errors exaggerate rates of molecular evolution, accumulation of deleterious mutations, and extreme base pair compositional biases (e.g. Douglas et al. 2001; Schelkunov et al. 2015; Waneka et al. 2021). Over time, accumulated mutations cause genes that underlie critical cellular metabolisms and functions to be lost, including the independent ability to regulate genome expression, synthesize membranes and transport metabolites and resources, and even synthesize essential resources required by the host and partner endosymbionts (Kuo et al. 2009; Bennett et al. 2016). These evolutionary processes take their toll on genome size and function, leading to the very smallest known genomes carved out of free-living ancestors (i.e. often just tens to hundreds of kilobases; Gray et al. 2001; Bennett and Moran 2013; Moran and Bennett 2014; Sibbald and Archibald 2020). They also create extreme situations where host lineages and species must adapt to the distinct molecular identities and needs of their endosymbionts through genomic compensation or the acquisition of novel partners (Bennett et al. 2016; Chong et al. 2019; Forsythe et al. 2021; Biot-Pelletier et al. 2023).

Host Evolution: Evolutionary Problem Solvers

Hosts need to overcome a few basic evolutionary challenges in order to sustain and integrate successful endosymbioses with ever-changing partners (Bennett and Moran 2015). These challenges generally include a way to stably exchange essential metabolic and cellular resources, communicate and regulate shared activities, and support ongoing endosymbiont genome degradation. The diverse ways in which hosts meet these challenges depend on the identity of the interacting partners, host anatomy and physiology (e.g. plants vs. animals), and the genetic and genomic constraints of each partner symbiont.

All endosymbioses depend on evolving a means of resource sharing. The sharing of essential metabolites and nutrition is critical to the function and maintenance of endosymbioses (e.g. insect dependence on essential amino acids; Russell et al. 2014; Spinelli and Haigis 2018). The evolution of exchange mechanisms has been accomplished in many ways. The solution depends on the nature of the endosymbiosis and whether endosymbionts retain membrane transport systems and other capabilities in their genomes. Endosymbionts with larger genomes, for example, retain transporters that can handle metabolite exchange (Toft and Fares 2008; Hehenberger et al. 2016). For ancient endosymbionts that no longer encode some or all transporters, hosts contribute them to their membranes and often the entire membrane as well (Price et al. 2011; Duncan et al. 2016; Cunningham and Rutter 2020). But, for unyielding endosymbionts with large independent genomes, such as those acquired from the environment, hosts can either sequester them to elicit and absorb excreted metabolites (e.g. root-nodulating rhizobia; Markmann and Parniske 2009) or simply consume them (e.g. chemosynthetic systems; Sogin et al. 2021).

Due to their tiny genomes, organelles and many endosymbionts also depend on the import of large host nuclear-encoded proteins to perform even their most basic cellular functions. For example, to share protein resources with mitochondria and plastids, eukaryotic hosts have evolved—independently—complex import systems (Tim–Tom and Tic–Toc, respectively; Soll and Schleiff 2004; Wiedemann and Pfanner 2017). In *Paulinella*—which has in many ways independently replayed chloroplast evolution—protein import re-evolved but the mechanisms appear to have distinct origins, possibly involving the Golgi apparatus (Singer et al. 2017). Similarly, the endosymbionts of invertebrate animals with highly degraded genomes also depend on protein imports, but the mechanisms are currently unclear (Nakabachi et al. 2014; Mao et al. 2018). Due to their many independent origins of endosymbioses in invertebrate animals, the mechanisms responsible for protein import across lineages may very well be cobbled together from distinct genes and cellular machineries.

Host endosymbiont cell–cell regulation and communication are essential for the stable long-term integration of successful endosymbioses. How this has been evolutionarily accomplished across the range of endosymbioses is diverse and complex, and much remains to be understood for many systems. Nevertheless, some mechanisms have been identified in key endosymbioses. In chloroplasts and mitochondria, for example, a process of retrograde signaling modulates host genome activities in response to a range of organelle functions, metabolite presence and abundances, and accumulation of reactive oxygen species (Wang et al. 2020). In plants, both mycorrhizae and rhizobia can use glycan signals to target root cells to establish endosymbiotic association and root nodule formation (Gough and Cullimore 2011). Plant hosts also excrete flavonoid signals that toggle bacterial expression of specific genes important in nodule formation and maintenance (Spaink 2000). After establishment, RNA appears to also play some regulatory roles in nutrient exchange (Xu et al. 2018). In marine endosymbioses between corals and algal dinoflagellates, a complex array of glycans, reactive oxygen species, RNA, and lipids are similarly involved in cell–cell

communication and symbiotic regulation (Rosset et al. 2021). For insects, less is currently known about how they regulate and communicate with their diverse nutritional endosymbionts. Some bacterial symbionts with larger genomes maintain complex abilities to monitor and respond to their environments in ways that influence their hosts' function. These bacterial capabilities include eukaryote targeting effectors and quorum sensing, which are mechanisms for attenuating the host-level fitness costs of harboring endosymbionts (Sanchez-Contreras et al. 2007; Enomoto et al. 2017; Hinzke et al. 2019). However, in the vast majority of ancient insect endosymbioses, these systems have long been lost. An emergent property of some systems is that host-level regulation of endosymbionts may be accomplished through nutrition and metabolite monitoring and exchange. In pea aphids, for example, metabolite transporters sense and regulate the flow of metabolites in response to essential amino acid concentrations (Wilkinson et al. 2007; Duncan et al. 2023).

Finally, maintaining endosymbionts, particularly as they age and degrade into complete dependence, requires the evolution of a multitude of support mechanisms. This process often leads to complex reconfiguration of host genomes. The most extreme is perhaps that of the eukaryotic nuclear genome, which is a core patchwork of archaeal, mitochondrial endosymbiont, and other prokaryotic genes (Ribeiro and Golding 1998; Pisani et al. 2007; Ku et al. 2015; Brueckner and Martin 2020). Accommodating and economizing the early endosymbioses with the mitochondria's ancestor was accomplished, in part, by absorbing and repurposing parts of its genome through EGT (Martin et al. 1998). A similar process permitted the capture and establishment of chloroplasts (Sibbald and Archibald 2020), as well as the continued acquisition of plastids through secondary endosymbioses (Ponce-Toledo et al. 2018).

For the establishment of later endosymbioses, such as those in insects, the core toolkit is the eukaryotic genome. However, endosymbionts are generally restricted to distinct cells and tissues (e.g. bacteriocytes) that undergo evolutionary modification into tailored support apparatuses (Buchner 1965). The gene expression of these cells is reprogrammed to differentially express thousands of genes responsible for the maintenance and regulation of endosymbionts (e.g. Sloan et al. 2014; Luan et al. 2015). These symbiont-support genes are derived from a range of origins to meet the specific needs of particular endosymbionts. For example, membrane transporters are typically lost from tiny endosymbiont genomes in insects. They often undergo extensive duplication in the host genome and reassignment to the host-endosymbiont interface (Price et al. 2011; Duncan et al. 2016). Another source of aid for later endosymbioses is the preexisting mitochondrial support genes the hosts acquired through ancient EGT. Up to hundreds of these genes are either dual-targeted to mitochondria and nutritional endosymbionts, or they have been duplicated and completely reassigned to support only the latter (Mao et al. 2018). Occasionally, however, the host genome alone cannot close gaps in the metabolic and cellular functions of their endosymbionts. When no other mechanisms are available, hosts resort to acquiring genes through horizontal gene transfer from *other* infecting bacteria (Sloan et al. 2014; Bublitz et al. 2019). They may also acquire additional symbionts along with their entire genomes (Deng et al. 2023). In essence, hosts pull every evolutionary trick they can to meet the needs of their ever-degrading endosymbionts.

Conclusion

The fields of molecular evolution—and SMBE journals—have tracked decades of scientific discoveries that have revealed the origins, evolution, and global impacts of major endosymbiotic events. This knowledge has invited the re-evaluation of long-held theories, including even the fundamental definitions of endosymbioses (i.e. organelles derived from endosymbioses vs. all other kinds of endosymbiotic interactions; see Husnik and Keeling 2019). While the classification of endosymbioses is partly a matter of semantics and theory, the evolutionary implications are important for understanding how endosymbiotic events have influenced biological diversity and how their interactions will persist and adapt to a changing planet. Our accumulated knowledge has revealed that there are indeed important categorical distinctions to make between endosymbiotic systems. We conclude by reviewing two of the more significant ones.

The Important Distinctions between Endosymbioses

The first major distinction between endosymbiotic events is their sharply contrasting scales of influence over the evolution of global biodiversity. At the broadest level, the endosymbiotic steps in eukaryogenesis permitted the adaptive radiation of single-celled eukaryotes, multicellularity, and the eventual evolution of plants and animals (Lane and Martin 2010). The early establishment of mitochondria and chloroplasts is also the essential foundation upon which all other endosymbioses are built. While the later endosymbioses that followed are certainly responsible for the global-scale diversification of many organismal groups, they are comparatively narrow in their host associations and are relatively plastic (Chomicki et al. 2019; Cornwallis et al. 2023). Nutritional endosymbioses in invertebrate animals, for example, have evolved repeatedly between a wide range of hosts, microbial partners, and environments (Sudakaran et al. 2017; Sogin et al. 2021). Many independent events have given rise to a wide diversity of host lineages over space and time. In contrast, mitochondria and chloroplasts are each derived from singular events

that enabled the evolution and diversification of everything that is not a prokaryote.

The second important distinction between endosymbioses is that the physical and cellular relationships between partners differentially influence their long-term evolution. Mitochondria, which evolved in single-celled hosts, have proliferated along with nearly every eukaryotic cell, including those comprising multicellular organisms. This is not the case for most later nutritional endosymbionts in multicellular hosts. These endosymbionts generally exist only in highly specialized organs and cells restricted from most others including the germline (Fronk and Sachs 2022). In vertically transmitted endosymbioses (e.g. those found in many insects), tissue and cellular restrictions put their endosymbionts in the perilous situation of having little to no control over their reproductive or evolutionary fates. Consequently, the cellular structures and genomes of vertically transmitted endosymbionts are whittled away to eventual extinction or replacement (McCutcheon et al. 2019). For partnerships where endosymbionts are acquired from the environment (e.g. plant–rhizobial and deep-sea animal–chemosynthetic bacterial endosymbioses), the evolutionary consequences of these associations may be comparatively less severe on endosymbiont genomes and their independent cellular capabilities (Young et al. 2006; Sogin et al. 2021).

Taken together, multicellular hosts that established additional endosymbioses long after eukaryogenesis may have the latitude to evolve away from endosymbioses acquire more partners or swap endosymbionts with better ones (Bennett and Moran 2015). They cannot as easily drop their dependence on mitochondria and, to a more limited extent, chloroplasts. These first endosymbionts have ensured their essentiality and near immortality among eukaryotic cells. Thus, it may be predicted that for as long as eukaryotes and photosynthesis persist on Earth, so too will mitochondria and chloroplasts. The same cannot be said for other kinds of endosymbioses.

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Author Contributions

G.M.B. devised the main conceptual ideas, outlined the structure and story, conducted an extensive *MBE* literature review, collated outstanding *MBE* papers, synthesized the information, wrote and revised the majority of the manuscript, and approved the final version for submission. Y.K. contributed to early discussions on manuscript concepts, conducted an extensive *GBE* literature review, collated outstanding *GBE* papers, synthesized the information, drafted sections on endosymbiont and host genome evolution, and revised the manuscript. R.M. synthesized and helped collect relevant papers, drafted sections on the origin and later endosymbioses in the initial manuscript, and revised the manuscript.

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Data Availability

No new data were produced.

Literature Cited

Archibald J. One plus one equals one: symbiosis and the evolution of complex life. Oxford (New York): Oxford University Press; 2014.

Archibald JM. Endosymbiosis and eukaryotic cell evolution. Curr Biol. 2015a:25(19):R911–R921. https://doi.org/10.1016/j.cub.2015.07.055.

Archibald JM. Genomic perspectives on the birth and spread of plastids. Proc Natl Acad Sci USA. 2015b:112(33):10147–10153. https://doi.org/10.1073/pnas.1421374112.

Baurain D, Brinkmann H., Petersen J., Rodriguez-Ezpeleta N., Stechmann A., Demoulin V., Roger A. J., Burger G., Lang B. F., Philippe H. Phylogenomic evidence for separate acquisition of plastids in cryptophytes, haptophytes, and stramenopiles. Mol Biol Evol. 2010:27(7):1698–1709. https://doi.org/10.1093/molbev/msq059.

Bennett AE, Groten K. The costs and benefits of plant-arbuscular mycorrhizal fungal interactions. Annu Rev Plant Biol. 2022:73 (1):649–672. https://doi.org/10.1146/annurev-arplant-102820-124504.

Bennett GM, McCutcheon JP, McDonald BR, Moran NA. Lineage-specific patterns of genome deterioration in obligate symbionts of sharpshooter leafhoppers. Genome Biol Evol. 2016:8(1):296–301. https://doi.org/10.1093/gbe/evv159.

Bennett GM, Moran NA. Heritable symbiosis: the advantages and perils of an evolutionary rabbit hole. Proc Natl Acad Sci USA. 2015:112(33): 10169–10176. https://doi.org/10.1073/pnas.1421388112.

Bennett GM, Moran NA. Small, smaller, smallest: the origins and evolution of ancient dual symbioses in a phloem-feeding insect. Genome Biol Evol. 2013:5(9):1675–1688. https://doi.org/10.1093/gbe/evt118.

Biot-Pelletier D, Bettinazzi S, Gagnon-Arsenault I, Dubé AK, Bédard C, Nguyen THM, Fiumera Heather L, Breton S, Landry CR. Evolutionary trajectories are contingent on mitonuclear interactions. Mol Biol Evol. 2023:40(4):msad061. https://doi.org/10.1093/molbev/msad061.

Bonfante P, Genre A. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun. 2010:1(1):48. https://doi.org/10.1038/ncomms1046.

Bright M, Bulgheresi S. A complex journey: transmission of microbial symbionts. Nat Rev Microbiol. 2010:8(3):218–230. https://doi.org/10.1038/nrmicro2262.

Brown AMV, Howe DK, Wasala SK, Peetz AB, Zasada IA, Denver DR. Comparative genomics of a plant-parasitic nematode endosymbiont suggest a role in nutritional symbiosis. Genome Biol Evol. 2015:7(9):2727–2746. https://doi.org/10.1093/gbe/evv176.

Brueckner J, Martin WF. Bacterial genes outnumber archaeal genes in eukaryotic genomes. Genome Biol Evol. 2020:12(4):282–292. https://doi.org/10.1093/gbe/evaa047.

Bublitz DC, Chadwick GL, Magyar JS, Sandoz KM, Brooks DM, Mesnage S, Ladinsky MS, Garber AI, Bjorkman PJ, Orphan VJ, et al. Peptidoglycan production by an insect-bacterial mosaic. Cell. 2019:179(3):703–712.e7. https://doi.org/10.1016/j.cell.2019.08.054.

Buchner P. Endosymbiosis of animals with plant microorganisms. Rev. Eng. ed. New York (NY), USA: Interscience Publishers; 1965.

Carrie C, Giraud E, Whelan J. Protein transport in organelles: dual targeting of proteins to mitochondria and chloroplasts. FEBS J. 2009:276(5):1187–1195. https://doi.org/10.1111/j.1742-4658.2009.06876.x.

Chomicki G, Weber M, Antonelli A, Bascompte J, Kiers ET. The impact of mutualisms on species richness. Trends Ecol Evol (Amst). 2019:34(8):698–711. https://doi.org/10.1016/j.tree.2019.03.003.

Chong RA, Park H, Moran NA. Genome evolution of the obligate endosymbiont Buchnera aphidicola. Mol Biol Evol. 2019:36(7):1481–1489. https://doi.org/10.1093/molbev/msz082.

Cornwallis CK, van 't Padje A, Ellers J, Klein M, Jackson R, Kiers ET, West SA, Henry LM. Symbioses shape feeding niches and diversification across insects. Nat Ecol Evol. 2023:7(7):1022–1044. https://doi.org/10.1038/s41559-023-02058-0.

Cunningham CN, Rutter J. 20,000 picometers under the OMM: diving into the vastness of mitochondrial metabolite transport. EMBO Rep. 2020:21(5):e50071. https://doi.org/10.15252/embr.202050071.

Dagan T, Roettger M, Stucken K, Landan G, Koch R, Major P, Gould SB, Goremykin VV, Rippka R, Tandeau de Marsac N, et al. Genomes of Stigonematalean cyanobacteria (subsection V) and the evolution of oxygenic photosynthesis from prokaryotes to plastids. Genome Biol Evol. 2013:5(1):31–44. https://doi.org/10.1093/gbe/evs117.

Dale C, Wang B, Moran N, Ochman H. Loss of DNA recombinational repair enzymes in the initial stages of genome degeneration. Mol Biol Evol. 2003:20(8):1188–1194. https://doi.org/10.1093/molbev/msg138.

de Oliveira AL, Mitchell J, Girguis P, Bright M. Novel insights on obligate symbiont lifestyle and adaptation to chemosynthetic environment as revealed by the giant tubeworm genome. Mol Biol Evol. 2022:39(1):msab347. https://doi.org/10.1093/molbev/msab347.

Delaux P-M, Schornack S. Plant evolution driven by interactions with symbiotic and pathogenic microbes. Science 2021:371(6531): eaba6605. https://doi.org/10.1126/science.aba6605.

Deng J, Bennett GM, Franco DC, Prus-Frankowska M, Stroiński A, Michalik A, Łukasik P. Genome comparison reveals inversions and alternative evolutionary history of nutritional endosymbionts in planthoppers (Hemiptera: Fulgoromorpha). Genome Biol Evol. 2023:15(7):evad120. https://doi.org/10.1093/gbe/evad120.

Dorrell RG, Howe CJ. Integration of plastids with their hosts: lessons learned from dinoflagellates. Proc Natl Acad Sci USA. 2015:112(33): 10247–10254. https://doi.org/10.1073/pnas.1421380112.

Douglas AE. Lessons from studying insect symbioses. Cell Host Microbe. 2011:10(4):359–367. https://doi.org/10.1016/j.chom.2011.09.001.

Douglas AE. Symbiosis as a general principle in eukaryotic evolution. Cold Spring Harb Perspect Biol. 2014:6(2):a016113. https://doi.org/10.1101/cshperspect.a016113.

Douglas AE. How multi-partner endosymbioses function. Nat Rev Microbiol. 2016:14(12):731–743. https://doi.org/10.1038/nrmicro.2016.151.

Douglas S, Zauner S, Fraunholz M, Beaton M, Penny S, Deng L-T, Wu X, Reith M, Cavalier-Smith T, Maier U-G. The highly reduced genome of an enslaved algal nucleus. Nature 2001:410(6832):1091–1096. https://doi.org/10.1038/35074092.

Douglas SE. Plastid evolution: origins, diversity, trends. Curr Opin Genet Dev. 1998:8(6):655–661. https://doi.org/10.1016/s0959-437x(98)80033-6.

Drew GC, Stevens EJ, King KC. Microbial evolution and transitions along the parasite–mutualist continuum. Nat Rev Microbiol. 2021:19(10): 623–638. https://doi.org/10.1038/s41579-021-00550-7.

Duncan RP, Anderson CMH, Thwaites DT, Luetje CW, Wilson ACC. Co-option of a conserved host glutamine transporter facilitates aphid/Buchnera metabolic integration. Proc Natl Acad Sci USA. 2023:120(43):e2308448120. https://doi.org/10.1073/pnas.2308448120.

Duncan RP, Feng H, Nguyen DM, Wilson ACC. Gene family expansions in aphids maintained by endosymbiotic and nonsymbiotic traits. Genome Biol Evol. 2016:8(3):753–764. https://doi.org/10.1093/gbe/evw020.

Enomoto S, Chari A, Clayton AL, Dale C. Quorum sensing attenuates virulence in Sodalis praecaptivus. Cell Host Microbe. 2017:21(5): 629–636.e5. https://doi.org/10.1016/j.chom.2017.04.003.

Fisher RM, Henry LM, Cornwallis CK, Kiers ET, West SA. The evolution of host-symbiont dependence. Nat Commun. 2017:8(1):15973. https://doi.org/10.1038/ncomms15973.

Fitzpatrick DA, Creevey CJ, McInerney JO. Genome phylogenies indicate a meaningful α-proteobacterial phylogeny and support a grouping of the mitochondria with the Rickettsiales. Mol Biol Evol. 2006:23(1):74–85. https://doi.org/10.1093/molbev/msj009.

Forsythe ES, Williams AM, Sloan DB. Genome-wide signatures of plastid-nuclear coevolution point to repeated perturbations of plastid proteostasis systems across angiosperms. Plant Cell. 2021:33(4):980–997. https://doi.org/10.1093/plcell/koab021.

Fronk DC, Sachs JL. Symbiotic organs: the nexus of host-microbe evolution. Trends Ecol Evol. 2022:37(7):599–610. https://doi.org/10.1016/j.tree.2022.02.014.

Garber AI, Kupper M, Laetsch DR, Weldon SR, Ladinsky MS, Bjorkman PJ, McCutcheon JP. The evolution of interdependence in a four-way mealybug symbiosis. Genome Biol Evol. 2021:13(8): evab123. https://doi.org/10.1093/gbe/evab123.

Geiger O, Sanchez-Flores A, Padilla-Gomez J, Degli Esposti M. Multiple approaches of cellular metabolism define the bacterial ancestry of mitochondria. Sci Adv. 2023:9(32):eadh0066. https://doi.org/10.1126/sciadv.adh0066.

Geurts R, Xiao TT, Reinhold-Hurek B. What does it take to evolve a nitrogen-fixing endosymbiosis? Trends Plant Sci. 2016:21(3): 199–208. https://doi.org/10.1016/j.tplants.2016.01.012.

Gossett JM, Porter ML, Vasquez YM, Bennett GM, Chong RA. Genomic comparisons reveal selection pressure and functional variation between nutritional endosymbionts of cave-adapted and epigean Hawaiian planthoppers. Genome Biol Evol. 2023:15(3):evad031. https://doi.org/10.1093/gbe/evad031.

Gould AL, Henderson JB, Lam AW. Chromosome-level genome assembly of the bioluminescent cardinalfish Siphamia tubifer: an emerging model for symbiosis research. Genome Biol Evol. 2022:14(4):evac044. https://doi.org/10.1093/gbe/evac044.

Gough C, Cullimore J. Lipo-chitooligosaccharide signaling in endosymbiotic plant-microbe interactions. Mol Plant-Microbe Interact. 2011:24(8):867–878. https://doi.org/10.1094/MPMI-01-11-0019.

Gray MW, Burger G, Lang BF. Mitochondrial evolution. Science 1999:283(5407):1476–1481. https://doi.org/10.1126/science.283.5407.1476.

Gray MW, Burger G, Lang BF. The origin and early evolution of mitochondria. Genome Biol. 2001:2(6):REVIEWS1018. https://doi.org/10.1186/gb-2001-2-6-reviews1018.

Hansen AK, Moran NA. The impact of microbial symbionts on host plant utilization by herbivorous insects. Mol Ecol. 2014:23(6): 1473–1496. https://doi.org/10.1111/mec.12421.

He C, Berkowitz O, Hu S, Zhao Y, Qian K, Shou H, Whelan J, Wang Y. Co-regulation of mitochondrial and chloroplast function: molecular components and mechanisms. Plant Commun. 2023:4(1): 100496. https://doi.org/10.1016/j.xplc.2022.100496.

Hehenberger E, Burki F, Kolisko M, Keeling PJ. Functional relationship between a dinoflagellate host and its diatom endosymbiont. Mol Biol Evol. 2016:33(9):2376–2390. https://doi.org/10.1093/molbev/msw109.

Hendry TA, De Wet JR, Dougan KE, Dunlap PV. Genome evolution in the obligate but environmentally active luminous symbionts of flashlight fish. Genome Biol Evol. 2016:8(7):2203–2213. https://doi.org/10.1093/gbe/evw161.

Hinzke T, Kleiner M, Breusing C, Felbeck H, Häsler R, Sievert SM, Schlüter R, Rosenstiel P, Reusch TBH, Schweder T, et al. Host-microbe interactions in the chemosynthetic riftia pachyptila symbiosis. mBio 2019:10(6):e02243-19. https://doi.org/10.1128/mbio.02243-19.

Hoefnagel MHN, Atkin OK, Wiskich JT. Interdependence between chloroplasts and mitochondria in the light and the dark. Biochim Biophys Acta Bioenerg. 1998:1366(3):235–255. https://doi.org/10.1016/S0005-2728(98)00126-1.

Husnik F, Keeling PJ. The fate of obligate endosymbionts: reduction, integration, or extinction. Curr Opin Genet Dev. 2019:58–59: 1–8. https://doi.org/10.1016/j.gde.2019.07.014.

Ip JC-H, Xu T, Sun J, Li R, Chen C, Lan Y, Han Z, Zhang H, Wei J, Wang H, et al. Host–endosymbiont genome integration in a deep-sea chemosymbiotic clam. Mol Biol Evol. 2021:38(2):502–518. https://doi.org/10.1093/molbev/msaa241.

Jiggins FM, Hurst GDD, Yang Z. Host-symbiont conflicts: positive selection on an outer membrane protein of parasitic but not mutualistic Rickettsiaceae. Mol Biol Evol. 2002:19(8):1341–1349. https://doi.org/10.1093/oxfordjournals.molbev.a004195.

Keeling PJ. The endosymbiotic origin, diversification and fate of plastids. Philos Trans R Soc Lond B Biol Sci. 2010:365(1541): 729–748. https://doi.org/10.1098/rstb.2009.0103.

Keeling PJ. The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. Annu Rev Plant Biol. 2013:64(1):583–607. https://doi.org/10.1146/annurev-arplant-050312-120144.

Kelly S. The economics of organellar gene loss and endosymbiotic gene transfer. Genome Biol. 2021:22(1):345. https://doi.org/10.1186/s13059-021-02567-w.

Khachane AN, Timmis KN, Martins dos Santos VAP. Dynamics of reductive genome evolution in mitochondria and obligate intracellular microbes. Mol Biol Evol. 2007:24(2):449–456. https://doi.org/10.1093/molbev/msl174.

Kiers ET, Rousseau RA, West SA, Denison RF. Host sanctions and the legume–rhizobium mutualism. Nature 2003:425(6953):78–81. https://doi.org/10.1038/nature01931.

Kinjo Y, Bourguignon T, Tong KJ, Kuwahara H, Lim SJ, Yoon KB, Shigenobu S, Park YC, Nalepa CA, Hongoh Y, et al. Parallel and gradual genome erosion in the *Blattabacterium* endosymbionts of *Mastotermes darwiniensis* and *Cryptocercus* wood roaches. Genome Biol Evol. 2018:10(6):1622–1630. https://doi.org/10.1093/gbe/evy110.

Koga R, Moran NA. Swapping symbionts in spittlebugs: evolutionary replacement of a reduced genome symbiont. ISME J. 2014:8(6): 1237–1246. https://doi.org/10.1038/ismej.2013.235.

Ku C, Nelson-Sathi S, Roettger M, Sousa FL, Lockhart PJ, Bryant D, Hazkani-Covo E, McInerney JO, Landan G, Martin WF. Endosymbiotic origin and differential loss of eukaryotic genes. Nature 2015:524(7566):427–432. https://doi.org/10.1038/nature14963.

Kuo C-H, Moran NA, Ochman H. The consequences of genetic drift for bacterial genome complexity. Genome Res. 2009:19(8):1450–1454. https://doi.org/10.1101/gr.091785.109.

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR. Systematic revision of symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol. 2018:28(16):2570–2580.e6. https://doi.org/10.1016/j.cub.2018.07.008.

Lane N, Martin W. The energetics of genome complexity. Nature 2010:467(7318):929–934. https://doi.org/10.1038/nature09486.

Leftwich PT, Edgington MP, Chapman T. Transmission efficiency drives host–microbe associations. Proc Biol Sci. 2020:287(1934): 20200820. https://doi.org/10.1098/rspb.2020.0820.

Levin RA, Beltran VH, Hill R, Kjelleberg S, McDougald D, Steinberg PD, van Oppen MJH. Sex, scavengers, and chaperones: transcriptome secrets of divergent symbiodinium thermal tolerances. Mol Biol Evol. 2016:33(9):2201–2215. https://doi.org/10.1093/molbev/msw119.

Liu H, Stephens TG, González-Pech RA, Beltran VH, Lapeyre B, Bongaerts P, Cooke I, Aranda M, Bourne DG, Forêt S, et al. Symbiodinium genomes reveal adaptive evolution of functions related to coral-dinoflagellate symbiosis. Commun Biol. 2018:1: 1–11. https://doi.org/10.1038/s42003-018-0098-3.

Luan J-B, Chen W, Hasegawa DK, Simmons AM, Wintermantel WM, Ling K-S, Fei Z, Liu S-S, Douglas AE. Metabolic coevolution in the bacterial symbiosis of whiteflies and related plant sap-feeding insects. Genome Biol Evol. 2015:7(9):2635–2647. https://doi.org/10.1093/gbe/evv170.

Lynch JB, Hsiao EY. Microbiomes as sources of emergent host phenotypes. Science 2019:365(6460):1405–1409. https://doi.org/10.1126/science.aay0240.

Manzano-Marín A, Oceguera-Figueroa A, Latorre A, Jiménez-García LF, Moya A. Solving a bloody mess: B-vitamin independent metabolic convergence among gammaproteobacterial obligate endosymbionts from blood-feeding arthropods and the leech *Haementeria officinalis*. Genome Biol Evol. 2015:7(10):2871–2884. https://doi.org/10.1093/gbe/evv188.

Mao M, Yang X, Bennett GM. Evolution of host support for two ancient bacterial symbionts with differentially degraded genomes in a leafhopper host. Proc Natl Acad Sci USA. 2018:115: E11691–E11700. https://doi.org/10.1073/pnas.1811932115.

Mao M, Yang X, Poff K, Bennett G. Comparative genomics of the dual-obligate symbionts from the treehopper, *Entylia carinata* (Hemiptera: Membracidae), provide insight into the origins and evolution of an ancient symbiosis. Genome Biol Evol. 2017:9(6): 1803–1815. https://doi.org/10.1093/gbe/evx134.

Marin BM, Nowack EC, Melkonian M. A plastid in the making: evidence for a second primary endosymbiosis. Protist 2005:156(4): 425–432. https://doi.org/10.1016/j.protis.2005.09.001.

Markmann K, Parniske M. Evolution of root endosymbiosis with bacteria: how novel are nodules? Trends Plant Sci. 2009:14(2): 77–86. https://doi.org/10.1016/j.tplants.2008.11.009.

Martin W, Müller M. The hydrogen hypothesis for the first eukaryote. Nature 1998:392(6671):37–41. https://doi.org/10.1038/32096.

Martin W, Stoebe B, Goremykin V, Hansmann S, Hasegawa M, Kowallik KV. Gene transfer to the nucleus and the evolution of

chloroplasts. Nature 1998:393(6681):162–165. https://doi.org/10.1038/30234.

Martin WF, Garg S, Zimorski V. Endosymbiotic theories for eukaryote origin. Philos Trans R Soc Lond B Biol Sci. 2015:370(1678): 20140330. https://doi.org/10.1098/rstb.2014.0330.

McBride HM, Neuspiel M, Wasiak S. Mitochondria: more than just a powerhouse. Curr Biol. 2006:16(14):R551–R560. https://doi.org/10.1016/j.cub.2006.06.054.

McCutcheon JP, Boyd BM, Dale C. The life of an insect endosymbiont from the cradle to the grave. Curr Biol. 2019:29(11):R485–R495. https://doi.org/10.1016/j.cub.2019.03.032.

McCutcheon JP, Moran NA. Functional convergence in reduced genomes of bacterial symbionts spanning 200 My of evolution. Genome Biol Evol. 2010:2:708–718. https://doi.org/10.1093/gbe/evq055.

McCutcheon JP, Moran NA. Extreme genome reduction in symbiotic bacteria. Nat Rev Microbiol. 2012:10(1):13–26. https://doi.org/10.1038/nrmicro2670.

McFadden GI. Chloroplast origin and integration1. Plant Physiol. 2001:125(1):50–53. https://doi.org/10.1104/pp.125.1.50.

McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, et al. Animals in a bacterial world, a new imperative for the life sciences. Proc Natl Acad Sci USA. 2013:110(9):3229–3236. https://doi.org/10.1073/pnas.1218525110.

McFall-Ngai MJ. Giving microbes their due—animal life in a microbially dominant world. J Exp Biol. 2015:218(12):1968–1973. https://doi.org/10.1242/jeb.115121.

Medina Munoz M, Pollio AR, White HL, Rio RVM. Into the wild: parallel transcriptomics of the tsetse-*Wigglesworthia* mutualism within Kenyan populations. Genome Biol Evol. 2017:9(9):2276–2291. https://doi.org/10.1093/gbe/evx175.

Mills DB, Boyle RA, Daines SJ, Sperling EA, Pisani D, Donoghue PCJ, Lenton TM. Eukaryogenesis and oxygen in Earth history. Nat Ecol Evol. 2022:6(5):520–532. https://doi.org/10.1038/s41559-022-01733-y.

Monnin D, Jackson R, Kiers ET, Bunker M, Ellers J, Henry LM. Parallel evolution in the integration of a co-obligate aphid symbiosis. Curr Biol. 2020:30(10):1949–1957.e6. https://doi.org/10.1016/j.cub.2020.03.011.

Moran NA. Accelerated evolution and Muller's rachet in endosymbiotic bacteria. Proc Natl Acad Sci U S A. 1996:93(7):2873–2878. https://doi.org/10.1073/pnas.93.7.2873.

Moran NA. Symbiosis as an adaptive process and source of phenotypic complexity. Proc Natl Acad Sci USA. 2007:104(Suppl 1): 8627–8633. https://doi.org/10.1073/pnas.0611659104.

Moran NA, Bennett GM. The tiniest tiny genomes. Annu Rev Microbiol. 2014:68(1):195–215. https://doi.org/10.1146/annurev-micro-091213-112901.

Moreira D, Le Guyader H, Philippe H. The origin of red algae and the evolution of chloroplasts. Nature 2000:405(6782):69–72. https://doi.org/10.1038/35011054.

Nakabachi A, Ishida K, Hongoh Y, Ohkuma M, Miyagishima S-Y. Aphid gene of bacterial origin encodes a protein transported to an obligate endosymbiont. Curr Biol. 2014:24(14):R640–R641. https://doi.org/10.1016/j.cub.2014.06.038.

Nikoh N, McCutcheon JP, Kudo T, Miyagishima S-y, Moran NA, Nakabachi A. Bacterial genes in the aphid genome: absence of functional gene transfer from *Buchnera* to its host. PLoS Genet. 2010:6(2): e1000827. https://doi.org/10.1371/journal.pgen.1000827.

Nowack ECM, Melkonian M, Glöckner G. Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. Curr Biol. 2008:18(6):410–418. https://doi.org/10.1016/j.cub.2008.02.051.

Nowack ECM, Vogel H, Groth M, Grossman AR, Melkonian M, Glockner G. Endosymbiotic gene transfer and transcriptional regulation of transferred genes in *Paulinella chromatophora*. Mol Biol Evol. 2011:28(1):407–422. https://doi.org/10.1093/molbev/msq209.

Oakeson KF, Gil R, Clayton AL, Dunn DM, von Niederhausern AC, Hamil C, Aoyagi A, Duval B, Baca A, Silva FJ, et al. Genome degeneration and adaptation in a nascent stage of symbiosis. Genome Biol Evol. 2014:6(1):76–93. https://doi.org/10.1093/gbe/evt210.

Oikawa K, Imai T, Thagun C, Toyooka K, Yoshizumi T, Ishikawa K, Kodama Y, Numata K. Mitochondrial movement during its association with chloroplasts in *Arabidopsis thaliana*. Commun Biol. 2021:4(1):292. https://doi.org/10.1038/s42003-021-01833-8.

Ozawa G, Shimamura S, Takaki Y, Takishita K, Ikuta T, Barry JP, Maruyama T, Fujikura K, Yoshida T. Ancient occasional host switching of maternally transmitted bacterial symbionts of chemosynthetic vesicomyid clams. Genome Biol Evol. 2017:9(9): 2226–2236. https://doi.org/10.1093/gbe/evx166.

Parniske M. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol. 2008:6(10):763–775. https://doi.org/10.1038/nrmicro1987.

Patiño-Navarrete R, Moya A, Latorre A, Peretó J. Comparative genomics of *Blattabacterium cuenoti*: the frozen legacy of an ancient endosymbiont genome. Genome Biol Evol. 2013:5(2):351–361. https://doi.org/10.1093/gbe/evt011.

Peek AS, Vrijenhoek RC, Gaut BS. Accelerated evolutionary rate in sulfur-oxidizing endosymbiotic bacteria associated with the mode of symbiont transmission. Mol Biol Evol. 1998:15(11):1514–1523. https://doi.org/10.1093/oxfordjournals.molbev.a025879.

Peeters N, Small I. Dual targeting to mitochondria and chloroplasts. Biochim Biophys Acta. 2001:1541(1-2):54–63. https://doi.org/10.1016/s0167-4889(01)00146-x.

Perreau J, Moran NA. Genetic innovations in animal–microbe symbioses. Nat Rev Genet. 2022:23(1):23–39. https://doi.org/10.1038/s41576-021-00395-z.

Pisani D, Cotton JA, McInerney JO. Supertrees disentangle the chimerical origin of eukaryotic genomes. Mol Biol Evol. 2007:24(8): 1752–1760. https://doi.org/10.1093/molbev/msm095.

Plaisance L, Caley MJ, Brainard RE, Knowlton N. The diversity of coral reefs: what are we missing? PLoS One. 2011:6(10):e25026. https://doi.org/10.1371/journal.pone.0025026.

Ponce-Toledo RI, López-García P, Moreira D. Horizontal and endosymbiotic gene transfer in early plastid evolution. New Phytol. 2019:224(2):618–624. https://doi.org/10.1111/nph.15965.

Ponce-Toledo RI, Moreira D, López-García P, Deschamps P. Secondary plastids of euglenids and chlorarachniophytes function with a mix of genes of red and green algal ancestry. Mol Biol Evol. 2018:35(9): 2198–2204. https://doi.org/10.1093/molbev/msy121.

Price DRG, Duncan RP, Shigenobu S, Wilson ACC. Genome expansion and differential expression of amino acid transporters at the aphid/*Buchnera* symbiotic interface. Mol Biol Evol. 2011:28(11): 3113–3126. https://doi.org/10.1093/molbev/msr140.

Raghavendra AS, Padmasree K. Beneficial interactions of mitochondrial metabolism with photosynthetic carbon assimilation. Trends Plant Sci. 2003:8(11):546–553. https://doi.org/10.1016/j.tplants.2003.09.015.

Raval PK, Martin WF, Gould SB. Mitochondrial evolution: gene shuffling, endosymbiosis, and signaling. Sci Adv. 2023:9(32):eadj4493. https://doi.org/10.1126/sciadv.adj4493.

Ribeiro S, Golding GB. The mosaic nature of the eukaryotic nucleus. Mol Biol Evol. 1998:15(7):779–788. https://doi.org/10.1093/oxfordjournals.molbev.a025983.

Rikkinen J. Cyanobacteria in terrestrial symbiotic systems. In: Hallenbeck PC, editors. Modern topics in the phototrophic prokaryotes. Cham: Springer International Publishing; 2017. p. 243–294.

Roger AJ, Muñoz-Gómez SA, Kamikawa R. The origin and diversification of mitochondria. Curr Biol. 2017:27(21):R1177–R1192. https://doi.org/10.1016/j.cub.2017.09.015.

Rogozin IB, Basu MK, Csürös M, Koonin EV. Analysis of rare genomic changes does not support the unikont–bikont phylogeny and suggests cyanobacterial symbiosis as the point of primary radiation of eukaryotes. Genome Biol Evol. 2009:1:99–113. https://doi.org/10.1093/gbe/evp011.

Romero Picazo D, Werner A, Dagan T, Kupczok A. Pangenome evolution in environmentally transmitted symbionts of deep-sea mussels is governed by vertical inheritance. Genome Biol Evol. 2022:14(7): evac098. https://doi.org/10.1093/gbe/evac098.

Rosset SL, Oakley CA, Ferrier-Pages C, Suggett DJ, Weiss VM, Davy SK. The molecular language of the cnidarian-dinoflagellate symbiosis. Trends Microbiol. 2021:29(4):320–333. https://doi.org/10.1016/j.tim.2020.08.005.

Russell CW, Poliakov A, Haribal M, Jander G, van Wijk KJ, Douglas AE. Matching the supply of bacterial nutrients to the nutritional demand of the animal host. Proc Biol Sci. 2014:281(1791): 20141163. https://doi.org/10.1098/rspb.2014.1163.

Sagan L. On the origin of mitosing cells. J Theor Biol. 1967:14(3): 255–274. https://doi.org/10.1016/0022-5193(67)90079-3.

Salem H, Florez L, Gerardo N, Kaltenpoth M. An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. Proceedings of the Royal Society B: Biological Sciences. 2015:282(1804):20142957. https://doi.org/10.1098/rspb.2014.2957.

Sanchez-Contreras M, Bauer WD, Gao M, Robinson JB, Allan Downie J. Quorum-sensing regulation in rhizobia and its role in symbiotic interactions with legumes. Philos Trans R Soc Lond B Biol Sci. 2007:362(1483): 1149–1163. https://doi.org/10.1098/rstb.2007.2041.

Santos-Garcia D, Latorre A, Moya A, Gibbs G, Hartung V, Dettner K, Kuechler SM, Silva FJ. Small but powerful, the primary endosymbiont of moss bugs, Candidatus Evansia muelleri, holds a reduced genome with large biosynthetic capabilities. Genome Biol Evol. 2014:6(7):1875–1893. https://doi.org/10.1093/gbe/evu149.

Schelkunov MI, Shtratnikova VY, Nuraliev MS, Selosse M-A, Penin AA, Logacheva MD. Exploring the limits for reduction of plastid genomes: a case study of the mycoheterotrophic orchids Epipogium aphyllum and Epipogium roseum. Genome Biol Evol. 2015:7(4): 1179–1191. https://doi.org/10.1093/gbe/evv019.

Shapiro LR, Scully ED, Straub TJ, Park J, Stephenson AG, Beattie GA, Gleason M, Kolter R, Coelho MC, De Moraes M, et al. Horizontal gene acquisitions, mobile element proliferation, and genome decay in the host-restricted plant pathogen Erwinia tracheiphila. Genome Biol Evol. 2016:8(3):649–664. https://doi.org/10.1093/gbe/evw016.

Shinzato C, Takeuchi T, Yoshioka Y, Tada I, Kanda M, Broussard C, Iguchi A, Kusakabe M, Marin F, Satoh N, et al. Whole-genome sequencing highlights conservative genomic strategies of a stress-tolerant, long-lived scleractinian coral, Porites australiensis Vaughan, 1918. Genome Biol Evol. 2021:13(12):evab270. https://doi.org/10.1093/gbe/evab270.

Sibbald SJ, Archibald JM. Genomic insights into plastid evolution. Genome Biol Evol. 2020:12(7):978–990. https://doi.org/10.1093/gbe/evaa096.

Singer A, Poschmann G, Mühlich C, Valadez-Cano C, Hänsch S, Hüren V, Rensing SA, Stühler K, Nowack ECM. Massive protein import into the early-evolutionary-stage photosynthetic organelle of the amoeba Paulinella chromatophora. Curr Biol. 2017:27(18):2763–2773.e5. https://doi.org/10.1016/j.cub.2017.08.010.

Sloan DB, Moran NA. The evolution of genomic instability in the obligate endosymbionts of whiteflies. Genome Biol Evol. 2013:5(5): 783–793. https://doi.org/10.1093/gbe/evt044.

Sloan DB, Nakabachi A, Richards S, Qu J, Murali SC, Gibbs RA, Moran NA. Parallel histories of horizontal gene transfer facilitated extreme reduction of endosymbiont genomes in sap-feeding insects. Mol Biol Evol. 2014:31(4):857–871. https://doi.org/10.1093/molbev/msu004.

Sogin EM, Kleiner M, Borowski C, Gruber-Vodicka HR, Dubilier N. Life in the dark: phylogenetic and physiological diversity of chemosynthetic symbioses. Annu Rev Microbiol. 2021:75(1):695–718. https://doi.org/10.1146/annurev-micro-051021-123130.

Sogin EM, Leisch N, Dubilier N. Chemosynthetic symbioses. Curr Biol. 2020:30(19):R1137–R1142. https://doi.org/10.1016/j.cub.2020.07.050.

Soll J, Schleiff E. Protein import into chloroplasts. Nat Rev Mol Cell Biol. 2004:5(3):198–208. https://doi.org/10.1038/nrm1333.

Spaink HP. Root nodulation and infection factors produced by rhizobial bacteria. Annu Rev Microbiol. 2000:54(1):257–288. https://doi.org/10.1146/annurev.micro.54.1.257.

Spaulding AW, von Dohlen CD. Phylogenetic characterization and molecular evolution of bacterial endosymbionts in psyllids (Hemiptera: Sternorrhyncha). Mol Biol Evol. 1998:15(11):1506–1513. https://doi.org/10.1093/oxfordjournals.molbev.a025878.

Spinelli JB, Haigis MC. The multifaceted contributions of mitochondria to cellular metabolism. Nat Cell Biol. 2018:20(7):745–754. https://doi.org/10.1038/s41556-018-0124-1.

Sudakaran S, Kost C, Kaltenpoth M. Symbiont acquisition and replacement as a source of ecological innovation. Trends Microbiol. 2017:25(5):375–390. https://doi.org/10.1016/j.tim.2017.02.014.

Sun Y, Sun J, Yang Y, Lan Y, Ip JC-H, Wong WC, Kwan YH, Zhang Y, Han Z, Qiu J-W, et al. Genomic signatures supporting the symbiosis and formation of chitinous tube in the deep-sea tubeworm Paraescarpia echinospica. Mol Biol Evol. 2021:38(10): 4116–4134. https://doi.org/10.1093/molbev/msab203.

Thiergart T, Landan G, Schenk M, Dagan T, Martin WF. An evolutionary network of genes present in the eukaryote common ancestor polls genomes on eukaryotic and mitochondrial origin. Genome Biol Evol. 2012:4(4):466–485. https://doi.org/10.1093/gbe/evs018.

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing N, Frei dit Frey N, Gianinazzi-Pearson V, et al. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. Proc Natl Acad Sci USA. 2013:110(50):20117–20122. https://doi.org/10.1073/pnas.1313452110.

Toft C, Fares MA. The evolution of the flagellar assembly pathway in endosymbiotic bacterial genomes. Mol Biol Evol. 2008:25(9): 2069–2076. https://doi.org/10.1093/molbev/msn153.

Uthanumallian K, Iha C, Repetti SI, Chan CX, Bhattacharya D, Duchene S, Verbruggen H. Tightly constrained genome reduction and relaxation of purifying selection during secondary plastid endosymbiosis. Mol Biol Evol. 2022:39(1):msab295. https://doi.org/10.1093/molbev/msab295.

Vogel KJ, Moran NA. Functional and evolutionary analysis of the genome of an obligate fungal symbiont. Genome Biol Evol. 2013:5(5): 891–904. https://doi.org/10.1093/gbe/evt054.

Waneka G, Vasquez YM, Bennett GM, Sloan DB. Mutational pressure drives differential genome conservation in two bacterial endosymbionts of sap-feeding insects. Genome Biol Evol. 2021:13(3): evaa254. https://doi.org/10.1093/gbe/evaa254.

Wang Y, Selinski J, Mao C, Zhu Y, Berkowitz O, Whelan J. Linking mitochondrial and chloroplast retrograde signalling in plants. Philos Trans R Soc B Biol Sci. 2020:375(1801):20190410. https://doi.org/10.1098/rstb.2019.0410.

Warshan D, Liaimer A, Pederson E, Kim S-Y, Shapiro N, Woyke T, Altermark B, Pawlowski K, Weyman PD, Dupont CL, et al.

Genomic changes associated with the evolutionary transitions of *Nostoc* to a plant symbiont. Mol Biol Evol. 2018:35(5): 1160–1175. https://doi.org/10.1093/molbev/msy029.

Weglarz KM, Havill NP, Burke GR, Von Dohlen CD. Partnering with a pest: genomes of hemlock woolly adelgid symbionts reveal atypical nutritional provisioning patterns in dual-obligate bacteria. Genome Biol Evol. 2018:10(6):1607–1621. https://doi.org/10.1093/gbe/evy114.

Wernegreen JJ. Endosymbiont evolution: predictions from theory and surprises from genomes. Ann N Y Acad Sci. 2015:1360(1):16–35. https://doi.org/10.1111/nyas.12740.

Wertheim JO, Murrell B, Smith MD, Kosakovsky Pond SL, Scheffler K. RELAX: detecting relaxed selection in a phylogenetic framework. Mol Biol Evol. 2015:32(3):820–832. https://doi.org/10.1093/molbev/msu400.

Wiedemann N, Pfanner N. Mitochondrial machineries for protein import and assembly. Annu Rev Biochem. 2017:86(1):685–714. https://doi.org/10.1146/annurev-biochem-060815-014352.

Wilkinson TL, Koga R, Fukatsu T. Role of host nutrition in symbiont regulation: impact of dietary nitrogen on proliferation of obligate and facultative bacterial endosymbionts of the pea aphid *Acyrthosiphon pisum*. Appl Environ Microbiol. 2007:73(4): 1362–1366. https://doi.org/10.1128/AEM.01211-06.

Williams LE, Wernegreen JJ. Purifying selection, sequence composition, and context-specific indel mutations shape intraspecific variation in a bacterial endosymbiont. Genome Biol Evol. 2012:4(1): 44–51. https://doi.org/10.1093/gbe/evr128.

Williams TA, Embley TM. Archaeal "dark matter" and the origin of eukaryotes. Genome Biol Evol. 2014:6(3):474–481. https://doi.org/10.1093/gbe/evu031.

Williams TA, Foster PG, Cox CJ, Embley TM. An archaeal origin of eukaryotes supports only two primary domains of life. Nature 2013:504(7479):231–236. https://doi.org/10.1038/nature12779.

Wilson ACC, Duncan RP. Signatures of host/symbiont genome co-evolution in insect nutritional endosymbioses. Proc Natl Acad Sci USA. 2015:112(33):10255–10261. https://doi.org/10.1073/pnas.1423305112.

Woolfit M, Bromham L. Increased rates of sequence evolution in endosymbiotic bacteria and fungi with small effective population sizes.

Mol Biol Evol. 2003:20(9):1545–1555. https://doi.org/10.1093/molbev/msg167.

Xu Y, Zhu S, Liu F, Wang W, Wang X, Han G, Cheng B. Identification of arbuscular mycorrhiza fungi responsive microRNAs and their regulatory network in maize. Int J Mol Sci. 2018:19(10):3201. https://doi.org/10.3390/ijms19103201.

Yang L-L, Jiang Z, Li Y, Wang E-T, Zhi X-Y. Plasmids related to the symbiotic nitrogen fixation are not only cooperated functionally but also may have evolved over a time span in family Rhizobiaceae. Genome Biol Evol. 2020:12(11):2002–2014. https://doi.org/10.1093/gbe/evaa152.

Yogev O, Pines O. Dual targeting of mitochondrial proteins: mechanism, regulation and function. Biochim Biophys Acta. 2011:1808(3): 1012–1020. https://doi.org/10.1016/j.bbamem.2010.07.004.

Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. A molecular timeline for the origin of photosynthetic eukaryotes. Mol Biol Evol. 2004:21(5):809–818. https://doi.org/10.1093/molbev/msh075.

Yoon HS, Hackett JD, Van Dolah FM, Nosenko T, Lidie KL, Bhattacharya D. Tertiary endosymbiosis driven genome evolution in dinoflagellate algae. Mol Biol Evol. 2005:22(5):1299–1308. https://doi.org/10.1093/molbev/msi118.

Young JPW, Crossman LC, Johnston AWB, Thomson NR, Ghazoui ZF, Hull KH, Wexler M, Curson ARJ, Todd JD, Poole PS, et al. The genome of *Rhizobium leguminosarum* has recognizable core and accessory components. Genome Biol. 2006:7(4):R34. https://doi.org/10.1186/gb-2006-7-4-r34.

Yutin N, Makarova KS, Mekhedov SL, Wolf YI, Koonin EV. The deep archaeal roots of eukaryotes. Mol Biol Evol. 2008:25(8):1619–1630. https://doi.org/10.1093/molbev/msn108.

Zhang X-P, Glaser E. Interaction of plant mitochondrial and chloroplast signal peptides with the Hsp70 molecular chaperone. Trends Plant Sci. 2002:7(1):14–21. https://doi.org/10.1016/s1360-1385(01)02180-x.

Zhao Y, Yu H, Zhou J-M, Smith SM, Li J. Malate circulation: linking chloroplast metabolism to mitochondrial ROS. Trends Plant Sci. 2020:25(5):446–454. https://doi.org/10.1016/j.tplants.2020.01.010.

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