

Using artificial systems to explore the ecology and evolution of symbioses

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Abstract The web of life is weaved from diverse symbiotic interactions between species. Symbioses vary from antagonistic interactions such as competition and predation to beneficial interactions such as mutualism. What are the bases for the origin and persistence of symbiosis? What affects the ecology and evolution of symbioses? How do symbiotic interactions generate ecological patterns? How do symbiotic partners evolve and coevolve? Many of these questions are difficult to address in natural systems. Artificial systems, from abstract to living, have been constructed to capture essential features of natural symbioses and to address these key questions. With reduced complexity and increased controllability, artificial systems can serve as useful models for natural systems. We review how artificial systems have contributed to our understanding of symbioses.

Keywords Synthetic biology · Artificial symbiosis · Ecology · Evolution

Introduction

Even though “symbiosis” is often used to describe mutually beneficial interactions between different species, the term was originally coined by de Bray in the mid-19th century to describe close and long-term associations between different organisms [1]. In this review, we will adopt and broaden the de Bray definition of symbiosis, encompassing interactions ranging from mutually beneficial mutualism to antagonistic predator–prey interactions, even if the association between species is not necessarily close. Two reasons are: (1) a given symbiotic interaction can vary from mutualism to antagonism depending on the environment [2]; and (2) the definition of “close and long-term association” suffers, like many terms in biology, from its qualitative nature. Except in extreme cases such as endosymbioses, it is difficult to determine whether an association qualifies as “close and long-term.”

Given the complexity of natural ecological networks [3, 4], one strategy is to resort to simpler systems. Artificial systems constructed from defined components, with reduced complexity and increased controllability, provide a platform to address many important questions about natural systems. For example, what are the potential origins of symbioses? What determines the persistence of symbioses? How do features of the environment, such as habitat structure, affect the ecology and evolution of symbioses [5, 6]? How do interactions at the individual level generate dynamics and patterns at the community level [7]? How might interacting species within a symbiotic system evolve and coevolve [8]? In “Artificial systems”, we review

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different types of artificial systems that have been constructed to study symbioses, as well as their strengths and limitations. In “[Origins and persistence of symbioses](#)”, we examine the potential origins of symbioses and mechanisms for their persistence, focusing on the special case of mutualism and cooperation. In “[Spatially structured habitats can promote the origin and persistence of diverse symbioses](#)”, we describe how a spatially structured habitat might impact the origin and persistence of a wide variety of symbioses through evolutionary and ecological mechanisms. In “[Symbiotic interactions generate ecological patterns](#)”, we focus on how symbiotic interactions generate community patterns and how to test whether a biological process is the underlying mechanism for the interaction and therefore the pattern. Finally, in “[Symbiosis and evolution](#)”, we discuss how symbioses affect evolution and coevolution. Unfortunately, the extensive nature of the literature precludes us from citing all relevant papers.

Artificial systems

From abstract to living

The most abstract artificial systems live in the realm of mathematics. In population-based systems, mathematical equations describe population-level quantities such as densities of populations and resources available to populations, assuming that a population can be approximated by the ensemble average of its individuals. A well-known example is the Lotka–Volterra differential equation model constructed to describe the population cycles observed in predator–prey interactions [9] (Fig. 1, i). In contrast,

individual-based models simulate consequences of local interactions among individual members of a population [10], such as cooperators and cheaters. A cooperator pays a fitness cost to produce a benefit for another individual, whereas a cheater receives the benefit without paying the cost of cooperation. When cooperating and cheating individuals are simulated to compete on a spatial grid according to their gains from interactions with other individuals, cooperators can avoid the demise of extinction if interactions are limited to between local neighbors [11].

In both population-based and individual-based mathematical systems, organisms have their properties represented by parameter values and their interactions specified by equations. Furthermore, stochasticity is often introduced to incorporate the probabilistic nature of processes such as mutation, drift, and birth and death [12].

To capture the effects of necessary conditions for evolution such as mutation and selection, digital organisms were invented [13]. Digital organisms are computer programs that self-replicate, mutate, and compete for computational resources. Avidians [14], digital organisms that perform various logic computations (Fig. 1, ii), can be “mixed” to form a symbiotic cross-feeding system in which one organism uses the byproducts of another organism [15]: simpler computations performed by organisms at the lower trophic level can be “excreted” as “byproducts” and utilized by “consumers” at upper levels to carry out more complex computations. This digital symbiotic system has been used to study the differences in recovery from extinction episodes that either instantly eliminated the vast majority of the population or gradually reduced the population size through prolonged restriction of resources [15].

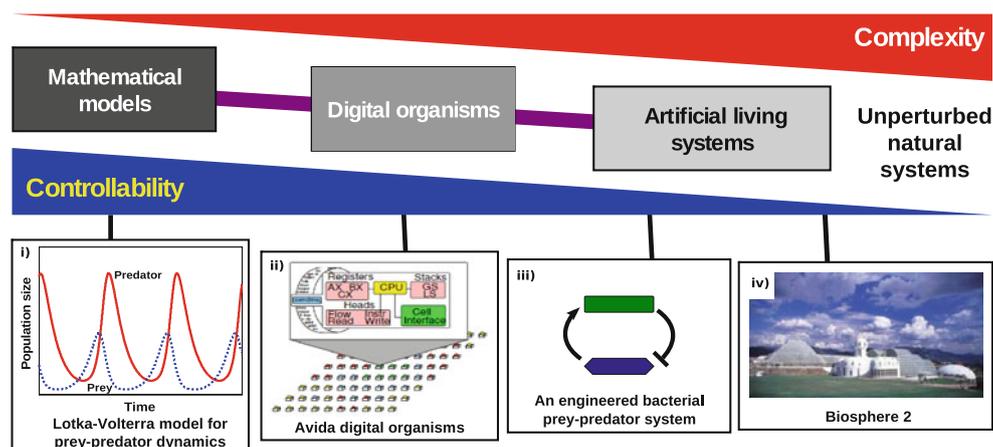


Fig. 1 Artificial systems for studying symbioses demonstrate the trade-off between controllability and complexity of a system. *Insets* show examples at different scales of controllability: i the Lotka–Volterra mathematical model for studying the dynamics of predator–prey systems [9]; ii Avida digital organisms [14] for studying

evolution; iii an engineered bacterial system for studying the predator–prey interactions [26]; and iv Biosphere 2 project, a synthetic ecosystem to study the earth biosphere [41]. Photo by C. Allan Morgan, courtesy of Global Ecotechnics

Mathematical and digital model systems are intended to abstract rules that govern ecological and evolutionary dynamics. Even though models are not intended to fully recapitulate details of living systems, they do provide powerful means to perform computational experiments for testing ecological [16] and evolutionary [15] hypotheses and to gain insights into processes that are otherwise difficult to observe [17, 18]. However, the convenience of computationally exploring the theoretical potential of system behavior has its tradeoff. It is sometimes unclear how realistic model assumptions are, and therefore, the conclusions derived from them can be unfounded.

Artificial systems composed of a small set of living organisms, termed microcosms, retain biological details of organisms and yet, compared to natural systems, have reduced network complexity in terms of the number of interacting species and the degree of connectivity between species. Thus, instead of a food web consisting of numerous types of predators and prey [19], an artificial system can feature one predator and one prey population such as *Didinium* and *Paramecium* [20] or phage and bacteria [21, 22].

Even though microcosms are greatly simplified compared to natural systems, the organisms themselves may be difficult to study and the interactions among them can be obscure. For instance, the artificial mutualistic system consisting of green algae and chick tissue culture cells [23] is likely to be challenging to study.

To establish experimentally tractable artificial living symbiotic systems, defined interactions can be engineered into “model organisms” such as bacterium and yeast [24, 25]. For instance, in an engineered bacterial predator–prey system, a quorum-sensing genetic circuit was adapted so that the predator induced the prey to express a suicidal toxin, while the prey rescued the predator by inducing the predator to express an antidote to the toxin [26] (Fig. 1, iii). A variety of population dynamics including oscillation could be observed in this system.

In summary, when choosing a model system, trade-offs among generality, realism, and precision will inevitably arise [27]. Mathematical models tend to abstract the most fundamental and general aspects of living systems. However, they are not able to (or intended to) thoroughly sample organismal properties and evolutionary changes. Mathematical models trade realism for generality. In contrast, artificial living systems retain the rich behavioral repertoire and evolutionary potential of a living entity without the network complexity typically found in natural systems. If comprised of “model” organisms, artificial systems will be particularly useful because of their short generation time, small genome size, ability to retain viability after freezing for evolution studies, and the availability of extensive molecular genetic tools [28, 29].

These systems can be experimentally manipulated, their dynamics can be quantified, and their evolutionary changes can be tracked at the molecular level. Artificial living systems trade generality for realism when compared to mathematical systems, and trade realism for precision of control when compared to natural systems. Thus, artificial living systems serve as the intermediate that fills the gap between abstract mathematical models and unperturbed natural systems (Fig. 1).

Idiosyncrasy versus generality

Can constructed artificial systems teach us principles that govern all, including natural, systems? Like natural systems, artificial systems have their idiosyncrasies. Thus, it is critical to study several systems so that we may discern idiosyncratic properties unique to a particular system from conserved properties that reveal general principles.

To illustrate how idiosyncrasy and generality may be teased apart, we compare three artificial microbial systems engaged in exchanges of essential metabolites. Viability of a system is defined as the ability of a coculture to grow from low to high density without external supplements of the essential metabolites. Metabolic exchanges have been observed in a wide variety of natural systems, such as the mutualism between legume and their nitrogen-fixing rhizobia [30], between coral and their endosymbiotic dinoflagellate [31], and between hosts and their gut microbial consortia [32–34]. The three artificial systems described below may be considered as models for these natural symbioses.

The first system (Fig. 2a, i) consisted of two auxotrophic (i.e., unable to synthesize certain compounds crucial for growth) *E. coli* strains. In certain combinations of auxotrophic strains, no mutations were necessary for viability of the system [35–37]. The second system (Fig. 2a, ii) started with a lysine-auxotrophic and an adenine-auxotrophic yeast strain. Unlike the *E. coli* system, the coculture was inviable unless an adenine-overproducing mutation was introduced into the lysine-requiring strain and a lysine-overproducing mutation was introduced into the adenine-requiring strain. Cells from neither strain released the overproduced metabolite until near cell-death [38]. The third system (Fig. 2a, iii) consisted of an *E. coli* strain auxotrophic for methionine and a *Salmonella* strain that consumed the metabolic waste of *E. coli* [39]. The initial mix of the methionine-requiring *E. coli* and its commensal *Salmonella* was not viable. After using chemical means to select for methionine overproduction mutants in *Salmonella*, the coculture still showed little visible growth. However, upon sub-culturing on agar, a second mutational event in *Salmonella* allowed excretion of a high level of methionine and consequently the system

became viable. Unlike the yeast system, methionine excretion was independent of cell death. Thus, how readily an artificial mutualistic system can be established and when the metabolites are released seem to be properties specific to a system. In contrast, system viability in every case required a sufficient level of nutrient to be supplied by each partner. Mathematically speaking, $S_1S_2 > C_1C_2$, where S_i ($i = 1, 2$) is the amount of metabolite supplied per type- i cell during its life time, and C_i is the amount of metabolite consumed to make a new type- i cell. The validity of this rule is independent of when cells release metabolites [37, 38] and is likely to be fundamental to all obligatory mutual cross-feeding systems. Thus, when multiple artificial systems are studied in parallel to distinguish general principles from special idiosyncrasies, we are likely to gain insights about ecology, evolution, and the interplay between the two [40].

Limitations

Artificial and natural systems may operate on drastically different scales of complexity and time. These differences in scales pose limitations on using artificial systems to understand natural systems.

On the scale of complexity. Limiting the types of participants in an artificial system may reduce complexity to the extent that important features of natural systems are missed. A well-known example is the Biosphere 2 project in Arizona [41] (Fig. 1, iv). Designed to model the earth biosphere, Biosphere 2 was a virtually airtight space frame and glass structure covering 12,700 m² with a volume of 180,000 m³. It harbored synthetically assembled tropical and subtropical ecosystems including those from rainforest, desert, and ocean. Could humans survive in this materially closed environment for a prolonged period of time? The initial closure experiment terminated after 16 months when the atmospheric concentration of O₂ dropped to a level that raised concerns about human health. Unlike the earth biosphere and despite its enormous operational cost, Biosphere 2 had failed to achieve the necessary “homeostasis” capable of supporting lives of the majority of introduced animals, including eight humans, for more than 2 years. The major conclusion from the Biosphere 2 experiment is that reconstituting a self-sustaining and life-supporting biosphere like the earth will be very challenging because of unexpected complexities of biological systems and their environments [42].

On the scale of time. The evolutionary possibilities an artificial system can explore over experimental time scales pale in comparison to those of natural systems that may have evolved for millions of years. Certain evolutionary innovations depend on prior genetic changes and can therefore only occur after a prolonged period of time. A beautiful example

of this type of “historical contingency” is illustrated in a long-term evolution experiment that has spanned the last two decades [43]. In this experiment, 12 initially identical populations were evolved in glucose-limited medium supplemented by citrate, a compound that *E. coli* cannot use. A citrate-using (Cit+) mutant eventually appeared after >30,000 generations in one of the populations, even though each population tested billions of mutations. In the Cit+ lineage, clones arising after 15,000 generations showed a significantly greater tendency to evolve Cit+, even though the overall mutation rate remained the same. Thus, it is likely that at least one “potentiating” change is required for evolution of the novel Cit+ trait. Since prior mutations can bias for or against a particular outcome [44, 45], and since complex features are generally built upon their simpler predecessors [18, 46, 47], evolution experiments require a large number of replicates and long duration to meaningfully sample potential trajectories.

Despite the limitations of artificial systems, we believe that they can yield valuable insights. First, artificial systems have been shown to recapitulate essential features of natural systems [48]. Second, they have been successfully used to test ecological and evolutionary hypotheses [49]. Third, fundamental differences between an artificial and a natural system can be educational, just as a mathematical model incapable of explaining experimental data may serve to illuminate faulty underlying assumptions and to prompt investigations into new hypotheses.

Origins and persistence of symbioses

It is challenging to deduce the origins of symbioses by relying only on observations of natural systems. Many extant symbioses are legacies of a history of interactions between their ancestral populations. These ancestral populations are not available for experimentation and the environmental conditions under which these symbioses first formed are unknown. In contrast, the early evolution of replicate artificial symbiotic systems in varying ecological conditions can be observed and their underlying genetic basis can be studied.

What are the origins of symbioses? One possibility is that different organisms make random encounters with each other as species migrate into or out of a community. These random interactions could develop into long-term associations if the migrating and the indigenous species adapt to each other. For example, invasive species quickly fit into new niches and interact with new partners [50], and new pathogens constantly infect hosts they have never encountered before [51]. These observations suggest that symbioses can be established quite rapidly. Once in geographical proximity, common conditions such as nutrient

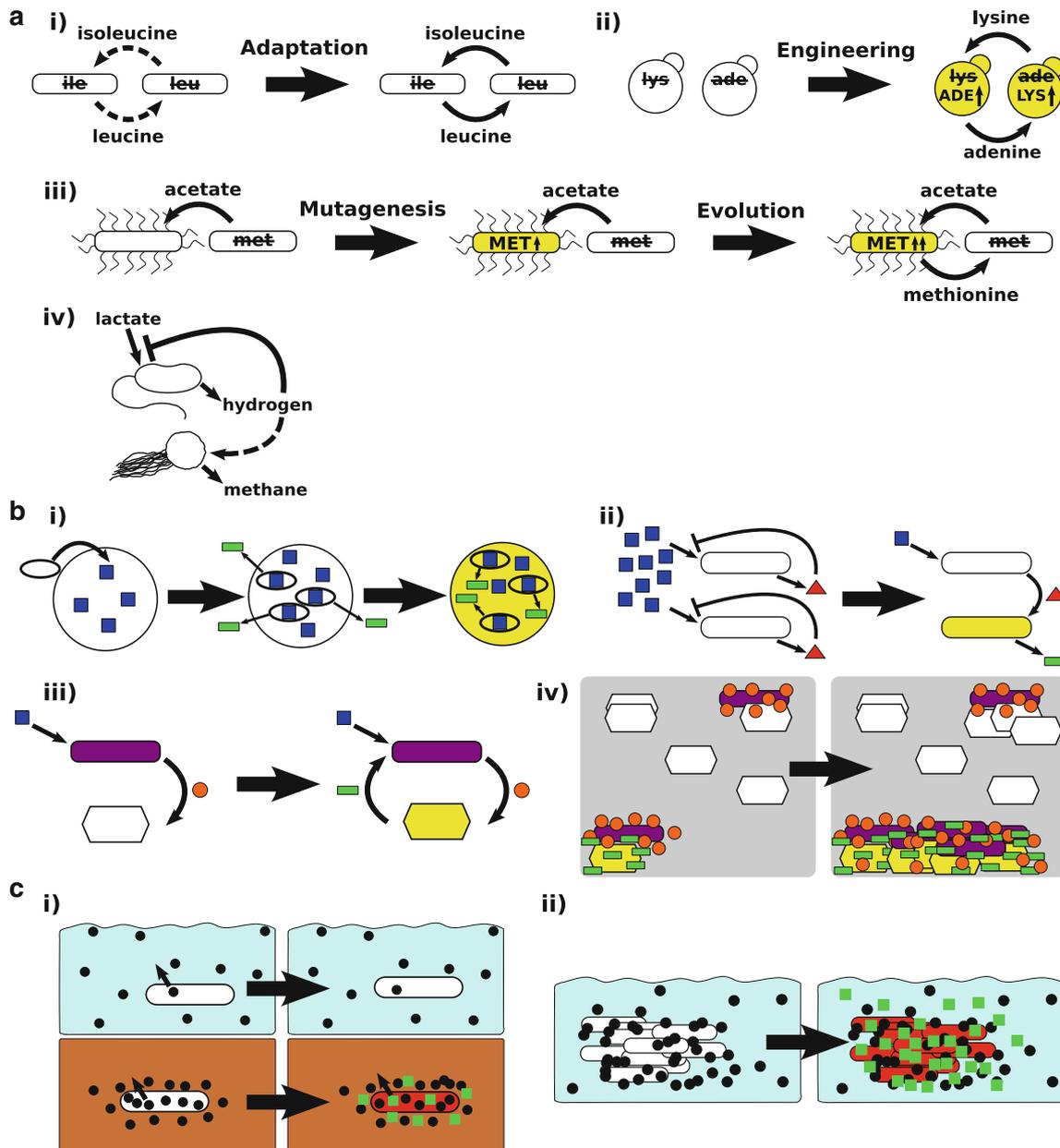


Fig. 2 Potential origins of mutualism and cooperation. *Yellow* indicates genetic changes. **a** In the laboratory environment, mutualism has been observed to arise spontaneously (iv, [68]), through rapid phenotypic adaptation (i, [37]), as the result of genetic engineering (ii, [38]) or evolution (iii, [39]). **b** In nature, mutualism can arise as the result of initially parasitic (i), competitive (ii), or commensal (iii, iv) interactions. i In an initially antagonistic host–parasite interaction, a parasite (*small oval*) invades its host (*large circle*), exploits host nutrients (*blue squares*), and produces a byproduct (*green rectangle*) that cannot be utilized by the host. Mutualism results if the host evolves to retain, utilize, and perhaps depend upon the byproduct of the parasite. ii Initially, organisms compete for a resource (*blue squares*) and convert it into a waste product (*red triangle*) that inhibits growth by reducing metabolic flux. Natural selection can favor the evolution of a type that is able to utilize the waste product as a primary source of energy, transforming competition into mutualism. iii, iv An initially commensal species (*hexagon*) can evolve to generate a benefit (*green rectangle*) for its partner (*magenta rod*), resulting in mutualism. If production of the

released product (*green rectangle*) is costly, a cooperator variant (*yellow hexagon*) can rise to high frequency in a spatially structured habitat [39] (iv). **c** Communication can be co-opted for cooperation. i A single cell (*rod*) produces an “inexpensive” membrane-permeable small molecule (*black circle*) that can, if at a high concentration, activate transcription of genes encoding expensive excreted molecules. The small molecule serves to monitor the diffusivity of the environment: in turbulent water (*top*), the molecule leaves rapidly and its low intracellular concentration is insufficient to activate gene expression. In soil (*bottom*), the molecule takes much longer to diffuse away, resulting in an intracellular concentration sufficient to induce (*red*) the production of expensive excreted molecules (*green squares*), which can be used, for example, to break down otherwise indigestible resources. ii Precisely the same mechanism could be used to allow a group of cells to sense when their local density is high enough to engage in cooperative group behavior such as synchronized release of excreted products (*green squares*). Group benefits are realized if a higher concentration of the excreted product generates a disproportionately larger benefit

limitation can favor the evolution of symbiosis, as has been observed between ciliates and green algae [52].

Symbioses can also develop from genetic diversification in an initially homogeneous population [43, 53–55]. Coexisting genotypes either use distinct metabolic strategies [43, 56, 57] or establish cross-feeding interactions [53, 54]. For example, the evolution of a cross-feeding symbiosis was repeatedly observed from initially isogenic populations of *E. coli* maintained in a glucose-limited environment [58]. Compared to the ancestor, all variants showed an increased affinity for and uptake rate of glucose. The variant most successful in taking up glucose also excreted a high level of acetate, a glucose metabolism byproduct. This was due in part to a null mutation in acetyl-CoA synthetase that converts acetate to acetyl-CoA. A different variant scavenged acetate due to a mutation that resulted in constitutive overexpression of acetyl-CoA synthetase [53]. These diversifications can mitigate competition between strains and lead to formation of new ecological niches, as also shown in digital organisms [59].

Once formed, symbiotic interactions can change rapidly with addition or removal of other species in the community. For instance, removing large mammals in an African savanna caused the breakdown of an ant–plant mutualism [60]. Symbiotic interactions can also rapidly evolve, as observed in laboratory experiments. Hansen et al. [61] observed that commensalism, in which one species benefits from the other while the other is unaffected, can evolve to exploitation in a biofilm. Harcombe observed evolution from commensalism to mutualism [39]. Thus, the nature of symbiosis may change rapidly. However, at least some species form stable symbioses that have persisted over millions of years [62]. For instance, analysis of the evolution of host use in a large and diverse group of interactions showed that symbiotic interactions are phylogenetically conserved to a high degree such that related species tend to interact with similar partners [63].

Of particular interest to evolutionary biology are the origin and persistence of mutualism. Mutualism can persist if it does not inflict a cost on any of its participants. However, many mutualistic interactions rely on cooperation that involves paying a cost to benefit a partner. Darwin stated, “Natural selection cannot possibly produce any modification in any one species exclusively for the good of another species” [46], which implies that cooperative traits cannot increase in frequency unless they somehow also benefit their bearers. Several mechanisms have been proposed to explain the origin of cooperation [64, 65]. For instance, in kin selection, an individual that enhances the fitness of its genetic relatives at a cost to itself may still be favored by natural selection [66]. However, kin selection does not directly promote inter-specific cooperation. Direct reciprocity can promote cooperation among non-relatives if interactions are

likely to be repeated and if partners employ strategies such as “tit-for-tat,” rewarding cooperators with cooperation and retaliating cheaters with cheating [67]. Below we showcase how artificial symbioses have suggested mechanisms for the origin and evolution of mutualism and cooperation.

Evolution of mutualism from byproduct exchange

The simplest scenario for the origin of mutualism is when behavior that is beneficial or at no cost to its executor happens to benefit another individual. This benefit could increase the likelihood of the recipient coexisting with, and thus adapting to, the donor. This type of commensalism can transition into mutualism when the recipient also returns benefits to the donor, constructing a positive feedback cycle [64] (Fig. 2b, iii).

Once formed, mutualism can strengthen, as observed in a nascent mutualism between the sulfate-reducing bacteria *Desulfovibrio vulgaris* and the methanogenic archaea *Methanococcus maripaludis* [68] (Fig. 2a, iv). When cultured in the absence of sulfate and hydrogen, *M. maripaludis* relies on *D. vulgaris* to produce the hydrogen it requires for growth, and in turn, provides energetically favorable conditions for the growth of *D. vulgaris* by removing inhibitory hydrogen. Prior to being isolated and propagated in pure culture in the laboratory, *D. vulgaris* and *M. maripaludis* may have relied on similar interactions with other methanogens or sulfate reducers in their respective communities [69]. However, the particular strains used in this mutualism were recently adapted to growth in pure culture, and have no known history of prior contact. Thus, the first steps in the evolution of this association are similar to those of two strains capable of, but not adapted to, byproduct exchange. These mutualisms were initially unstable, causing two out of 24 cocultures to go extinct. However, the remaining replicates developed into stable and productive associations within 300 generations. The potential role of each species in promoting cooperation was tested by using a technique that would be extremely difficult, if not impossible, to use in natural systems. By mixing evolved and ancestral mutualists and comparing their biomass production, the authors showed that the improvements of many of the evolved mutualisms were less than the sum of contributions from individual populations. Thus, either some form of competition for common resources also evolved, or improvements in the mutualistic traits of the two species have some degree of redundancy.

Origin and persistence of costly cooperative mutualism

In many types of mutualism, participation is costly. Nectar generated to attract pollinators and carbon compounds fed to rhizobia are costly to produce. These costs affect the viability of cooperation in two ways. First, if the benefits of

cooperation are less than the costs or if the benefits are not delivered in time, cooperation will not be viable [37, 38, 70]. Second, “cheaters” that reap the benefits without paying the costs of cooperation will have a fitness advantage over cooperators. If unchecked, cheaters will increase in frequency and the cooperative system will cease to be viable.

Costly cooperation may develop from no-cost mutualism. For example, as mentioned in the previous section, there is no cost in mutualism based on byproduct exchange, and therefore the concept of “cheating” does not apply. However, as a byproduct exchange mutualism evolves, populations could invest more resources in producing more byproducts or additional products in order to exchange with and extract more benefits from the partner. If the investment in producing resources is costly, byproduct mutualism can evolve into cooperation and become vulnerable to cheaters.

Costly cooperative mutualism could have also evolved from exploitation [2] (Fig. 2b, i). Exploitation of one organism by another sets the stage for close interactions between the two. Mutualism would be established if the exploited organism evolved to exploit the original exploiter. For instance, Jeon observed that when amoeba was initially infected with a strain of bacteria, the parasitic bacteria proliferated within and were harmful to the host. After years of infection, the infective bacteria became harmless, and the nucleus of the host cell became dependent on the infective organisms for its normal functions [51]. The host seemed to have evolved the ability to extract benefits from the parasite, thereby turning a harmful parasite into a beneficial endosymbiont.

Conflict between the host and the symbiont can be mitigated when transmission of the symbiont is vertical, i.e., from parent to progeny. In contrast, horizontal transmission among unrelated individuals destabilizes cooperation because the chance of repeated encounters and thus the possibility of direct reciprocity is reduced [71]. Indeed, vertical propagation of filamentous phages selected for variants that were least harmful to the host [72]. A cooperative algae symbiont in jellyfish host became parasitic when the mode of transmission was experimentally changed from vertical to horizontal [73].

Originally competitive interactions can turn into mutualism and cooperation (Fig. 2b, ii). Sachs and Bull [74] observed the evolution of cooperation between co-infecting phages through co-transmission, which represses competition. Two phages, each carrying a different antibiotic resistance gene, were propagated using *E. coli* as host. The two phages had to alternate between competing for hosts and cooperating to provide both types of antibiotic resistance to enable the host to grow. The two phages evolved to be co-packaged in the same protein coat, thereby ensuring that both phages would always infect the host

together to furnish resistance to both antibiotics. During the process of evolution, both phages accumulated multiple mutations, with one phage experiencing extreme genome reduction and loss of independence.

Finally, cooperator mutations with high fitness cost can arise and increase in frequency in a non-cooperating population if the cooperators are preferentially rewarded with the benefits of cooperation (Fig. 2b, iv). In Harcombe’s artificial *E. coli*–*Salmonella* system discussed earlier [39], a mutant *Salmonella* overproducing methionine at a great fitness cost to itself evolved only when *E. coli* and *Salmonella* were cocultured on an agar plate instead of a well-mixed culture. On plates, methionine released by a *Salmonella* overproducer primarily benefited neighboring *E. coli* whose metabolic byproduct primarily benefited neighboring methionine-overproducing *Salmonella*. In contrast, in well-mixed cultures, cooperative *Salmonella* would promote *E. coli* growth, but the increased supply from *E. coli* would be evenly distributed rather than being directed toward cooperators who had paid the fitness cost of methionine overproduction. Thus, costly cooperation evolved only in a spatially structured habitat that facilitated direct reciprocity. The importance of direct reciprocity is further supported by research on cell–cell signaling systems in which the specificity of signaling molecule targets is found to be positively correlated with the metabolic cost to produce them [75].

Interestingly, the stability of cooperation can quickly improve even under well-mixed conditions [38]. Metabolite overexpression was found to be costly in the yeast system of Shou et al. [38] (WS unpublished data, Fig. 2a, ii), although the cost was much smaller than that of methionine overproduction in *Salmonella*. Within 100 generations, the well-mixed yeast system evolved 100-fold lower cell-density requirements for supporting viable cooperation. Unlike the *E. coli*–*Salmonella* system in which rare cooperator mutants must “invade” a cheating population, the engineered yeast system consisted purely of cooperators, at least initially. Whether initially cooperative systems will inevitably accumulate cheaters over a longer period of time awaits further experimentation.

Communication and cooperation

Communication among bacteria through the production and detection of extracellular chemicals for monitoring population density [76, 77] is thought to coordinate cooperative group behavior [78]. Can communication—the display of an inexpensive signal indicating the ability to perform expensive cooperative acts such as supplying costly shared goods—facilitate the evolution of cooperation?

Interestingly, in both individual-based models and robot populations, honest communication between individuals

enables direct reciprocity and the evolution of cooperation [78, 79]. In the robot experiment, robots were equipped with panoramic vision cameras and light-emitting diodes. Signal production through light emission was initially random, but could become associated with food or poison location. This association was more likely to evolve if successful groups rather than successful individuals were selected to propagate, or if group members were highly related by sharing a similar algorithm. In other words, “signals” in communication originally for other uses may be adopted to facilitate the evolution of cooperation under proper selective conditions. This is consistent with the “efficiency sensing” hypothesis [80], which argues that quorum sensing signaling molecules were originally used for individual benefit, for example to predict whether it is efficient to produce expensive excreted molecules that may diffuse away from the cell, before being adopted to orchestrate cooperative group behavior (Fig. 2c).

The cooperation-promoting mechanisms mentioned above are not mutually exclusive. For example, biofilms are established by bacteria that secrete various signals and effectors to cooperate. These cooperators may over time diverge in the resources they use, leading to decreased competition for resources and increased resistance to cheaters, thus further stabilizing cooperation [81]. Are some mechanisms more accessible than others? Do these mechanisms correlate with each other such that some enhance or impair others? Answers to these questions await future experimental studies.

Spatially structured habitats can promote the origin and persistence of diverse symbioses

A spatially structured habitat facilitates the generation of spatial heterogeneity, i.e., non-uniform spatial distribution of individuals or resources [82], by restricting mixing. A spatially structured habitat can generate and modify symbioses through evolutionary mechanisms, and maintain symbioses through ecological mechanisms.

Evolutionary mechanisms for generating and modifying symbioses

Habitat heterogeneity creates different ecological niches that promote the evolution of multiple “niche-specialists,” each adapting to its local environment and together forming new symbioses. For instance, when the aerobic bacterium *Pseudomonas fluorescens* was switched from a homogeneous well-mixed to a heterogeneous static-broth environment, variants quickly evolved [83]. In contrast to cells from the homogeneous environment which, when plated on agar, formed smooth colonies, those from the

heterogeneous environment repeatedly evolved into cells that formed “wrinkly spreader” or “fuzzy spreader” colonies. Importantly, wrinkly and fuzzy spreaders occupied the top (oxygen-rich) and the bottom (oxygen-poor) layer of the static environment respectively, suggesting that they had adapted to different ecological niches [83].

Certain evolutionary changes that alter the nature of symbiosis can only occur in a spatially structured environment. When a biofilm consisting of soil-dwelling bacteria *Acinetobacter* and *Pseudomonas putida* was grown on benzyl alcohol as the sole carbon source, *Acinetobacter* transformed the carbon source to benzoate and excreted it, which was metabolized by *P. putida*. When this commensal community was propagated in a spatially structured environment consisting of a glass surface within a flow chamber, a heritable rough colony-variant of *P. putida* evolved which formed a mantle covering *Acinetobacter* [61]. This rough colony-variant was caused by a mutation in a gene involved in the biosynthesis of a lipopolysaccharide. Wild-type *P. putida* detached from the biofilm when the O₂ level was low, while mutant *P. putida* adhered to and competed for O₂ with *Acinetobacter*. Consequently, mutant *P. putida* obtained more benzoate and grew to a higher density to the detriment of *Acinetobacter*. This evolution from commensalism to exploitation occurred in a structured environment but was not observed in the unstructured environment of a chemostat [61].

Spatial heterogeneity can be diminished by migration. To understand how patterns of migration between fragmented habitats might affect the persistence of a “rock-paper-scissors” symbiotic community, bacteria and phages were propagated in microtiter plates [84]. Specifically, wells were filled with bacteria, phage, or sterile media. Migrating bacteria “beat” media wells through colonization; migrating phage “beat” bacteria through infection and lysis; and migrating media “beat” phage through dilution. Migration was implemented by transfer of a fraction of the content of one well to another well, and could occur between neighboring wells (restricted migration) or random wells (global migration). Individual-based computer modeling predicted that both the phage and bacteria densities should differ significantly between local and global migrations. However, no such difference was observed in the experiments. This discrepancy between model and experiment prompted the discovery of two types of evolved phage, both more virulent than the ancestor but each adapted to the particular pattern of migration. Global migration selected for “rapacious” phage that reproduce relatively quickly but “waste” bacteria resources, while local migration selected for “prudent” phage that reproduce relatively slowly but reach a higher titer through efficient usage of bacteria. This occurred because, when migration was local, the probability of having to compete

with rapacious phage was reduced. The increased virulence of evolved phages and the tradeoff between competitive ability and productivity were due to mutations in a single locus encoding an inhibitor of bacteria lysis [85]. Thus, local migration encouraged the evolution of slower-reproducing, higher-yield variants while global migration favored the evolution of faster-reproducing, lower-yield variants [56, 84].

Ecological mechanisms for maintaining symbioses

Theoretical investigations have shown that under most circumstances, spatial heterogeneity boosts coexistence of species and thus symbioses. In competitive symbiosis, a uniform environment with one limiting resource can, at equilibrium, support only one consumer species [86]. In contrast, in a spatially structured habitat, aggregation of organisms that enhance intraspecific and weaken interspecific competition allows coexistence of multiple species [87]. Pre-existing resource heterogeneity in a spatially structured habitat can support the coexistence of resource-specialists. Even when the spatially structured habitat is physically homogeneous and dispersal is random, a more competitive species with less colonization ability can coexist with a less competitive species with better colonization ability [88]. Similarly, in predator–prey symbioses, populations with discrete generations are prone to local extinction due to population oscillations to low minima. However, if the spatial distribution of predators around each prey is significantly heterogeneous [89] or if populations are connected through limited local migration [90], spatially structured habitats are predicted to facilitate the coexistence of predator and prey. Finally, mathematical models show that spatially structured habitats allow coexistence of cooperators and cheaters [11, 91].

Experiments supporting or refuting theories for maintenance of biodiversity have been far scarcer. However, artificial symbiotic systems are increasingly deployed to test hypotheses generated from mathematical models. To understand how spatial configuration affects competition, random or conspecifically aggregated spatial arrangements were imposed on four different plant species [92]. Compared to a random distribution, self-aggregation caused poor competitors to perform better and strong competitors to perform worse. Stronger intraspecific competition can improve the chance of coexistence of different species. In fact, geneticists have customarily taken advantage of spatially structured habitats to increase biodiversity in their mutagenesis screens: cell populations exposed to a mutagen are usually transferred onto solid agar plates instead of liquid medium to prevent slow-growing mutants from being outcompeted and to allow isolation of independent mutants.

Experiments on predator–prey symbioses have shown that spatially structured habitats facilitate the coexistence of predator and prey [84, 93–95]. When herbivorous mites and their predator mites were placed on a single undivided habitat of plants, both species went extinct. However, when the habitat was divided into islands of plants connected by bridges to allow migration of mites, both populations persisted [93]. Because the coexistence of prey and predator depends on heterogeneous local environments in which not all patches of resources are simultaneously exploited by prey and not all patches of prey are simultaneously consumed by predators, the spatial environment must be sufficiently large and complex to ensure a sufficient degree of heterogeneity [94].

To study how spatially structured habitats might influence coexistence in a multispecies bacterial community, three types of soil bacteria were studied using microfluidics [96]. *Azotobacter vinelandii* fixes gaseous nitrogen into amino acids, *Bacillus licheniformis* degrades antibiotics, and *Paenibacillus curdlanolyticus* produces a carbon energy source. The three types of cells were cultured in wells connected by fluidic communication channels that allowed the diffusion of chemicals, but not migration of bacteria, among wells. The community was viable only under intermediate spatial separation, because when the bacteria species were too close together, the most competitive species would deplete the shared resource and drive the other species to extinction and when they were too far away, the supply rate of essential metabolites was too slow to keep the community viable. These findings again highlight the importance of spatially structured habitats in the biodiversity of multispecies communities.

In summary, spatial heterogeneity generally favors the evolution and maintenance of biodiversity, which in turn fuels the formation and persistence of new symbioses.

Symbiotic interactions generate ecological patterns

Temporal and spatial patterns at the ecosystem level are generated by interactions among organisms and between organisms and the environment [16, 97, 98]. However, the numerous types of organisms in a natural system and the complexity of their biology pose significant obstacles in identifying and quantifying interactions. For these reasons, ecology has traditionally been mostly observational and theoretical, and less experimental [99].

In the early 20th century, Gause [20] pioneered using artificial systems to study the ecology of competition and predation. Recently, biological engineering has allowed a wide variety of artificial systems to be implemented [24, 49]. Artificial systems offer experimentalists unsurpassed control over aspects such as interactions between

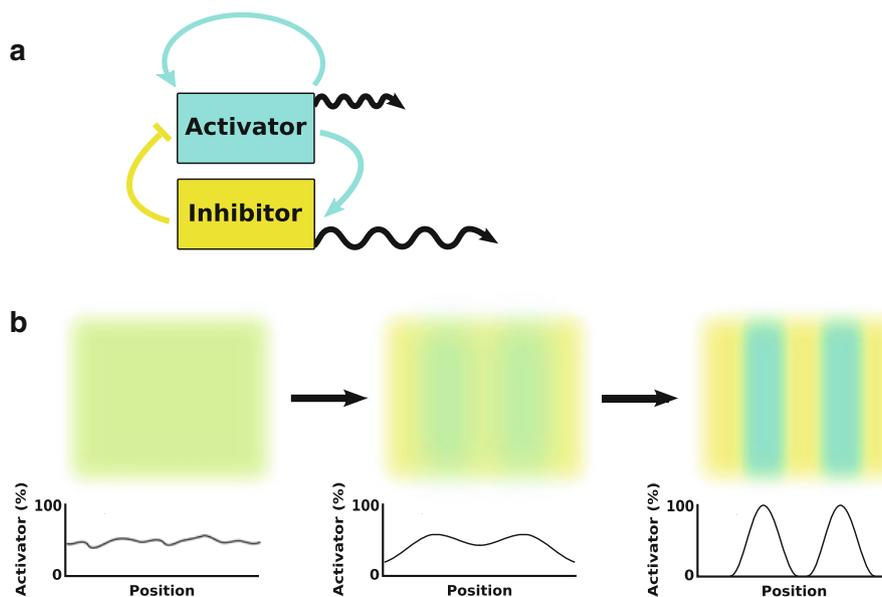
organisms, initial conditions of the system, and nutrient supply rates. Simplified artificial systems facilitate quantification of both the local interactions and the global ecosystem patterns, thereby allowing a mechanistic linkage between the two.

To understand consequences of interspecies interactions, a traditional approach is to construct a mathematical model to probe the potential range of spatial–temporal patterns [100]. These theoretical patterns may in turn be used as clues to infer the properties of species interactions from observed ecological patterns. A well-known example is the spatially periodic Turing patterns [101], similar to those found in animal skins or coats [102]. Turing’s mathematical model showed that in an initially homogeneous environment, reaction and diffusion of chemicals can, under certain conditions, generate periodic spatial patterns. The formation of Turing patterns relies on a self-activating activator that also generates an inhibitor with a higher diffusivity than the activator (Fig. 3). Regular patterns observed in arid, wetland, and savanna ecosystems have been attributed in part to the Turing mechanism [103]. For instance, plant growth promotes water infiltration into the soil, which in turn facilitates plant growth. This short-range self-activation depletes water from the surrounding area, in essence creating a long-range inhibition of plant growth. When advection such as water flowing downhill is also included, a Turing pattern can appear in which stripes of vegetation alternate with patches of barren soil [104].

The challenge of inferring mechanisms from patterns is that different mechanisms can generate similar patterns. Turing mechanisms can generate stripes and spots on animal coats, but so can biological morphogens [105]. How do

we investigate whether a hypothesized mechanism is responsible for the observed pattern? The most direct approach is to perturb the system and test whether the pattern is altered as predicted by the hypothesized mechanism. Such a task may be difficult or unethical to perform in natural ecosystems, but is achievable in experimental systems. For instance, in a classic study on inter-specific competition between yeasts *Schizosaccharomyces* and *Saccharomyces* [20], Gause first examined intra-specific competition: what prevented monocultures from growing indefinitely? Gause postulated that under anaerobic growth conditions in the presence of a high level of sugar, accumulation of alcohol, a fermentation product, eventually inhibited yeast growth and thus set the maximum yield of a culture. To test this hypothesis, different amounts of alcohol were added to yeast cultures and indeed, corresponding reductions in the maximum yields were observed. Thus, the mechanism for growth inhibition seemed to result from accumulation of an inhibitory compound rather than depletion of resources. If so, the same mechanism should also operate when two species competed in a coculture. In this case, the reduction in final yield of a species should be fully accounted for by alcohol produced by the competitor species. To test this hypothesis, Gause competed the two species in a coculture. From the resulting growth kinetics, Gause determined the “empirical coefficients of the struggle for existence,” which quantified the reduction in growth of each species per individual of its competitor species. For each species, Gause independently calculated the coefficients of the struggle for existence from measurements of alcohol production: the more alcohol produced per individual of a species, the more competitive

Fig. 3 **a** A Turing pattern can result from an autocatalytic “activator” that promotes the formation of an “inhibitor” with a greater diffusivity (*wavy arrow*) than the activator. **b** Random small disturbances in an initially homogenous mixture of activator and inhibitor can result in the spontaneous formation of highly ordered patterns, such as stripes



the species is—even if it grows slowly. The coefficients measured from the two methods were similar, supporting alcohol production as the mechanism of growth limitation.

Gause's work and other contemporary studies [38, 106–111] highlight the potentials of artificial systems for observing, quantifying, and mathematically understanding the consequences of symbiotic interactions in controlled environments. In contrast, inferring mechanisms from patterns has so far been rare [112, 113], and could be a challenging but fruitful avenue of investigation.

Symbiosis and evolution

Symbiosis has long been thought to impose selection on organisms, resulting in adaptations such as nodules on legume roots and the extremely long spur of the Christmas orchid (*Angraecum sesquipedale*) predicted by Darwin to require a specialized pollinator [114]. Besides mutualism [115–117], competitive [118], predator–prey [119], and host–parasite [120, 121] interactions all affect the evolution of populations.

To understand how ecological symbioses affect evolutionary adaptations, we cannot rely entirely on fossils and natural populations. Organisms co-localized in fossil beds did not necessarily live together when alive, making it difficult to infer whether they had interacted and the types of interactions that occurred, if any. Comparative studies of extant species may help us reconstruct their phylogenetic relationships and infer the differences between those that participated in a specific type of symbiosis and those that did not [122]. However, it is important to recognize that the differences may be due to reasons other than symbiosis, such as historical accidents or organismal constraints [123].

With artificial symbiosis, it is possible to examine the effects of symbiosis on evolution. First, it is possible to evolve replicate populations in the same ecological conditions. If most of the independently evolved populations acquired the same characteristics within a limited time-frame, it likely resulted from natural selection rather than stochastic processes [124, 125]. Second, artificial symbioses can be formed from species with small genomes that can be easily resequenced [35] and genetically manipulated. Each mutation or mutation combination can be introduced into the ancestor to assess its adaptive value [44, 45, 126]. Third, artificial systems utilizing microbes can be frozen and revived, offering the opportunity to replay the tape of symbiotic evolution and coevolution. Fourth, microcosms of previously non-interacting species allow us to observe the evolution of incipient symbioses. Fifth, in artificial symbioses, it is often possible to separate the evolved symbionts and study them in non-symbiotic conditions or in alternative pairings [68, 127]. This can be crucial for testing which

evolved participant is responsible for community-level changes and whether an evolved trait is tailored to a specific partner. Finally, if multiple artificial symbioses composed of diverse taxa are studied, it may be possible to identify general rules about how symbioses affect evolution and to predict, at least to some extent, how symbioses may evolve.

Here, we describe how artificial systems have been used to address the following questions: How does symbiosis affect evolution? How might symbiotic coevolution affect the evolutionary dynamics and outcomes relative to what might occur if only one species was allowed to evolve or if the species had evolved in isolation (Fig. 4)?

How does symbiosis affect evolution?

Artificial symbioses allow us to test hypotheses about how symbioses may affect evolutionary outcomes. This is demonstrated by several researchers that tested foraging theories by observing the evolution of predators [128–131]. Foraging theory is a set of models predicting the optimal behavior of predators or grazers with respect to the density and quality of prey [132, 133]. These models assume that a predator has evolved to maximize its rate of energy intake and a resulting adaptation is its tendency to, for example, fully consume a prey patch when prey are scarce but not when they are common. Foraging theories have typically been tested by observing predator behavior in environments varying in prey composition and comparing that behavior to model predictions [134]. More recently, when evolution of a predator was directly observed, some but not all foraging theory predictions were confirmed. Observing the evolution of the predator *Myxococcus xanthus* on *E. coli* prey distributed at high and low density, Hillesland et al. [131] confirmed the foraging theory prediction that low prey density would select for faster searching behavior in the predator. However, unlike predictions from foraging models, predators evolving at high prey density could not attack and kill prey faster than predators evolving at low prey density or the ancestor. Similarly, the phage ϕ X174 did not evolve the specialization or expansion of host range at high or low host density, respectively, that would be predicted by foraging theory [130]. Heineman et al. [129] observed the evolution of discrimination by phage T7 between high- and low-quality *E. coli* prey. The evolved phage chose high-quality prey when the prey were abundant, but did not perform as predicted when poor-quality prey were abundant. In another study, T7 evolved a shorter latent period at high host density, as predicted by foraging theory, but the latent period did not evolve as expected at low host density [128]. Together, these studies show that some evolutionary outcomes can be predicted from theories, but they also emphasize the confounding effects of organismal constraints.

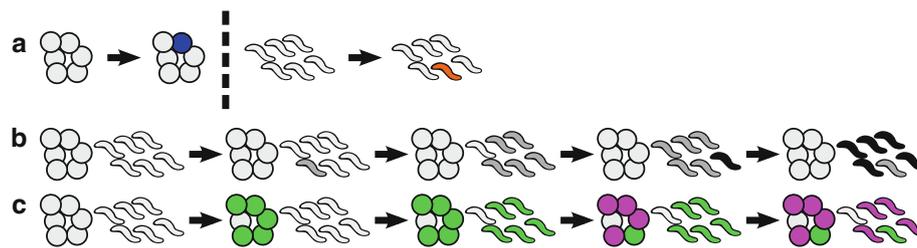


Fig. 4 If two interacting species, such as host (grey circles) and parasite (grey squiggles), are propagated separately (a), mutations that allow the host to escape parasite infection (blue) and mutations that allow the parasite to increase infection of host (orange) are not selected for. If the parasite is allowed to evolve against a constant host at the ancestral state (b), mutations in the parasite that increase its

ability to infect the host are selected for (light grey, dark grey, and black indicate increasing levels of infectivity). Evolutionary arms race in coevolution (c) results in a faster rate of evolution and increased genetic diversity for both partners, as the host evolves to evade parasite invasion (changing host color) and the parasite evolves to attack the host in a frequency-dependent manner (changing parasite color)

Evolution and coevolution

Symbiosis sets the stage for coevolution, defined as evolutionary changes as the result of interactions between species. Coevolution is a major driving force in evolution [8], because it leads to species diversification [114, 135]. With artificial symbioses, it is possible to test the effects of coevolution by observing evolution with and without the coevolutionary partner or by preventing the partner from evolving. For example, competition can cause character displacement, which refers to the divergence of traits between species in response to competition. Experiments conducted in stickleback fish (*Gasterosteus aculeatus*) showed that coexistence with a competitor species occupying similar niches resulted in selection for individuals most different from the competitor, both morphologically and ecologically [136]. In contrast, when the fish was evolved alone, traits varied more or less randomly. Similarly, species diversification has been observed in other competitive systems [137–139]. Predator–prey [140, 141] and host–parasite interactions [142–146] have also been shown to cause genetic divergence.

Coevolution can result in a constantly changing selection environment that is distinct from that imposed by abiotic variables. Abiotic variables such as temperature may vary dramatically in a seasonal environment, but these variations occur in a predictable sequence. Moreover, after a species adapts to a change in the abiotic environment, no evolutionary response in the abiotic environment is elicited. In contrast, organisms can continuously evolve a seemingly endless succession of phenotypes that challenge their coevolving partner. For instance, in a host–parasite “arms race,” the parasite evolves to attack the host more effectively while the host evolves to evade parasite invasion (Fig. 4c). Because of these dynamics, the Red Queen hypothesis posits that antagonistic coevolution increases evolutionary rates [147]. Furthermore, during coevolution, parasites adapt to more efficiently infect and thus reduce the abundance of the most common host genotype, allowing other host genotypes

to flourish. In this case, the fitness of an allele depends on its frequency in a population. This frequency-dependent selection hypothesis predicts that genetic diversity will be higher during coevolution.

To test the frequency-dependent selection hypothesis, snail evolution in replicated experimental tanks with a coevolving trematode parasite was compared to evolution without the parasite. In support of the hypothesis, the snail clone that was initially most common in all tanks became much less frequent in coevolving tanks but not in tanks without parasites. In addition, initially rare clones tended to increase in frequency in coevolving tanks but not control tanks. Finally, the common clone in coevolution treatments declined in abundance due to its greater tendency of being infected by the coevolved compared to the unevolved trematodes [142–146].

The rate of change in coevolving populations can be compared with that of populations where only one species is allowed to evolve while the other is kept in the ancestral state or is excluded from the environment altogether (Fig. 4). Using this approach, Paterson et al. [144] and Schulte et al. [145] observed increased rates of evolution during the coevolution of *Pseudomonas fluorescens* SBW35 with a viral parasite and the coevolution of *Caenorhabditis elegans* with the parasite *Bacillus thuringiensis*, respectively. In both systems, greater within- and between-population diversities were observed during coevolution than in static-partner or no-partner controls, suggesting that antagonistic coevolution leads to diversification, as predicted by theory.

What are the genetic mechanisms of coevolution? This question can be answered using a combination of genome sequencing and partner swapping among independently evolved lines. In the case of *C. elegans* and *B. thuringiensis*, coevolving parasites and hosts consistently exhibited higher virulence and resistance than the respective solitary evolution controls. Toxin genes in the parasite and microsatellite sequences in several locations of the host genome were found to exhibit higher within- and among-population genetic diversities during coevolution [145]. Sequencing of the phage infecting *P. fluorescens* showed

that during coevolution, a tail fiber gene responsible for host infection had a higher density of mutations than in treatments where the host remained evolutionarily static [144]. In addition, the host range of phage from independently coevolved lines mirrored their genetic similarity, suggesting that the evolutionary responses of the phage were to some extent specific to particular evolutionary changes in the host population.

Due to genetic constraints, evolutionary arms races do not always proceed at an uninterrupted pace. For instance, a strain of *E. coli* eventually evolved a resistance that phage T7 could not overcome [148], and phage T2 seems unable to evolve host-range mutations [149].

Are coevolutionary dynamics in mutually beneficial interactions different from coevolution in antagonistic interactions? The effect of mutualism on evolutionary dynamics may vary depending on the type of mutualism involved [150]. In the simplest possible mutualism, an improvement in the fitness of one species may result in a higher production of benefits to its coevolving partner, thereby increasing the growth of both species. In this scenario, it is not clear that a reciprocal evolutionary response necessarily ensues. Some even predict that mutualism will decrease the rate of evolution—dubbed as the Red King hypothesis [151]: if mutualists are ‘bargaining’ over the exchange rate of benefits, the mutualist that does not rapidly respond to ‘demands’ for higher payment will have an advantage. Before it evolves to acquiesce, the ‘demanding’ partner may evolve back to ‘accept’ the original, lower price. In mutualisms that need to fend off cheaters, reciprocal adaptations in partners that enhance the specificity of symbiosis, such as those observed between certain species of flowers and pollinators [114], may occur rapidly. Future research involving a variety of artificial symbioses will be necessary to develop a good understanding of the mechanisms and rates of coevolution in mutualisms.

Summary

Symbiotic associations between species are ecologically and taxonomically ubiquitous and often play key roles in shaping ecology and evolution. However, it is often not possible to manipulate and control symbiotic species or their habitats, or to directly observe their early evolution. Thus, many hypotheses about the formation, persistence, and evolution of symbioses cannot be thoroughly tested without the aid of artificial systems. Here, we described a diverse array of artificial symbioses that have been developed, including robotic, digital, and genetically engineered organisms, as well as more natural interactions that have been adapted to a laboratory environment. These artificial systems have been used to study the ecology and evolution

of diverse symbioses and the environmental conditions that may promote more intimate associations. Research with artificial symbioses confirmed theoretical predictions that antagonistic interactions can increase both diversification of species and the rates of evolution compared to what happens if the same species does not evolve with its antagonistic symbiont. Other experiments provided a first glimpse of evolution at the origin of symbiosis and provided empirical tests of the conditions permitting cooperative relationships. Manipulation of spatial habitat and migration patterns in several different artificial symbioses showed that spatial heterogeneity allows for the generation and maintenance of diversity and enhances the stability of costly cooperation. These results may explain how symbioses form and why some species develop tight physical associations. Once formed, symbiotic interactions generate ecological patterns, and quantitative studies in artificial systems have helped to reveal how. As more artificial symbioses are developed and used to test a range of hypotheses about species interactions, it will be possible to discern general ecological and evolutionary principles affecting taxonomically diverse symbioses. These principles may eventually be used to interpret and predict the behavior of more complex natural symbioses of medical, agricultural, and environmental interests.

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