

Convergent evolution of signal-structure interfaces for maintaining symbioses

Reed M Stubbendieck, Hongjie Li and Cameron R Currie



Symbiotic microbes are essential to the ecological success and evolutionary diversification of multicellular organisms. The establishment and stability of bipartite symbioses are shaped by mechanisms ensuring partner fidelity between host and symbiont. In this minireview, we demonstrate how the interface of chemical signals and host structures influences fidelity between legume root nodules and rhizobia, Hawaiian bobtail squid light organs and *Allivibrio fischeri*, and fungus-growing ant crypts and *Pseudonocardia*. Subsequently, we illustrate the morphological diversity and widespread phylogenetic distribution of specialized structures used by hosts to house microbial symbionts, indicating the importance of signal-structure interfaces across the history of multicellular life. These observations, and the insights garnered from well-studied bipartite associations, demonstrate the need to concentrate on the signal-structure interface in complex and multipartite systems, including the human microbiome.

Address

Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, United States

Corresponding author: Currie, Cameron R (currie@bact.wisc.edu)

Current Opinion in Microbiology 2019, 50:71–78

This review comes from a themed issue on **Microbiota**

Edited by **Karen Guillemin** and **Julia A Segre**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 7th November 2019

<https://doi.org/10.1016/j.mib.2019.10.001>

1369-5274/© 2019 Elsevier Ltd. All rights reserved.

Introduction

The diversity and complexity of plants and animals have been shaped, at least in part, through the formation of beneficial associations with symbiotic microbes [1]. The importance of beneficial symbioses in the evolution of plants and animals is illustrated by the virtual ubiquity of symbiotic microbes in aiding digestion across metazoans and by many plants engaging in mutualisms with mycorrhizal fungi. Despite the crucial role symbiotic microbes play in shaping the biology of their hosts, our understanding of the formation, maintenance, and codiversification of symbiotic associations is limited, and largely informed

by our understanding of a relatively small number of symbioses.

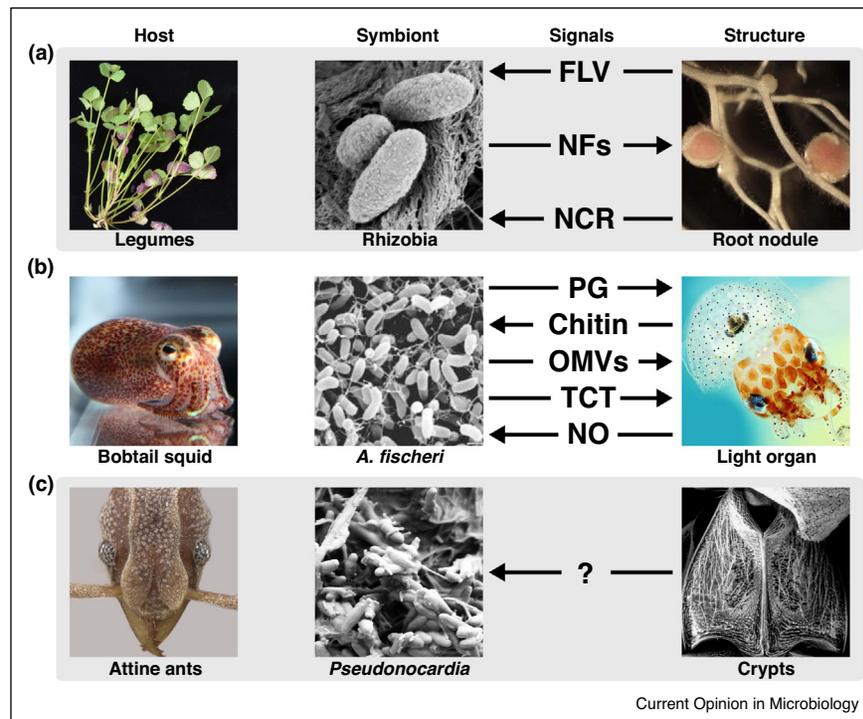
Symbiotic associations, by definition, are interactions that involve two or more species living together in an intimate association. Thus, the host-microbe interface — where the host and symbiont interact directly and exchange complex chemical signals — is fundamental for the establishment and maintenance of the symbiosis. The importance of this interface has been demonstrated through extensive studies of several model bipartite symbiosis, where high host specificity of single symbiont strains greatly facilitates studying how partner fidelity is shaped by chemical signaling and host physiology, behavior, and morphology. Herein, we illustrate the importance of the host-microbe interface in shaping the establishment and maintenance of symbiosis by reviewing three well-studied symbiotic systems involving elaborate structures: legume root nodules and rhizobia, Hawaiian bobtail squid light organs and *Allivibrio fischeri*, and fungus-farming ant crypts and *Pseudonocardia*. Finally, we document the widespread distribution of convergently evolved specialized structures for maintaining microbial symbionts across animals and plants and argue that deeper understanding of the general principles of symbiosis are possible through determining the chemical and physical underpinnings that shape the interface between hosts and symbiotic microbes.

Partner fidelity is maintained through cognate signal exchange in the legume root nodule-rhizobia symbiosis

Many legumes acquire nitrogen through symbiosis with nitrogen-fixing rhizobia, which are housed in specialized structures called root nodules (Figure 1a). These structures accommodate the rhizobial symbionts in an anoxic environment suitable for nitrogenase activity [2]. During the establishment of this symbiosis, the rhizobia gain access into inner cortical root cells through infection threads and are able to directly exchange molecules with the legume host [3,4]. Consequently, partner fidelity is established through legumes screening rhizobial symbionts from the milieu of incompatible microbes and potential pathogens occupying the rhizosphere [5].

Nodulation begins when legume roots release flavonoids, which function as chemoattractants for rhizobia. Notably, different legume species produce distinct suites of flavonoids that attract coadapted rhizobial symbionts (recently reviewed in Ref. [6]). In turn, the coevolved rhizobia

Figure 1



Signal-structure interfaces in bipartite associations between (a) legumes and rhizobia, (b) Hawaiian bobtail squid and *A. fischeri*, and (c) attine ants and *Pseudonocardia*. The arrows indicate the direction of signals from producer to respondent and are arranged from top to bottom in each panel to indicate the order of signaling. FLV, flavonoids; NF, nodulation factors; NCR, nodule-specific cysteine-rich peptides; PG, peptidoglycan; OMVs; outer membrane vesicles; TCT, tracheal cytotoxin; NO, nitric oxide. Image credits: legume, rhizobia, and root nodule, Jean Michel Ané; bobtail squid, Mark Mandell; *A. fischeri* from [28]; light organ from Spencer Nyholm, under the terms of the Creative Commons Attribution License; attine ant, Ted Schultz; *Pseudonocardia*, Cameron Currie; ant crypt, Cameron Currie.

respond to the flavonoid signal by activating expression of *nod* genes to produce lipochitooligosaccharides called Nodulation Factors (NFs). NFs stimulate root hairs to curl and enclose the rhizobia, leading to infection thread formation, differentiation of the rhizobia into bacteroids, and ultimately to root nodulation (reviewed in [7,8]). Rhizobia produce specific NFs consisting of variable numbers of *N*-acetylglucosamine units of variable numbers of *N*- that may be further modified by the addition of acetyl, carbamoyl, methyl, or variable acyl groups to the terminal non-reducing sugar and addition of acetate, D-arabinose, fucose, glycerol, or sulfate groups to the terminal reducing sugar [9]. Because NFs directly bind to legume membrane receptors called NF Receptors 1 and 5 [10,11], incompatible NF-receptor pairs will fail to initiate signal transduction in the host legume and prevents incompatible symbionts from stimulating nodule formation.

In addition to providing the rhizobia with an environment for nitrogen fixation, the root nodule facilitates the transfer of nodule-specific cysteine-rich (NCR) peptides from the host to the symbiont. These peptides consist of 30–50 variable amino acids with 4 or 6 conserved cysteine residues

[12]. As many NCR peptides exhibit antimicrobial activity against rhizobia *in vitro*, it was initially thought NCR peptides function solely in rhizobia population control [13]. However, mutants of the model legume *Medicago truncatula* that are unable to produce specific NCR peptides form defective root nodules and are unable to fix nitrogen [14], suggesting that NCR peptides have additional functions in the symbiosis. Indeed, the antimicrobial activity of NCR peptides occurs at high concentrations [15^{••}], but at sublethal concentrations these peptides alter the bacterial transcriptional program. Specifically, NCR peptides activate regulons that are crucial for symbiosis [15^{••},16], essential for rhizobia to adapt to the intracellular environment of their legume hosts, and necessary for their transition into differentiated bacteroids [17]. Furthermore, variation in NCR peptide sequence coupled with observations that differences in peptide regioisomeric and reduction state modulate the biological activity of these peptides [15^{••},18[•]]. These various modifications bestow NCR peptides with strain-specific activity toward different rhizobial species, which is exemplified by the large number of predicted NCR peptides encoded per genome (often >100) that control the resulting bacteroid's morphology and size [19]. In summary, root nodule symbioses are

formed, and partner fidelity is maintained through production of highly variable and specific chemical signals by both legume hosts and their coevolved rhizobial symbionts.

Non-specific signals and structural adaptations maintain partner fidelity in Hawaiian bobtail squid light organ symbiosis

The nocturnal Hawaiian bobtail squid (*Euprymna scolopes*) masks its shadow from predators using counter-illumination produced by the bioluminescent Gram-negative bacterium *Aliivibrio fischeri* (formerly *Vibrio fischeri*), which colonize a specialized structure called the light organ [20] (Figure 1b). Similar to the legume-rhizobia symbiosis, acquisition of *A. fischeri* by squid is horizontal and dependent upon screening to select symbionts from the greater ocean bacterial community [21]. As the bioluminescence produced by *A. fischeri* is essential for camouflage and bacterial colonization of the light organ is concomitant with irreversible morphological changes [22], there is strong selective pressure for squid to maintain partner fidelity and occlude non-bioluminescent bacteria from persistently colonizing the light organ.

Colonization of the light organ by *A. fischeri* occurs in three phases [23]. In the first phase, newly hatched squid pass microbe-laden seawater through their mantle cavity and over a pair of mucus-coated organs called the ciliated epithelial fields. In response to peptidoglycan derivatives, this appendage secretes mucus stores that facilitate bacterial aggregation on the ciliated epithelial fields [24]. During the second phase of colonization, the squid uses ciliary motions to draw bacterial aggregates toward pores at the base of the ciliated epithelial field and into portal ducts [24]. The final colonization phase occurs when *A. fischeri* migrate in response to a gradient of chitin oligosaccharides (chitobiose) produced in the duct and into the deep crypts of the light organ [23]. Analogous to bacteroid differentiation in the root nodule, when *A. fischeri* cells colonize the light organ, they proliferate and undergo transcriptional reprogramming [21], which results in the loss of flagella [25^{*}] and the release a diaminopimelic acid-type peptidoglycan fragment called tracheal cytotoxin and lipid A. Together these molecules act as synergistic signals to trigger widespread morphological changes that include apoptosis and regression of the ciliated fields [22]. It was recently discovered that during proliferation in the light organ *A. fischeri* shed outer membrane vesicles (OMVs) that contain lipid A, but are devoid of tracheal cytotoxin. These OMVs are sufficient to trigger equivalent morphological differentiation in the squid [26,27]. Moreover, exposure to OMVs shed by non-symbiotic Gram-negative bacteria induces hemocyte migration in squid, which is a hallmark for light organ morphogenesis [26]. Together, the non-specific signal exchange between *A. fischeri* and squid suggest that mechanisms to maintain partner fidelity must occur upstream of light organ colonization.

A major determinant of squid colonization specificity is biofilm formation by *A. fischeri* [28,29]. Furthermore, squid also contribute to partner fidelity with *A. fischeri* through specialized mucosal structures that function as intrinsic physical barriers to colonization [30] and by generating environmental conditions to select *A. fischeri* over non-symbiotic bacteria [31]. For instance, though peptidoglycan isolated from distantly related bacteria indiscriminately induces mucus secretion by the squid, these secretions contain galaxin protein EsGal1, which contains an antimicrobial repeat domain that inhibits the growth of Gram-positive bacteria *in vitro* [32]. During colonization, bacteria are exposed to nitric oxide produced by squid [33]. By producing flavohemoglobin [34] and an alternative oxidase [35] *A. fischeri* survive oxygen radical stress, which inhibits competing bacteria. In addition, nitric oxide may disperse *A. fischeri* biofilms and promote entry of *A. fischeri* into the light organ ducts [36]. In specific response to small aggregates of *A. fischeri* (≤ 5 cells) the squid produce and secrete an enzyme called chitotriosidase into the mucus fields and ducts. This enzyme is an endochitinase that produces chitobiose, which primes *A. fischeri* for chemotaxis toward light organ ducts [37]. In conclusion, by coupling signaling to specialized structures, the squid maintains partner fidelity with *A. fischeri*, despite the reliance on generic microbial signals, such as lipopolysaccharide and peptidoglycan in the microbial world.

Behavioral and structural adaptations maintain partner fidelity in the Attine ant-*Pseudonocardia* symbiosis

Attine ants engage in an ancient, coevolved, and obligate mutualism with fungi they cultivate for food in specialized gardens. The cultivated fungi are host to coevolved and specialized fungal parasites in the genus *Escovopsis* [38], which function as ‘crop diseases’, directly consuming the fungal cultivar [39,40]. To help defend their fungus garden from *Escovopsis*, many attine ants engage in a mutualism with bacteria in the genus *Pseudonocardia*, which occur as exosymbionts, growing directly on the cuticle of worker ants and queens [41,42] (Figure 1c). *Pseudonocardia* produce antifungal antibiotics with potent and directed antagonistic properties towards *Escovopsis* [43,44].

New adults emerge from their pupal casing aposymbiotic, immediately after which *Pseudonocardia*-covered workers inoculate the new adult’s exoskeleton with the symbiont [45]. If a new adult worker does not acquire *Pseudonocardia* within the first two hours after emerging, it is no longer able to associate with the exosymbiont [45]. Thus, the specialized transmission behavior and narrow temporal window for acquisition helps ensure the specificity and maintenance of ant-*Pseudonocardia* symbiosis, as the chance for introduction of other microbial symbionts or opportunists is greatly reduced. Indeed, there is high

partner fidelity between attine ants and *Pseudonocardia*: a single strain of *Pseudonocardia* colonizes worker ants and queens within an individual colony [46,47]. Across generations, partner fidelity is supported by maternal transmission: *Pseudonocardia* are transferred vertically between generations by colony-founding queens [41]. As predicted by vertical transmission, there is high relatedness of *Pseudonocardia* both between ant colonies within populations as well as across species within the attine ant genera [48,49], indicating that this partner fidelity is maintained over large evolutionary scales.

Just as with legumes and squid, attine ants have specialized structures and glands associated with the ant-*Pseudonocardia* interface. The structures can be elaborate, including large crypts or invaginations in the ant exoskeleton [42,50**]. In most genera, the structures include tubercles, on which *Pseudonocardia* directly grow. These tubercles are connected to internal glands that provide nutrients for *Pseudonocardia* growth [51]. Although the chemical signals involved at the ant-*Pseudonocardia* interface remain to be determined, it is clear that this interface is crucial to the stability and fidelity of the association. First, amino acid based stable isotope studies indicate that *Pseudonocardia* obtain all their nutrition directly from ants [51]. Second, by restricting the temporal window for *Pseudonocardia* acquisition before newly emerged adult workers leave the relatively hygienic environment of the fungus garden, it is improbable that these ants will become colonized by non-partner *Pseudonocardia* or other non-symbiotic microbes. Though the precise mechanisms are still under investigation, this narrow acquisition window is likely controlled by a programed disruption of gland cells that feed the *Pseudonocardia*. Third, ant-*Pseudonocardia* switching experiments have demonstrated significant reductions in *Pseudonocardia* acquisition in non-native pairings [52], indicating nutritional and glandular secretion coadaptation at finer phylogenetic levels. Fourth, phylogenomic analyses show that the anatomical modification to house *Pseudonocardia* has arisen independently at least three times throughout evolutionary history of attine ants [50**]. Taken together, the above evidence indicates that the maintenance, stability, and fidelity of the ant-*Pseudonocardia* association is largely mediated by the chemical signaling occurring embedded within the interface of the symbiosis on the ant exoskeleton.

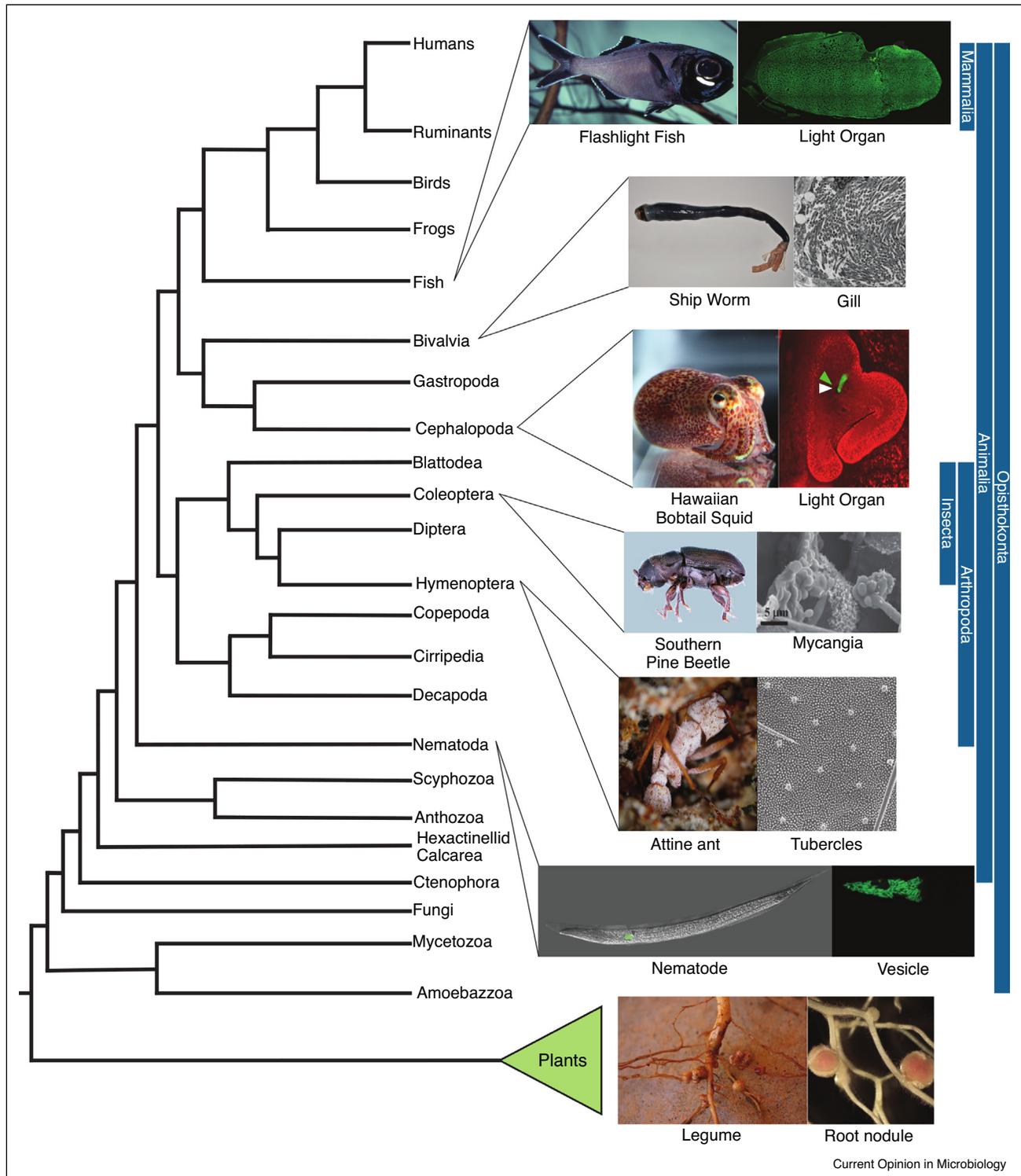
Conclusion

Signal-structure interfaces between hosts and their microbial symbionts are crucial for maintaining legume-rhizobia, squid-*A. fischeri*, and ant-*Pseudonocardia* partner fidelity (Figure 1). Integral to each symbiosis is the presence of modified morphological structures that are essential for maintaining the microbial symbionts and mediating host-symbiont signal exchange. Similar to these three well-studied bipartite associations highlighted above, elaborate structural modifications for housing microbial

symbionts occur across plants and animals (Figure 2). For example, crypt structures that house fungal symbionts — mycangia — have evolved numerous times within beetles [53] and the crypts that house *Pseudonocardia* in attine ants evolved at least three independent times [50**]. Similar to the light organ of bobtail squid, an analogous structure has convergently evolved in flashlight fish for harboring bioluminescent bacteria, including *A. fischeri* [54]. Notably, biofilm formation by *A. fischeri* is required for successful colonization of both squid and fish light organs. However, production of biofilm exopolysaccharide is regulated by distinct *A. fischeri* lineage-specific pathways, suggesting specific adaptations have arisen to modulate the signal-structure interface across different hosts [55**]. In addition, there are many more examples of structural modifications that house symbiotic microbes across eukaryotes, including the gills of shipworms [56] and the vesicles of nematodes [57]. Taken together, this suggests host structural modification are crucial to the host-microbe interface within bipartite symbioses.

Beyond bipartite host-microbe systems, within the widespread occurrence of microbiomes associating with diverse hosts, the presence of structural modifications at the host-microbe interface is much less recognized. These modifications may not be as well characterized in part because the associations between hosts and their microbiomes appear more plastic than bipartite associations. In animals, the majority of microbe-host interfaces occur in the gastrointestinal tract, which may harbor structural modifications to accommodate microbial symbionts that are analogous to the modifications observed in bipartite systems [58–60,61*,62,63**,64]. For example, both ruminants (Class Mammalia) and the hoatzin (Class Aves) have convergently evolved specialized structures, the rumen and the crop, respectively, in their foreguts that facilitate the growth of communities of fermentative microbes [65]. Furthermore, examples showing concordance between host phylogeny and microbiome composition [66] and co-diversification of microbial symbionts with their hosts [67] suggest that there are suitable frameworks in place for the evolution of signal-structure interfaces in all animals. For instance, it is tempting to hypothesize that the suite of antimicrobial peptides produced by the human immune system may be analogous to NCR peptides in modulating the physiology of microbes and to promote the growth of specific symbionts, as recently uncovered in amphibian systems [68]. Nevertheless, it is clear that signal-structure interfaces influence partner fidelity across the tree of life and these interfaces likely occur not only in bipartite associations, but also within more complex microbiomes. We believe more concerted efforts examining the signal-structure interfaces that occur across phylogenetically diverse host-microbe associations will help identify the general principles that govern the maintenance, stability, and coevolution of symbiotic systems.

Figure 2



Convergent evolution of structures to maintain microbial symbionts across the eukaryotic tree of life. The phylogenetic tree shows the relationships between different eukaryotic lineages. Each inset represents an example of a eukaryotic host (left) from the specified lineage that uses a specialized structure (right) to establish and maintain a bipartite association with a microbial symbiont. Image credits: flashlight fish, Stefan Herlitze; flashlight fish light organ adapted from [69] under the terms of the Creative Commons Attribution License; ship worm and gill, Margo Haygood; bobtail squid and light organ, Mark Mandel; southern pine beetle, Erich Vallery under the terms of the Creative Commons Attribution License; mycangia, Kier Klepzig [70]; attine ant, Don Parsons; ant crypt, Cameron Currie; nematode and vesicle modified from [71] under the terms of the Creative Commons Attribution License; legume and root nodule, Jean Michel Ané.

Funding

This project was supported through National Institutes of Health (NIH) U19 AI109673, NIH U19 TW009872). Reed M. Stubbendieck was supported by a National Library of Medicine training grant to the Computation and Informatics in Biology and Medicine Training Program (NLM 5T15LM007359).

Acknowledgements

We thank Caitlin Carlson for assistance in generating figures. We thank Jean-Michel Ané and Mark Mandel for providing images of legume root nodules and the bobtail squid light organ, respectively. We thank Heidi Horn, Daniel May, Mark Mandel, and Margaret Thairu for critical reading of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Kiers ET, West SA: **Evolutionary biology. Evolving new organisms via symbiosis.** *Science* 2015, **348**:392-394.
 2. Ott T, van Dongen JT, Günther C, Krusell L, Desbrosses G, Vigeolas H, Bock V, Czechowski T, Geigenberger P, Udvardi MK: **Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development.** *Curr Biol* 2005, **15**:531-535.
 3. Clarke VC, Loughlin PC, Day DA, Smith PMC: **Transport processes of the legume symbiosome membrane.** *Front Plant Sci* 2014, **5**:699.
 4. Clarke VC, Loughlin PC, Gavrin A, Chen C, Brear EM, Day DA, Smith PMC: **Proteomic analysis of the soybean symbiosome identifies new symbiotic proteins.** *Mol Cell Proteomics* 2015, **14**:1301-1322.
 5. Marchetti M, Capela D, Glew M, Cruveiller S, Chane-Woon-Ming B, Gris C, Timmers T, Poinsoy V, Gilbert LB, Heeb P *et al.*: **Experimental evolution of a plant pathogen into a legume symbiont.** *PLoS Biol* 2010, **8**:e1000280.
 6. Liu C-W, Murray JD: **The role of flavonoids in nodulation host-range specificity: an update.** *Plants (Basel, Switzerland)* 2016, **5**.
 7. Desbrosses GJ, Stougaard J: **Root nodulation: a paradigm for how plant-microbe symbiosis influences host developmental pathways.** *Cell Host Microbe* 2011, **10**:348-358.
 8. Parniske M: **Uptake of bacteria into living plant cells, the unifying and distinct feature of the nitrogen-fixing root nodule symbiosis.** *Curr Opin Plant Biol* 2018, **44**:164-174.
 9. Long SR: **Rhizobium symbiosis: nod factors in perspective.** *Plant Cell* 1996, **8**:1885-1898.
 10. Madsen EB, Madsen LH, Radutoiu S, Olbryt M, Rakwalska M, Szczyglowski K, Sato S, Kaneko T, Tabata S, Sandal N *et al.*: **A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals.** *Nature* 2003, **425**:637-640.
 11. Broghammer A, Krusell L, Blaise M, Sauer J, Sullivan JT, Maolanon N, Vinther M, Lorentzen A, Madsen EB, Jensen KJ *et al.*: **Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding.** *Proc Natl Acad Sci U S A* 2012, **109**:13859-13864.
 12. Alunni B, Kevei Z, Redondo-Nieto M, Kondorosi A, Mergaert P, Kondorosi E: **Genomic organization and evolutionary insights on GRP and NCR genes, two large nodule-specific gene families in *Medicago truncatula*.** *Mol Plant Microbe Interact* 2007, **20**:1138-1148.
 13. Haag AF, Balaban M, Sani M, Kerscher B, Pierre O, Farkas A, Longhi R, Boncompagni E, Héroutart D, Dall'angelo S *et al.*: **Protection of *Sinorhizobium* against host cysteine-rich antimicrobial peptides is critical for symbiosis.** *PLoS Biol* 2011, **9**:e1001169.
 14. Kim M, Chen Y, Xi J, Waters C, Chen R, Wang D: **An antimicrobial peptide essential for bacterial survival in the nitrogen-fixing symbiosis.** *Proc Natl Acad Sci U S A* 2015, **112**:15238-15243.
 15. Shabab M, Arnold MFF, Penterman J, Wommack AJ, Bocker HT, Price PA, Griffiths JS, Nolan EM, Walker GC: **Disulfide cross-linking influences symbiotic activities of nodule peptide NCR247.** *Proc Natl Acad Sci U S A* 2016, **113**:10157-10162.
- This study takes a systematic structure-activity relationship approach to characterize the function of the conserved cysteine residues in NCR peptides and found that each peptide variant had different biological activities, suggesting that legume-rhizobia interactions may be modulated by peptide modifications.
16. Penterman J, Abo RP, De Nisco NJ, Arnold MFF, Longhi R, Zanda M, Walker GC: **Host plant peptides elicit a transcriptional response to control the *Sinorhizobium meliloti* cell cycle during symbiosis.** *Proc Natl Acad Sci U S A* 2014, **111**:3561-3566.
 17. Horváth B, Domonkos Á, Kereszt A, Szűcs A, Ábrahám E, Ayaydin F, Bóka K, Chen Y, Chen R, Murray JD *et al.*: **Loss of the nodule-specific cysteine rich peptide, NCR169, abolishes symbiotic nitrogen fixation in the *Medicago truncatula* *dnf7* mutant.** *Proc Natl Acad Sci U S A* 2015, **112**:15232-15237.
 18. Wang Q, Liu J, Li H, Yang S, Körmöczy P, Kereszt A, Zhu H: **Nodule-specific cysteine-rich peptides negatively regulate nitrogen-fixing symbiosis in a strain-specific manner in *Medicago truncatula*.** *Mol Plant-Microbe Interact* 2018, **31**:240-248.
- Using crosses of recombinant inbred lines, this study shows that legume-rhizobia partner fidelity at the level of nitrogen fixation is controlled by NCR peptides. Wang *et al.* validate these findings using chemically synthesized NCR peptides.
19. Herczeg R, Montiel J, Bálint B, Downie JA, Farkas A, Mergaert P, Bihari P, Kondorosi E, Kereszt A: **Morphotype of bacteroids in different legumes correlates with the number and type of symbiotic NCR peptides.** *Proc Natl Acad Sci U S A* 2017, **114**:5041-5046.
 20. Jones BW, Nishiguchi MK: **Counterillumination in the Hawaiian bobtail squid, *Euprymna scolopes* Berry (Mollusca: Cephalopoda).** *Mar Biol* 2004, **144**:1151-1155.
 21. Thompson LR, Nikolakakis K, Pan S, Reed J, Knight R, Ruby EG: **Transcriptional characterization of *Vibrio fischeri* during colonization of juvenile *Euprymna scolopes*.** *Environ Microbiol* 2017, **19**:1845-1856.
 22. Koropatnick T, Goodson MS, Heath-Heckman EAC, McFall-Ngai M: **Identifying the cellular mechanisms of symbiont-induced epithelial morphogenesis in the squid-*Vibrio* association.** *Biol Bull* 2014, **226**:56-68.
 23. Mandel MJ, Schaefer AL, Brennan CA, Heath-Heckman EAC, Deloney-Marino CR, McFall-Ngai MJ, Ruby EG: **Squid-derived chitin oligosaccharides are a chemotactic signal during colonization by *Vibrio fischeri*.** *Appl Environ Microbiol* 2012, **78**:4620-4626.
 24. Nyholm SV, Stabb EV, Ruby EG, McFall-Ngai MJ: **Establishment of an animal-bacterial association: recruiting symbiotic vibrios from the environment.** *Proc Natl Acad Sci U S A* 2000, **97**:10231-10235.
 25. Ruby EG, Asato LM: **Growth and flagellation of *Vibrio fischeri* during initiation of the sepiolid squid light organ symbiosis.** *Arch Microbiol* 1993, **159**:160-167.
- This is the first study to demonstrate that OMVs shed by *A. fischeri* during normal growth are sufficient to trigger morphological differentiation of the squid host, independent of tracheal cytotoxin.
26. Aschtgen M-S, Wetzel K, Goldman W, McFall-Ngai M, Ruby E: ***Vibrio fischeri*-derived outer membrane vesicles trigger host development.** *Cell Microbiol* 2016, **18**:488-499.

27. Aschtgen M-S, Lynch JB, Koch E, Schwartzman J, McFall-Ngai M, Ruby E: **Rotation of *Vibrio fischeri* flagella produces outer membrane vesicles that induce host development.** *J Bacteriol* 2016, **198**:2156-2165.
 28. Yip ES, Grublesky BT, Hussa EA, Visick KL: **A novel, conserved cluster of genes promotes symbiotic colonization and α 54-dependent biofilm formation by *Vibrio fischeri*.** *Mol Microbiol* 2005, **57**:1485-1498.
 29. Mandel MJ, Wollenberg MS, Stabb EV, Visick KL, Ruby EG: **A single regulatory gene is sufficient to alter bacterial host range.** *Nature* 2009, **458**:215-218.
 30. Nyholm SV, Deplancke B, Gaskins HR, Apicella MA, McFall-Ngai MJ: **Roles of *Vibrio fischeri* and nonsymbiotic bacteria in the dynamics of mucus secretion during symbiont colonization of the *Euprymna scolopes* light organ.** *Appl Environ Microbiol* 2002, **68**:5113-5122.
 31. Nyholm SV, McFall-Ngai MJ: **Dominance of *Vibrio fischeri* in secreted mucus outside the light organ of *Euprymna scolopes*: the first site of symbiont specificity.** *Appl Environ Microbiol* 2003, **69**:3932-3937.
 32. Heath-Heckman EAC, Gillette AA, Augustin R, Gillette MX, Goldman WE, McFall-Ngai MJ: **Shaping the microenvironment: evidence for the influence of a host galaxin on symbiont acquisition and maintenance in the squid-*Vibrio* symbiosis.** *Environ Microbiol* 2014, **16**:3669-3682.
 33. Davidson SK, Koropatnick TA, Kossmehl R, Sycuro L, McFall-Ngai MJ: **NO means "yes" in the squid-*Vibrio* symbiosis: nitric oxide (NO) during the initial stages of a beneficial association.** *Cell Microbiol* 2004, **6**:1139-1151.
 34. Wang Y, Dunn AK, Wilneff J, McFall-Ngai MJ, Spiro S, Ruby EG: ***Vibrio fischeri* flavohaemoglobin protects against nitric oxide during initiation of the squid-*Vibrio* symbiosis.** *Mol Microbiol* 2010, **78**:903-915.
 35. Dunn AK: **Alternative oxidase activity reduces stress in *Vibrio fischeri* cells exposed to nitric oxide.** *J Bacteriol* 2018, **200**:1-11.
 36. Thompson CM, Tischler AH, Tarnowski DA, Mandel MJ, Visick KL: **Nitric oxide inhibits biofilm formation by *Vibrio fischeri* via the nitric oxide sensor HnoX.** *Mol Microbiol* 2019, **111**:187-203.
 37. Kremer N, Philipp EER, Carpentier M-C, Brennan CA, Kraemer L, Altura MA, Augustin R, Häsler R, Heath-Heckman EAC, Peyer SM *et al.*: **Initial symbiont contact orchestrates host-organ-wide transcriptional changes that prime tissue colonization.** *Cell Host Microbe* 2013, **14**:183-194.
 38. Currie CR, Wong B, Stuart AE, Schultz TR, Rehner SA, Mueller UG, Sung G-H, Spatafora JW, Straus NA: **Ancient tripartite coevolution in the attine ant-microbe symbiosis.** *Science* 2003, **299**:386-388.
 39. Reynolds HT, Currie CR: **Pathogenicity of *Escovopsis weberi*: the parasite of the attine ant-microbe symbiosis directly consumes the ant-cultivated fungus.** *Mycologia* 2004, **96**:955-959.
 40. Custodio BC, Rodrigues A: ***Escovopsis kreiselii* specialization to its native hosts in the fungiculture of the lower attine ant *Mycetophylax morschi*.** *Antonie Van Leeuwenhoek* 2019, **112**:305-317.
 41. Currie CR, Scott JA, Summerbell RC, Malloch D: **Fungus-growing ants use antibiotic-producing bacteria to control garden parasites.** *Nature* 1999, **398**:701-704.
 42. Currie CR, Poulsen M, Mendenhall J, Boomsma JJ, Billen J: **Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants.** *Science* 2006, **311**:81-83.
 43. Oh DC, Scott JJ, Currie CR, Clardy J: **Mycangimycin, a polyene peroxide from a mutualist *Streptomyces* sp.** *Org Lett* 2009, **11**:633-636.
 44. Van Arnam EB, Ruzzini AC, Sit CS, Horn H, Pinto-Tomás AA, Currie CR, Clardy J: **Selvamicin, an atypical antifungal polyene from two alternative genomic contexts.** *Proc Natl Acad Sci U S A* 2016, **113**:12940-12945.
 45. Marsh SE, Poulsen M, Pinto-Tomás A, Currie CR: **Interaction between workers during a short time window is required for bacterial symbiont transmission in *Acromyrmex* leaf-cutting ants.** *PLoS One* 2014, **9**:e103269.
 46. Poulsen M, Cafaro M, Boomsma JJ, Currie CR: **Specificity of the mutualistic association between actinomycete bacteria and two sympatric species of *Acromyrmex* leaf-cutting ants.** *Mol Ecol* 2005, **14**:3597-3604.
 47. Andersen SB, Hansen LH, Sapountzis P, Sørensen SJ, Boomsma JJ: **Specificity and stability of the *Acromyrmex-Pseudonocardia* symbiosis.** *Mol Ecol* 2013, **22**:4307-4321.
 48. Cafaro MJ, Poulsen M, Little AEF, Price SL, Gerardo NM, Wong B, Stuart AE, Larget B, Abbot P, Currie CR: **Specificity in the symbiotic association between fungus-growing ants and protective *Pseudonocardia* bacteria.** *Proc Biol Sci* 2011, **278**:1814-1822.
 49. McDonald BR, Chevrette MG, Klassen JL, Horn HA, Caldera EJ, Wendt-Pienkowski E, Cafaro MJ, Ruzzini AC, Arnam EB, Van Weinstock GM *et al.*: **Biogeography and microscale diversity shape the biosynthetic potential of fungus-growing ant-associated *Pseudonocardia*.** *bioRxiv* 2019 <http://dx.doi.org/10.1101/545640>.
 50. Li H, Sosa-Calvo J, Horn HA, Pupo MT, Clardy J, Rabeling C, Schultz TR, Currie CR: **Convergent evolution of complex structures for ant-bacterial defensive symbiosis in fungus-farming ants.** *Proc Natl Acad Sci U S A* 2018, **115**:10720-10725 <http://dx.doi.org/10.1073/pnas.1809332115>
- By reconstructing the evolutionary history of the ant-*Pseudonocardia* defensive symbiosis, this study reveals the convergent evolution of anatomical adaptations for maintaining beneficial symbionts in fungus-growing ants.
51. Steffan SA, Chikaraishi Y, Currie CR, Horn H, Gaines-Day HR, Pauli JN, Zalapa JE, Ohkouchi N: **Microbes are trophic analogs of animals.** *Proc Natl Acad Sci U S A* 2015, **112**:15119-15124.
 52. Armitage SAO, Broch JF, Marín HF, Nash DR, Boomsma JJ: **Immune defense in leaf-cutting ants: a cross-fostering approach.** *Evolution* 2011, **65**:1791-1799.
 53. Hulcr J, Stelinski LL: **The ambrosia symbiosis: from evolutionary ecology to practical management.** *Annu Rev Entomol* 2017, **62**:285-303.
 54. Haygood MG: **Light organ symbioses in fishes.** *Crit Rev Microbiol* 1993, **19**:191-216.
 55. Rotman ER, Bultman KM, Brooks JF, Gyllborg MC, Burgos HL, Wollenberg MS, Mandel MJ: **Natural strain variation reveals diverse biofilm regulation in squid-colonizing *Vibrio fischeri*.** *J Bacteriol* 2019, **201**:1-20
- This study surveys *A. fischeri* isolates worldwide, finds that biofilm formation is a requirement for successful squid colonization, and shows that distinct lineages of *A. fischeri* have evolved alternative mechanisms to regulate the biofilm formation pathway.
56. Distel DL, Altamia MA, Lin Z, Shipway JR, Han A, Forteza I, Antemano R, Limbaco MGJP, Tebo AG, Dechavez R *et al.*: **Discovery of chemoautotrophic symbiosis in the giant shipworm *Kuphus polythalamia* (Bivalvia: Teredinidae) extends wooden-steps theory.** *Proc Natl Acad Sci U S A* 2017, **114**:E3652-E3658.
 57. Martens EC, Goodrich-Blair H: **The *Steinernema carpocapsae* intestinal vesicle contains a subcellular structure with which *Xenorhabdus nematophila* associates during colonization initiation.** *Cell Microbiol* 2005, **7**:1723-1735.
 58. Kikuchi Y, Hosokawa T, Fukatsu T: **An ancient but promiscuous host-symbiont association between *Burkholderia* gut symbionts and their heteropteran hosts.** *ISMEJ* 2011, **5**:446-460.
 59. Ohbayashi T, Takeshita K, Kitagawa W, Nikoh N, Koga R, Meng X-Y, Tago K, Hori T, Hayatsu M, Asano K *et al.*: **Insect's intestinal organ for symbiont sorting.** *Proc Natl Acad Sci U S A* 2015, **112**:E5179-88.
 60. Alonso-Pernas P, Arias-Cordero E, Novoselov A, Ebert C, Rybak J, Kaltenpoth M, Westermann M, Neugebauer U, Boland W: **Bacterial community and PHB-accumulating bacteria associated with the wall and specialized niches of the hindgut**

- of the forest cockchafer (*Melolontha hippocastani*). *Front Microbiol* 2017, **8**:291.
61. Oishi S, Moriyama M, Koga R, Fukatsu T: **Morphogenesis and development of midgut symbiotic organ of the stinkbug *Plautia stali* (Hemiptera: Pentatomidae)**. *Zool Lett* 2019, **5**:16
Examining midgut morphogenesis in the stinkbug *Plautia stali*, Oishi *et al.* show the close integration of microbial symbionts with host gut development.
 62. Vaishnava S, Behrendt CL, Ismail AS, Eckmann L, Hooper LV: **Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface**. *Proc Natl Acad Sci U S A* 2008, **105**:20858-20863.
 63. Earle KA, Billings G, Sigal M, Lichtman JS, Hansson GC, Elias JE, Amieva MR, Huang KC, Sonnenburg JL: **Quantitative imaging of gut microbiota spatial organization**. *Cell Host Microbe* 2015, **18**:478-488
This study demonstrates the importance of spatial segregation of microbes within microhabitats in the gastrointestinal tract, and suggests that distinct signal-structure interfaces may exist in the gastrointestinal tract.
 64. Mark Welch JL, Hasegawa Y, McNulty NP, Gordon JI, Borisy GG: **Spatial organization of a model 15-member human gut microbiota established in gnotobiotic mice**. *Proc Natl Acad Sci U S A* 2017, **114**:E9105-E9114.
 65. Godoy-Vitorino F, Goldfarb KC, Karaoz U, Leal S, Garcia-Amado MA, Hugenholtz P, Tringe SG, Brodie EL, Dominguez-Bello MG: **Comparative analyses of foregut and hindgut bacterial communities in hoatzins and cows**. *ISME J* 2012, **6**:531-541.
 66. Brooks AW, Kohl KD, Brucker RM, van Opstal EJ, Bordenstein SR: **Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history**. *PLoS Biol* 2016, **14**:e2000225.
 67. Moeller AH, Caro-Quintero A, Mjunga D, Georgiev AV, Lonsdorf EV, Muller MN, Pusey AE, Peeters M, Hahn BH, Ochman H: **Cospeciation of gut microbiota with hominids**. *Science* 2016, **353**:380-382.
 68. Woodhams DC, Rollins-Smith LA, Reinert LK, Lam BA, Harris RN, Briggs CJ, Vredenburg VT, Patel BT, Caprioli RM, Chaurand P *et al.*: **Probiotics modulate a novel amphibian skin defense peptide that is antifungal and facilitates growth of antifungal bacteria**. *Microb Ecol* 2019 <http://dx.doi.org/10.1007/s00248-019-01385-9>.
 69. Hellinger J, Jägers P, Donner M, Sutt F, Mark MD, Senen B, Tollrian R, Herlitz S: **The flashlight fish *Anomalops katoptron* uses bioluminescent light to detect prey in the dark**. *PLoS One* 2017, **12**:e0170489.
 70. Yuceer C, Hsu C-Y, Erbilgin N, Klepzig KD: **Ultrastructure of the mycangium of the southern pine beetle, *Dendroctonus frontalis* (Coleoptera: Curculionidae, Scolytinae): complex morphology for complex interactions**. *Acta Zool* 2011, **92**:216-224.
 71. Stilwell MD, Cao M, Goodrich-Blair H, Weibel DB: **Studying the symbiotic bacterium *Xenorhabdus nematophila* in individual, living *Steinernema carpocapsae* nematodes using microfluidic systems**. *mSphere* 2018, **3**.