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Review

Symbiotic organs: the nexus of host–microbe evolution

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Diverse plants and animals have evolved specialized structures to filter and house beneficial microbes. These symbiotic organs form crucial points of exchange between host and symbiont, are often shaped by both partners, and exhibit features that facilitate a suite of microbial services. While symbiotic organs exhibit varied function, morphology, and developmental plasticity, they share core features linked to the evolutionary maintenance of beneficial symbiosis. Moreover, these organs can have a significant role in altering the demographic forces that shape microbial genomes, driving population bottlenecks and horizontal gene transfer (HGT). To advance our understanding of these 'joint phenotypes' across varied systems, future research must consider the emergent forces that can shape symbiotic organs, including fitness feedbacks and conflicts between interacting genomes.

Highlights

Symbiotic organs are host structures that house populations of beneficial microbes.

Microbes within symbiotic organs provide a core set of mutualism services to hosts, including nitrogen fixation, bioluminescence, antimicrobial protection, nutrition, and vertical transmission to new hosts.

Microbial communities are filtered by symbiotic organs, mediating which genotypes interact with hosts, gain host resources, and are subsequently passed on to new hosts.

Growth and development of symbiotic organs imposes selection on microbial populations, altering their population structure, effective population size, opportunity for gene exchange, and genome architecture.

Cooperation and conflict between microbes and host shape the development, morphology, and functions of symbiotic organs, and have emergent effects on individuality, heritability, and the stability of microbial mutualisms.

Diverse symbiotic organs have evolved in animal and plant hosts

Animals and plants thrive, reproduce, and evolve in a microbe-dominated world [1,2], with dense and diverse communities of microbes that coat host surfaces and mucosa, colonize intercellular spaces, and gain access to protected intracellular host habitats [2]. An immense diversity of these microbes are archaea and bacteria, taxa that preceded eukaryotic origins by hundreds of millions of years [3]. Thus, eukaryotes have evolved since their origins in the continual presence of archaeal and bacterial microbes in associations that exhibit diverse effects on host fitness. A feature that is common to host-associated microbes, including pathogens and mutualists, is that they invariably consume host resources to establish on the host, reproduce in host tissues, and gain transmission to new hosts or habitats [4,5]. Host defense mechanisms against pathogenic microbes, including innate and adaptive immunity, have been studied in depth and display features that can be traced back over deep evolutionary time [6,7]. Less attention has focused on host traits that house, support, feed, regulate, and transmit beneficial microbial partners. Paramount among these traits are **symbiotic organs** (see Glossary), encompassing diverse host structures that house beneficial microbes. Symbiotic organs are densely colonized by distinct communities of microbes, providing a mutualist service to the host through direct cellular contact or indirectly through the transmission of metabolites or other biologically important compounds or services.

Symbiotic organs have been described in both plants and animals and are extraordinarily varied in structure, phylogenetic origin, and microbial taxa [8–11] (Figure 1), including root nodules in plants, which house nitrogen-fixing **rhizobia** [10,12], light organs in bobtail squid (*Euprymna scolopes*), which support bioluminescent bacteria [8,13], exoskeletal **crypts** in Hymenoptera, which accommodate antibiotic-producing bacteria [9,14,15], pit-like **mycangia** in ambrosia beetles (Scolytidae and Platypodinae), which carry fungal symbionts [16], symbiont-sorting organs of hemipteran midguts [17], and **bacteriomes**, membrane-bound clusters of host cells that house intracellular, nutrient-provisioning bacteria [18] (Box 1). Symbiotic organs provide a variety of fitness-enhancing functions for hosts, including biological nitrogen fixation, bioluminescence,

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chemical protection, assistance with nutrition and digestion, and **vertical transmission** of symbionts. Despite this incredible diversity, symbiotic organs share a suite of core features, including singular or modular structures that house microbes, capacity to filter or constrain the microbial genotypes that gain access to the host, and the provisioning of sustenance to the microbes that are housed within (Box 2). In many cases, the morphological development of symbiotic organs is **phenotypically plastic**, responding elegantly to stimuli from compatible microbes, whereas, in other cases, symbiotic organs are **canalized**, hence their ontology is unchanged by infection (Table 1).

Symbiotic organs facilitate a suite of microbial services

Symbiotic nitrogen fixation

Plants experience nitrogen-limiting conditions in most soils. Nitrogen fixation by bacteria can provide substantial fitness benefit to plants by supplying a continuous source of nitrogen and expanding the host niche. Nitrogenase, which reduces dinitrogen, has evolved in disparate bacterial taxa, but requires low oxygen concentrations, conditions that are provided and regulated within a variety of plant symbiotic organs. Simple symbiotic organs, ones that house nitrogen-fixing bacteria in extracellular structures, have evolved recurrently across plants, including waterferns, liverworts, and cycads [6] (Figure 1). More complex symbiotic organs, in the form of root or stem nodules that house intracellular bacteria, have evolved in legumes (e.g., *Medicago* and *Lotus*), actinorhizal plants (e.g., *Alnus*), and charcoal trees (i.e., *Parasponia*; Figure 1). Nodule organogenesis is phenotypically plastic, being induced by the presence of compatible symbionts and requiring molecular communication between symbiont and host [19]. Despite this specificity, root nodules can occasionally be colonized by bacteria that lack the canonical *nod* loci as well as rhizobia that carry *nod* genes but do not provide any nitrogen fixation for the host [20]. Thus, while symbiotic organs likely evolved in the context of housing beneficial microbes, they are vulnerable to colonization by nonbeneficial bacteria. Given the potential for costly infections, hosts that produce symbiotic organs must engage in post-infection **host control** mechanisms to minimize exploitation by ineffective symbionts [21].

Bioluminescence

Bacterial luminescence can provide associated hosts with various advantages, including camouflage and prey detection [22,23]. As such, light organs that house bioluminescent bacteria have evolved at least twice within marine invertebrates [24] and at least 17 times in marine fish [25]. These organs show considerable evolutionary convergence in structure and function. For instance, the bobtail squid, flashlight fish (*Anomalops katoptron*), and the diverged host lineages of deep-sea anglerfish (Lophiiformes) all use similar mechanisms to manipulate light produced by the symbiotic organ and house their symbiotic bacteria. All three host taxa house their symbionts in internal crypts that are open to the environment and, once hosts are infected, reflectors and pigments combined with muscular contractions can be used by hosts to control light emission (although lenses and filters may also be present) [23,26–28]. In flashlight fish, light is used to detect prey [22] and allows for social signaling while schooling [27]. Conversely, bobtail squid use light emission to evade detection via counterillumination, disrupting their silhouette when foraging [23]. The light organ of bobtail squid is one of the best characterized, comprising both internal crypts that house symbionts and external structures that are lost after symbiont colonization [23,29]. The crypts are acidic and enriched with host antimicrobials, selecting for light-producing *Vibrio fischeri,* creating an environment that is inhospitable to most other microbes, and increasing the availability of oxygen for light production [23,29,30].

In both vertebrate and invertebrate hosts with light organs, symbiont acquisition is enabled by bacterial **chemotaxis** and host signal recognition (Box 3). Both the symbionts of flashlight fish

Glossary

Bacteriocytes: specialized host cells that house endosymbiotic microbes.
Bacteriome: cluster of bacteriocytes that house vertically transmitted symbionts. These structures are usually surrounded by a host-derived membrane.
Canalized: organ or structure the developmental outcome of which is not changed due to external stimuli, such as the presence of symbionts.
Chemotaxis: movement in response to a chemical stimulus.
Crypt: hollowed-out region of host tissue, often surrounded by host epithelium, and used in symbiotic organs to house associated symbionts (also referred to as a tubule).
Environmentally acquired symbionts: symbionts that follow horizontal transmission; symbiont recruitment is mediated through a combination of host signaling and or chemical/mechanical filtering.
Genotype × genotype interactions: phenotypic or fitness outcomes between species that depend on their respective genotypes.
Horizontal gene transfer (HGT): movement of genetic material between different organisms, other than between parent and offspring.
Host control: trait exhibited by the host to reward beneficial symbionts or sanction ineffective or harmful symbionts.
Horizontal/vertical transmission: acquisition of symbionts from an environmental source or an unrelated host (horizontal), or from a genetically related host, most often a parent (vertical).
Intergenomic epistasis: epistatic effect of two genotypes (host and symbiont) interacting, producing phenotypic outcomes that depend on the genetic backgrounds of host and symbiont.
Joint phenotype: trait that is dependent on the genotype of at least two interacting species.
Mycangium (mycangia, plural): animal structure adapted for transport of symbiotic fungi
Phenotypically plastic: refers to a phenotype that changes in response to external stimuli, such as the presence of symbionts
Population bottleneck: dramatic reduction in effective population size, resulting in a reduced efficiency of

and deep-sea anglerfish maintain genes for chemotaxis despite significantly reduced genomes, suggesting that recognition of host signals is required for light organ colonization [31–33]. Similarly, the symbionts of the bobtail squid undergo chemotaxis toward host-produced *N*-acetylneuraminic acid and chitin oligosaccharides, and mutant strains with defects in flagella or chemotaxis regulators exhibit decreased colonization [23,29].

The open nature of light organs allows hosts to seed their environment with symbionts. Bobtail squid expel symbionts daily, ensuring that dark mutants are removed and beneficial symbionts are plentiful [23]. Similarly, whereas deep-sea anglerfish symbionts are found at multiple ocean depths, they are most abundant in the mesopelagic zone, associated with adult hosts [26]. Finally, as a strong case for the effect of host specificity on environmental symbionts, a study of the coral reef fish *Siphamia tubifer* and its light organ-associated symbiont *Photobacterium mandapamensis* suggested that host populations separated by tens of kilometers can create genetically distinct symbiont communities [34].

Chemical defense and antimicrobial protection

Diverse animals house bacteria within symbiotic organs that offer chemical protection against parasites and pathogens. For attine ants, bacteria in the genus *Pseudonocardia* are maintained in cuticular crypts supported by associated exocrine glands, producing antibiotics that protect the fungal gardens of the ants from microfungal parasites [15,35]. While attine ants use their symbionts to protect their food, bobtail squid and beewolves (Crabronidae) use symbionts to defend offspring. The accessory nidamental gland of bobtail squids houses a consortium of antifungal and antimicrobial-producing bacteria, with different crypts generally dominated by individual strains [36,37]. This consortium is deposited on the jelly coat of freshly laid eggs, protecting them from fouling by fungal and bacterial contamination [38,39]. Beewolves house their symbiont *Streptomyces philanthi* in crypts within antennal glands, the contents of which, including the symbionts, are excreted into brood cells for developing larvae and their food [14]. Larvae incorporate the material into their cocoons, providing protection while ensuring symbiont colonization upon emergence [14,40].

For symbiotic organs that provide antimicrobial protection, the evolution of resistance can negate their function, but these organs exhibit features that can minimize this possibility. The accessory nidamental gland of the bobtail squid acquires symbionts by sampling a diverse subset of the microbial community of the environment [37]. This broad sampling, compared with the singular host–symbiont pairings of other systems, allows the acquisition of diverse symbionts that produce an array of metabolites to protect the offspring of the host [38,39]. By contrast, beewolves and attine ants maintain antimicrobial protection with single symbiont taxa. In beewolves, *S. philanthi* shows considerable stability in a broad array of antibiotics across host genera [40]. This robust suite of metabolites with antibiotic properties appears sufficient to prevent multidrug resistance from evolving in host pathogens. In attine ants, the exposed nature of the symbiont allows for **HGT** with other bacteria on the host, such as *Streptomyces*, allowing for acquisition of novel antibiotic gene clusters that can enhance symbiont protection [35,41].

Chemical defense systems can also extend to protecting the host from predators and competitors. In leaf nodules of Bwanashupa shrubs (*Psychotria kirkii*), bacterial symbionts (*Candidatus Burkholderia kirkii*) provide the host with chemicals toxic to arthropods or that impede root elongation in competitors, preventing seed plants from growing near the host [42]. Although the symbiont has experienced significant genomic erosion associated with vertical transmission, it maintains two gene clusters not found in free-living *Burkholderia*, which are involved in the synthesis of these chemicals [43].

natural selection due to the loss of genetic diversity.
Rhizobia: polyphyletic lineages of alpha- and betaproteobacteria that have acquired the capacity to trigger nodule formation on legume roots (and sometimes stems) and to fix atmospheric nitrogen.
Symbiotic organ: evolutionarily derived host structure that contributes to a symbiotic interaction with one or more phylogenetically distinct symbionts and the functions of which are affected by the presence or absence of those symbionts.

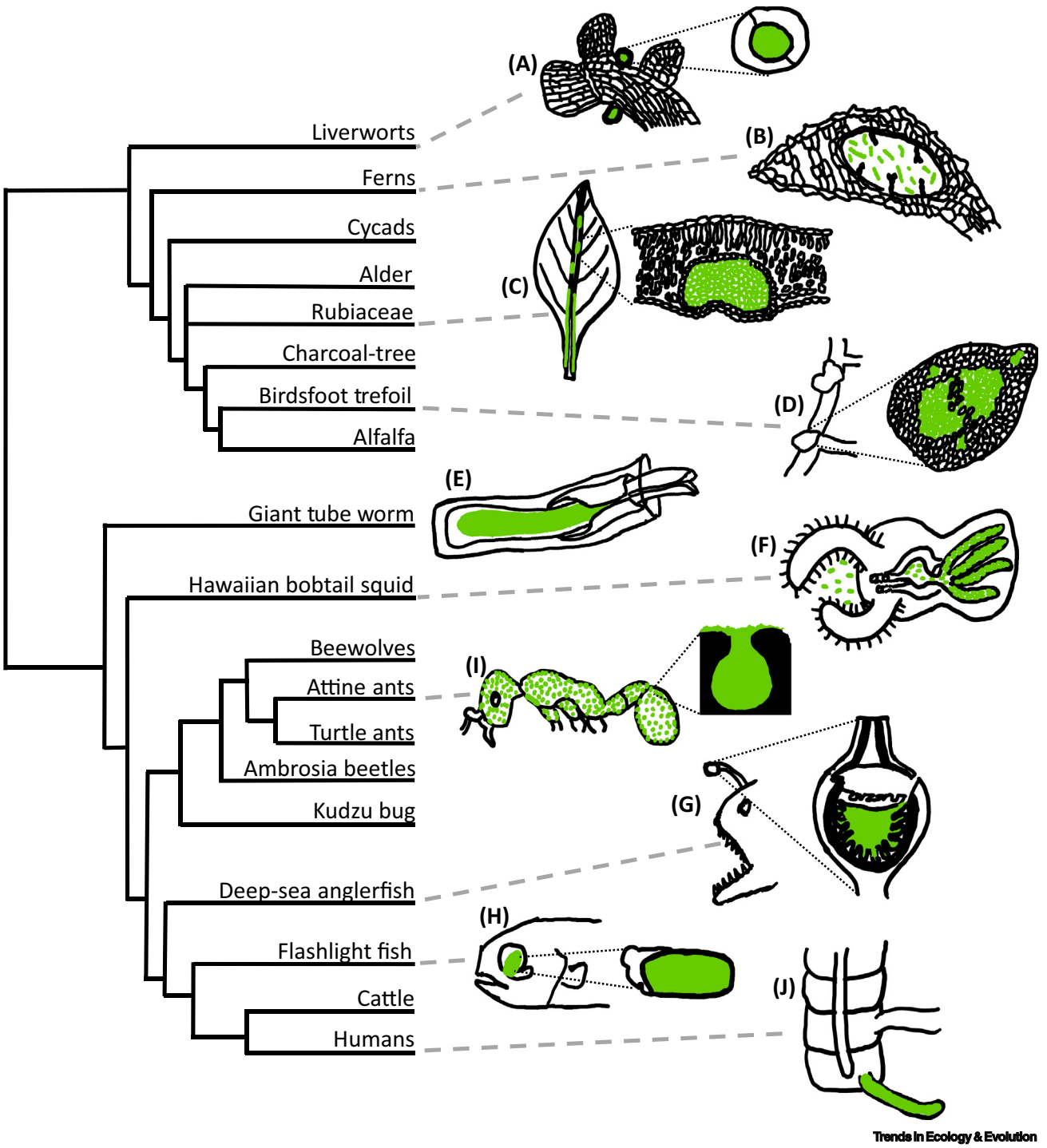

Figure 1. Symbiotic organs in plants and animals. Host tissues are indicated in black and symbionts are indicated in green. In plants, organs have evolved that house diverse bacteria, including: (A) auricles in liverworts and (B) leaf cavities in the waterfern *Azolla*, both of which fix nitrogen; (C) leaf nodules in Bwanashupa (*Psychotria kirkii*), which produce toxins against competitors and predators; and (D) determinate root nodules in legumes, which also fix nitrogen. In animals, a more diverse set of services has been uncovered including: (E) the trophosome in tubeworms, which houses carbon-fixing bacteria; light organs with bioluminescent bacteria in (F) squids, (G) anglerfish, and (H) flashlight fish; (I) exoskeletal crypts in ants that house antibiotic-producing bacteria; and (J) the cecal appendix in humans. Adapted from [6] (A), [92] (B), [43] (C), [46] (E), [23] (F), [28] (G), [22] (H), and [9] (I).

Box 1. What features promote the origins of symbiotic organs?

Symbiotic organs are not required for microbial mutualism. However, these organs have evolved multiple times across the tree of life (see Figure 1 in the main text). The origins of symbiotic organs imply substantial evolutionary changes in host development that allow for housing and resource provisioning for symbiotic microbes [6,8]. Research suggests a set of five key features that can promote these changes, as discussed below.

Intracellular accommodation

Symbiotic organs often accommodate growth of microbes within a living host cell, which is thought to be an independent step from the formation of the organ itself [10]. Such intimate association can be a key factor allowing the efficient transmission of services from symbiont to host.

Microbial induction of host cell division

Symbiotic organs often require direct microbial induction of host cell division, which involves both the production of mobile microbial signals and host cells that are developmentally permissive in their response [94]. The intricacies of the signaling required can restrict the origins of these associations [104].

Infection specificity

Host mechanisms that promote specificity are particularly critical in symbiotic organs that are open to the environment during part or all of their development [11,47].

Compartmentalization

The compartmentalization of symbiotic organs can promote the imposition of host specificity mechanisms, because each individual structure allows for an independent action of host control against harmful strains [5,77].

Complementarity

In intimate symbioses, the microbial partners often express metabolic pathways that are lacking in the host [2], and such provisioning is of particular prominence in symbiotic organs (see Table 1 in the main text). More broadly, symbiotic organs are predicted to be associated with fitness benefit for the host, otherwise costs involved in the maintenance of novel structures would favor their loss [105].

Nutrition

Symbiotic organs associated with host nutrition can supplement the host diet with required amino acids [44] and by expanding the diversity of carbon catabolism [45]. More drastically, nutritional symbiotic organs can also supplant the host digestive system [46]. The functional diversity of these nutritional services is reflected in the varied host structures, with some animal host species modifying relatively small sections of their gastrointestinal system [17,47], whereas others display wholesale morphological restructuring [48].

In stinkbugs (superfamily Pentatomoidea) and turtle ants (*Cephalotes* spp.), the gastrointestinal tract is modified with a selective valve, a symbiotic organ that regulates the flow of bacterial taxa and prevents the passage of large particles downstream [17,47,49]. For the brown-winged green bug (*Plautia stali*), the valve divides the midgut itself and ensures that only the compatible symbiont (*Pantoea* spp.) reaches and colonizes crypts in the fourth section of the host midgut [17]. For turtle ants, this structure divides the crop and midgut, maintaining a midgut microbial community distinct from the upstream gastrointestinal tract and from the external host habitat [47]. Hosts from both groups are herbivorous and rely on their symbionts for growth and survival: the symbiont community in turtle ants generates amino acids by recycling nitrogenous waste products [44], a process important for host cuticle development [50], whereas the symbiont in stinkbugs is critical to host health, because removal results in high mortality, with no offspring reaching adulthood [49]. Both systems show evidence of host behavior that

Table 1. Genotype × genotype interactions: plasticity and canalization in symbiotic organs[a]

Host	Symbiotic organ	Developmental evidence	Refs
Symbiotic organ morphology altered by symbiont colonization			
Legumes, actinorhizal plants, *Gunnera*	Tumorous or glandular organs that house nitrogen-fixing bacteria	Symbionts and hosts influence the development and function of nitrogen-fixation organs	[20,94,95]
Stinkbug: *Riptortus pedestris*	Midgut crypts and partitions that house bacteria for host nutrition	Closing of midgut constricted region in *R. pedestris* in response to symbiont infection	[96]
Stinkbug: *Plautia stali*	Midgut crypts that house nutritional symbionts	Modifications to midgut occur after symbiont exposure	[17]
Bobtail squid *Euprymna scolopes*	Light organ that houses luminescent bacteria	Modification to internal crypt epithelia and environment as well as apoptosis of external ciliated epithelium in response to colonization	[97]
	Accessory nidamental gland that houses antibiotic-producing bacteria	Symbionts colonize organ during early development of crypts	[98]
Hydrothermal-vent tubeworm	Trophosome that houses chemoautotrophic bacteria, which provide host nutrition	Formation of trophosome induced by symbiont colonization	[18]
Ruminant mammals	Stomach structure for microbial fermentation by diverse community	Stomach development continues postnatally, during symbiont exposure, suggesting potential for symbiont influence	[99]
Ambrosia beetles	Glandular mycangium to transport fungal cultivars to newly established galleries	Mycangial inflation only occurs if symbiont is present	[100]
Flashlight fish	Light organ housing luminescent bacteria	Organ degradation seen in individuals lacking symbionts	[22]
Symbiotic organ development appears canalized (i.e., no genotype × genotype interactions)			
Beewolves	Antennal glands that house antibiotic producing bacteria	Symbionts are acquired after gland is fully formed	[14]
Attine ants	Exoskeletal crypts with antibiotic producing bacteria	Symbiont exposure occurs after host eclosion and crypt formation	[101]
Turtle ants	Proventriculus filters midgut microbial community	Symbiont exposure occurs after organ formation	[102]
Deep-sea anglerfish	Light organ that houses luminescent bacteria	Symbiont exposure occurs after light organ is formed	[26]

[a]Symbiotic organs often express phenotypic plasticity, wherein organ development responds to microbial signals and varies dependent on the host and symbiont genotype (i.e., genotype × genotype interactions). Conversely, some symbiotic organs complete development before symbiont colonization or otherwise exhibit canalized development that is unaffected by symbiont colonization.

ensures vertical transmission of symbionts, including trophallaxis in turtle ants [44] and egg-smearing and similar behaviors in stinkbugs [49,51].

More extensive changes in the gastrointestinal tract are seen in the hydrothermal vent tube worm, *Riftia pachyptila*, and in ruminant species. The gastrointestinal tract of adult *Riftia* is lost during the formation of its symbiotic organ, the trophosome [48]. The trophosome houses the **environmentally acquired symbiont** *Candidatus Endoriftia persephone* in **bacteriocytes** supplied with reduced sulfur [52]. The symbionts use sulfide oxidation to drive carbon fixation and growth, which benefits the host because a portion of symbionts are consumed [46]. By contrast, ruminants have modified portions of their gastrointestinal tract into a multistomach symbiotic organ housing a diverse microbial consortium [53,54]. Cross-feeding between symbionts produces volatile fatty acids from the fermentation of cellulose, which can then be catabolized by the host [45]. Ruminants also show evidence of symbiont consumption, because genes associated with microbial degradation (lysozyme c family) are expanded in copy number compared with other mammals [55].

Vertical transmission

Some symbiotic organs are critical to the vertical transmission of the symbionts themselves, ensuring maintenance of a host–symbiont interaction across generations. In some cases,

Box 2. Core features that are common across diverse symbiotic organs

The services and structures of symbiotic organs are strikingly diverse (see Table 1 in the main text) but nonetheless can be defined and categorized by considering three prominent features: their structure, capacity for filtering microbial partners, and ability to maintain beneficial partnerships.

Structure of symbiotic organs

Symbiotic organs exhibit structures in which microbes interact directly with host cells. These structures range in complexity from a simple invagination, to differentiated, modular crypts, to complex multitissue organs [17,19,54,93]. These structures can restrict symbiont access to certain host tissues, and are often characterized by being modular and, thus, are repeated in multiple locations on the host [5,77]. By isolating symbionts from other host systems, these structures reduce symbiont interference with other host processes while creating microenvironments that optimize the services provided by symbionts [19,47,52]. The structure might be canalized and unaffected by symbiont colonization, or phenotypically plastic with considerable modifications once symbiosis is established (see Table 1 in the main text).

Filtering of microbial partner

Symbiotic organs filter microbial partners, using mechanical structures that spatially and or temporally restrict access to these spaces, chemical attractants that help recruit symbionts, and antibiotic selectors that remove nonsymbiotic strains, all of which might be supplemented by the behavioral traits of the host [14,17,29,47]. Each filter selects for genetically compatible symbionts and determines the establishing members of the microbial community of each symbiotic organ. Regardless of whether symbionts are horizontally acquired from the environment or vertically inherited from parent to offspring, filtering traits help define the genotypic and phenotypic diversity of symbionts that thrive in the symbiotic organ and, thus, are critical for the establishment and specificity of symbiosis.

Ability to maintain beneficial partnerships

Symbiotic organs provide for the maintenance of microbial services, referring to host investment that generates nutritional support to beneficial symbionts and host sanctioning of strains that disrupt the symbiotic organ or its function [19,23,54]. This might also extend to other changes in the symbiotic organ that enable effective symbiosis, including modifying the concentration of various molecules that inhibit or enhance the role of the symbionts in the host. Maintenance often involves regulation of host gene expression to facilitate the optimal function of an organ, and has important fitness effects on the survival, fitness, and transmission of the *in hospite* symbionts.

this involves transporting symbionts that provide benefit while outside the host. For example, fungus-farming ambrosia beetles (Scolytidae and Platypodinae) rely on their symbiotic organ (mycangium) to carry spores of their symbionts (*Ambrosiella*) as hosts migrate to new locations and establish fungal gardens [56–58]. Mycangia are cavities or pockets in the host surface or oral space that allow spore packing. These structures have evolved at least 13 times independently within ambrosia beetles [57] and vary among species in size, tissue of origin, and complexity [56,58]. Evidence for coarse phylogenetic congruence between host and symbiont clades suggests some host–symbiont specificity has been maintained over long periods of evolutionary time [16]. Specificity of symbionts to particular aspects of the host symbiotic organ is further supported by cross-infection experiments using the black twig borer host (*Xylosandrus compactus*) and four broadly sampled non-native *Ambrosiella* symbionts, which demonstrated significant decreases in mycangium packing with distantly related symbionts [16].

Vertical transmission organs can also ensure maintenance of symbionts that benefit the host, whether in host-derived crypts or bacteriocytes. In stinkbugs (Pentatomoidea), which derive nutritional benefit from associated symbionts, vertical transmission organs have evolved multiple times [51]. In some of these taxa, derivations of the midgut-associated crypts facilitate vertical transmission, including enlarged crypts that regularly shed symbionts of pentatomid stinkbugs for egg smearing [59], and specialized posterior midgut sections in kudzu bugs (Plataspidae), which create symbiont capsules on which nymphs feed [60]. Analogous adaptations to symbiont capsules can be found in Coleoptera, where female thistle tortoise beetles

Box 3. Host mechanisms mediating microbial transmission in symbiotic organs

Host signaling

Hosts often release signals to attract compatible symbionts to the site of infection from a broader environmental pool of microbes. This mechanism to enhance acquisition relies on symbionts recognizing specific host metabolites and migrating toward the host or its symbiotic organ specifically. Host signaling occurs in both marine and terrestrial systems, as seen by the attraction of *Vibrio* to light organ pores in bobtail squid [29] and the attraction of rhizobia to flavonoids released by legume roots [19] (Figure IA).

Mechanical and chemical filtering

Hosts exhibit mechanisms to filter compatible symbionts from a wider environmental pool, and this filtering often occurs within the symbiotic organ. These filters can restrict which microbes reach the symbiotic organ, such as the constricted midgut region in stinkbug *Riptortus pedestris* [11,103] (Figure IB). The microenvironment of the symbiotic organ itself can also select for symbionts and, thus, acts as a chemical filter, as seen in the bobtail squid light organ [23] and in the gastrointestinal tract of ruminants [45].

Physical transfer

Some hosts have evolved specific behaviors to transfer symbionts to offspring. This can include incorporating symbionts into physical structures that the offspring interact with, such as beewolf cocoons [91] or symbiont capsules in Plataspidae stinkbugs [60], or direct parent–offspring interaction, such as turtle ant trophallaxis [44] (Figure IC) and attine ant exposure to exosymbionts [41].

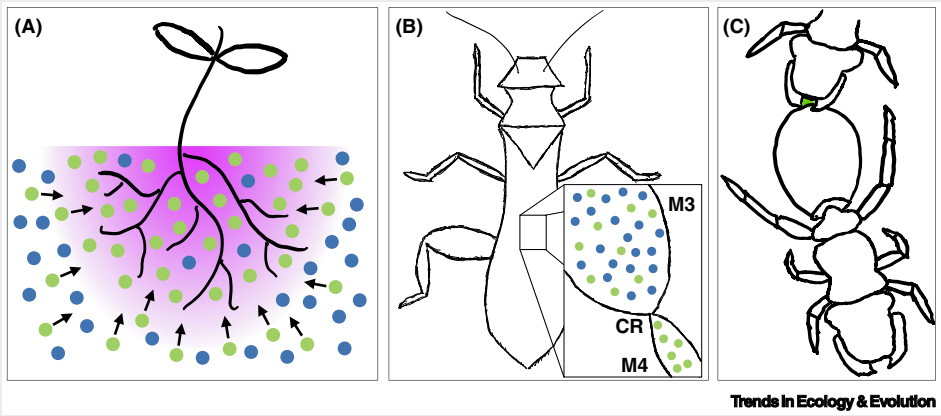

Figure I. Modes of symbiont acquisition. Symbionts are in green, nonsymbionts are in blue, and hosts are in black. (A) Chemotaxis of rhizobia toward legume roots in response to host flavonoid signals [20]. (B) Filtering the broader pool of midgut 3 (M3) in *Riptortus pedestris* using a constricted region (CR) to ensure only symbionts reach midgut 4 (M4) [103]. (C) Behavior-based transfer of symbionts via oral–anal trophallaxis in turtle ants [44].

(*Cassida rubiginosa*) generate symbiont-housing egg caplets from glandular reservoirs that open into their genital tract [61]. In other Pentatomoidea, midgut crypts are closed off and not accessible, leading to the formation of additional symbiotic organs, such as the egg smearing-lubricating organs of shield bugs (Acanthosomatidae) [62] and the anatomically distinct symbiont jelly-producing ovipositor-associated organs of basal stinkbugs (Urostylididae) [63]. Vertical transmission organs can also benefit hosts beyond the transfer and movement of symbionts, as seen in tsetse flies, in which the milk gland ensures bacteriocyte development in offspring [64]. These bacteriocytes contain the obligate bacterial symbiont *Wigglesworthia glossinidia*, which provides B vitamins lacking from the blood-feeding diet of the host and which are needed for female fecundity [64,65]. The milk gland produces an immune regulatory peptidoglycan recognition protein (PGRP-LB), suppressing immune function in offspring to facilitate symbiont uptake as well as providing a physical path for symbiont transfer [65,66].

Symbiotic organs are joint phenotypes

Host and microbe partners often both contribute to structural and functional variation of symbiotic organs [67] through **genotype × genotype interactions** (Table 1), thus defining them as **joint phenotypes** [68]. Given this mutuality, emergent effects, such as **intergenomic epistasis**, can shape trait outcomes. Thus, we must expand our view of trait evolution to consider phenotypic variance driven by both the host and symbiont genotype (and their heritability values), as well as environmental pressures that contribute to partner phenotypes [67–71]. One of the novel predictions that has arisen from these models is that the striking variation in fitness outcomes seen in mutualism services is driven by diverse host × symbiont genotypic interactions [67,69,72,73].

Core features of symbiotic organs, such as their growth, development, maintenance, and senescence, can be affected by both host and microbial genotypes (Table 1). Given this joint control over phenotypic outcomes, natural selection on each partner can lead to evolutionary conflict, wherein each party is predicted to gain from pushing these joint phenotypes in opposite directions [71]. However, host–symbiont interactions are highly asymmetric; microbial associates have a tremendous evolutionary advantage over their hosts in terms of generation time and population size [20]. With their rapid adaptation to novel conditions, microbes are predicted to evolve to exploit host resources and manipulate host traits toward selfish fitness interests, potentially altering the host phenotype and its interaction with the environment. For example, some nonfixing rhizobia induce root nodule development and proliferate within host tissues by expressing enzymes that interfere with the capacity of the host to optimally regulate rhizobial differentiation within nodules [74]. More broadly, diverse bacterial symbionts have evolved to mimic eukaryotic signals, apparently to manipulate access to, and benefits from, the host [75].

Whether cooperation or conflict is the outcome in a symbiotic association can depend on the degree of overlap between host and symbiont for the fitness optima of joint phenotypes [20,67]. How or if this conflict is resolved might depend on the degree to which the host can regulate the development of symbiotic organs, control their function postcolonization, and minimize the capacity of the microbial partner to extract host resources [76]. To minimize conflict, symbiotic organs often exhibit structural and physiological features that allow the host to control microbial colonization, specificity, and proliferation within host tissues, often by regulating nutritional provisioning to microbes, and controlling vertical or **horizontal transmission** of microbes to new hosts [4,5,18,77]. Central among these structural features is modularity, wherein symbiotic organs are repeated and compartmentalized across the host, thus allowing the host to more efficiently impose host control, favoring cooperative symbionts and punishing harmful genotypes [5,77]. Conversely, if conflict is not resolved, symbiotic organs can become lost over evolutionary time, because the costs of interspecific conflict outweigh the benefits of mutualism [78].

Symbiotic organs drive emergent features of microbial evolution and genomic architecture

Host-associated microbes can experience cyclical phases of infection, proliferation within host tissues, and transmission to the next host generation [4]. For microbes that inhabit symbiotic organs, each of these phases can provide for different selective regimes that can reshape the population structure and genomic architecture of the symbionts.

Microbial colonization of symbiotic organs allows host to impose selection on the genotypes of symbionts that infect the host and proliferate within it. This selection is predicted to reshape symbiont populations and favor beneficial symbionts [79]. Conversely, environmental phases and transmission to new hosts can favor a different set of microbial traits [4], because selection on

microbes in these different contexts need not be aligned with host fitness [30,80]. Few analyses have compared the effects of selection inside verses outside of the host, but work along these lines can highlight the differential selective forces that can shape symbionts over their multipartite lifecycle [29,81]. In some cases, selection inside and outside the host shapes different regions of symbiont genomes, suggesting that modular genome architectures help to optimize fitness in varied host and environmental settings [82].

Symbiotic organs establish predictable and rich environments that facilitate rapid microbial growth, providing the microbes with reliable nutrient sources, reducing exposure to competitors, and often producing compounds at levels not readily seen outside the host [23,52]. Given these features, symbionts often compete to gain access to host tissues, a process that can favor strains that are superior competitors irrespective of their degree of benefit to the host [20,74,83]. After infection is established, hosts can impose selective rewards and punishment that are thought to restructure local microbial communities, enriching the environment with beneficial taxa and reducing overall strain diversity [34,84]. Competition and spatial clustering during infection of the symbiotic organ can promote HGT between symbionts, which can allow strains to acquire novel traits that facilitate host–symbiont relations [85]. For instance, in some bacteria, HGT is enhanced by host signals during colonization of symbiotic organs [86], suggesting that the symbionts have evolved to respond to these high-density competitive scenarios.

Finally, the processes of transmission and host infection can also create **population bottlenecks**, wherein a small subset of an external pool of symbionts is able to gain access to the host [87,88]. This bottleneck is likely to be more extreme during the colonization of restricted host structures, such as symbiotic organs, but this would depend on the number of colonizing symbiont cells and the opportunity for continued colonization. By reducing effective population size, population bottlenecks can diminish the efficiency of natural selection and allow for the accumulation of mildly deleterious mutations via drift [89,90]. Effects of drift are reflected in the degraded symbiont genomes of deep-sea anglerfish, flashlight fish, and beewolves [32,33,91]. The common feature in these cases is that transmission bottlenecks are thought to have a role in reducing the effective population size of the symbiont pools [33,91].

Concluding remarks

Symbiotic organs represent a significant energy expenditure for the host in their formation and maintenance, as well as considerable developmental complexity, which can make the host vulnerable to exploitation. Despite these hurdles, symbiotic organs have evolved recurrently across deeply divergent plant and animal lineages. Although diverse in form and function, the shared mechanistic constraints of symbiotic organs create common selective pressures on hosts and symbionts and reflect the joint nature of these phenotypes. Although there have been many recent research advances, our understanding of symbiotic organs is far from complete (see Outstanding questions). With continued examination of the systems presented here, as well as the discovery of as yet uncharacterized symbiotic organs, we may better perceive their role in the evolution of hosts, microbes, and the instigation of novel joint phenotypes.

Authors' contributions

D.C.F. and J.L.S. collaborated on the ideas and writing in this manuscript.

Declaration of interests

None declared by authors.

Outstanding questions

How do symbiotic organs evolve? Do the organs evolve from other structures or *de novo*? To what degree does association with symbionts promote the origins of symbiotic organs?

What are the genetic underpinnings of symbiotic organs? By uncovering the genes essential for the development and function of symbiotic organs, we can better understand their evolutionary origins, selective pressures that shape them, and how their variation mirrors both environmental and biotic parameters.

How variable are symbiotic organs within host species? Characterizing both phenotypic and genotypic variation in symbiotic organs is important to understanding how they can be shaped by natural selection.

How do symbiotic organs shape population genetic structure of symbionts? Infection and proliferation within symbiotic organs can reshape population structure of symbionts via bottleneck events, promotion of HGT, and recombination of disparate genotypes. However, these effects of evolution within symbiotic organs remains poorly characterized.

Do symbiotic organs enhance beneficial microbes in the environment? In some cases, hosts are thought to impose intense natural selection on symbionts by favoring beneficial services. In facultative associations, in which hosts often release symbionts back into the environment, how does host selection affect the diversity and relative frequencies of local symbiont genotypes?

How do symbionts respond to diverse pressures in the host versus environment? For symbionts that survive both within and outside the host, to what extent is their evolution driven by selection imposed by the host (i.e., top-down) versus pressures that are shaped in the environment (i.e., bottom-up)?

What signals do symbionts express to induce symbiotic organ formation? Are there commonalities in the production and detection of signals across taxa?

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What factors promote the evolutionary loss of symbiotic organs? One prediction is that the loss of symbiotic organs corresponds purely to the cost versus benefit of the organs. Another prediction is that the loss of symbiosis co-occurs with the loss of the symbiotic organ. Comparative genomic analyses could provide evidence of genes that are degraded in taxa that have lost symbiotic organs and insight into which genes are potentially necessary for symbiotic organs to function.

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