It Takes a Village: Ecological and Fitness Impacts of Multipartite Mutualism

Elizabeth A. Hussa and Heidi Goodrich-Blair

Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin 53706; email: hussa@wisc.edu, hgblair@bact.wisc.edu

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Abstract

Microbial symbioses, in which microbes have either positive (mutualistic) or negative (parasitic) impacts on host fitness, are integral to all aspects of biology, from ecology to human health. In many well-studied cases, microbial symbiosis is characterized by a specialized association between a host and a specific microbe that provides it with one or more beneficial functions, such as novel metabolic pathways or defense against pathogens. Even in relatively simple associations, symbiont-derived benefits can be context dependent and influenced by other host-associated or environmental microbes. Furthermore, naturally occurring symbioses are typically complex, in which multiple symbionts exhibit coordinated, competing, or independent influences on host physiology, or in which individual symbionts affect multiple interacting hosts. Here we describe research on the mechanisms and consequences of multipartite symbioses, including consortia in which multiple organisms interact with the host and one another, and on conditional mutualists whose impact on the host depends on additional interacting organisms.

Contents

INTRODUCTION

Impressive strides have been made in understanding molecular, cellular, and evolutionary aspects of microbial symbiosis by focusing on relatively "simple" associations between one host and one microbe. However, we must also consider that most (if not all) organisms engage in symbioses with more than one partner. In some cases, multiple partners interact to govern a single symbiotic trait, whereas in other cases, multiple independent relationships control distinct traits. These complex relationships have important organismal and ecological impacts and are at the forefront of current symbiosis research. In this review we focus on multipartite microbial mutualisms: long-term associations among three or more species wherein at least two partners benefit from the interaction (**Table 1**).

Health and Fitness Impacts of Multipartite Symbiosis

Multipartite mutualism: a prolonged association among more than two partners in which at least two organisms benefit

Consortial symbiotic systems: association in which more than one symbiont is maintained within a single host, often within the same tissue or organ

Microbial symbionts can fundamentally alter the physiology of a host organism, in many cases imparting new or optimized abilities that result in increased organismal fitness. These fitness impacts include ecological niche expansion, which allows the host organism to utilize (or better utilize) a food source and conveys resistance to environmental stresses, predators, or parasites. Regardless of the fitness impact, both simple (two-partner) and multipartite mutualisms are based on mutual exploitation in which each organism incurs a cost of contributing to the interaction but receives a net positive benefit.

Some hosts garner expanded benefits by associating with multiple different microbial symbionts that provide either independent or redundant beneficial traits. The latter is likely the case in consortial symbiotic systems such as the human gut microbiota, in which multiple symbionts likely contribute to host digestion and immune development (11, 32). Multiple symbionts present within a single host compete for host-derived benefits (e.g., nutrients), and the increased draw on host resources can shift the net effect of the interaction toward the negative. This challenge is circumvented if each individual symbiont provides independent benefits to the host, increasing the net positive balance of the interaction. Alternatively, competition may be ameliorated if symbionts with mutually redundant beneficial functions fluctuate in their relative abundance (e.g., based on

Table 1 Multipartite mutualism model systems

Benefit category	Host(s)	Symbiont(s)	Specific benefit
Niche adaptation (nutrient acquisition)	Higher termites	Bacteria,[a] archaea[a]	Lignocellulose digestion
Niche adaptation (nutrient acquisition)	Lower termites	Protists,[a] bacteria,[a] archaea[a]	Lignocellulose digestion
Niche adaptation (nutrient acquisition)	Entomopathogenic nematodes, insect larvae[a]	Bacteria (*Xenorhabdus* or *Photorhabdus* species)	Accessing and processing insect host tissues
Niche adaptation (nutrient acquisition)	Mealybugs	Bacteria (*Moranella* and *Tremblaya*)	Metabolic patchwork
Niche adaptation (nutrient acquisition)	Glassy-winged sharpshooters	Bacteria (*Baumania* and *Sulcia*)	Metabolic patchwork
Niche adaptation (nutrient acquisition)	Tsetse flies	Bacteria (*Wigglesworthia* and *Sodalis glossinidius*)	Metabolic patchwork (potential)
Niche adaptation (nutrient acquisition)	Oligochaete worms	Bacteria (four unknown species)	Metabolic syntrophy in oxic and anoxic conditions
Niche adaptation (environmental tolerance)	Sea anemone	Zooxanthellae and/or zoochlorellae	Photosynthetic carbon fixation in different light and/or temperature conditions
Niche adaptation (environmental tolerance)	Mountain pine beetle	Fungi (*Grosmannia clavigera* and *Ophiostoma montium*)	Nutritional supplementation across a variety of temperatures
Niche adaptation (environmental tolerance)	Tropical panic grass	Fungus (*Curvularia protuberata*) and *Curvularia thermal tolerance virus*	Temperature resistance
Defensive	Arthropods[a]	Bacteria (*Wolbachia* spp.)	Defense against viral infection
Defensive	Attine ants	Fungus (Lepiotaceae family) and bacteria (*Pseudonocardia* spp.)	Defense against parasitic fungus
Defensive	Mice, humans	Herpesvirus	Activation of generalized immune response by latent virus

[a]Various species.

differences in environmental preference) or evolve toward codependency, such as shared pathways of resource production or management.

In some multipartite associations, one microbial (mutualistic) partner provides the host with defense against the other (parasitic) partner. In these cases, the mutualistic microbe may produce immune effectors, such as antimicrobial agents, that kill or deter parasites. Alternatively, the mutualist may induce low-level activation of the host immune response, resulting in decreased susceptibility to parasitism. Defensive symbioses face distinct challenges in maintaining a net positive benefit. In an environment where the parasite is ubiquitous or the mutualist protects against a variety of pathogens, selective pressure ensures symbiont maintenance. However, when parasitism is rare, selective pressure preserving the symbiosis is low, and to be maintained in this context the symbiont must use negligible host resources or provide additional benefit to the host. The multipartite model systems highlighted in the following sections demonstrate not only the advantages of complex multipartite interactions, but also the ways in which they circumvent challenges to their stability.

CORAL

As the primary producers of many marine ecosystems, the fragile nature of the coral holobiont is of great concern in the face of increasing environmental change. Although studies focused on coral microbiome diversity are still in their infancy, the increasing prevalence of coral bleaching and disease has intensified the importance of understanding and maintaining healthy coral holobionts. Defined as the loss of photosynthetic algae (zooxanthellae), coral bleaching is thought to be an adaptive response of corals to environmental stress, potentially enabling them to take up different species or strains of algae better suited for that environment. Other coral diseases are caused by bacterial, viral, and fungal infection, which may occur due to environmental stress-related shifts in microbial and metagenomic content. Although these initial studies greatly contribute to our knowledge of the effects of environmental change on coral reef ecosystems, the key to saving them may lie in the study of inter-symbiont interactions within the holobiont system itself.

Ecological Impacts of Multipartite Symbiosis

Holobiont: an organism and its associated community of microbes

Multipartite mutualisms have a major impact on both structure and preservation of the ecological landscape. One of the best examples, reviewed elsewhere in detail, is the reef-building coral symbiosis, which is a keystone species in ocean ecology (72, 74). The coral holobiont (host and all associated symbionts) comprises numerous microorganisms, including bacteria, archaea, viruses, and photosynthetic algae (zooxanthellae), together functioning as the major primary producer of marine ecosystems. Disturbances within the symbiotic association, particularly the loss of zooxanthellae (i.e., coral bleaching), therefore are a significant threat not only to coral survival but also to ocean ecology as a whole (see sidebar, Coral). The coral symbiosis highlights the importance of multipartite symbioses as cornerstones of ecological networks.

Variation in, or acquisition of, new symbiont partners can drastically change or expand the ecological range of the holobiont and its contribution to the ecological network by conferring innovative traits such as utilization of novel food sources or tolerance of environmental stress. Many of the best-studied multipartite holobionts, including coral and plant rhizospheres, exhibit stress-mediated changes in their microbial content that are believed to aid in coping with environmental fluctuation (44, 48). Given the looming threat of climate change, understanding the role of symbiosis in environmental adaptation is of major importance to ecological threat assessment and conservation efforts. Multipartite symbioses are also proving to be an important component of biocontrol strategies (e.g., manipulating microbial symbionts) to prevent ecological devastation. In the remainder of this review, we use specific examples to describe recent advances in our understanding of the organismal and ecological impacts of multipartite symbioses.

TO BOLDLY GO: NICHE ADAPTATION THROUGH MULTIPARTITE MUTUALISM

Microbial mutualists can optimize host adaptation to, or allow host occupation of, otherwise uninhabitable ecological niches. As revealed by the specific examples detailed below, multipartite interactions contribute to niche expansion in a variety of ways. In many cases, multiple symbiont species are present within a host and exert synergistic, redundant, or independent effects on niche range. Alternatively, the presence of a single symbiont may enable the host to parasitize or resist parasitism by a third symbiotic partner.

Let Them Eat Cake: Symbiotic Adaptation to Nutrient Acquisition

Metabolic provisioning is one of the most common fitness benefits derived from mutualism. For example, sap-feeding insects rely on bacterial endosymbionts to provide essential amino acids lacking in their diet (20, 84). It is therefore not surprising that some of the best-understood multipartite interactions revolve around the optimization of nutrient acquisition.

GHFs: glycosyl hydrolase families

The termite microbiome. Microbial symbiosis enables termites to subsist on recalcitrant cellulosic plant materials, such as wood. The termite gut is inhabited by several archaeal and bacterial species and, in some cases, protistan species carrying their own bacterial symbionts (31). These microbial inhabitants contribute to host lignocellulose digestion. However, functional analyses are limited because most of the symbionts are not yet cultured (98). The distinct phylogenetic group known as lower termites contain more than 10^3 protistan cells, representing as many as 20 distinct species, within their hindgut (31). Higher termites appear to lack protists, but both lower and higher termites harbor more than 10^6 bacterial and archaeal cells in the gut, with hundreds of distinct phylotypes in a single termite species and more than 1,500 phylotypes across multiple termite species (31).

It is not known which or how many termite gut microbial symbionts contribute to lignocellulose digestion. Metatranscriptomics of the protistan symbionts from four lower termite species suggested that the symbionts encode a diverse array of lignocellulose-degrading enzymes of both protistan and bacterial origin, representing between five and seven distinct glycoside hydrolase families (GHFs) within each termite species (96). Similarly, pyrosequencing analysis of the prokaryotic gut symbionts of the higher termite *Nasutitermes takasagoensis* revealed the presence of cellulose-degrading enzymes from 45 distinct GHFs (104). The rich coding potential of microbial GHFs indicates diversity in the types of glycosidic bonds that can be hydrolyzed in the termite gut, likely contributing to host range with respect to meal variety. The higher-termite host genome also encodes its own cellulose-degrading enzymes, complicating the inference of microbial contribution to cellulose digestion. However, a recent study demonstrated synergy in cellulase activity among host- and symbiont-encoded enzymes (79). The authors of this study also observed end-product inhibition of recombinant host-encoded cellulases, leading them to propose that GHF diversity prevents buildup of these products, resulting in the observed synergy while also potentially limiting competition among symbionts. Thus, termite gut microbial symbiont diversity contributes to both the efficiency and range of host metabolism via synergistic collaboration.

Like termites, leafcutter ants use a complex microbial symbiosis to degrade plant material that supports growth of their fungus garden (discussed in further detail below) (10). This community encodes diverse GHFs that contribute to plant degradation (93). Understanding termite and ant multispecies collaboration in lignocellulose digestion has broad implications in fields such as conservation biology, human health, and bioenergy. Because cellulose processing is the rate-limiting factor in biofuel production (43), characterizing holobiont synergy among cellulase enzymes may prove critical in using either natural or engineered celluloytic systems in manufacturing bioethanol (75, 93). Also, studying the cellulase enzymatic activity in the termite and ant systems may inform studies of the carbohydrate-active enzymes (CAZymes) encoded within the human microbiome.

Entomopathogenic nematodes. Multipartite symbioses include those with multiple hosts and one microbe, such as those in which the microbial symbiont expands the ecological niche of one host by aiding in parasitism of another. Examples include filarial nematodes that utilize *Wolbachia* endosymbionts to cause disease and reproduce within mammalian hosts (94), and tree-killing bark beetles that associate with phytopathogenic fungal symbionts that may aid in avoiding

tree immunity (39). Similarly, steinernematid and heterorhabditid nematodes mutualistically associate with *Xenorhabdus* and *Photorhabdus* bacterial symbionts, respectively, which allow them to exploit insect hosts as a nutritional and reproductive niche (26). The bacteria provide several beneficial activities to the symbiosis, including virulence factors that modulate insect immunity and cause insect death (26). *Xenorhabdus* species actively suppress the induction of insect-encoded cationic antimicrobial peptides via an unknown mechanism (35, 58). *Photorhabdus* species lack this suppressive function but resist killing by these antimicrobial factors (23). Both species of bacteria can suppress activation of the insect eicosanoid pathway and thus the cellular immune response, likely protecting both bacterial and nematode parasites (37). Further, within the insect cadaver the bacterial symbionts support nematode reproduction (26). This contribution to host fitness is at least partly nutritional: The bacterial cells themselves likely are a direct food source for the nematodes and also express factors that release the energy within insect tissues (13, 36, 65–67). Whether the bacteria have nutrition-independent effects on nematode reproduction (e.g., influencing nematode development) remains to be elucidated.

A characteristic of multihost microbial symbioses is that the microbial partner expresses specific and appropriate symbiotic traits (e.g., beneficial or harmful) depending on host context. A recent study revealed that *Photorhabdus* bacteria have evolved a stochastic genetic switch that causes the cells to alternate between mutualistic (nematode-supportive) and pathogenic (insect-virulent) states (90). A similar mechanism may be involved in regulating context-dependent traits in *Xenorhabdus* species (58). Such regulatory mechanisms may be common among multihost symbionts and vectored pathogens, and continued investigation is poised to reveal unifying themes in symbiont host-dependent regulation of gene expression. The study of tripartite insect-nematode-bacteria symbioses also has potential applied impacts in agriculture. For example, our understanding of independent and synergistic activities of nematodes and bacteria during insect killing would facilitate the use of these symbionts as biocontrol agents to control insect pests (27).

Necessity Is the Mother of Invention: Multipartite Symbiotic Adaptation to Environmental Change

Microbes exhibit remarkable diversity in their ability to withstand environmental pressures. Many hosts engaging in multipartite mutualism appear to take advantage of this diversity by recruiting microbial colonizers with distinct environmental tolerance characteristics as a means to adapt to environmental change.

Sea anemones and algal symbionts. Like many aquatic invertebrate species, sea anemones of the genus *Anthopleura* harbor unicellular algal symbionts in a nutritional (photosynthetic) symbiosis (18). The anemones associate with dinoflagellates (called zooxanthellae, ZX), chlorophytes (called zoochlorellae, ZC), or both. Although individual anemones can contain a mixture of ZX and ZC, environmental surveys indicate that the variation in the distribution of these photosynthetic symbionts depends on environmental conditions (53, 55). *Anthopleura* species found at higher shore height or southerly latitudes tend to carry ZX, whereas those at lower shore height or northerly latitudes tend to carry ZC (4, 81). In addition, laboratory analyses indicate that ZX have higher photosynthetic productivity at higher temperatures and irradiances than ZC do (6, 52). These correlative studies suggest that sea anemones may preferentially associate with different symbionts with optimal performance under specific temperature and/or light conditions.

ZX: zooxanthellae

ZC: zoochlorellae

The impact of environmental conditions on symbiont carriage was revealed by transplantation experiments in a variety of natural habitats. Transplantation of aposymbiotic anemones along a light (and temperature) gradient within a cave exhibited colonization by ZX at high irradiance

and ZC at moderate irradiance; anemones at low irradiance remained uncolonized (82). These results indicate that algal distribution is environmentally controlled, though it is not yet known whether changes in symbiont dominance occur due to de novo colonization or to outgrowth of a minority symbiont population. Thus, the algal symbionts of sea anemones can be changed in an environmentally dependent manner. Similar observations were made regarding *Symbiodinium* species distribution in scleractinian corals, providing evidence for the argument that coral bleaching (and recolonization) may in fact be a means to adapt to changing environmental conditions and/or stress factors (48).

Mountain pine beetles and fungal symbionts. All mutualisms are subject to instability due to the potential for cheaters to shift the cost/benefit balance; however, multipartite mutualisms face the unique challenge of potential competition among symbionts, especially when the symbionts exhibit redundancy in their contributions to the host. The mountain pine beetle (MPB), *Dendroctonus ponderosae*, consistently associates with two co-occurring ophiostomatoid fungi, *Ophiostoma montium* and *Grosmannia clavigera*, in a seemingly redundant nutritional symbiosis (85). Bark beetles such as the MPB feed off nutrient-poor phloem of parasitized trees and deposit the fungi into egg galleries to supplement larval nutritional intake (39, 76).

A possible explanation for the co-occurrence of two fungal symbionts within one host is that they make independent contributions to beetle fitness. In feeding experiments in which only one or the other fungus was provided to the beetle, *G. clavigera* supported the production of greater numbers of MPB, which also tended to be larger than those produced in the presence of *O. montium* (7). Despite this observation, developing larvae preferred phloem containing both fungal species to phloem colonized by either species alone, suggesting selection for behavior that promotes dual symbiont carriage (7). Echoing the anemone symbiosis described above, dual symbiont carriage may be maintained due to differences in temperature tolerance between the symbionts; *G. clavigera* exhibits a faster growth rate than *O. montium* at low temperatures, but only *O. montium* can grow at high temperatures (87, 89). Consistent with these growth differences, in nature *G. clavigera* is more abundant than *O. montium* at lower temperatures, whereas *O. montium* dominates at higher temperatures (86). Seasonal changes in symbiont predominance may provide a mechanism by which the two fungal species avoid direct competition while still imparting environmental flexibility on the host beetle (8). In fact, the fungus *Leptographium longiclavatum*, which is closely related to *G. clavigera* and exhibits similar high-temperature growth constraints, does compete with *G. clavigera* for MPB colonization (71).

The constituents of host-associated consortial microbiomes may also adapt to environmental changes. Herbivorous woodrats (*Neotoma* spp.) feed on plants that produce toxic compounds, such as the creosote bush (*Larrea tridentata*), and are thought to rely on their microbial contents to reduce the toxicity of this nutrient source. Recent work revealed that adaptations of the woodrat gut microbiota enable detoxification of creosote bush secondary metabolites (40). Seasonal and stress-mediated differences in the bacterial communities within Caribbean reef sponges and reef-building coral, respectively, also have been identified, though the potential functional distinctions in community composition have yet to be experimentally analyzed (99, 107). Taken together, if changes in microbiota community composition do in fact correlate with functional differences, these studies indicate the ability of symbiont and consortial communities to adapt to environmental challenges for the benefit of the host organism.

Panic grass. Plants maintain ancient associations with endophytic fungi (92). Although the benefits of these associations can be diverse, symbionts characterized as class 2 endophytes aid in plant resistance to abiotic, habitat-specific stress factors such as pH, salinity, and temperature (70). One

class 2 endophyte, *Curvularia protuberata*, associates with the tropical grass species *Dichanthelium lanuginosum* (panic grass) in geothermal soils reaching temperatures as high as ~50°C (62). Symbiosis is essential for high-temperature survival; neither species can survive independently (63). The presence of a fungal virus, *Curvularia thermal tolerance virus* (CThTV), is required to confer heat resistance to the plant-endophyte symbiotic system (45). It is unclear how the presence of the virus affects the system. However, the fact that the *C. protuberata*–CThTV combination conferred heat tolerance to both monocot (*D. lanuginosum*) and eudicot (*Solanum lycopersicon*) plants suggests a general mechanism, potentially low-level activation of the host stress response (45). This tripartite symbiosis provides the potential for applied use of specific fungal endosymbionts to allow agriculturally relevant crops to tolerate and adapt to higher temperatures, which may be essential in ameliorating climate change issues. In addition, this system highlights the potential for viruses to function as mutualists, especially considering their rapid mutation rates and consequent adaptability.

A similar tritrophic association involving a viral partner occurs in the pea aphid, *Acyrthosiphon pisum*. Aphids engage in a nutritional symbiosis with a so-called primary symbiont, *Buchnera aphidicola*, but also harbor a secondary bacterial symbiont, *Hamiltonella defensa*, which confers protection against parasitoid wasps in a manner that depends on the presence of a toxin-encoding bacteriophage, *A. pisum* secondary endosymbiont (APSE) (105). A recent publication revealed that the presence of APSE also inversely correlates with bacterial symbiont abundance and directly correlates with aphid host fitness. The authors suggest that in the absence of APSE-mediated lysis, the *H. defensa* symbiont becomes sufficiently abundant to siphon nutrients from both the aphid and *Buchnera*, resulting in decreased fitness of the holobiont (105). Similarly, the presence of bacteriophages can influence the composition of bacteria within consortial systems, such as the human gut microbiota, with consequent impacts on holobiont fitness (49). Additional examples of viral mutualism are outlined below and are the subject of a recent review (73).

If You Can't Beat Them, Join Them: Multipartite Interdependence

In many two-partner symbioses, the microbial symbiont exhibits reduced genome complexity as a consequence of long-term maintenance within the host. Microbial genes are either transferred to the host genome or the genes are eliminated due to functional redundancy with host gene products. In the case of multipartite relationships, a picture is emerging in which each interacting organism exhibits genomic interdependence, potentially as a means to increase the overall fitness of the holobiont.

Hemipteran insects (mealybugs and glassy-winged sharpshooters). Insects of the order Hemiptera are characterized by the ability to feed on plant sap. Although nutrient rich, plant sap is lacking in essential amino acids, which instead are provided by bacterial symbionts such as *Buchnera aphidicola* in the pea aphid, *Acyrthosiphon pisum* (20, 84). Mealybugs (Pseudococcidae), however, demonstrate a unique adaptation to phloem sap feeding, carrying a "nested set" of symbionts: *Gammaproteobacteria* living within *Betaproteobacteria* in the insect bacteriocyte cells (100). The *Betaproteobacteria* (primary endosymbionts) found in various mealybugs belong to a single species, "*Candidatus* Tremblaya princeps" (41). However, the *Gammaproteobacteria* (secondary endosymbionts) isolated from different mealybugs form several clades, implying multiple independent acquisition events (41).

Recently, McCutcheon & von Dohlen (47) probed the unusual relationship between the nested symbionts of the *Planococcus citri* mealybug by analyzing the complete genomes of each symbiont. They found that "*Ca.* Tremblaya princeps" has an extremely limited genome; in fact, at only

~139 kbp, it is the smallest genome discovered to date. The *Tremblaya* genome even lacks its own aminoacyl tRNA synthetase genes, and the authors suggest these bacteria may rely on synthetases or tRNAs released by lysed gammaproteobacterial symbionts. The genomes of both *Tremblaya* and the gammaproteobacterium "*Candidatus* Moranella endobia" revealed a complex interdependency in symbiosis. Although *Tremblaya* dedicates 22% of its genomic contents (29 genes) to essential amino acid synthesis, it does not encode a single complete biosynthesis pathway. Similarly, the *Moranella* genome also lacks complete pathways but encodes 15 genes involved in essential amino acid production, only 3 of which overlap with those of *Tremblaya*. Overall, the symbiosis comprises a metabolic patchwork between the two bacteria for the production of tryptophan and threonine. Further, production of phenylalanine, arginine, and isoleucine appears to require genes from both the bacterial symbionts and the host, which likely provides branched-chain amino acid aminotransferase activities (28). The loss of large coding regions from the symbiont genome, known as reductive evolution, likely occurs due to reduced selective pressures associated with intracellular symbiosis, with the added benefit of promoting interdependence and countering competition among multipartite symbionts (51).

Reductive evolution: reduction in genome content due to relaxed selective pressure, typically exhibited by endosymbionts

Bacteriome: an organ found in insects that houses symbionts, typically within host cells (bacteriocytes)

The glassy-winged sharpshooter [*Homalodisca vitripennis* (formerly known as *H. coagulata*)] feeds on xylem sap, which lacks essential amino acids and also has extremely limited nitrogen and carbon contents. Similar to the mealybug, sharpshooters house two obligate symbionts within their bacteriome, the gammaproteobacterium "*Candidatus* Baumannia cicadellinicola" and the *Bacteroidetes* species "*Candidatus* Sulcia muelleri," though these endosymbionts do not exhibit the nested architecture of the mealybug symbionts (50). Genomic analysis suggests a slightly different division of labor between the sharpshooter symbionts and the mealybug symbionts. *Baumannia* contains 83 genes (almost 14% of its genome) that encode proteins involved in vitamin and cofactor production, yet lacks complete amino acid biosynthesis pathways, except for histidine (110). *Sulcia* encodes complete pathways for threonine, leucine, valine, isoleucine, phenylalanine, and tryptophan production, as well as additional components required for lysine and arginine biosynthesis; however, it lacks genes for vitamin and cofactor production (46, 110). The insect likely provides additional amino acids, as well as the resources necessary for nitrogen acquisition among the symbionts, via either nonessential amino acids present in sap feed or the production of proteins required for ammonium assimilation (46, 110).

Tsetse flies. While a primary focus of tsetse fly research is on its role as an obligate vector of the human-pathogenic African trypanosomes, a new story is emerging regarding the interdependent relationship of two of its bacterial symbionts: a *Wigglesworthia* species and *Sodalis glossinidius*. The primary, obligate mutualist *Wigglesworthia* resides in the bacteriome and has a reduced genome (1). It contributes to tsetse reproduction and nutrition likely through production of B vitamins such as thiamine that supplement the vertebrate host blood meal (1, 69). *Sodalis* is most abundant in the fly midgut, and there is some controversy with regard to its symbiotic function. Loss of *Sodalis* results in reduced tsetse fly longevity, suggesting an essential contribution to the host life cycle. However, the symbiont may also increase the competency of the fly to serve as a trypanosome vector (91).

Despite the fact that *Sodalis* exhibits signs of genome decay, its genome has a large amount of functional overlap with the *Wigglesworthia* genome (5). The one notable exception is that *Sodalis* does not encode the genes necessary for thiamine biosynthesis but does encode a putative thiamine transporter, suggesting that *Wigglesworthia* supplies thiamine both to the host and to *Sodalis*. In support of this hypothesis, *Wigglesworthia* density within the tsetse fly decreases when the insect is fed thiamine-supplemented blood, and flies lacking *Wigglesworthia* eventually lose their *Sodalis* symbionts (88, 103). Together, these results support a role for *Wigglesworthia*-mediated

thiamine production as a key selective factor for *Wigglesworthia*–tsetse fly interactions, as well as *Wigglesworthia-Sodalis* interactions, in an arrangement that likely minimizes competition between the two symbionts. Given that association of tsetse flies with *Wigglesworthia* is ancient, whereas *Sodalis* was recently acquired, this system may represent an interdependent collaboration that is early in its evolutionary progress and thus may eventually evolve further genome reduction and shared resource management (97).

Syntrophy: mutual dependence of two organisms for nutrition

Oligochaete worms. Reductive genome evolution is not the only means by which symbiont interdependence can be achieved; metabolic syntrophy also can stabilize symbiont coexistence within a host. Such is the case in the gutless marine worm *Olavius algarvensis*, which inhabits Mediterranean sediment and relies on at least four symbiotic bacterial species for both nutrition and waste recycling (21, 109). According to metagenomic sequencing data, the symbionts (two *Gammaproteobacteria* and two *Deltaproteobacteria*) do not have the reduced genomes characteristic of obligate intracellular symbionts (109). The gammaproteobacterial symbionts of *O. algarvensis* (γ1 and γ3) are thought to be sulfur-oxidizing autotrophs capable of fixing CO_2 using oxygen (γ1 only), fumarate, or nitrate (γ3 only) as electron acceptors (22, 38, 109). This metabolic diversity may allow the worm to thrive in both oxic and anoxic conditions. The *Deltaproteobacteria* (δ1 and δ4) encode and express genes involved in the reduction of sulfate to toxic sulfide (38). The current model regarding symbiotic function in oligochaete worms states that the symbionts exchange oxidized ($\gamma \rightarrow \delta$) and reduced ($\delta \rightarrow \gamma$) sulfur in a metabolic syntrophy that ultimately provides organic nutrients to the worm. Maintenance of all four symbionts despite their genomic similarity may be supported by differences in gene expression (38) or functional differences in preferred electron acceptors and carbon or energy sources. This condition-dependent metabolic diversity may reduce competitive opportunity among symbionts while expanding the worm's ecological range. The combined effect of these symbionts likely results in metabolic synergy, similar to the contributions of the termite gut microbiota to cellulose digestion (described above).

THE ENEMY OF MY ENEMY IS MY FRIEND: DEFENSIVE MUTUALISM

Multipartite mutualisms in which a symbiotic interaction reduces parasitism of the host are examples of defensive symbioses. As discussed above, the defensive phenotype typically occurs either by symbiont-mediated activation of host immunity or through symbiont-produced inhibitory factors, such as antimicrobial agents. Here, we discuss a variety of well-characterized mutualisms that represent these approaches to host defense.

Wolbachia and Various Host Species

Wolbachia endosymbionts are noted for their effects as reproductive parasites in arthropods (106). Recent evidence indicates that *Wolbachia* may have evolved to engage in mutualistic interactions in some arthropod hosts, independent of its role as a reproductive parasite (78). As of now, all the identified mutualistic functions of arthropod *Wolbachia* endosymbionts involve protection against invading parasitic species, likely through modulation of the host immune response.

Wolbachia is maintained in natural populations of *Drosophila melanogaster* despite lacking any strong reproductive effects in this host, suggesting a mutualistic interaction (29). Indeed, recent studies indicate that *Wolbachia* infection protects *D. melanogaster* from infection by RNA viruses, such as *Drosophila* C virus, Nora virus, West Nile virus, and Flock House virus (25, 30, 95). To date, this defensive function is the only known role of *Wolbachia* in *D. melanogaster*, and it also may occur in *Wolbachia* associations with other natural arthropod host species. Indeed, another

natural *Wolbachia* host, *Drosophila simulans*, can be protected against Flock House virus infection, although protection depends on the strain of *Wolbachia* used, and at least one strain (*w*Ri) induces moderate protection against viral infection and functions as a reproductive parasite within the host, indicating these activities are not mutually exclusive (57).

Defensive symbiosis: interaction in which a symbiont prevents parasitism by one or many additional organisms on the host

The mechanism of defensive symbiosis by *Wolbachia* in natural hosts is not known. Generalized immune activation is unlikely in this case, as *Wolbachia* does not induce expression of genes encoding antibacterial effectors in these hosts and accordingly does not protect against bacterial infection (9, 108). Recent evidence suggests that a rapidly replicating *Wolbachia* strain (*w*MelPop) can induce autophagy in *Drosophila* (102). Given that at least two RNA viruses, the arboviruses dengue and Chikungunya, also induce autophagy and require autophagosomes for replication and transmission, *Wolbachia* may somehow compete or otherwise interfere with viral replication via its manipulations of autophagy (42). The protective effects of *Wolbachia* extend to nonnative hosts, though the mechanism of protection may differ. In fact, the potential use of *Wolbachia* as a biocontrol agent in mosquito-vectored diseases is an active area of research (34).

Fungus-growing ants. For over one hundred years, researchers have studied the obligate mutualism between ants of the tribe Attini and the fungi (mostly within the family Lepiotaceae) they cultivate as a food source (10). These ant colonies maintain monocultures of fungi vertically transmitted by foundress queens (33, 101). Workers within the ant colony forage for nutrients to support the growth of the fungal garden, which is tended by additional workers. The foraged nutrient varies among ant genera, with the aptly named leafcutter ants using small leaf cuttings to feed their fungal gardens. The ant-fungus mutualism comprises only part of a complex symbiotic system involving defensive mutualism. The horizontally transmitted parasitic fungi of the genus *Escovopsis* can overtake colonies by degrading and absorbing nutrients from the cultivated fungus (15, 64). Ant-mediated resistance to parasitism is due, at least in part, to the presence of actinobacteria of the genus *Pseudonocardia*, found in association with exocrine glands on the ant surface (16, 17). The vertically transmitted *Pseudonocardia* produces antifungal substances that prevent the growth of *Escovopsis* but does not affect growth of the mutualist fungal gardens (14, 17).

The antifungal compound produced by one *Pseudonocardia* strain, isolated from the ant *Apterostigma dentigerum*, was identified and named dentigerumycin (56). However, variability in parasite–bacterial pathogen interactions was observed by crossing different bacterial and parasite strains both in vitro and in vivo, suggesting that different *Pseudonocardia* strains produce distinct antibiotics (61). In some cases, *Escovopsis* strains exhibit resistance to the defenses produced by the symbionts of their natural host (61). In light of these data, resistant *Escovopsis* is being investigated as a potential biocontrol agent to control the populations of leafcutter ants in the Neotropics, where they are significant agricultural pests (24).

Many of the well-studied multipartite symbioses reveal additional levels of complexity, in which more than one type of mutualism is exhibited within the system. Therefore, it is not surprising that recent studies indicate the presence of multiple additional bacterial symbionts, with the predominant residents belonging to the *Klebsiella* and *Pantoea* genera (60, 93), residing within the fungal cultivars of attine ants. Metagenomic and functional data indicate that these symbionts participate in nitrogen fixation and carbohydrate metabolism in the community to supplement the nutritional content of the fungal cultivar (2).

Members of another genus of antibiotic-producing actinobacteria, *Streptomyces*, also appear to engage in defensive symbioses with a variety of plant, fungus, and animal hosts, as outlined in a recent review (83). Two studies established the association of actinobacteria with two different species of bark beetles, *Dendroctonus frontalis* (southern pine beetle) and *Dendroctonus rufipennis* (spruce beetle) (12, 80). Bacteria from a clade related to *Streptomyces thermosacchari* were found

within the galleries of southern pine beetles (80). The actinobacterium produces an antifungal compound, mycangimycin, that is active against the antagonistic fungal species *Ophiostoma minus*, but not the mutualistic fungus *Entomocorticium* sp. A, which is essential for normal larval development (80). Many bacteria with antifungal activity, including the actinobacterium *Micrococcus luteus*, were isolated from galleries and oral secretions of spruce beetles released during infection by four susceptible antagonistic fungal species (12). These studies indicate the widespread use of symbionts, particularly actinobacteria, in defensive symbioses.

Herpesvirus latency. Microbes considered primarily pathogenic also can exhibit context-dependent mutualism within a host. Infection by various herpesviruses is ubiquitous among animals and is typically characterized by acute infection and subsequent latency with the lifelong potential for viral reactivation. In an attempt to study the effects of viral latency using murine gammaherpesvirus 68 and murine cytomegalovirus, Barton et al. (3) observed that lysogenic, but not acute, infection of mice results in activation of host macrophages via increased production of gamma interferon (IFN-γ) and tumor necrosis factor alpha. Latent infection also rendered mice resistant to infection by the gram-positive bacterial pathogen *Listeria monocytogenes* and the gram-negative *Yersinia pestis*, but it did not result in resistance to West Nile virus infection (3). Other examples of cross protection have been observed in mammalian models. Latency-mediated protection is remarkably long lasting, functioning at least five months after initial infection, suggesting that these interactions represent symbiotic partnerships (111).

Recent experiments in humans suggest that the defensive function of herpesvirus infection observed in mice may also extend to human viruses such as human cytomegalovirus (HCMV) and Epstein Barr virus (EBV). Researchers have observed activation of HCMV- and EBV-specific CD8 T cells upon acute infection with a variety of unrelated viruses (77). Although the mechanism of heterologous activation of herpesvirus-specific CD8 T cells is not known, these T cells exhibit increased IFN-γ production relative to CD8 T cells specific for other virus types (77). Taken together, data from mice and human models suggest that latent herpesvirus infection may induce generalized heterologous immunity mediated by IFN-γ.

It stands to reason that any parasite that engages in a long-term relationship with a host and induces an innate immune response is capable of mediating defensive mutualism. Infection of *Anopheles gambiae* mosquitoes with *Plasmodium* results in the induction of immune factors active against both *Plasmodium* and bacteria (*Escherichia coli* and *Staphylococcus aureus*), as well as factors active against bacteria alone, suggesting the potential for defensive mutualism (19, 68). The widespread human gastric pathogen *Helicobacter pylori* and the intracellular parasite *Toxoplasma gondii* also generate IFN-γ-mediated protection against other pathogens such as *Mycobacterium tuberculosis* and H5N1 influenza virus, respectively (54, 59). Further examination of pathogen-induced defensive immunity may result in protection and control strategies for a variety of infectious agents and disease vectors, bringing new meaning to "keep your friends close, but your enemies closer."

FUTURE DIRECTIONS

As we move toward the holobiont perspective of symbiosis research, we are beginning to appreciate and understand the complexity of symbiotic interaction networks. Multipartite mutualisms such as those described in this review serve more as the rule rather than the exception in nature. In fact, some organisms exhibit layered mutualisms in which independent multipartite interactions mediate multiple symbiotic traits. For example, the bark beetle associates with various fungal species to mediate nutritional supplementation across a range of temperatures and potentially to

aid in tree killing. At the same time, the beetles maintain bacteria as defensive symbionts to protect against antagonistic fungal species. Defining the nature of symbiont-symbiont and symbiont-host interactions within a multipartite mutualism such as that of the bark beetle often represents a significant challenge. This is especially true when the symbiont(s) cannot be independently cultured. However, the insights gained from studying these systems have far-reaching benefits, ranging from a basic understanding of their ecological and fitness impacts to the potential for manipulation of multipartite relationships in biotechnology (i.e., biocontrol and bioenergy). Further, given that the majority of organisms associate with more than one microbial partner, multipartite interactions are poised to become the vanguard of modern symbiosis research.

SUMMARY POINTS

1. Many mutualisms involve coordinated interactions among three or more partners.
2. These multipartite mutualisms can involve many symbionts carried within a single host or a single symbiont that modulates host interactions with other organisms.
3. Many multipartite mutualisms impart a novel trait that increases host fitness in a given ecological niche, such as aiding in nutrient acquisition and/or persistence under environmental stress.
4. When multiple symbionts are maintained in a single host, the evolution of functional and/or genomic interdependence optimizes efficiency and minimizes competition among symbionts.
5. Defensive mutualism occurs when a symbiont protects the host from parasitism or predation. This can occur through symbiont-mediated activation of the host's immune response or by production of antagonistic, defensive effectors.
6. The ecological impacts of multipartite mutualism extend far beyond the organismal level, affecting population and niche-wide stability in the face of a changing global environment.
7. The knowledge gained from investigating multipartite mutualisms has broad applications in agriculture, industry, and medicine.

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Annual Review of Microbiology

Volume 67, 2013

Contents

Index

Errata

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