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Opinion

Tracking the early events of photosymbiosis evolution

Loïc Quevarec,^{1,2} Gaël Brasseur,² Denise Aragnol,¹ and Christophe Robaglia^{1,*}

Oxygenic photosynthesis evolved in cyanobacteria around 3.2 giga-annum (Ga) ago and was acquired by eukaryotes starting around 1.8 Ga ago by endosymbiosis. Photosymbiosis results either from integration of a photosynthetic bacteria by heterotrophic eukaryotes (primary photosymbiosis) or by successive integration of photosymbiotic eukaryotes by heterotrophic eukaryotes (secondary photosymbiosis). Primary endosymbiosis is thought to have been a rare event, whereas secondary and higher-order photosymbiosis evolved multiple times independently in different taxa. Despite its recurrent evolution, the molecular and cellular mechanisms underlying photosymbiosis are unknown. In this opinion, we discuss the primary events leading to the establishment of photosymbiosis, and we present recent research suggesting that, in some cases, domestication occurred instead of symbiosis, and how oxygen and host immunity can be involved in symbiont maintenance.

Highlights

Photosymbiosis evolved recurrently within the tree of life.

Oxygen could have provided the primary selection pressure for photosymbiont acquisition.

Host recognition and immunity are involved in photosymbiont maintenance.

Intracellular pathogens can trigger photosymbiont maintenance.

Genetics will open up the analysis of the cellular mechanisms of photosymbiont maintenance.

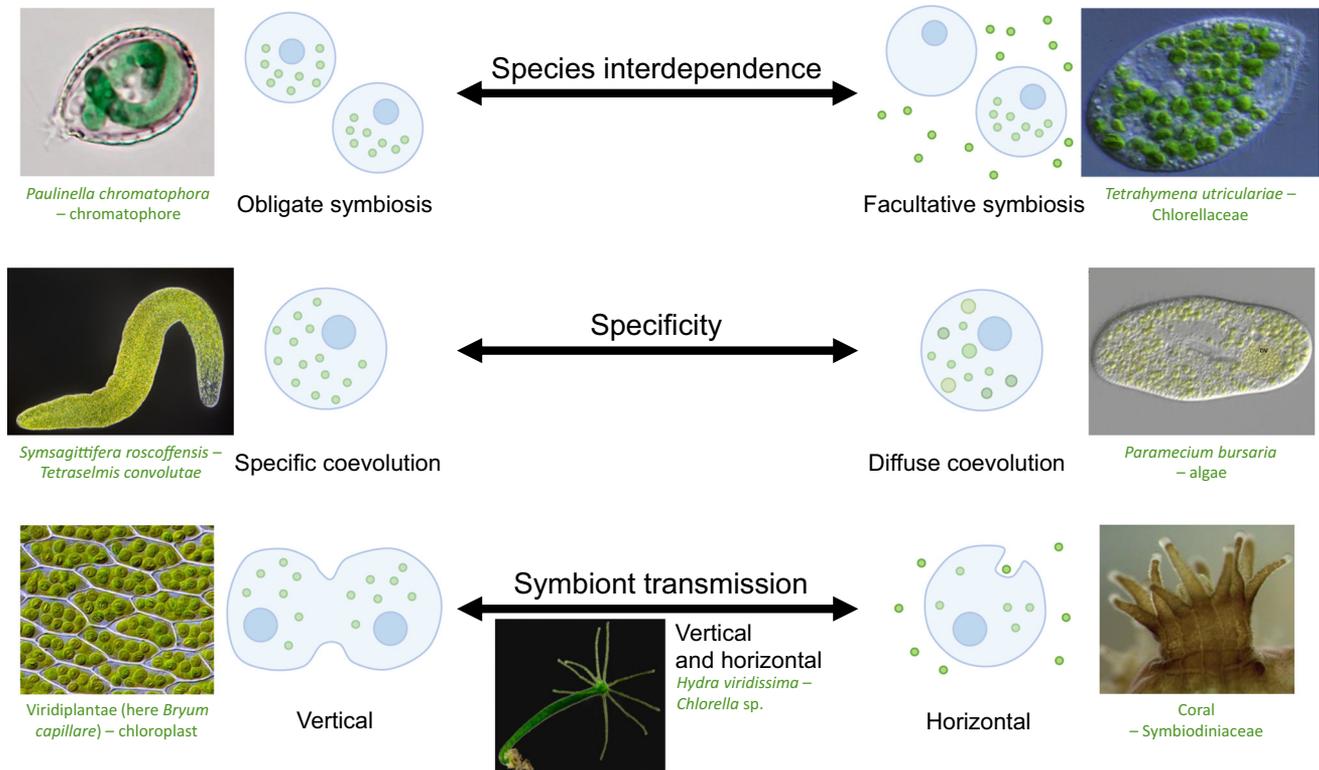
Photosymbiosis evolved multiple times independently

Oxygenic photosynthesis, which uses water photochemically oxidized by photosystems and generating O₂ as a by-product [$n\text{CO}_2 + \text{H}_2\text{O} + \text{light} \rightarrow (\text{CH}_2\text{O})_n + \text{O}_2$] evolved in the ancestors of modern cyanobacteria, leading at least 3 Ga ago to a progressive oxygenation of the Earth and widespread environmental changes [1,2], causing, in particular, about 2.4 Ga ago, the ‘great oxidation event’ [3,4]. Organisms had to adapt to the increased oxygen level, and its potential toxicity, particularly for anaerobic organisms. Oxidative metabolism allows a more efficient combustion of organic molecules and increases the availability of cell energy [5]. Among the many innovations at the origin of the eukaryotic cell, the evolution of phagocytosis and aerobic energetics benefitted from the acquisition of mitochondria and may have promoted the emergence of predatory eukaryotes [6]. Around 1.5 Ga, a eukaryote that possessed mitochondria most likely engulfed a cyanobacteria and evolved towards its cytoplasmic retention. This new organelle ultimately became the plastid and defined the kingdom Archaeplastida [7–9]. This is known as primary endosymbiosis [10]. Throughout the evolutionary history of the kingdom Archaeplastida, a large part of the cyanobacterial genome was transferred to the host’s eukaryotic nucleus, enhancing the molecular dependence between the hosts and the obligatory symbionts [11,12]. At present, groups within Archaeplastida, such as the Glaucophyta, the Rhodophyta (red algae), and the Viridiplantae (green algae and land plants), represent all the extant photosynthetic eukaryotes with primary plastids, including terrestrial plants, with the exception of the genus *Paulinella chromatophora* (Figure 1) [12], which underwent an independent shift to primary photosymbiosis around 90–140 million years ago [13,14]. It is unknown whether other cases of primary photosymbiosis occurred or still exist. Their scarcity may be linked to the major evolutionary innovations required for both organisms [15], such as the difficulty of establishing a metabolic connection between prokaryotic photosynthetic and eukaryotic sugar metabolism. In the ‘ménage à trois’ hypothesis, it is proposed that connecting

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Trends In Plant Science

Figure 1. Representation of the diversity of photosymbiotic associations between the host (blue) and the symbiont (green) according to three arbitrary independent axes. (Top) Species interdependence: interdependence of species in symbiosis can vary from obligate symbiosis (highly dependent) to facultative symbiosis (low dependency). (Middle) Specificity: the host–symbiont association can be very restrictive in terms of species (specific coevolution) or conversely, the two partners may associate with different species (diffuse coevolution). (Bottom) Symbiont transmission: symbionts can be transmitted from one generation to the next between the mother cell and the daughter cell (vertical transmission), or the newly formed organisms must acquire the symbiont from their environment in each generation (horizontal transmission). The transmission of the symbiont can be both vertical and horizontal in some species. A representative example is given to illustrate each case. Photograph credits: *Paulinella chromatophora* (Ferry Siemensma, www.arcella.nl), *Tetrahymena utriculariae* (John Wiley and Sons, <https://doi.org/10.1111/jeu.12368>), *Symsagittifera roscoffensis* (Michael Plewka, www.plingfactory.de), *Paramecium bursaria* (<https://doi.org/10.3390/d12080287>), *Bryum capillare* (Des Callaghan), *Hydra viridissima* (Frank Fox), and coral (National Coral Reef Institute).

enzymes could have been provided by another, possibly pathogenic, intracellular prokaryote such as Chlamydiae [16].

In striking contrast to the scarcity of primary endosymbiosis, a wealth of independent, secondary, and tertiary endosymbiosis events have since occurred, where nonphotosynthetic eukaryotes engulfed already photosynthetic unicellular eukaryotes [17]. These events have deeply impacted the evolution of unicellular eukaryotes belonging to several groups, namely Cryptophyta, Haptophyta, Ochrophyta, Dinophyta, Chlorarachniophyta, or Euglenozoa [12]. There is great diversity in the modalities of endosymbiosis (Figure 1). In addition to the preceding cases where the symbiont is stably inherited, some organisms acquire a photosynthetic symbiont from the environment. In obligatory endosymbiosis, the host does not survive without the symbiont, as is the case for many corals [18], the katablepharid *Hatena arenicola* [19], and the acoel worm *Symsagittifera roscoffensis* (Figure 1) [20]. By contrast, organisms can facultatively, and sometimes transiently, acquire photosymbiosis by engulfing a specific or nonspecific phototrophic eukaryote (Figure 1). Acquired photosymbiosis can be viewed as the initial step towards more stable associations, leading to plastids [21].

Secondary and higher-order photosymbiosis evolved independently in several lineages of hosts and with several different lineages of green or brown algae as symbionts. Altogether, its large distribution and recurrent occurrence imply that the cellular features required for the initiation of photosymbiosis are relatively widespread in the tree of life and that the environmental conditions favoring the acquisition of photosynthetic symbionts are common. However, the molecular mechanisms and the selective pressures leading to the early events of photosymbiosis remain unknown. Here, we discuss some aspects of endosymbiosis that we think are interesting to explore further, because they could help to identify the evolutionary processes that led to the associations among organisms and the consequences of these changes.

Photosymbiosis or photodomestication?

Mutualistic symbiosis supposes a reciprocal fitness benefit for both organisms [22]. This benefit does not need to be symmetrical or simultaneous. From the symbiont's point of view, the host can provide diverse organic and inorganic compounds, such as amino acids and carbon dioxide, or a physical shelter from predators [23]. Therefore, escape from digestion caused, for example, by specific cell wall features may have an immediate advantage for a 'would-be' symbiont. Supporting this scenario, symbiotic *Chlorella* sp. of the ciliate *Paramecium bursaria* (Figure 1) are not digested, although the ciliate can still feed on other prey. However, in *P. bursaria*, algal digestion occurs when photosynthesis is stopped by prolonged periods of darkness or in the presence of photosynthesis inhibitors, demonstrating host control over the symbiont's survival and growth [24]. Investigation of the symbiont's metabolism in the facultative associations between *P. bursaria* and *Chlorella* sp. and in the obligatory symbiosis between *Acantharia* sp. and *Phaeocystis* sp. also showed that algae suffer from strong physiological stress and are nutrient-starved in a way that favors photosynthesis over growth [25,26]. In addition, it was shown that the host controls and limits the population of symbionts by restricting their division, consumption, or ejection [27–29]. All these mechanisms are likely to decrease the fitness of the photosynthetic symbiont, questioning its advantage in the symbiotic relationship [25,30]. Although they may not hold for all photosymbiotic associations, these examples suggest that the balance of exchange primarily benefits the host. If this is the case, primary genetic (or epigenetic) changes underlying intracellular maintenance or the recognition of foreign photosynthetic cells may have occurred first in the hosts. The situation can be compared with that of the coevolution loop between plants and humans known as domestication, where the order of events is difficult to assess. In this case, archeological data show that cultivation of wild plant species preceded the appearance of the morphological changes that are, hallmarks of domestication, implying that the primary events were modifications in the behavior of the domesticator [31]. The photosymbiont can also have a strong influence on its host, particularly on its reproduction [32,33]. For example, oogenesis in *Hydra viridissima* (Figure 1) is favored by the symbiont *Chlorella* sp. [32]. Since *Chlorella* sp. are transmitted vertically by the host's oocytes to the next generation, the symbiont favors its own transmission to the new generations [32]. Interestingly, *Hydra* tends to reproduce sexually under stressful conditions [34], in the same way as other species with both sexual and asexual reproduction (e.g., *Daphnia magna* [35], *Strongyloides ratti* [36], *Caenorhabditis elegans* [37], and *Tetrahymena thermophila* [38]). In this case, *Chlorella* sp. may induce stress in its host to promote its own transmission and, therefore, its fitness. Furthermore, the photosymbiont can play a 'dual game', depending on the environmental conditions. When the environment becomes unfavorable for the symbiont, it can move from a mutualistic to a more parasitic symbiotic relationship. For example, in the gastropod *Strombus gigas* that hosts Symbiodiniaceae, during the growth of the gastropod's shell, light transmission decreases, reducing the photosynthesis of the symbiont. To compensate for the loss, Symbiodiniaceae draws on the resources of its host and is more parasitic [39]. Similarly, it appears that the symbiotic diatom *Melosira* sp. of the sponge *Scolymastra joubini* becomes parasitic when photosynthesis is limited [40–42].

The question of selective pressure leading to photosymbiosis establishment

Considering the host as the primary beneficiary would shift the search for the causes of photosymbiosis establishment towards the advantages of the host rather than those of the symbiont. The primary products of photosynthesis are reduced carbon and oxygen. Carbon supply is thought to be a strong selective force for the acquisition of photosymbionts, especially in nutrient-poor habitats [43], and the extant stable endosymbiosis in plants and algae is clearly based on autotrophy of carbon [44,45]. However, the full digestion of prey provides more immediate carbon than that made available through its photosynthesis, mitigating the advantage of keeping the engulfed cells alive instead of consuming them, at least in the initial state. Oxygen supply, as a driving force in the evolution of photosymbiosis, is obviously also possible, although this hypothesis has been little studied. The supply of oxygen to the host by the photosymbionts may represent a strong and fast advantage, including allowing expansions of the host's niche into anaerobic habitats, providing access to new resources and refuge from aerobic competitors or predators [10,46]. The intracellular retention of phototrophs for supplying oxygen to the host may therefore represent a selective advantage in this case. For example, in a hypereutrophic pond, the production of oxygen by algal symbionts was sufficient to allow the host ciliates to live in anoxic water [46]. Other studies have shown that thanks to the oxygen produced by the symbiont, the host can survive in a hypoxic environment [47,48]. Moreover, oxygen supply may improve host life history traits. For example, Small *et al.* [49] showed that the oxygen produced by the photosymbiotic green algae *Oophila amblystomatis* influenced the embryonic growth of the salamander *Ambystoma maculatum*. In long-standing stable associations, the oxygen produced by the plastid's photosynthesis can be used by terminal cytochrome *c* oxidase in the mitochondria inside the same algal cell [50] and, in plants submitted to hypoxic, photosynthetic oxygen contributes to stress tolerance [51]. The recently discovered *Tetrahymena utriculariae* (Figure 1) is a photosymbiotic ciliate species which lives within the traps of the aquatic carnivorous plant *Utricularia reflexa* [52]. This particular ecosystem is rich in dissolved carbon and other nutrients but is almost completely anoxic [53], further supporting the idea that intracellular algae provide oxygen to their ciliate *Tetrahymena* host [52]. Under aerobic conditions with bacterial food, *T. utriculariae* gradually loses its algal symbionts. Pitsch *et al.* [52] proposed that the lack of oxygen in the traps was the main selective force driving the evolution of this alga–ciliate symbiosis. Altogether, resistance to hypoxia might have been an initial selective pressure promoting photosymbiosis, while the supply of reduced carbon was a bonus that subsequently became pre-eminent.

Genetic events underlying photosymbiont maintenance

Decades of research on stable primary endosymbiosis, mostly in plants and green algae, have enlightened the intimate integration between the symbiont (the plastid) and the host cell, resulting from billion years of coevolution [54]. The association is irreversible and the trace of early evolutionary paths leading to acquisition of the symbiont cannot be discerned or may even have disappeared. By contrast, facultative associations can be established or reversed in the laboratory and open a window on the cellular mechanisms of acquiring symbionts. Full genome sequences and large-scale gene expression profiles are useful for studying the physiological and molecular changes associated with photosymbiosis in the host and in the symbiont [55–59]. In the coral–Symbiodiniaceae association (Figure 1), comparisons of the transcriptome between the symbiotic and aposymbiotic hosts and RNA interference studies have suggested the modulation of the host's immunity to accept an incoming cell [60–63]. This was further confirmed in the sea anemone *Aiptasia pallida*, where a pharmacogenetic analysis demonstrated that the host's immunity was altered to avoid symbiont rejection [64] and that the host's thrombospondin type 1 repeat (TSR) proteins and lectins, a family of recognition proteins, were involved in the colonization by dinoflagellates [65]. However, a limitation in establishing causal relationships between

molecular changes in the host and their impact on the acquisition of symbionts, is that most hosts used in photosymbiosis research are not yet fully genetically tractable [42,66]. The intraspecific diversity related to symbiosis, if any, is often unknown, and genetic crosses are, in most cases, not possible, leading to the intractability of mutations. In this light, the development of RNA interference tools in *P. bursaria*, *A. pallida*, and *Xenia* sp. represent an opportunity to validate the functions of the genes involved in photosymbiosis [67]. In many cases, the acquisition of algae occurs by predation, and the ingested cells survive in a distinct vesicle, the symbiosome, which is often cortically disposed. The molecular mechanisms allowing the routing of specific phagocytosed algae from the phagosome to the symbiosome are unknown but could be explored, thanks to expanded knowledge on vesicular trafficking and associated proteins in genetically tractable hosts [68].

Expanding the ‘ménage à trois’ hypothesis

The ‘ménage à trois’ hypothesis posits that primary endosymbiosis was caused by the simultaneous presence, within the same eukaryotic cell, of a cyanobacteria and a pathogenic intracellular bacterium related to Chlamydiae. The pathogen can protect the cyanobacteria from host immunity within its induced membrane vesicle and produce effector proteins, allowing a metabolic connection between the host and the cyanobacteria [16,69]. This pioneering concept of a third partner that is possibly pathogenic, that allows the installation of a symbiont can be extended. Many kinds of intracellular pathogens, including viruses, prokaryotes, and eukaryotes can evade host digestion and immunity [70–73]. This is often caused by the production of effector molecules with highly diverse functions that interfere with the host’s cellular defenses at many levels [74]. The coexistence of pathogens with independently phagocytosed organisms can improve their intracellular maintenance, providing an initial state that enables further symbiosis evolution, if a fitness advantage is gained. Of course, pathogenesis may counteract this advantage, but this is not necessarily an issue, because some species of nonpathogenic bacteria were found to evade digestion by predatory ciliates. Those bacteria are not digested and are instead packaged into membranous structures and expelled as fecal pellets, improving their survival in the environment [75,76]. Tripartite associations can be a transitory state, requiring a particular environmental context to persist, and may be favored by further lateral gene transfer between the pathogen and the host.

Concluding remarks and future perspectives

In this opinion, we have describes how recent methodological developments have contributed to unravelling the underlying molecular and cellular basis for the recurrent acquisition of photosymbionts throughout the tree of life. These developments include advances in microscopy and other nondestructive measurements of the fate of intracellular phototrophs and genetic toolkits for the study of hosts and symbionts (RNA interference, CRISPR-Cas mutagenesis, positional cloning). The field will also be fueled by expanding the knowledge concerning the mechanisms of immunity and self versus non-self recognition in eukaryotic hosts and the interference that can be caused by intracellular parasites. If the environmental pressures that make it advantageous to maintain (or domesticate) a symbiont are identified, experimental evolutionary approaches could reveal the genetic or epigenetic changes leading to the intracellular establishment of symbiosis. Comparative genomics will then allow the identification of selection signatures, if any, in parallel groups in which photosymbiosis has evolved (see [Outstanding questions](#)).

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Outstanding questions

Do we have a complete picture of the diversity of photosymbiosis that occurs in the protist world?

In addition to the primary events that gave rise to *Archaeplastidia* and *Paulinella chromatophora* independently, do other cases of primary photosymbiosis exist?

What is the cellular and molecular basis for the maintenance of photosymbionts? Are there common mechanisms despite the diversity of photosymbiotic events?

What are the molecular mechanisms allowing the recognition and sorting of prey for the establishment of photosymbionts?

To what extent can intracellular pathogens trigger the maintenance of symbionts by interfering with phagocytosis and immunity?

Can we evolve photosymbiosis in the laboratory to experimentally test the underlying selective pressures and early molecular events?

Declaration of interests

The authors have no interests to declare.

References

- Crowe, S.A. *et al.* (2013) Atmospheric oxygenation three billion years ago. *Nature* 501, 535–538
- Fischer, W.W. *et al.* (2016) Evolution of oxygenic photosynthesis. *Annu. Rev. Earth Planet. Sci.* 44, 647–683
- Gumsley, A.P. *et al.* (2017) Timing and tempo of the Great Oxidation Event. *Proc. Natl. Acad. Sci. U.S.A.* 114, 1811–1816
- Hodgskiss, M.S.W. *et al.* (2019) A productivity collapse to end Earth's Great Oxidation. *Proc. Natl. Acad. Sci. U.S.A.* 116, 17207–17212
- Lane, N. (2020) How energy flow shapes cell evolution. *Curr. Biol.* 30, R471–R476
- Cavaliere-Smith, T. (2009) Predation and eukaryote cell origins: a co-evolutionary perspective. *Int. J. Biochem. Cell Biol.* 41, 307–322
- Archibald, J.M. (2015) Endosymbiosis and eukaryotic cell evolution. *Curr. Biol.* 25, R911–R921
- Ochoa de Alda, J.A.G. *et al.* (2014) The plastid ancestor originated among one of the major cyanobacterial lineages. *Nat. Commun.* 5, 4937
- Reyes-Prieto, A. *et al.* (2007) The origin and establishment of the plastid in algae and plants. *Annu. Rev. Genet.* 41, 147–168
- Gavelis, G.S. and Gile, G.H. (2018) How did cyanobacteria first embark on the path to becoming plastids?: lessons from protist symbioses. *FEMS Microbiol. Lett.* 365, fny209
- Martin, W. (2003) Gene transfer from organelles to the nucleus: frequent and in big chunks. *Proc. Natl. Acad. Sci. U.S.A.* 100, 8612–8614
- Ponce-Toledo, R.I. *et al.* (2019) Horizontal and endosymbiotic gene transfer in early plastid evolution. *New Phytol.* 224, 618–624
- Delaye, L. *et al.* (2016) How really ancient is *Paulinella chromatophora*? *PLoS Curr.* 8 Published online March 15, 2016. <https://doi.org/10.1371/currents.tol.e68a099364bb1a1e129a17b4e06b0c6b>
- Marin, B. *et al.* (2005) A plastid in the making: evidence for a second primary endosymbiosis. *Protist* 156, 425–432
- Dorrell, R.G. and Howe, C.J. (2012) What makes a chloroplast? Reconstructing the establishment of photosynthetic symbioses. *J. Cell Sci.* 125, 1865–1875
- Ball, S.G. *et al.* (2013) Metabolic effectors secreted by bacterial pathogens: essential facilitators of plastid endosymbiosis? *Plant Cell* 25, 7–21
- Archibald, J.M. (2012) *The evolution of algae by secondary and tertiary endosymbiosis*. Adv. Bot. Res. 64. Elsevier, pp. 87–118
- Allemand, D. and Furla, P. (2018) How does an animal behave like a plant? Physiological and molecular adaptations of zooxanthellae and their hosts to symbiosis. *C. R. Biol.* 341, 276–280
- Okamoto, N. and Inouye, I. (2005) A secondary symbiosis in progress? *Science* 310, 287
- Arboleda, E. *et al.* (2018) An emerging system to study photosymbiosis, brain regeneration, chronobiology, and behavior: the marine acael *Symsagittifera roscoffensis*. *Bioessays* 40, e1800107
- Bhattacharya, D. *et al.* (2007) How do endosymbionts become organelles? Understanding early events in plastid evolution. *Bioessays* 29, 1239–1246
- Gil, R. and Latorre, A. (2019) Unity makes strength: a review on mutualistic symbiosis in representative insect clades. *Life* 9, 21
- Jeon, K.W. (2004) Genetic and physiological interactions in the amoeba–bacteria symbiosis. *J. Eukaryot. Microbiol.* 51, 502–508
- Kodama, Y. and Fujishima, M. (2010) Secondary symbiosis between *Paramecium* and *Chlorella* cells. *Int. Rev. Cell Mol. Biol.* 279, 33–77
- Lowe, C.D. *et al.* (2016) Shining a light on exploitative host control in a photosynthetic endosymbiosis. *Curr. Biol.* 26, 207–211
- Decelle, J. *et al.* (2019) Algal remodeling in a ubiquitous planktonic photosymbiosis. *Curr. Biol.* 29, 968–978
- Uwizeye, C. *et al.* (2021) Cytokleptin in the plankton: a host strategy to optimize the bioenergetic machinery of endosymbiotic algae. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2025252118
- Davy, S.K. *et al.* (2012) Cell biology of cnidarian–dinoflagellate symbiosis. *Microbiol. Mol. Biol. Rev.* 76, 229–261
- Minter, E.J.A. *et al.* (2018) Variation and asymmetry in host–symbiont dependence in a microbial symbiosis. *BMC Evol. Biol.* 18, 108
- Dean, A.D. *et al.* (2016) Host control and nutrient trading in a photosynthetic symbiosis. *J. Theor. Biol.* 405, 82–93
- Weiss, E. *et al.* (2006) Anthropology. Autonomous cultivation before domestication. *Science* 312, 1608–1610
- Habetha, M. *et al.* (2003) The *Hydra viridis/Chlorella* symbiosis. Growth and sexual differentiation in polyps without symbionts. *Zoology* 106, 101–108
- Michalek-Wagner, K. and Willis, B.L. (2001) Impacts of bleaching on the soft coral *Lobophytum compactum*. I. Fecundity, fertilization and offspring viability. *Coral Reefs* 19, 231–239
- Quinn, B. *et al.* (2012) Hydra, a model system for environmental studies. *Int. J. Dev. Biol.* 56, 613–625
- Rider, C.V. *et al.* (2005) Stress signaling: coregulation of hemoglobin and male sex determination through a terpenoid signaling pathway in a crustacean. *J. Exp. Biol.* 208, 15–23
- Gemmill, A.W. *et al.* (1997) Host immune status determines sexuality in a parasitic nematode. *Evolution* 51, 393–401
- Quevarec, L. *et al.* (2022) Male frequency in *Caenorhabditis elegans* increases in response to chronic irradiation. *Evol. Appl.* 15, 1331–1343
- Loicil, J. (2021) *Tetrahymena* meiosis: simple yet ingenious. *PLoS Genet.* 17, e1009627
- Banaszak, A.T. *et al.* (2013) The symbiosis between the gastropod *Strombus gigas* and the dinoflagellate *Symbiodinium*: an ontogenetic journey from mutualism to parasitism. *J. Exp. Mar. Biol. Ecol.* 449, 358–365
- Bavestrello, G. *et al.* (2000) Parasitic diatoms inside Antarctic sponges. *Biol. Bull.* 198, 29–33
- Cerrano, C. *et al.* (2000) Diatom invasion in the antarctic hexactinellid sponge *Scolymastra joubini*. *Polar Biol.* 23, 441–444
- Melo Clavijo, J. *et al.* (2018) Polymorphic adaptations in metazoans to establish and maintain photosymbioses: evolution of photosymbiosis. *Biol. Rev.* 93, 2006–2020
- Venn, A.A. *et al.* (2008) Photosynthetic symbioses in animals. *J. Exp. Bot.* 59, 1069–1080
- Harii, S. *et al.* (2010) The relative contribution of dinoflagellate photosynthesis and stored lipids to the survivorship of symbiotic larvae of the reef-building corals. *Mar. Biol.* 157, 1215–1224
- Hoefnagel, M.H. *et al.* (1998) Interdependence between chloroplasts and mitochondria in the light and the dark. *Biochim. Biophys. Acta Bioenerg.* 1366, 235–255
- Finlay, B.J. *et al.* (1996) Spectacular abundance of ciliates in anoxic pond water: contribution of symbiont photosynthesis to host respiratory oxygen requirements. *FEMS Microbiol. Ecol.* 20, 229–235
- Malcolm, J.M. and Brown, W.I. (1977) Zooxanthellae-produced O₂ promotes sea anemone expansion and eliminates oxygen debt under environmental hypoxia. *J. Exp. Zool.* 201, 149–155
- Rands, M.L. *et al.* (1992) Avoidance of hypoxia in a cnidarian symbiosis by algal photosynthetic oxygen. *Biol. Bull.* 182, 159–162
- Small, D.P. *et al.* (2014) The roles of oxygen and ammonia in the symbiotic relationship between the spotted salamander *Ambystoma maculatum* and the green alga *Clophila amblystomatis* during embryonic development. *Symbiosis* 64, 1–10
- Lavergne, J. (1989) Mitochondrial responses to intracellular pulses of photosynthetic oxygen. *Proc. Natl. Acad. Sci. U. S. A.* 86, 8768–8772
- Winkel, A. *et al.* (2013) Internal aeration of paddy field rice (*Oryza sativa*) during complete submergence importance of light and floodwater O₂. *New Phytol.* 197, 1193–1203
- Pitsch, G. *et al.* (2017) The green *Tetrahymena utriculariae* n. sp. (Ciliophora, Oligohymenophorea) with its endosymbiotic algae (*Micractinium* sp.), living in traps of a carnivorous aquatic plant. *J. Eukaryot. Microbiol.* 64, 322–335
- Adamec, L. (2007) Oxygen concentrations inside the traps of the carnivorous plants *Utricularia* and *Genlisea* (Lentibulariaceae). *Ann. Bot.* 100, 849–856

54. Gould, S.B. *et al.* (2008) Plastid evolution. *Annu. Rev. Plant Biol.* 59, 491–517
55. Burns, J.A. *et al.* (2017) Transcriptome analysis illuminates the nature of the intracellular interaction in a vertebrate–algal symbiosis. *eLife* 6, e22054
56. González-Pech, R.A. *et al.* (2017) Signatures of adaptation and symbiosis in genomes and transcriptomes of *Symbiodinium*. *Sci. Rep.* 7, 15021
57. Hamada, M. *et al.* (2018) Metabolic co-dependence drives the evolutionarily ancient *Hydra–Chlorella* symbiosis. *eLife* 7, e35122
58. Kodama, Y. *et al.* (2014) Comparison of gene expression of *Paramecium bursaria* with and without *Chlorella variabilis* symbionts. *BMC Genomics* 15, 183
59. Lehnert, E.M. *et al.* (2012) Developing the anemone *Aiptasia* as a tractable model for cnidarian–dinoflagellate symbiosis: the transcriptome of aposymbiotic *A. pallida*. *BMC Genomics* 13, 271
60. Hu, M. *et al.* (2023) Coral–algal endosymbiosis characterized using RNAi and single-cell RNA-seq. *Nat. Microbiol.* 8, 1240–1251
61. Mansfield, K.M. and Gilmore, T.D. (2019) Innate immunity and cnidarian–*Symbiodiniaceae* mutualism. *Dev. Comp. Immunol.* 90, 199–209
62. Rivera, H.E. and Davies, S.W. (2021) Symbiosis maintenance in the facultative coral, *Oculina arbuscula*, relies on nitrogen cycling, cell cycle modulation, and immunity. *Sci. Rep.* 11, 21226
63. Yuyama, I. *et al.* (2018) Transcriptomic changes with increasing algal symbiont reveal the detailed process underlying establishment of coral–algal symbiosis. *Sci. Rep.* 8, 16802
64. Jacobovitz, M.R. *et al.* (2021) Dinoflagellate symbionts escape vomocytosis by host cell immune suppression. *Nat. Microbiol.* 6, 769–782
65. Neubauer, E.-F. *et al.* (2017) A diverse host thrombospondin-type-1 repeat protein repertoire promotes symbiont colonization during establishment of cnidarian–dinoflagellate symbiosis. *eLife* 6, e24494
66. Rola, M. *et al.* (2022) *Cladobranchia* (Gastropoda, Nudibranchia) as a promising model to understand the molecular evolution of photosymbiosis in animals. *Front. Mar. Sci.* 8, 745644
67. Dunn, S.R. *et al.* (2007) Knockdown of actin and caspase gene expression by RNA interference in the symbiotic anemone *Aiptasia pallida*. *Biol. Bull.* 212, 250–258
68. Fujishima, M. and Kodama, Y. (2022) Mechanisms for establishing primary and secondary endosymbiosis in *Paramecium*. *J. Eukaryot. Microbiol.* 69, e12901
69. Cenci, U. *et al.* (2017) Biotic host–pathogen interactions as major drivers of plastid endosymbiosis. *Trends Plant Sci.* 22, 316–328
70. Lopez-Gomollon, S. and Baulcombe, D.C. (2022) Roles of RNA silencing in viral and non-viral plant immunity and in the crosstalk between disease resistance systems. *Nat. Rev. Mol. Cell. Biol.* 23, 645–662
71. Cui, H. *et al.* (2015) Effector-triggered immunity: from pathogen perception to robust defense. *Annu. Rev. Plant Biol.* 66, 487–511
72. Ge, J. and Shao, F. (2011) Manipulation of host vesicular trafficking and innate immune defence by *Legionella* Dot/Icm effectors. *Cell. Microbiol.* 13, 1870–1880
73. Hayn, M. *et al.* (2021) Systematic functional analysis of SARS-CoV-2 proteins uncovers viral innate immune antagonists and remaining vulnerabilities. *Cell Rep.* 35, 109126
74. Uribe-Querol, E. and Rosales, C. (2017) Control of phagocytosis by microbial pathogens. *Front. Immunol.* 8, 1368
75. Amaro, F. and Martín-González, A. (2021) Microbial warfare in the wild—the impact of protists on the evolution and virulence of bacterial pathogens. *Int. Microbiol.* 24, 559–571
76. Rehfuss, M.Y.M. *et al.* (2011) *Salmonella* transcriptional signature in *Tetrahymena* phagosomes and role of acid tolerance in passage through the protist. *ISME J.* 5, 262–273