Trends in Microbiology

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Review

Currency, Exchange, and Inheritance in the Evolution of Symbiosis

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Symbiotic interactions between eukaryotes and prokaryotes are widespread in nature. Here we offer a conceptual framework to study the evolutionary origins and ecological circumstances of species in beneficial symbiosis. We posit that mutual symbiotic interactions are well described by three elements: a currency, the mechanism of currency exchange, and mechanisms of symbiont inheritance. Each of these elements may be at the origin of symbiosis, with the other elements developing with time. The identity of currency in symbiosis depends on the ecological context of the symbiosis, while the specificity of the exchange mechanism underlies molecular adaptations for the symbiosis. The inheritance regime determines the degree of partner dependency and the symbiosis evolutionary trajectory. Focusing on these three elements, we review examples and open questions in the research on symbiosis.

Origins of Beneficial Symbioses

Beneficial symbioses between eukaryotic and prokaryotic organisms have evolved multiple times across the eukaryotic domain. From an evolutionary perspective, the establishment of a stable interaction with bacterial symbionts is comparable to the evolution of a novel beneficial trait [1]. Hence, the association with beneficial symbionts plays an important role in the evolution of their host. The evolution of beneficial symbiosis is thus accompanied by natural selection for maintenance of the interaction, that is, for aspects of the species interaction that constitute selectable traits. We propose that the constituents of species interactions that may be subject to natural selection require three basic components: a currency, mechanisms of currency exchange, and the mode of the interaction inheritance over generations (Figure 1A). By currency we refer to the specific nature of resources that species may gain from other species in the environment. The presence of a mechanism of exchange enables the currency to become a medium of exchange between the partners, and a heritable interaction between the partners enables the continuity of the interaction over generations. We posit that each of these constituents may supply an alternative basis for the origin of beneficial symbioses.

Currency in Symbiosis

Here we classify the currency in beneficial symbioses into nutritional or defensive types (Table 1, Figure 1B). An important determinant in the evolution of symbioses based on nutritional currencies is the prevalence of a specific resource in the biosphere. For example, many environments are oligotrophic for biologically accessible nitrogen (N), and indeed, many symbioses found in nature are characterized by the lack of bioavailable N. N is most prevalent in the form of N_2, and only prokaryotes are able to assimilate N into biologically accessible forms [2]. In N-currency symbioses, hosts commonly obtain assimilated N in the form of amino acids (Table 1), and symbionts obtain sources of organic carbon (C), such as malate and succinate, in exchange (Table 1). An example is the symbiosis between Leguminosae plants and the Rhizobiales, which is widely studied due to its relevance in crop growth (Figure 1B). The association with N_2-fixing

Highlights

Inspired by the evolution of eukaryotic organelles, we propose a conceptual framework to study the evolutionary and ecological drivers of symbiosis, including three main elements: a currency, mechanisms of currency exchange, and inheritance.

Currency in symbiosis is the type resources that species in a beneficial symbiosis gain from their partner.

Currency exchange is a complex process that requires molecular adaptations in one or both partners.

We identify two distinct but not mutually exclusive initial evolutionary imperatives for the establishment of symbiosis, termed *currency first*, in which the initial interaction stems from a common currency exchange between the interacting partners to complement their environmental requirements, and *transmission first*, in which stable transgenerational transmission precedes the evolution of currency exchange.

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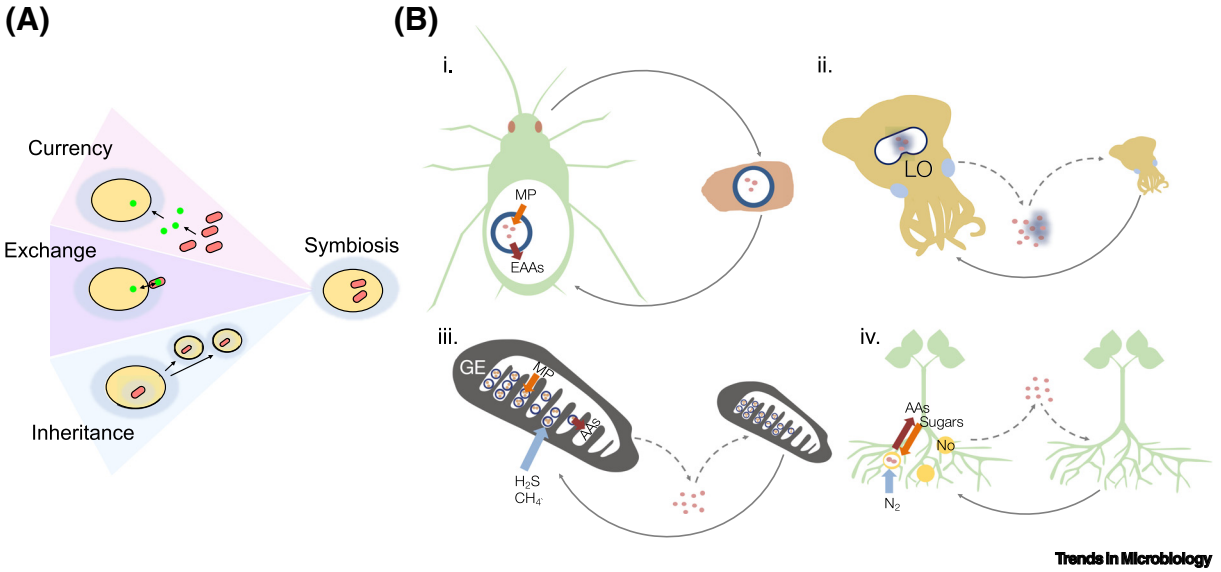

Figure 1. Currency, Exchange, and Inheritance in the Evolution of Symbiosis. (A) An illustration of the three elements. (B) Demonstrative examples of the three elements in symbiosis. Currency supplied by the host is indicated by green arrows. Currency supplied by the symbiont is indicated by orange arrows. Environmental uptake of inorganic compounds is indicated by blue arrows. (i) The currency in the aphid–*Buchnera* symbiosis is essential amino acids (EAAs). (ii) In the squid–*Vibrio* symbiosis a protective currency is provided by the symbiont, which is indicated by glowing bacterial cells that selectively colonize the host light organ (LO). (iii) Deep-sea mussels harbor symbiotic chemosynthetic bacteria in their gill epithelium (GE) where the currency is nutritional (MP = metabolic precursor). (iv) The currency in the legume–*Rhizobium* symbiosis is fixed N_2 (i.e., nutritional; No, nodules, AAs, amino acids). Gray arrows show symbiont transmission mode, where dashed lines represent the connectivity with an environmental pool in horizontal transmission. Blue circles in (i) and (ii) represent bacteriocytes.

bacteria allows the plant to inhabit N-depleted environments [3]. Symbioses based on biologically accessible N as a currency are also common in aquatic environments; for example, the association between the unicellular cyanobacterium *Candidatus* Atelocyanobacterium thalassa and the single-celled eukaryote prymnesiophyte [4,5]. Furthermore, the fixation of inorganic N by chemosynthetic symbionts of deep-sea marine nematodes and bivalves was reported [6] (Table 1). Another frequent currency in symbiosis is organic C. For example, the ancient acquisition of plastids by green plants allows for the introduction of inorganic compounds into the C cycle via photosynthesis (Box 1). Another example of photosynthesis-based symbiosis is lichens, in which the association of fungi with green algae or cyanobacteria allows for the fixation of inorganic C. Animals that colonize dark habitats, such us hydrothermal vents, are often found in C-based symbioses with chemosynthetic bacteria. Examples are the tube worms or deep-sea mussels: these organisms harbor methanotrophic and sulfur-oxidizing bacteria (Figure 1B). Chemosynthetic bacteria use inorganic compounds such as methane or hydrogen sulfide as an alternative inorganic electron donor for the fixation of inorganic C [7].

In several symbioses the nutritional currency is a waste product of one partner that is a beneficial resource for the other partner. In symbioses where the waste product is harmful to the host, its elimination by the symbiont becomes beneficial for the host. For instance, in the coral–*Symbiodinium* symbiosis, the coral produces ammonium as a harmful waste product that is sequestered by *Symbiodinium* as an N source [8]. Another example is the syntrophic symbiosis between rumen protozoa and methanogens, where H_2 is released during fermentation performed by the protist. The released H_2 constitutes a waste product since fermentation is stoichiometrically possible only if excess H_2 is removed. The methanogenic archaeon uses H_2 as an inorganic electron donor, where constant H_2 consumption enables continued fermentation [9,10].

In addition to exchanged nutritional currencies, symbiotic partners can perform other exchanges of currencies, such as defensive or protective functions. In many symbioses, the host provides the symbiont with a suitable habitat, where in addition to host-derived nutrients, the symbiont benefits from reduced competition and predation [11]. Additionally, the microbial partners may protect their hosts against pathogens or predators via the production of toxins (Table 1). Protective currencies have been described in insects, plants, and marine organisms. One example is the symbiosis between grasses and endophytic fungi. The endophytic fungus secretes alkaloids that are toxic to insects and defend the host against herbivory [12]. Protection can also occur without the production of harmful molecules. An example is the symbiosis between the bobtail squid *Euprymna scolopes* and the luminescent bacterium *Vibrio fischeri*; the symbiotic bacteria are harbored in a specialized tissue called the light organ (Figure 1B). The bacteria emit luminescence, which enables the squid to mimic the moonlight and avoid predation [13]. The symbiosis between *Pantoea agglomerans* with plants is another example for protective currency; here, the protection is provided to the host by the competition for nutrients between *Pantoea* and phytopathogenic fungi [14].

Defining the currency in symbiosis is not only helpful for studying the nature of the symbiotic interaction, but also aids understanding of the evolutionary and ecological circumstances of the symbiosis.

Mechanisms of Exchange

The mechanism of resource exchange is the second major determinant in the evolution of symbiosis, and constitutes a selectable trait for both partners. How resources are exchanged between organisms is dependent upon their nature and proximity, spatial structure, and the goods being exchanged. Some exchanges are small scale and occur across very small distances between organisms that are contained within the cells of the other (e.g., endosymbiotic bacteria in insects) [15]; in close proximity in space and time (e.g., gut microbes in vertebrates) [16]; or donors and recipients that are distant in terms of space, time, or both (e.g., detritivores and colonizing plants) [17]. Each spatial setting presents a novel set of physical barriers and physiological conditions which must be surpassed for goods to be produced by the donor and received by the recipient.

Membrane transport is an essential component of all direct currency exchanges between symbiotic partners, independently of the nature of goods. Biological membranes comprise a phospholipid monolayer or bilayer, which separates the contents of the cell from the environment and generates distinct subcellular compartments in eukaryotes [18,19]. Proteins that traffic molecules between compartments decorate the surface and interior of membranes, whether the membranes are subcellular or delineate cell contents from the surrounding environment. Some of these proteins function in the biogenesis, structure, or functioning of membranes, while others are involved in the bidirectional movement of molecules across membranes in order to maintain cellular homeostasis. Membrane proteins evolve at accelerated rates in comparison to cytosolic proteins, illustrating the strong selection pressure acting on cellular machinery for goods exchange with the environment, whether that be within or between organisms [20]. The composition and turnover of the lipid layer can also facilitate the movement of molecules from one side of a membrane to the other, for example by the generation of vesicles or lipid rafts [21,22]. Thanks to this diversity of mechanisms, and unique membrane biochemistry and metabolic requirements, different organisms have variable complements of molecular machinery for membrane transport, which constrains the establishment and subsequent evolution of symbioses.

Table 1. Currency in Symbiosis

Host				Symbiont							
Organism	Type of currency supplied	Currency supplied	Dependency	Organism	Type of currency supplied	Currency supplied	Dependency	Transmission mode	Localization	Trophic state	Refs
Legumes	Nutritional	Organic acids, Iron, sugars	Facultative	*Rhizobium*	Nutritional	NH_3	Facultative	Horizontal	Intracellular, Nodules	N-depleted environment	[3,95]
Gunnera	Nutritional	Organic acids	Facultative	Nostoc	Nutritional	NH_3	Facultative	Horizontal	Intracellular, Stolones	N-depleted environment	[96,97]
Protists in the termite gut	Nutritional	Sugars, urinates, urea, H_2, NH_3	?	*Treponema* Spirochete	Nutritional	Amino acids	?	?	Intracellular	N-depleted environment	[98]
Marine bivalves	Nutritional	Organic compounds	Facultative	Chemosynthetic bacteria	Nutritional	Amino acids/cofactors	Facultative[a]	Horizontal and vertical	Intracellular, gill	Light and N-depleted environment	[99–101]
Marine stilbonematid nematodes	Nutritional	Organic compounds	?	Chemosynthetic bacteria	Nutritional	Amino acids	?	Horizontal	Extracellular	Light and N-depleted environment	[6,102]
Single-cell eukaryotic algae	Nutritional	Organic compounds	Obligate	Cyanobacteria	Nutritional	Amino acids	Obligate	Horizontal	Unicellular	N-depleted environment	[4,5]
Corals	Nutritional	Wasted N (NH_4)	Obligate	Dinoflagellate algae *Symbiodinium*	Nutritional	Organic compounds	Obligate	Horizontal	Intracellular Symbiosome (endoderm)	N-depleted environment	[8]
Corals	Nutritional	Wasted N (NH_4)	Obligate	Dinoflagellate algae *Symbiodinium*	Defensive	Diterpenes	Obligate	Horizontal	Intracellular Symbiosome (endoderm)	NA	[103]
Hydrogenosome-containing cilliate	Nutritional	Wasted H_2	?	Methanogen	Nutritional	Cellular macromolecules	?	Vertical, multiple replacements	Intracellular	O_2-depleted environment	[104,105]
Hydrogenosome-containing protozoa from rumen	Nutritional	Wasted H_2	?	Methanogen	Nutritional	Cellular macromolecules	?	?	Intracellular	O_2-depleted environment	[10]
Wasp	?	?	Obligate	*Wolbachia*	Defensive	Bacterioferritin[b]	Obligate	Mostly vertical	Intracellular	NA	[106,107]

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Squid	Nutritional	Amino acids, fatty acids	Obligate	*Vibrio*	Defensive	Light	Facultative	Horizontal	Extracellular Light organ	NA	[108–110]
Nematodes	Habitat-based	Habitat	Obligate	*Xenorhabdus* and *Photorhabdus* spp.	Defensive	Toxin against insect host	Obligate	Vertical	Intracellular	NA	[111–113]
Grasses	Habitat-based	Habitat	Facultative	Fungal endophytes	Defensive	Alkaloids and antioxidants[c]	?	Vertical and horizontal	Extracellular, between plant cells	NA	[12,114]
Tsetse fly	Habitat-based	Habitat	Obligate	*Wigglesworthia glossinidia*	Nutritional	Vitamins	Obligate	Vertical	Intracellular	NA	[115–117]
Aphids	Nutritional	Metabolic precursors	Obligate	*Buchnera*	Nutritional	Essential amino acids	Obligate	Vertical	Intracellular	Nutrient-depleted environment	[118]
Fungus	Habitat-based	Habitat	Obligate	Green algae/ cyanobacteria	Nutritional	Fixed C	?	Vertical and Horizontal	Extracellular	Nutrient-depleted environment	[119]
Aphids	Nutritional	Amino-acids	Facultative	*Hamiltonella defensa*	Defensive	Bacteriophage--encoded toxins	Obligate	Vertical and Horizontal	Intracellular and extracellular	NA	[120–123]
Paederus beetle	?	?	?	Uncultired γ-proteobacterium	Defensive	Pederins	?	?	?	NA	[124]
Wheat	Habitat-based	Habitat	Facultative	*Pantoea agglomerans*	Defensive	NA[d]	Facultative	?	Extracellular and intercellular	NA	[14,125]
Moss animals	?	?	Obligate	*Candidatus* Endobugula sertula	Defensive	Bryostatins	Obligate	Vertical	Extracellular	NA	[126]
Amoebae	Nutritional	Organic compounds	Facultative	*Protochlamydia amoebophila.*	Defensive	?	Obligate	Vertical and Horizontal	Intracellular	NA	[127,128]
Paramecium	Habitat-based	Habitat	Obligate	Habitat	Defensive[e]	?	Facultative	Horizontal	Intracellular	NA	[129]

?, Aspects of the symbiosis that are currently unknown.
NA, not assigned.
[a]Bacteria most likely remain dormant outside the host. Yet, because the symbionts are horizontally acquired, the possibility of facultative symbiosis cannot be rejected.
[b]Note that the synthesis of bacterioferritin has been suggested to protect the host against ROS production caused by the establishment of symbiosis, and thus, it does not imply a clear benefit to the host.
[c]Alkaloids protect the plant against herbivory and act as antioxidants of ROS produced during photosynthesis.
[d]*Pantoea* protective mechanism has been suggested to be based on the competition for nutrients with phytopathogenic fungi.
[e]Cells of *Paramecium* infected with the endosymbiont grow slower, and are more prone to be eaten by predators, but on the other hand they are protected against populations of competitor strains which do not harbor the symbiont. Therefore, the symbiont confers an advantage on its host only when host populations are infected by the symbiont.

One example of membrane transport machinery mediating the establishment of symbiosis is implicit in the hydrogen hypothesis for the origin of mitochondria: metabolic machinery for the expulsion of hydrogen generated as a metabolic by-product by the ancestral alphaproteobacterial symbiont existed prior to its association with the archaeon. The archaeon could use the resulting hydrogen for methanogenesis as intracellular hydrogen build-up is toxic for the host, thus, the export machinery had already evolved (Box 1). Similarly, the pre-existing ability of the ancestral proteobacterial symbiont to generate outer membrane vesicles (OMVs) is hypothesized to have enabled the formation of subcellular organelles and protein targeting – a critical step in the evolution of multicellular organisms [23,24]. Similar molecular mechanisms mediate the exchange of goods between extracellular symbionts. For example, *V. fischeri* produces OMVs containing peptidoglycan (PG) and lipopolysaccharide (LPS) molecules – components of the outer

Box 1. Currency, Exchange, and Inheritance in the Evolution of Eukaryotic Organelles

The most extreme outcome of bacterial endosymbiosis is exemplified by the organelles known as mitochondria and plastids, and their derivatives (Table I). All plastids, as hallmarks of photoautotrophy, were originally acquired by eukaryotes via endosymbiosis of a cyanobacterial ancestor [58,59], approximately 1.2 billion years ago [60]. The origin of mitochondria, as 'powerplants' of the cell, is tightly bound with the emergence of the first eukaryotes at least 1.6 billion years ago [61]. There is common agreement that the mitochondria evolved from an alpha-proteobacterium ancestor that was in a symbiosis with an archaea-like host [62,63]. Further derivatives of mitochondria are hydrogenosomes, characterized by the production of hydrogen [64,65], and mitosomes [64,66]; both are devoid of a genome.

The currency of plastids and mitochondria as endosymbionts is mainly determined by their central functionality in host metabolism, which is still localized to the compartment: photoautotrophy for functional plastids, and energy conservation for mitochondria. They resemble nutritional symbioses via direct currency exchange with the host (see main text). The host provides most of the proteins and nutrients to fuel the organelle's machinery, while metabolic products are transported back to the host cytoplasm (i.e., organic compounds or the cellular unit of energy: adenosine triphosphate, ATP). However, the organelle-associated currencies we observe today might not reflect those of the original endosymbiont and may have changed over the course of evolution. Symbioses between cyanobacteria or algae and eukaryotic hosts are widespread in nature and rely on similar currencies as observed for plastids (Table 1). In such photosynthetic symbioses, the autotrophic symbiont supplies the host with fixed inorganic compounds (e.g., C or N), while the host provides organic compounds or a competition-free environment (niche). In plants, the metabolic pathways associated with the fixation of C and N are predominantly localized to the plastid, suggesting that they originated from the cyanobacterial plastid ancestors and that the basic currencies have not changed since then. In addition, this indicates that symbiotic relationships reminiscent of plastid evolution may be repeatable, as is suggested for the plastid-like cyanobacterial endosymbiont of *Paulinella chromatophora* [67]. Another example is *Hatena arenicola*, which forms obligate, but unstable, associations with *Nephroselmis* algae that potentially resemble the ancestors of complex plastids [68]. In contrast to plastids, the currency of the mitochondrion ancestor is less easy to compare to symbioses observed today. It is likely that the original endosymbiosis leading to mitochondria did not depend on ATP production, as it does today, but rather on other factors. Releasing such energy-rich molecules to the environment would unlikely be favorable for the endosymbiont [69]. Thus, the hydrogen hypothesis, one of the most popular hypotheses on the origin of mitochondria, postulates that hydrogen produced as a waste product of the alphaproteobacterial endosymbiont was used by the methanogenic host [69,70]. A similar type of symbiotic association is described in hydrogen-producing ciliates (Table 1). The evolution from the endosymbiont to the mitochondrial organelle was accompanied by adaptation to the host and a change in currency. Currency modifications following the transition to an organelle are well described in apicoplasts, the plastid derivates found in most nonphotosynthetic apicomplexan parasites [71], and mitosomes. Both organelles lost their fundamental functions in the energy metabolism of the host, but are still retained and function in diverse metabolic pathways, including the synthesis of iron–sulfur clusters and haem [72–74].

Mechanisms for the exchange of goods between organelles and their host environment are complex, covering various ions and metabolites, and are mainly governed by transport proteins (reviewed in [75,76]). Most transporters of the inner membrane of mitochondria belong to a large protein family, termed mitochondrial carriers, displaying diverse substrate specificities [77]. This family has ~50 members in humans alone, and many still lack a functional classification. Notably, several aspects of the organelles' transport systems remain to be elucidated. For example, it is currently unknown how most amino acids are transported from the cytosol to the mitochondria or plastids where protein synthesis is located in the organelles. Unique to organelles, in contrast to bacterial endosymbioses, are protein import mechanisms allowing host proteins encoded in the nucleus to function in other cellular compartments. Both mitochondria and plastids convergently evolved similar transport mechanisms (translocon of the inner/outer mitochondrial/chloroplast membrane; TIM/TOM & TIC/TOC, respectively; reviewed in [78,79]) guided by target peptide sequences of precursor proteins. Even more advanced transport systems are found in algae with complex plastids [80]. As a consequence of protein transport, gene content of the endosymbiotic organelle ancestors could be integrated into the nuclear genome (termed endosymbiotic gene transfer; EGT), but still function in the organelle [63,81]. EGT has been demonstrated to dominate long-term gene content evolution in eukaryotes. The number of genes encoded in the genomes of the organelles (3 to ~200) is diminishingly small in comparison to those of free-living bacteria (≫5000) [82]. The vast majority of nuclear genes appears to be of endosymbiotic ancestry [63], while nuclear genomes of plants include ca. 20% genes that descended from the plastid ancestor [83].

Transmission mechanisms for organelles are not always tightly controlled with the host cell cycle. For mitochondria in multicellular organisms, for example, no segregation mechanisms are known, suggesting that the presence of numerous organelles in the cell results in stochastic distribution to both daughter cells following cell division [84,85]. However, this scenario may randomly result in the loss of mitochondria in one of the daughter cells, and thus cell death. Such scenarios can be tolerated in multicellular organisms but are less likely in single-celled ancestors of eukaryotes. Mitochondrial segregation in asymmetric cell division of yeast is tightly controlled [84]. Many algae harbor only a single plastid per cell (termed monoplastidy), of which division is tightly controlled with the host cell cycle [85–87]. Monoplastidy is suggested to represent the ancestral state of algal endosymbiosis and a prerequisite of plastid emergence [85–87]. Therefore, controlled segregation mechanisms were likely required to sustain stable transmission of plastids and mitochondria, or at least inheritance of their ancestors within unicellular hosts.

Table I. Currency in Endosymbiosis[a]

Host				Symbiont					
Organism	Currency type	Currency	Dependence	Organelle	Currency type	Currency	Dependence	Transmission mode	Example
Archaplastidae	Nutrient	Anorganic compounds, proteins	Obligate	Primary plastid (cyanobacterium)	Niche	Organic compounds	Obligate	Vertical	*Arabidopsis thaliana* [88]
Algal eukaryote	Nutrient	Anorganic compounds, proteins	Obligate	Complex plastid (algal eukaryote)	Niche	Organic compounds	Obligate	Vertical	Diatoms [89]
Apicomplexa	Nutrient	Anorganic compound (?), proteins	Obligate	Apicoplast (complex plastid)	Niche	Specific metabolites?	Obligate	Vertical	*Plasmodium falciparum* [90]
Paulinella chromatophora	Nutrient	Anorganic compounds	Obligate	Chromatophore (cyanobacterium)	Niche	Organic compounds	Obligate	Vertical	*Paulinella chromatophora* [91]
Hatena arenicola	Nutrient	Anorganic compounds, proteins	Obligate	*Nephroselmis* algae	Niche	Organic compounds	?	Horizontal & Vertical	*Hatena arenicola* [68]
Eukaryote	Nutrient?	Organic compounds, proteins	Obligate	Mitochondrion (α-proteobacterium)	Nutrient?	Energy (ATP)	Obligate	Vertical	*Homo sapiens* [92]
Anaerobic eukaryote	Nutrient?	Organic compounds, proteins	Obligate	Hydrogenosome (mitochondrion)	Nutrient?	Energy (ATP)	Obligate	Vertical	*Trichomonas vaginalis* [93]
Anaerobic eukaryote	Nutrient?	Organic compounds?, proteins	Obligate	Mitosome (mitochondrion)	Nutrient?	Specific metabolites?	Obligate	Vertical	*Giardia lamblia* [72]
Rhopalodiaceae diatoms	Nutrient	Organic compounds?, proteins	Obligate	Spheroid body (Cyanobacterium)	Niche?	N-fixation	Obligate	Vertical	*Rhopalodia gibbs* [94]

[a]? indicates that information is missing.

membrane of Gram-negative bacteria which are recognized by epithelial receptors of the squid light organ, and trigger host development [25,26]. The latter demonstrates the co-option of pre-existing transport mechanisms for the maintenance of an interaction, specifically in this system, where *V. fischeri* cells must be horizontally acquired from the environment during the juvenile phase [27].

The presence of nonspecific exchange mechanisms may hinder the establishment of a stable symbiosis between two organisms. For example, developmental cues in *Aedes aegypti* mosquitoes are triggered by the consumption of oxygen by aerobic bacteria in the gut, allowing mosquito development to pupation. The identity of the bacteria performing the function varies and does not influence the net effect of the interaction; the gut is permissive of microbes as long as they consume oxygen [28]. Despite the integration of this microbially mediated function into host development (i.e., making it essential), no stable interaction has formed – presumably because oxygen consumption is ubiquitous or very common in colonizing microbes. This has been posited as one explanation for the lack of uniformity amongst gut bacterial communities of larval mosquitoes developing in different environments, and provides an explanation for the lack of specific gut symbioses in this species [28,29]. Similarly, the roots of land plants acquire arbuscular mycorrhizal (AM) fungi from the soil at each generation, and these fungi colonize the cortical cells of plant roots and establish a symbiont interface for direct nutrient exchange using complex signaling

mechanisms. Plants provide the colonizing AM fungi with C, and the fungi reciprocate by providing mineral nutrients, mainly phosphorus (P) [30,31]. The acquisition of AM fungi is horizontal, and partner fidelity is not uniform between generations, with the same plant forming interactions with multiple fungi, and fungi able to simultaneously colonize the roots of several different plant partners [32,33]. As a result, elaborate policing mechanisms have evolved to regulate the exchange of goods to avoid exploitation by cheaters [34]: plants can increase the transport of C to cooperative individuals that provide the host with more P, and fungi can respond by increasing P flux to the plant, ensuring that investment is managed in both partners [35].

Pre-existing mechanisms for the translocation of proteins, metabolites or nucleic acids have the potential to evolve into specific exchange mechanisms depending on the currency. The nature and chemistry of the material being exchanged, and the environmental context in which the exchange occurs, generates constraints on the evolution and maintenance of mechanisms for currency exchange in symbioses.

Inheritance

The establishment of stable symbiotic relations critically depends on the persistence of the interaction over the course of evolution. Thus, the maintenance of symbionts throughout generations is essential for the evolution of a common currency and mechanisms of exchange in the symbiosis. Two fundamentally different symbiont transmission modes are distinguished in the literature: vertical inheritance, where the symbionts are transmitted from ancestor to descendants, and horizontal transmission where the symbionts are acquired from the environment (reviewed in [36]). Transmission fidelity is crucial for the long-term establishment of the symbiosis because the symbiont is at risk of loss in every generation and, in cases of obligate dependency, lethality (and extinction) of both partners. The evolution of transmission modes and stable interactions between the partners as well as the mechanisms that lead to lineage-specific symbiosis remain understudied. Here we propose two routes for the establishment and evolution of long-term symbiosis. In the first route, which we term *currency first*, stable symbiosis is established upon the provision of an essential resource for the host (i.e., currency). In this route, the exchange of currencies is beneficial for the partners involved, hence the symbiosis evolves under positive selection for maintenance of the interaction. In the second route, which we term *transmission first*, stable inheritance or horizontal transmission in each host generation precedes the evolution of currency exchange. In this route, the interaction may have emerged under neutral or nearly neutral conditions where the constant presence of the symbiont over generations may evolve into a beneficial interaction (through currency exchange) and finally symbiosis (Figure 2).

In the currency first scenario, the symbiosis provides a fitness advantage to the partners by making an essential currency available. This implies that the mechanisms of currency exchange were already in place prior to the onset of the interaction. It is likely that many symbiotic interactions that we listed here (Table 1) may have evolved along that route. This includes symbioses in which the currency constitutes the product of metabolic pathways that are abundant in prokaryotic organisms and absent in eukaryotic organisms (e.g., nitrate; Table 1) as well as rare metabolites such as vitamins or amino acids that might be difficult for the host to produce (e.g., essential amino acids in the *Buchnera aphidicola* and aphid symbiosis; Table 1). We note that beneficial symbioses with a mixed microbial community may be accompanied by extensive functional redundancy, where the currency exchange is nonspecific with regard to the partner identity and can be satisfied by community-level processes (e.g., developmental stage triggered by symbiont oxygen consumption; [28]). These nonspecific interactions may evolve into symbioses with specific partners under positive selection for the interaction. The evolution of a specific partner can be advantageous for the fidelity of the currency supply because it enables partner recognition and

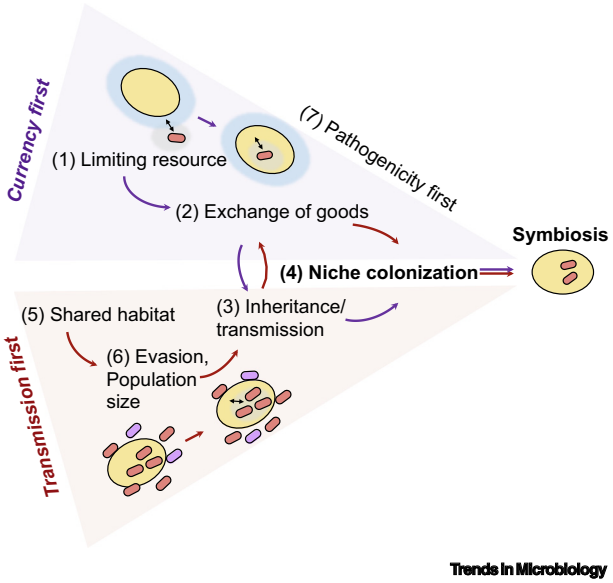

Figure 2. Evolutionary Routes to Symbiosis. Under the *currency first* scenario (purple panel and arrows), limiting factors in the environment (1) constitute currencies that lead to the exchange of currency (2) which is followed by stable inheritance (3) and stable niche colonization (4). In the *transmission first* route (red panel and arrows), the evolution of symbiosis is initiated by co-occurrence in the ecosystems (5), and neutral (6), but stable inheritance (3) prior to the development of currency exchange (2) and stable niche colonization (4). The scenario of *pathogenicity first* (7) involves a unidirectional currency transfer and a stable transmission. Several studies have suggested that, under certain conditions, pathogenic symbionts may evolve a mutual or commensal interaction with their host [49,50].

policing mechanisms through mutual dependency. If the reproductive success of individuals that maintain the symbiosis is higher than those that do not, the ability to maintain the symbiosis becomes an advantageous trait. Hence, the mechanism of symbiont inheritance (or acquisition) constitutes a selectable trait, which may subsequently be fixed in the lineage.

As a second route for the evolution of stable symbioses we propose the transmission first scenario. In this route, stable transmission of the symbiont (vertical or horizontal) may precede the evolution of currency exchange. We propose that the host–symbiont interaction may emerge from random associations under conditions that may be neutral for the partners (i.e., no partner benefits from a specific resource). Many associations between eukaryotes and microorganisms are described as transient, namely, they are variable across individuals and life span. For example, part of the human microbiota is transient, yet may be stably transmitted from mothers to babies (e.g., [37]). In addition, many anaerobic ciliates are associated with methanogens, which thrive upon hydrogen production by the ciliate [38]. These interactions are typically thought to be transient, as the methanogenic bacteria interaction with the ciliate is not obligatory (i.e., they are found also as free living, e.g., in the rumen [39]). Nonetheless, a stable vertical inheritance may occur with every cell division. The stable transmission of bacteria can rely on the large bacterial population size and rapid growth rate in comparison to the eukaryotic host. Furthermore, bacterial symbionts have the ability to evade digestion or defense systems of the host (e.g., evasion strategies in inhabitants of protists [40–42]) and thus gain an opportunity to persist in the host environment over multiple life cycles. Highly persistent intracellular bacteria may evolve stable transmission with the host and so gain a constant presence in the lineage. Stable transmission of intracellular bacteria may be facilitated by several strategies: (i) a high abundance in the host cell can ensure inheritance during cell division (as is the inheritance strategy of mitochondria of multicellular organisms; Box 1); (ii) active symbiont segregation, where the allocation of symbionts to the offspring is well coordinated with host cell division (as the inheritance strategy of plastids in many algae; Box 1), e.g., by hitchhiking with the host cytoskeleton or membrane-bound organelles. An example is *Wolbachia* that hitchhikes with the segregation apparatus by attachment to the spindle filaments in *Drosophila melanogaster* oocytes during mitosis [43]. As a result, *Wolbachia* segregates along with the host chromosomes, thereby ensuring its stable inheritance. The

evolution of stable transmission may be followed by the evolution of currency exchange. The latter evolutionary development may be driven, for example, by a change in the environmental conditions that render a neutral association into an advantageous one (e.g., [44]). Another possibility is the loss of a host function that may be compensated for by the symbiont. An example of this scenario is described for the interaction between the leaf beetle *Conistra rubiginosa* and bacteria of the genus *Stammera*, which express pectinases to aid in the digestion of pectin-rich plant material [45]. In this family of phytophagous beetles, *C. rubiginosa* is the only one that acquired a symbiont species to perform this function and lost the metabolic pathway from its own genome [46]. Another example of the evolution of currency in symbiosis following a stable association is the evolution of heterotrophic plants: the ability to perform photosynthesis was lost in the ghost orchid *Epipogium aphyllum*, and it has been suggested that the requirement for fixed C is supplied by fungi associated with the orchid root [47]. In addition, *transmission first* may enable a constant uptake of environmental bacteria that utilize the existing transmission machinery of the residing symbionts. One example is the stinkbug *Plautia stali* and its symbiosis with different *Pantoea* strains. These strains were shown to be replaceable, and thus functionally equivalent for their host, yet they had distinct evolutionary histories [48].

An additional route to symbiosis is *pathogenicity first*, which is found in the twilight zone of the two other routes (Figure 2). In this route, the partners are already in a stable interaction; however, only one partner – the pathogen – is benefiting from a host resource (i.e., in this scenario currency is not exchanged, rather it is taken). Such a relationship may change while moving across the parasitism–mutualism continuum. Several examples can be found in the gut microbiome of animals. For example, in the gut of *Caenorhabditis elegans*, a mildly pathogenic strain of *Enterococcus faecalis* rapidly evolved in about 15 host generations to defend their hosts against infection by a more virulent pathogen via an increase of antimicrobial superoxides production [49]. Another recent study showed that the opportunistic fungal pathogen *Candida albicans* in the mouse gut could be evolved into a beneficial partner for the host. The selected fungus was able to protect the host from multiple systemic infections [50]. Thus, for both the microbes and hosts the interaction is advantageous, which may hint at the onset of a long-term stable symbiosis. At the other end of this continuum, the ciliate protozoan *Paramecium tetraurelia* is known to carry the intracellular, vertically transmitted *Caedibacter taeniospiralis* that confers the 'killer trait', killing other protozoa lacking the symbiont. A recent study showed that the fitness advantage conferred by the symbiont can shift towards parasitism depending on the *Paramecium* growth conditions [51]. Thus, pathobionts may arise from stable mutualistic or commensal interactions where the switch into a new lifestyle is triggered by a change in environmental conditions.

A stable inheritance is an important prerequisite for the evolution of symbiosis, yet the nature of the interaction may range from commensalism to pathogenicity. The difference between pathogenicity and beneficial symbiosis may be smaller than we think and largely depend on the contemporary environmental conditions.

Concluding Remarks

Recognizing the basic ingredients for the development of species interactions into a stable symbiosis – currency, exchange, and inheritance – supplies a useful framework for future investigations of symbioses. For example, if a limiting resource becomes abundant in the environment it is no longer considered as currency, and supplementation may become neutral or even harmful. What happens when a symbiotic interaction is no longer beneficial? Under such a scenario the beneficial trait (i.e., the symbiosis) would be nonfunctionalized, and evolutionary theory predicts that it is no longer under stabilizing selection. If a mechanism of stable inheritance is already in

Outstanding Questions

What are the currencies in the symbiosis, and what is their ecological context?

Is there a functional redundancy within the symbiotic community with regard to the currencies?

What are the exchange mechanisms between host and symbiont?

Does the fitness effect of the symbiosis depend on the environmental context?

What are the mechanisms of stable association over generations?

How do symbiont strains establish a competitive advantage within the host habitat?

What is the chronology of the development of currency, currency exchange, and stable association in the symbiosis evolution?

place, the loss of the symbiosis largely depends on the fitness effect of the interaction. When the interaction is neutral to the fitness of the partners, the symbiosis might persist for a while. In contrast, if the symbiotic interaction has a deleterious effect on the fitness of one of the partners, the interaction may be quickly lost. Nonetheless, also in this scenario, the stability of the inheritance mechanism is expected to play an important role, as the loss of symbiosis requires the emergence of hosts that lost the symbiont (or host-free symbionts) which subsequently outcompete symbiotic hosts. We note that symbiotic interactions may involve multiple symbiotic species (i.e., a symbiotic community) rather than a single symbiont. Thus, in symbiotic interactions with a mixed microbial community, where multiple strains harbor the core symbiont currency exchange mechanisms (e.g., [52]), functional redundancy may lead to a transient interaction rather than stable inheritance. In such symbioses, colonization history can result in alternative symbiont community composition that nonetheless secures the host requirements (e.g., [53]). From a host-centric point of view, it is likely more informative to study the mechanisms and role of currency exchange rather than the specific symbiont strain identity. From the symbiont point of view, an open question is how do symbiont strains establish a competitive advantage within the host habitat and develop stable inheritance despite their functional redundancy (e.g., priority effects in colonization [54–56] or intraspecific competition [57])? Are certain hosts more prone to be in symbiotic relations with bacteria and vice versa? These are topics that require much more research and a synthesis with existing ecological theory (see Outstanding Questions). Modern techniques allow the analysis of specific bacterial strains in their association with a host at the single-cell level, and comparative genomics with closely related free-living species could uncover the genomic basis for the emergence of beneficial interactions.

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References

1. Douglas, A.E. (2014) Symbiosis as a general principle in eukaryotic evolution. *Cold Spring Harb. Perspect. Biol.* 6, a016113
2. Kneip, C. *et al.* (2007) Nitrogen fixation in eukaryotes – new models for symbiosis. *BMC Evol. Biol.* 7 55–12
3. Jones, K.M. *et al.* (2007) How rhizobial symbionts invade plants: the *Sinorhizobium–Medicago* model. *Nat. Rev. Microbiol.* 5, 619–633
4. Thompson, A.W. *et al.* (2012) Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. *Science* 337, 1546–1550
5. Cabello, A.M. *et al.* (2016) Global distribution and vertical patterns of a prymnesiophyte-cyanobacteria obligate symbiosis. *ISME J.* 10, 693–706
6. Petersen, J.M. *et al.* (2016) Chemosynthetic symbionts of marine invertebrate animals are capable of nitrogen fixation. *Nature Microbiol.* 2, 1–11
7. Dubilier, N. *et al.* (2008) Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nat. Rev. Microbiol.* 6, 725–740
8. Rädecker, N. *et al.* (2015) Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends Microbiol.* 23, 490–497
9. Ushida, K. *et al.* (1997) Interspecies hydrogen transfer between the rumen ciliate *Polyplastron multivesiculatum* and *Methanosarcina barkeri*. *J. Gen. Appl. Microbiol.* 43, 129–131
10. Finlay, B. (1994) Some rumen ciliates have endosymbiotic methanogens. *FEMS Microbiol. Lett.* 117, 157–161
11. Garcia, J.R. and Gerardo, N.M. (2014) The symbiont side of symbiosis: do microbes really benefit? *Front. Microbiol.* 5, 1103
12. Omacini, M. *et al.* (2001) Symbiotic fungal endophytes control insect host-parasite interaction webs. *Nature* 409, 78–81
13. McFall-Ngai, M.J. (2014) The importance of microbes in animal development: lessons from the squid-*Vibrio* symbiosis. *Ann. Rev. Microbiol.* 68, 177–194
14. Poppe, L. *et al.* (2003) Modes of action of *Pantoea agglomerans* CPA-2, an antagonist of postharvest pathogens on fruits. *Eur. J. Plant Pathol.* 109, 963–973
15. Douglas, A.E. (1989) Mycetocyte symbiosis in insects. *Biol. Rev. Camb. Philos. Soc.* 64, 409–434
16. Ley, R.E. *et al.* (2008) Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* 6, 776–788
17. Chapin, S. *et al.* (2007) *Principles of Terrestrial Ecosystem Ecology,* Springer
18. Edidin, M. (2003) The state of lipid rafts: from model membranes to cells. *Annu. Rev. Biophys. Biomol. Struct.* 32, 257–283
19. Gould, S.B. (2018) Membranes and evolution. *Curr. Biol.* 28, R381–R385
20. Sojo, V. *et al.* (2016) Membrane proteins are dramatically less conserved than water-soluble proteins across the Tree of Life. *Mol. Biol. Evol.* 33, 2874–2884
21. Kulp, A. and Kuehn, M.J. (2010) Biological functions and biogenesis of secreted bacterial outer membrane vesicles. *Ann. Rev. Microbiol.* 64, 163–184
22. Lingwood, D. and Simons, K. (2010) Lipid rafts as a membrane-organizing principle. *Science* 327, 46–50
23. Gould, S.B. *et al.* (2016) Bacterial vesicle secretion and the evolutionary origin of the eukaryotic endomembrane system. *Trends Microbiol.* 24, 525–534

24. Soubannier, V. *et al.* (2012) A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. *Curr. Biol.* 22, 135–141
25. Aschtgen, M.-S. *et al.* (2015) *Vibrio fischeri*-derived outer membrane vesicles trigger host development. *Cell. Microbiol.* 18, 488–499
26. Aschtgen, M.-S. *et al.* (2016) Rotation of *Vibrio fischeri* flagella produces outer membrane vesicles that induce host development. *J. Bacteriol.* 198, 2156–2165
27. Nyholm, S.V. and McFall-Ngai, M. (2004) The winnowing: establishing the squid–*Vibrio* symbiosis. *Nat. Rev. Microbiol.* 2, 632–642
28. Coon, K.L. *et al.* (2017) Bacteria-mediated hypoxia functions as a signal for mosquito development. *Proc. Natl. Acad. Sci. U. S. A.* 114, E5362–E5369
29. Coon, K.L. *et al.* (2016) Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. *Mol. Ecol.* 25, 5806–5826
30. Smith, S.E. and Smith, F.A. (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annu. Rev. Plant Biol.* 62, 227–250
31. Smith, S.E. and Smith, F.A. (1990) Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. *New Phytol.* 114, 1–38
32. Mikkelsen, B.L. *et al.* (2008) Underground resource allocation between individual networks of mycorrhizal fungi. *New Phytol.* 180, 890–898
33. Vandenkoornhuyse, P. *et al.* (2007) Active root-inhabiting microbes identified by rapid incorporation of plant-derived carbon into RNA. *Proc. Natl. Acad. Sci. U. S. A.* 104, 16970–16975
34. Douglas, A.E. (2008) Conflict, cheats and the persistence of symbioses. *New Phytol.* 177, 849–858
35. Kiers, E.T. *et al.* (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333, 880–882
36. Bright, M. and Bulgheresi, S. (2010) A complex journey: transmission of microbial symbionts. *Nat. Rev. Microbiol.* 8, 218–230
37. Ferretti, P. *et al.* (2018) Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host Microbe* 24, 133–145
38. Embley, T.M. and Finlay, B.J. (1994) The use of small subunit rRNA sequences to unravel the relationships between anaerobic ciliates and their methanogen endosymbionts. *Microbiology* 140, 225–235
39. Levy, B. and Jami, E. (2018) Exploring the prokaryotic community associated with the rumen ciliate protozoa population. *Front. Microbiol.* 9, 663
40. Matz, C. and Kjelleberg, S. (2005) Off the hook-how bacteria survive protozoan grazing. *Trends Microbiol.* 13, 302–307
41. Gong, J. *et al.* (2016) Protist–bacteria associations: Gammaproteobacteria and Alphaproteobacteria are prevalent as digestion-resistant bacteria in ciliated protozoa. *Front. Microbiol.* 7, 498
42. Brock, D.A. *et al.* (2018) Diversity of free-living environmental bacteria and their interactions with a bactivorous amoeba. *Front. Cell. Inf. Microbiol.* 8, 411
43. Ferree, P.M. *et al.* (2005) *Wolbachia* utilizes host microtubules and Dynein for anterior localization in the *Drosophila* oocyte. *PLoS Pathog.* 1, 0111–0124
44. Osmanovic, D. *et al.* (2018) Darwinian selection of host and bacteria supports emergence of Lamarckian-like adaptation of the system as a whole. *Biol. Direct* 13, 24
45. Salem, H. *et al.* (2017) Drastic genome reduction in an herbivore's pectinolytic symbiont. *Cell* 171, 1–12
46. Kirsch, R. *et al.* (2014) Horizontal gene transfer and functional diversification of plant cell wall degrading polygalacturonases: Key events in the evolution of herbivory in beetles. *Insect Biochem. Mol. Biol.* 52, 33–50
47. Liebel, H.T. and Gebauer, G. (2011) Stable isotope signatures confirm carbon and nitrogen gain through ectomycorrhizas in the ghost orchid *Epipogium aphyllum* Swartz. *Plant Biol.* 13, 270–275
48. Hosokawa, T. *et al.* (2016) Obligate bacterial mutualists evolving from environmental bacteria in natural insect populations. *Nature Microbiol.* 1, 1–7
49. King, K.C. *et al.* (2016) Rapid evolution of microbe-mediated protection against pathogens in a worm host. *ISME J.* 10, 1915–1924
50. Tso, G.H.W. *et al.* (2018) Experimental evolution of a fungal pathogen into a gut symbiont. *Science* 362, 589–595
51. Schu, M.G. and Schrallhammer, M. (2018) Cultivation conditions can cause a shift from mutualistic to parasitic behavior in the symbiosis between *Paramecium* and its bacterial symbiont *Caedibacter taeniospiralis*. *Curr. Microbiol.* 75, 1099–1102
52. Ansorge, R. *et al.* (2019) Diversity matters: Deep-sea mussels harbor multiple symbiont strains. *bioRxiv* Published online January 26, 2019. https://doi.org/10.1101/531459
53. Romero Picazo, D. *et al.* (2019) Horizontally transmitted symbiont populations in deep-sea mussels are genetically isolated. *bioRxiv* Published online February 1, 2019. https://doi.org/10.1101/536854
54. Wein, T. *et al.* (2018) Carrying capacity and colonization dynamics of *Curvibacter* in the *Hydra* host habitat. *Front. Microbiol.* 9, 443
55. Bongrand, C. *et al.* (2016) A genomic comparison of 13 symbiotic *Vibrio fischeri* isolates from the perspective of their host source and colonization behavior. *ISME J.* 10, 2907–2917
56. Bongrand, C. and Ruby, E.G. (2018) Achieving a multi-strain symbiosis: strain behavior and infection dynamics. *ISME J.* 13, 698–706
57. Speare, L. *et al.* (2018) Bacterial symbionts use a type VI secretion system to eliminate competitors in their natural host. *Proc. Natl. Acad. Sci. U. S. A.* 115, E8528–E8537
58. Giovannoni, S.J. *et al.* (1988) Evolutionary relationships among cyanobacteria and green chloroplasts. *J. Bacteriol.* 170, 3584–3592
59. Martin, W. and Kowallik, K. (1999) Annotated English translation of Mereschkowsky's 1905 paper 'Über Natur und Ursprung der Chromatophoren im Pflanzenreiche'. *Europ. J. Phycol.* 34, 287–295
60. Parfrey, L.W. *et al.* (2011) Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proc. Natl. Acad. Sci. U. S. A.* 108, 13624–13629
61. Knoll, A.H. (2014) Paleobiological perspectives on early eukaryotic evolution. *Cold Spring Harb. Perspect. Biol.* 6, 1–16
62. Yang, D. *et al.* (1985) Mitochondrial origins. *Proc. Natl. Acad. Sci. U. S. A.* 82, 4443–4447
63. Esser, C. *et al.* (2004) A genome phylogeny for mitochondria among alpha-proteobacteria and a predominantly eubacterial ancestry of yeast nuclear genes. *Mol. Biol. Evol.* 21, 1643–1660
64. Embley, T.M. and Martin, W. (2006) Eukaryotic evolution, changes and challenges. *Nature* 440, 623–630
65. Lindmark, D.G. and Müller, M. (1973) Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate *Tritrichomonas foetus*, and its role in pyruvate metabolism. *J. Biol. Chem.* 248, 7724–7728
66. Tovar, J. *et al.* (1999) The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. *Mol. Microbiol.* 32, 1013–1021
67. Kies, L. (1974) Elektronenmikroskopische Untersuchungen an *Paulinella chromatophora* Lauterborn, einer Thekamöbe mit blau-grünen Endosymbionten (Cyanellen). *Protoplasma* 80, 69–89
68. Okamoto, N. and Inouye, I. (2006) *Hatena arenicola* gen. et sp. nov., a katablepharid undergoing probable plastid acquisition. *Ann. Anat.* 157, 401–419
69. Martin, W. and Müller, M. (1998) The hydrogen hypothesis for the first eukaryote. *Nature* 392, 37–41
70. Martin, W.F. *et al.* (2015) Endosymbiotic theories for eukaryote origin. *Phil. Trans. R. Soc. B* 370 20140330–18
71. McFadden, G.I. (2011) The apicoplast. *Protoplasma* 248, 641–650
72. Tovar, J. *et al.* (2003) Mitochondrial remnant organelles of *Giardia* function in iron-sulphur protein maturation. *Nature* 426, 172–176
73. Mi-ichi, F. *et al.* (2009) Mitosomes in *Entamoeba histolytica* contain a sulfate activation pathway. *Proc. Natl. Acad. Sci. U. S. A.* 106, 21731–21736

74. Lim, L. and McFadden, G.I. (2010) The evolution, metabolism and functions of the apicoplast. *Phil. Trans. R. Soc. B* 365, 749–763
75. Palmieri, F. *et al.* (2011) Evolution, structure and function of mitochondrial carriers: a review with new insights. *Plant J.* 66, 161–181
76. Marchand, J. *et al.* (2018) Ion and metabolite transport in the chloroplast of algae: lessons from land plants. *Cell. Mol. Life Sci.* 75, 2153–2176
77. Palmieri, F. (2004) The mitochondrial transporter family (SLC25): physiological and pathological implications. *Pflugers Arch.* 447, 689–709
78. Neupert, W. and Herrmann, J.M. (2007) Translocation of proteins into mitochondria. *Ann. Rev. Biochem.* 76, 723–749
79. Shi, L.-X. and Theg, S.M. (2013) The chloroplast protein import system: from algae to trees. *BBA – Mol. Cell Res.* 1833, 314–331
80. Sommer, M.S. *et al.* (2007) Der1-mediated preprotein import into the periplastid compartment of chromalveolates? *Mol. Biol. Evol.* 24, 918–928
81. Timmis, J.N. *et al.* (2004) Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nat. Rev. Genet.* 5, 123–135
82. Maier, U.-G. *et al.* (2013) Massively convergent evolution for ribosomal protein gene content in plastid and mitochondrial genomes. *Gen. Biol. Evol.* 5, 2318–2329
83. Dagan, T. *et al.* (2013) Genomes of Stigonematalean cyanobacteria (subsection V) and the evolution of oxygenic photosynthesis from prokaryotes to plastids. *Gen. Biol. Evol.* 5, 31–44
84. Mishra, P. and Chan, D.C. (2014) Mitochondrial dynamics and inheritance during cell division, development and disease. *Nat. Rev. Mol. Cell Biol.* 15, 634–646
85. de Vries, J. and Gould, S.B. (2018) The monoplastidic bottleneck in algae and plant evolution. *J. Cell Science* 131 jcs203414–13
86. Hashimoto, H. (2005) The ultrastructural features and division of secondary plastids. *J. Plant Res.* 118, 163–172
87. Sumiya, N. *et al.* (2016) Chloroplast division checkpoint in eukaryotic algae. *Proc. Natl. Acad. Sci. U. S. A.* 113, E7629–E7638
88. Deusch, O. *et al.* (2008) Genes of cyanobacterial origin in plant nuclear genomes point to a heterocyst-forming plastid ancestor. *Mol. Biol. Evol.* 25, 748–761
89. Woehle, C. *et al.* (2017) Expansion of the redox-sensitive proteome coincides with the plastid endosymbiosis. *Nature Plants* 3, 17066
90. Arisue, N. *et al.* (2012) The *Plasmodium* apicoplast genome: conserved structure and close relationship of *P. ovale* to rodent malaria parasites. *Mol. Biol. Evol.* 29, 2095–2099
91. Nowack, E.C.M. *et al.* (2008) Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Curr. Biol.* 18, 410–418
92. Esser, C. *et al.* (2004) A genome phylogeny for mitochondria among alpha-proteobacteria and a predominantly eubacterial ancestry of yeast nuclear genes. *Mol. Biol. Evol.* 21, 1643–1660
93. Burstein, D. *et al.* (2012) A machine learning approach to identify hydrogenosomal proteins in *Trichomonas vaginalis*. *Euk. Cell* 11, 217–228
94. Kneip, C. *et al.* (2008) The cyanobacterial endosymbiont of the unicellular algae *Rhopalodia gibba* shows reductive genome evolution. *BMC Evol. Biol.* 8, 30
95. Brear, E.M. *et al.* (2013) Iron: an essential micronutrient for the legume-rhizobium symbiosis. *Front. Plant Sci.* 4, 359
96. Santi, C. *et al.* (2013) Biological nitrogen fixation in non-legume plants. *Ann. Bot.* 111, 743–767
97. Mus, F. *et al.* (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Appl. Envir. Microbiol.* 82, 3698–3710
98. Ohkuma, M. *et al.* (2015) Acetogenesis from H_2 plus CO_2 and nitrogen fixation by an endosymbiotic spirochete of a termite-gut cellulolytic protist. *Proc. Natl. Acad. Sci. U. S. A.* 112, 10224–10230
99. Wentrup, C. *et al.* (2014) Forever competent: deep-sea bivalves are colonized by their chemosynthetic symbionts throughout their lifetime. *Environ. Microbiol.* 16, 3699–3713
100. Russell, S.L. and Cavanaugh, C.M. (2017) Intrahost genetic diversity of bacterial symbionts exhibits evidence of mixed infections and recombinant haplotypes. *Mol. Biol. Evol.* 34, 2747–2761
101. Ponnudurai, R. *et al.* (2017) Metabolic and physiological interdependencies in the *Bathymodiolus azoricus* symbiosis. *ISME J.* 11, 463–477
102. Nussbaumer, A.D. *et al.* (2004) Attachment mechanism in a highly specific association between ectosymbiotic bacteria and marine nematodes. *Aquatic Microbiol. Ecol.* 35, 239–246
103. Boehnlein, J.M. *et al.* (2005) Diterpene biosynthesis by the dinoflagellate symbiont of the Caribbean gorgonian *Pseudopterogorgia bipinnata*. *Mar. Ecol. Prog. Ser.* 303, 105–111
104. Edgcomb, V.P. *et al.* (2011) Structured multiple endosymbiosis of bacteria and archaea in a ciliate from marine sulfidic sediments: a survival mechanism in low oxygen, sulfidic sediments? *Front. Microbiol.* 2, 55
105. van Hoek, A.H. *et al.* (2000) Multiple acquisition of methanogenic archaeal symbionts by anaerobic ciliates. *Mol. Biol. Evol.* 17, 251–258
106. Huigens, M.E. *et al.* (2004) Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in *Trichogramma* wasps. *Proc. R. Soc. B Biol. Sci.* 271, 509–515
107. Kremer, N. *et al.* (2009) *Wolbachia* interferes with ferritin expression and iron metabolism in insects. *PLoS Pathog.* 5, e1000630
108. Graf, J. and Ruby, E.G. (1998) Host-derived amino acids support the proliferation of symbiotic bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 95, 1818–1822
109. Wier, A.M. *et al.* (2010) Transcriptional patterns in both host and bacterium underlie a daily rhythm of anatomical and metabolic change in a beneficial symbiosis. *Proc. Natl. Acad. Sci. U. S. A.* 107, 2259–2264
110. McFall-Ngai, M. (2014) Divining the essence of symbiosis: insights from the Squid-*Vibrio* model. *PLoS Biol.* 12, e1001783
111. Bowen, D. *et al.* (1998) Insecticidal toxins from the bacterium *Photorhabdus luminescens*. *Science* 280, 2129–2132
112. Ciche, T.A. *et al.* (2008) Cell invasion and matricide during *Photorhabdus luminescens* transmission by *Heterorhabditis bacteriophora* nematodes. *Appl. Envir. Microbiol.* 74, 2275–2287
113. Chaston, J.M. *et al.* (2011) The entomopathogenic bacterial endosymbionts *Xenorhabdus* and *Photorhabdus*: convergent lifestyles from divergent genomes. *PLoS One* 6, e27909–e27913
114. Clay, K. and Schardl, C. (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am. Nat.* 160, S99–S127
115. Akman, L. *et al.* (2002) Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature Genet.* 32, 402–407
116. Pais, R. *et al.* (2008) The obligate mutualist *Wigglesworthia glossinidia* influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Appl. Envir. Microbiol.* 74, 5965–5974
117. Bing, X. *et al.* (2017) Unravelling the relationship between the tsetse fly and its obligate symbiont *Wigglesworthia*: transcriptomic and metabolomic landscapes reveal highly integrated physiological networks. *Proc. R. Soc. B* 284, 20170360
118. Douglas, A.E. and Prosser, W.A. (1992) Synthesis of the essential amino acid tryptophan in the pea aphid (*Acyrthosiphon pisum*) symbiosis. *J. Insect Physiol.* 38, 565–568
119. Aschenbrenner, I.A. *et al.* (2016) Understanding microbial multi-species symbioses. *Front. Microbiol.* 7, 180
120. Sandström, J.P. *et al.* (2001) Independent origins and horizontal transfer of bacterial symbionts of aphids. *Mol. Ecol.* 10, 217–228
121. Oliver, K.M. *et al.* (2009) Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science* 325, 992–994

122. Tsuchida, T. *et al.* (2005) Characterization of a facultative endosymbiotic bacterium of the pea phid *Acyrthosiphon pisum*. *Microb. Ecol.* 49, 126–133
123. Degnan, P.H. *et al.* (2009) *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. *Proc. Natl. Acad. Sci. U. S. A.* 106, 9063–9068
124. Piel, J. (2002) A polyketide synthase-peptide synthetase gene cluster from an uncultured bacterial symbiont of *Paederus* beetles. *Proc. Natl. Acad. Sci. U. S. A.* 99, 14002–14007
125. Ruppel, S. *et al.* (1992) Settlement of the diazotrophic, phytoeffective bacterial strain *Pantoea agglomerans* on and within winter wheat: an investigation using ELISA and transmission electron microscopy. *Plant Soil* 145, 261–273
126. Lopanik, N. *et al.* (2004) Potent cytotoxins produced by a microbial symbiont protect host larvae from predation. *Oecologia* 139, 131–139
127. König, L. *et al.* (2019) Symbiont-mediated defense against *Legionella pneumophila* in amoebae. *mBio* 10, e00333-19
128. Heinz, E. *et al.* (2010) Inclusion membrane proteins of *Protochlamydia amoebophila* UWE25 reveal a conserved mechanism for host cell interaction among the *Chlamydiae*. *J. Bacteriol.* 192, 5093–5102
129. Kusch, J. *et al.* (2002) Competitive advantages of *Caedibacter*-infected paramecia. *Protist* 153, 47–58