
13 Microbial determinants of folivory in insects

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13.1 Introduction

A centerpiece to ecosystem productivity, land plants are key drivers of energy conversion and carbon fixation (Zelitch 1975; Kroth 2015). As the primary photosynthetic organs of plants, the bulk of Earth's carbon reservoirs are fixed and stored by leaves (Vogelman, Nishio, and Smith 1996). Abundant in energy-rich sugars, and endowed with a steady supply of essential nutrients, peptides, lipids, and cofactors, leaves share many features of a nutritionally balanced diet. Obligate folivory, however, evolved a limited number of times throughout the metazoan tree of life (McNab 1988; Chivers 1989; Rand et al. 1990; Currano, Labandeira, and Wilf 2010).

Despite the extensive radiation of land plants across every major continent and the ubiquity of foliage as a seemingly accessible resource, most animals lack the metabolic and physiological adaptations necessary to subsist on leaves as a sole source of nutrition. First, and most prominently, animals largely lack the enzymatic circuitry necessary to maximize the dietary value of ingested foliage. The highly recalcitrant polysaccharides that define the fibrous features of leaves can only be hydrolyzed by a specific range of enzymes that are ancestrally encoded by plants and their specialized pathogens (Walton 1994; Kubicek, Starr, and Glass 2014). Towards accessing the nutritionally rich cytosol, folivores must first contend with the main structural polysaccharide components of the plant cell wall, despite generally lacking an endogenous repertoire of essential digestive enzymes. Second, leaves are typically enriched with a range of constitutive and induced plant secondary metabolites evolved to mitigate the incidence and impact of herbivory (Levin 1976; Stotz et al. 2000; Wittstock and Gershenson 2002). Ranging from terpenoids to alkaloids, these defensive compounds disrupt the integrity of the digestive epithelial lining of animals and compromise the functionality of neuronal networks when ingested. Finally, foliage can be transient, as with deciduous trees and shrubs spanning Earth's

temperate and polar regions (Jackson 1967). The total abscission of leaves poses as a considerable hurdle for folivores during the winter season (Giron et al. 2007), necessitating the evolution of strategies to survive in the absence of a specialized diet for months on end. Towards mitigating these challenges, strikingly convergent adaptations arose in independent folivorous lineages, many of which are mediated through symbioses with metabolically dynamic microbial communities.

With the advent of sequencing technologies and metabolic modeling, coupled with the development of conceptual frameworks to study the functionality of the metaorganism (McFall-Ngai et al. 2013), microbes are increasingly recognized as an essential source of adaptations for animals (Douglas 2015; Chomicki et al. 2019). By contributing complementary metabolic profiles, symbioses can upgrade the phenotypic complexity of both partners (Moran 2007), spurring their radiation into novel ecological niches and triggering their diversification. Microbial symbionts are especially recognized for fueling their hosts' specializations on nutritionally challenging diets (Douglas 2015). Sap-feeding invertebrates are consistently demonstrated to partner with symbionts that supplement the essential amino acids lacking in their diet (Hansen and Moran 2014; Baumann 2005). Haematophagous animals contend with the B-vitamin deficiency of their bloodmeals through nutritional partnerships with endosymbionts contributing these cofactors (Akman et al. 2002; Rio, Attardo, and Weiss 2016; Duron et al. 2018). Wood feeding is made possible through the digestive range of their lignocellulolytic symbionts across a number of metazoan taxa (Brune 2014; Brune and Dietrich 2015). Here, we argue that leaves constitute an equally specialized diet, necessitating innovations that extend well beyond the metabolic range of most animals. In outlining the convergent metabolic features of folivore microbiomes, we point towards the outsized role microbes play towards upgrading the dietary value of ingested leaves. Given the compositionally simple, experimentally tractable partnerships folivorous insects form with their microbial partners, we emphasize the unique suitability of these study systems to pursue hypothesis-driven research into the adaptive impact of symbiosis in its intersection with the evolution of leaf-feeding behavior (Figure 13.1).

13.2 Deconstructing the plant cell wall

Serving as the largest reservoir of organic carbon on Earth, plant cell walls are metabolically inaccessible to most animals (Rose 2003). Composed primarily of cellulose, hemicellulose, lignin, and pectin, these polysaccharides define the mechanical properties and endow the wall with its characteristic rigidity (Mohnen 2008; Burton, Gidley, and Fincher 2010). Instrumental towards ensuring the structural integrity of the plant cell, the polysaccharidic matrix also contributes towards adhesion and signal transduction (Burton, Gidley, and Fincher 2010). As the foremost barrier separating the nutritionally rich cytosol from the extracellular matrix, the plant cell wall safeguards against intracellular infection by pathogens and parasites (Underwood 2012). For folivores, the benefits of degrading the plant cell wall are thus twofold: (1) an upgraded carbon economy by tapping into a recalcitrant source of energetically valuable sugars, and (2) mediating an efficient extraction of limiting nitrogenous content and lipids from the cytosol for a more balanced diet.

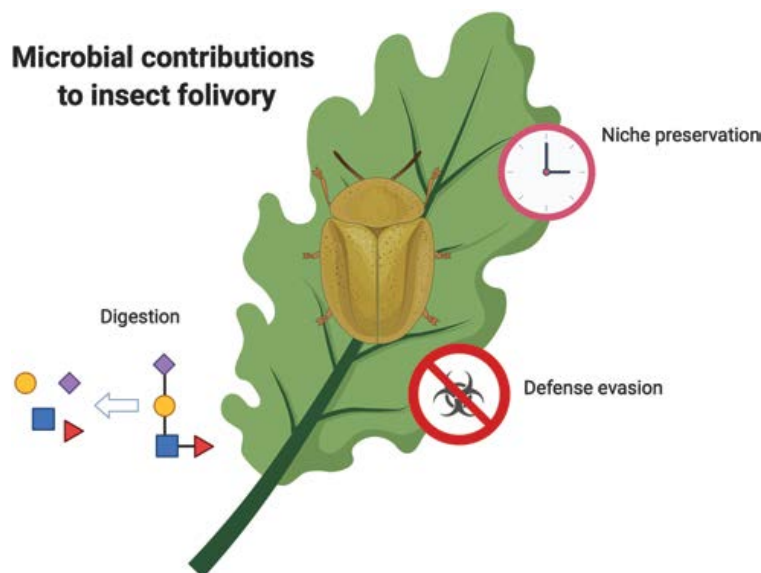


FIGURE 13.1 Microbial contributions to insect folivory include the degradation of recalcitrant plant cell wall polysaccharides (e.g. pectin, cellulose, and hemicellulose), the avoidance of noxious secondary compounds, and the preservation of leaves as their ecological niche. Overcoming these obstacles allow folivores to exploit an otherwise imbalanced nutritional resource.

Towards deconstructing the plant cell wall, a conserved battery of enzymes (e.g. glucoside hydrolases, polysaccharide lyases, etc.) are necessary to transform representative polysaccharidic sequences into simple sugars that can be metabolized throughout the digestive tract (Kubicek, Starr, and Glass 2014). Complementary to a range of endogenous digestive enzymes (Calderón-Cortés et al. 2012; McKenna et al. 2019), functional descriptions across a myriad of herbivore gut microbiomes also revealed the importance of the resident community in mediating plant biomass degradation (Martens et al. 2011; Engel, Martinson, and Moran 2012; Pope et al. 2012; Patel et al. 2014). In ruminants, relatively stable gut microbiomes serve as bioreactors for plant cell wall degradation and fermentation (Dai et al. 2015). Koalas and wombats—generalist and specialist herbivores, respectively—harbor highly convergent microbial communities enriched for the production of cellulases and xylanases to process a leafy diet (Shiffman et al. 2017). While the consistent annotation of carbohydrate-active enzymes may highlight the adaptive potential of gut microbiomes to their herbivorous hosts, assigning specific functions to individual taxa within these communities remains challenging given their complexity. As highlighted earlier, these limitations are less pronounced in the partnerships insects form with their symbionts (Douglas 2015). Stabilized through millions of years of coevolution between host and microbe, the symbioses that foliage-feeding ants and beetles engage in with microorganisms serve as some of the most streamlined partnerships both compositionally and functionally (Figure 13.2).

Leaf-cutting ants (Hymenoptera: Formicidae: Attini) are among the most prolific defoliators in tropical forests (Mueller et al. 2001; Wirth et al. 2003). Conspicuous and widespread in the New World tropics, members of the Attini tribe form

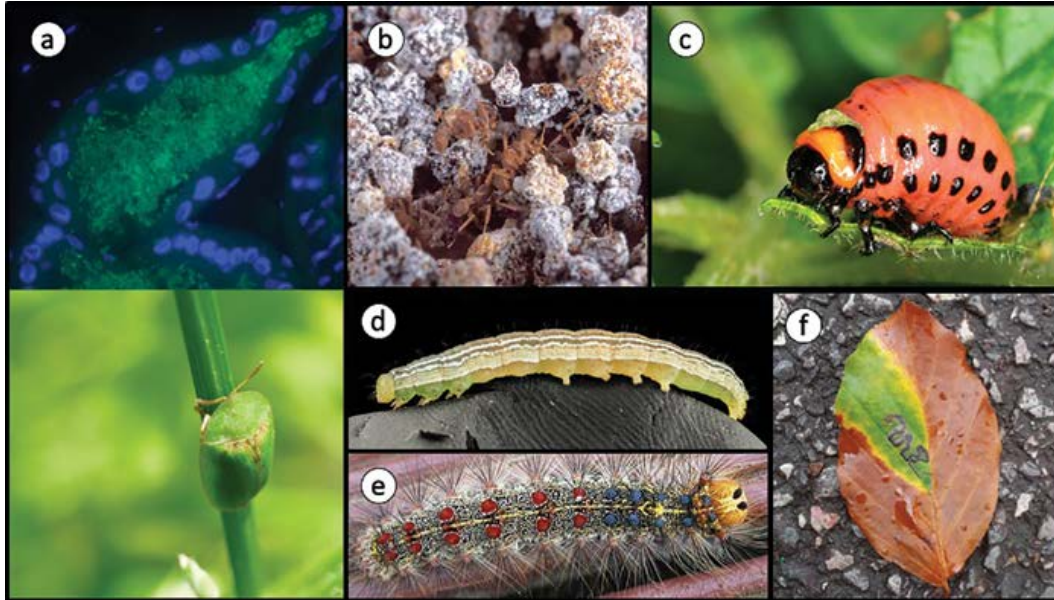


FIGURE 13.2 Microbial symbionts of folivores mediate host plant use across a diversity of insect hosts. (a) *Ca Stammera capleta* (above) produces two pectinolytic enzymes that facilitate degradation of plant cell walls, making the cytosolic content available for its tortoise beetle hosts (below). (b) The fungal partner of leaf-cutting ants functions as an external digestive system, both degrading most types of recalcitrant sugars present in leaves, as well as detoxifying plant phenolics. (c) Bacteria in the oral secretions of the Colorado potato beetle deceive tomato plants into perceiving herbivore attack as microbial, diverting plant resources into mounting an incorrect defense strategy (credit Tavo Romann). (d) The velvet bean caterpillar seems to avoid plant protease inhibitors by associating with gut bacteria whose proteases are unaffected by plant inhibitors. (e) Gypsy moth caterpillars feeding on quaking aspen acquire a gut microbiome able to degrade their host plant salicinoids. (f) *Wolbachia* symbionts allow leaf miners to maintain a green island in yellowing leaves, preserving their food source despite a decaying environment (credit Dr. Susannah Lydon).

enormous colonies that can host millions of workers and feature nests that subsist for decades (Wirth et al. 2003). Displaying a division of labor characteristic of other social insects, colonies host reproductively active queens, and differentially sized morphological worker castes divided to fulfill an array of specialized tasks (Weber 1966; Currie and Stuart 2001). This includes defending the colony, clearing the nest of debris and waste, and foraging for freshly cut leaves (Currie and Stuart 2001). As individual colonies are capable of foraging hundreds of kilograms of leaves each year, leaf-cutting ants drastically alter forest ecosystems and drive nutrient cycling (Wirth et al. 2003). But at the center of the ants' remarkable capacity to deconstruct massive amounts of plant biomass is a specialized, cultivated leucocoprineous fungus that the colony depends on for nutrition (Weber 1966; Mueller et al. 2001). A partnership dated at ~60 million years old, attini ants and their cultivars have diversified rapidly since the origin of the symbiosis, totaling 220 known species (Mueller et al. 2001). Provisioning their cultivars with fresh leaves, the ants manage their fungiculture in ways that parallel human agricultural practices (Mueller et al. 2005). Here, the ants inoculate, manipulate and transplant their cultivars to

maximize growth. Carefully tended and continuously manured through fecal fluids, the fungus is cultivated for several weeks ahead of harvesting the mature mycelium and its associated gongylidia (specialized nodules) (Mueller et al. 2005). Rich in nutrients, the gongylidia serve as vacuolized bundles that nourish the colony. Larval development depends entirely on consuming the cultivated fungus (Quinlan and Cherrett 1978), while adult workers supplement their cultivar meals with sugary extracts from floral nectaries and other plant juices (Murakami and Higashi 1997). Given the elemental role of the cultivar, attine ants transmit the fungus vertically through trophophoresy (Wirth et al. 2003). Acquiring inocula from their natal gardens, reproductive females transfer the fungal cultivar via specialized pockets towards establishing a starter culture (Mueller et al. 2005).

As the main food source for the colony, the cultivar's primary function is to convert plant biomass into nutrients for the ants (Suen et al. 2010). Serving as an external digestive system, the central metabolic features of the fungal gardens within the nest functionally resemble the gut microbiomes of bovines and other folivorous ruminants (Suen et al. 2010; Aylward et al. 2015). Structurally differentiated into distinct strata, fresh foliar material is introduced only to the top layer ahead of stepwise degradation process that lasts for six weeks and concludes with the removal and expulsion of degraded biomass by worker ants into refuse dumps (Wirth et al. 2003). Throughout this process, the quantification of plant cell wall polysaccharides revealed marked reduction in cellulose, hemicellulose and pectin. Most prominently, cellulose content decreased by 30% following passage through the fungal garden (Suen et al. 2010). Genome annotation of cultivars isolated from *Atta cephalotes* and *Acromyrmex echinatio*r identified 145 predicted plant biomass-degrading enzymes, including 81 glycoside hydrolases, 6 polysaccharide lyases and nine carbohydrate esterases (Aylward et al. 2013). Complementary metaproteomic characterization predicted many of these enzymes hydrolyze the complete spectrum of polysaccharides that comprise the plant cell wall. Gene expression analyses of the cultivar revealed that genes encoding plant cell wall degrading enzymes reached their highest expression in the bottom section of the fungal garden (Grell et al. 2013). This is consistent with the observed physical transformation of plant substrates from green, leafy material on the top layer, to an amalgam of degraded biomass and mature fungal cells. Strikingly, many of these glycolytic enzymes are characterized in the fecal droplets the ants deposit to manure the garden throughout the cultivation process (Schiøtt et al. 2010). This suggests that the proteins survive the ants' digestive system ahead of reapplication to fresh plant material, possibly as a secondary adaptation to prolong the deconstructive efficacy of enzymes within the gardens (Schiøtt et al. 2010).

While enormous colony sizes and a clear division of labor allows for leaf-cutting ants to engage in an ancient agricultural practice that culminates with outsourcing essential digestive processes to an external partner, other insects coopt microbial metabolic diversity by engaging in highly intimate nutritional symbioses. One clear example involves tortoise leaf beetles (Coleoptera: Chrysomelidae: Cassidinae) and their symbiont, *Candidatus Stammera capleta* (henceforth *Stammera*). With ~3000 described species arranged in 170 genera and 24 tribes, cassidines are an exceptionally diverse group of herbivorous beetles (Chaboo 2007). Despite their cosmopolitan

distribution, cassidines nonetheless diversified most rapidly in the tropics where their biogeography closely aligns with their host plants (Windsor 1987; Windsor, Riley, and Stockwell 1992; Morrison and Windsor 2018). Towards acquiring essential digestive enzymes to process a strictly folivorous diet, tortoise leaf beetles engage in a highly streamlined symbiosis with *Stammera*.

Localized extracellularly in symbiotic organs connected to the foregut, *Stammera* populations are maintained as monocultures resulting in low strain diversity across host populations (Salem et al. 2017). Strikingly, given the microbe's localization, *Stammera* possesses a drastically reduced genome (0.27 Mb) that is largely dedicated to informational processing (transcription, translation), replication, and the production essential digestive enzymes. Encoded within *Stammera*'s limited metabolism are two pectinolytic enzymes, polygalacturonase and rhamnogalacturonan lyase (Salem et al. 2017). The former is an endo-active glycoside hydrolase (family 28) that cleaves homogalacturonan, nature's most abundant pectic class; while the latter is a polysaccharide lyase (family 4) that hydrolyzes the heteropolymeric backbone of pectin through a beta-elimination reaction (Salem et al. 2017). Collectively, both symbiont-derived enzymes are responsible for the insect's pectinolytic phenotype, an essential adaptation given the high abundance of pectin in foliage (Burton, Gidley, and Fincher 2010) and the recalcitrant complexity of the polysaccharide (Mohnen 2008). Consistent with the specialized role of *Stammera*, symbiont elimination diminishes the digestive capacity of cassidines, notably in relation to the two pectic classes (Salem et al. 2017). This corresponds with low larval survivorship and slow developmental times relative to symbiotic insects. Given the mutualistic impact of *Stammera*, female leaf beetles ensure a stable continuum of the symbiosis through strict vertical transmission by packaging the microbe into caplet-like structures deposited individually over the anterior pole of each egg. Upon hatching, emerging larva consume the caplets, and in the process, acquire their starting inoculum of a pectinase-producing partner.

In line with the assessment that *Stammera* possesses the smallest known genome of any extracellular microbe (Salem et al. 2017), transcriptional profiling revealed a tightly regulated and metabolically integrated symbiosis, expanding our view of the minimal metabolism required to sustain life outside of a host cell (Bauer et al. 2020). In contrast to endosymbionts with highly reduced genomes, *Stammera* does not utilize aerobic respiration for energy generation. Rather, energy production and the recovery of reducing equivalents are achieved through the oxidation of sugars to pyruvate via glycolysis, followed by the fermentation of pyruvate to lactate through the activity of lactate dehydrogenase (Bauer et al. 2020). Aerobic respiration is typically conserved in most nutritional endosymbionts since amino acid and vitamin biosynthesis relies on precursors produced through the citric acid cycle, tying respiratory energy generation with the mutualistic factors that underlie the host-symbiont partnership (McCutcheon and Moran 2012). But since *Stammera*'s mutualistic role does not lie in supplementing micronutrients, selection to maintain a complex respiratory apparatus is relaxed in favor of fermentative one. While less efficient in terms of ATP output per unit of glucose, the symbiont's obligate reliance on fermentation for energy generation is unlikely to be costly given the host's carbohydrate-rich diet. This is supported by the upregulation of sugar transport into

the symbiotic organ to fuel the minimal fermentative metabolism of an essential digestive symbiont (Bauer et al. 2020).

Broad-scale genome sequencing of representative *Stammera* strains revealed that the differential distribution of symbiont-encoded pectinolytic enzymes can drastically shape the digestive physiology of Cassidinae beetles with direct implications on host plant use (Salem et al. In press). Conserved across the *Stammera* pangenome is the ability to produce and supplement polygalacturonase, highlighting the homogalacturonan-targeting pectinase as a foundational enzyme for the stability of the symbiosis with cassidines. In contrast, the annotation of rhamnogalacturonan lyase is limited to a subset of *Stammera* strains, as is the ability to deconstruct the heteropolymeric sequence of pectin (Salem et al. In press). Consistent with *in silico* predictions, beetles harboring *Stammera* encoding polygalacturonase and rhamnogalacturonan lyase display a greater pectinolytic range relative to cassidines whose symbionts only supplement the former of the two digestive enzymes (Salem et al. In press). Matching an ability to metabolize a greater diversity of universal plant polysaccharides, cassidines deploying both pectinases have radiated to exploit a wider range of host plants. In reconciling detailed records of life history traits with comparative genomics, transcriptomics and biochemical assays, the symbiosis between tortoise leaf beetles and *Stammera* serves as an example of how small changes to a symbiont's metabolic range can drastically impact the phenotypic complexity and the adaptive potential of its metazoan host.

13.3 Symbiont-mediated evasion of plant defenses

Plants counter challenges from herbivores and pathogens through the production of noxious secondary metabolites; some are constitutively expressed (Wittstock and Gershenson 2002) while others can be induced upon attack (Stotz et al. 2000). Enriched within leaves, plant toxins include metabolites as chemically diverse as alkaloids, cyanogenic glycosides, phenolics, terpenes, benzoxazinoids, and glucosinolates, among others (Fürstenberg-Hägg, Zagrobelny, and Bak 2013). Their mode of action remains elusive in many cases, but these compounds are often involved in the disruption of gut membranes, hindering metabolism and preventing normal molecular signaling, ion and nutrient transport, as well as triggering the interruption of hormone-controlled physiological processes (Mithöfer and Boland 2012). Thus, folivores are under a strong selective pressure to evolve strategies to overcome these compounds. While many of these adaptations are endogenously encoded in a folivore's metabolic repertoire (Zhu-Salzman, Bi, and Liu 2005; Després, David, and Gallet 2007), it is now evident that microbial symbionts play a central role in mediating host plant use by interfering with and degrading plant chemical defenses.

Contingent on the nature of the attack, plants induce different defense responses. Following microbial infection, plants activate salicylic acid (SA)-dependent defenses, whereas herbivory induces jasmonic acid (JA) synthesis. Often, these pathways negatively cross talk. Towards feeding on tomato plant (Solanaceae) leaves, the Colorado potato beetle (*Leptinotarsa decemlineata*) secretes bacteria-containing saliva into the wound. Detecting the threat as microbial instead of herbivorous, the

plant mounts a defense response based on SA instead of JA. This deception benefits the insect, which avoids antiherbivore defenses and experiences higher larval growth (Chung et al. 2013). Among the several bacterial taxa present in the oral secretion, only three isolates, *Pseudomonas*, *Enterobacter*, and *Stenotrophomonas*, suppress JA-dependent defenses. In particular, among the different bacterial components in these three isolates, flagellin isolated from *Pseudomonas*, was identified as one of the effectors that downregulate JA synthesis (Chung et al. 2013). These strategies are conserved in other herbivorous insect lineages. For instance, Aster Yellows (AY) phytoplasmas, a plant pathogen, could be considered a facultative symbiont of its vector leafhopper *Macrostelus quadrilineatus*. AY excretes an effector protein (SAP11) that modulates host plant (*Arabidopsis*) defense responses, downregulating the production of JA-derived metabolites to the benefit of the insect, which experiences higher fecundity (Sugio et al. 2011).

While the aforementioned examples feature symbionts protecting their insect host against plant allelochemicals prior to their synthesis, most known examples involve protection against metabolites that have already been produced, either by symbiont-mediated avoidance or breakdown of these compounds. Upon herbivorous attack, soybean plants induce the production of protein inhibitors targeting folivore proteinases, consequently hindering insect digestion (Carlini and Grossi-de-Sá 2002). Despite this, the velvet-bean caterpillar, *Anticarsia gemmatalis*, represents a major pest of soybeans. Antibiotic treatment of these insects disrupts its gut bacterial community and results in lower caterpillar growth and survival, suggesting a beneficial role of microbes. Subsequent enzymatic assays demonstrate that proteolytic and lipolytic activities are significantly affected by symbiont loss (Visôto et al. 2009). Interestingly, some members of the gut microbiome are able to synthesize proteinases that are immune to soybean proteinase inhibitors (Pilon et al. 2013), suggesting that microbial proteases may serve as a secondary set of enzymes that mediate the insect host circumventing plant defenses.

The gypsy moth (*Lymantria dispar*), a generalist folivore and pest, is well known for population outbreaks that drastically alter forest ecosystems (Liebhold et al. 1994). Given its broad host plant range, *L. dispar* caterpillars tolerates an equally diverse assortment of plant secondary metabolites. The gypsy moth's preferred host plant, the quaking aspen (*Populus tremuloides*), is rich in terpenes, phenolic glycosides (salicinoids), and tannins (Lindroth and St. Clair 2013). Earlier descriptions of *L. dispar* gut bacterial community found members of the *Rhodococcus* genus; noted for their production of terpene-degrading enzymes (van der Vlugt-Bergmans and van der Werf 2001; Broderick et al. 2004). Salicinoids lower growth and development in gypsy moth caterpillars (Hemming and Lindroth 1995). While *L. dispar* has evolved some adaptations to overcome plant toxins, such as a highly alkaline midgut, and a battery of detoxification enzymes, these strategies can be overwhelmed by high concentrations of phenolic glycosides. However, gypsy moth larvae harbor gut bacterial communities that can degrade salicinoids, leading to increased larval growth following chemical challenges by the secondary metabolite (Mason, Couture, and Raffa 2014). As described in other lepidopterans, the gut microbiome composition in this species is largely shaped by the microbial community present in ingested leaves, whereas its structure is determined by insect physiology (Mason and Raffa 2014).

Accordingly, caterpillars harboring an aspen-derived community are better adapted to tolerate aspen-derived defenses than those harboring nonaspen associated bacteria (Mason, Couture, and Raffa 2014). It appears that the benefit of these gut symbionts to their insect hosts may be derived from their ability to defend themselves against plant secondary metabolites in their primary environment as leaf-associated microbes.

Among fungus-growing ants, the transition to folivory corresponded with the necessity to contend with a wide diversity of plant secondary metabolites (Berenbaum 1988). Although leaf cutting ants can exploit nearly 75% of all plants present in the New World tropical forests, they avoid foraging on some species (Howard, Cazin, and Wiemer 1988), presumably those that contain toxins with detrimental effect to themselves and their symbiotic fungal partner (Seaman 1984). The metabolic repertoire of the fungal cultivar, which includes the ability to detoxify some plant toxins, may have been a key factor for leaf-cutting ants in becoming dominant folivores in the New World. Towards degrading phenols, the fungal cultivar of attini ants produces several phenol-oxidizing enzymes of the laccase family. One of them, LgLcc1 is highly expressed in the gongylidia and is ingested by ant workers, surviving digestion before being defecated on top of the garden. This mechanism ensures that laccase activity is highest in the garden top layers where new leaf material is being deposited and detoxification is most needed (De Fine Licht et al. 2013). Additionally, the presence of the laccase in the gut of foragers may aid in detoxifying phenolics ants may drink when cutting and chewing leaves to manure the fungal garden. Analyses of orthologous genes encoding this phenol-oxidizing enzyme demonstrate that LgLcc1 has been selected for in gongylidia-producing fungal gardens. Consistent with this observation, laccase activity is highest in the nests of leaf-cutting ants than in their noncutting counterparts (De Fine Licht et al. 2013).

Microbial degradation of plant noxious compounds is not unique to folivorous insects. Folivorous vertebrates such as cows, sheep and rats, as well as birds, harbor bacterial communities with the ability to degrade plant secondary metabolites (García-Amado et al. 2007; Kohl and Dearing 2012; Kohl et al. 2016). Similarly, other herbivorous insects besides folivores also outsource some of their adaptations against plant toxins to microbial symbionts (Barr et al. 2010; Boone et al. 2013; Hammerbacher et al. 2013; Ceja-Navarro et al. 2015; Welte et al. 2016; Berasategui et al. 2017). Thus, there is increasing evidence that symbiotic microbes can mediate host plant use through the manipulation, degradation and inactivation of plant defenses, and is possibly representative of a widespread occurrence (Shen and Dowd 1990).

13.4 Niche preservation

Dietary specialization carries the risk of temporal instability. This is most evident in the challenges faced by folivores in their coevolution with deciduous plants, where resource quality and accessibility are directly influenced by seasonality and abiotic conditions (Giron et al. 2007; Kaiser et al. 2010; Gutzwiller et al. 2015; Zhang et al. 2017). Despite a leaf's inevitable developmental progression, from initiation to senescence (Bar and Ori 2014), numerous herbivorous lineages have nonetheless evolved strategies to stall that process to continue exploiting foliage nutritionally

well beyond seasonal fall. Endophagous herbivores, such leaf miners and insect gallers, can achieve this by stalling morphogenesis and coopting plant architecture to generate structures that buffer against environmental change, protect from natural enemies, and, ultimately, extend the lifespan of a dietary niche. By coopting their host plant's metabolism, endophagous herbivores can actively trigger the differentiation of their microhabitat to shape its chemical composition and dietary value *in situ*. Among leaf-miners, this process is mediated by the bacterial symbiont, *Wolbachia*.

Responsible for their spectacular induction of "green islands" on yellowing leaves during autumn, the leaf-miner moth *Phyllonorycter* (Lepidoptera: Gracillariidae) is able to preserve a photosynthetically active patch embedded within an otherwise decaying leaf (Giron et al. 2007). Defined by elevated cytokinins, these patches reflect the continued maintenance of chlorophyll and the inhibited progression of senescence. Infection by *Wolbachia* directly impacts the ability of *Phyllonorycter* to induce green islands, since symbiont loss corresponds with the absence of the phenotype in yellowing leaves (Kaiser et al. 2010). Responding to a deteriorating ecological niche, aposymbiotic insects exhibit compensatory feeding and higher levels of mortality relative to *Phyllonorycter* infected by *Wolbachia* (Kaiser et al. 2010). By mediating its host's manipulation leaf tissues vis-à-vis cytokinin production, *Wolbachia* ensures that the insect's access to sugar-rich, metabolically active habitat despite a rapidly decaying environment (Zhang et al. 2017). While symbiont-induced nutritional homeostasis is estimated to be widespread across the Gracillariidae, the identification of two separate *Wolbachia* strains suggests several independent origins of green-island induction within this insect family (Gutzwiller et al. 2015).

13.5 Conclusions

Symbiont acquisition and replacement are essential processes, coinciding with the integration of novel metabolic features and the ability to exploit previously inaccessible niches (Moran 2007). Among insects exploiting highly specialized diets, microbes serve as an important source of metabolites and supplements towards upgrading their hosts' nutritional ecology. This is best documented in the evolutionary independent associations between sap-feeding hemipterans and nutritional endosymbionts that supplement the essential amino acids lacking in their diet (Bennett and Moran 2015). Similarly, convergent mutualistic factors are enriched in the primary endosymbionts of blood-feeding insects. Across ticks (Duron et al. 2018), bedbugs (Hosokawa et al. 2010), and tsetse flies (Akman et al. 2002), haematophagy is made possible through symbiont-encoded B vitamin contributions to balance a diet that is highly deficient in these cofactors. This chapter outlines that leaf feeding poses an inherently different set of challenges for obligate folivores. Challenges largely countered by animals engaging in stable symbioses with microbes that contribute towards the degradation of complex polymers, detoxification of noxious compounds, and niche preservation. While many of the examples highlighted here feature taxonomically diverse microbes, the range of services endowed to the insect host are nonetheless conserved. Given that conservation, and the experimental tractability of insect symbioses to pursue novel and emerging questions within the field of microbiome research, we

emphasize the suitability of folivorous insects as dynamic study systems to illustrate the metaorganismal basis of adaptation in animals.

References

- Akman, L., Yamashita, A., Watanabe, H., Oshima, K., Shiba, T., Hattori, M., Aksoy, S. 2002. Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature Genetics* 32(3):402-407. doi:10.1038/ng986.
- Aylward, F.O., Burnum-Johnson, K.E., Tringe, S.G., Teiling, C., Tremmel, D.M., Moeller, J.A., Scott, J.J. et al. 2013. *Leucoagaricus gongylophorus* produces diverse enzymes for the degradation of recalcitrant plant polymers in leaf-cutter ant fungus gardens. *Applied Environmental Microbiology* 79: 3770–3778. doi: 10.1128/AEM.03833-12.
- Aylward, F. O., Khadempour, L., Tremmel, D. M., McDonald, B. R., Nicora, C. D., Wu, S., Moore, R.J. et al. 2015. Enrichment and broad representation of plant biomass-degrading enzymes in the specialized hyphal swellings of *Leucoagaricus gongylophorus*, the fungal symbiont of leaf-cutter ants. *PLoS ONE* 10(8): e0134752. doi:10.1371/journal.pone.0134752.
- Bar, M., and Ori, N. 2014. Leaf development and morphogenesis. *Development*, 141:4219-4230. doi:10.1242/dev.106195.
- Barr, K.L., Hearne, L.B., Briesacher, S., Clark, T.L., Davis, G.E. 2010. Microbial symbionts in insects influence down-regulation of defense genes in maize. *PLoS ONE* 5(6): e11339. doi:10.1371/journal.pone.0011339
- Bauman, P. 2005. Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annual Reviews of Microbiology* 59:155–189. doi: 10.1146/annurev.micro.59.030804.121041.
- Bauer, E., Kaltenpoth, M., Salem, H. 2020. Minimal fermentative metabolism fuels extracellular symbiont in a leaf beetle. *The ISME Journal* 14:866–870. doi.org/10.1038/s41396-019-0562-1.
- Bennett, G.M., and Moran, N.A. 2015. Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole. *Proceedings of the National Academy of Sciences USA* 112(33):10169-10176. doi: 10.1073/pnas.1421388112.
- Berasategui, A., Salem, H., Paetz, C., Santoro, M., Gershenzon, J., Kaltenpoth, M., Schmidt, A. 2017. Gut microbiota of the pine weevil degrades conifer diterpenes and increases insect fitness. *Molecular Ecology* 26(15):4099–4110. doi:10.1111/mec.14186.
- Berenbaum, M.R. 1988. Allelochemicals in insect-microbe-plant interactions; agents provocateurs in the coevolutionary arms race. In: *Novel Aspects of Insect-Plant Interactions* (Barbosa P, Letourneau DK), pp. 97–123. John Wiley & Sons, New York.
- Boone, C.K., Keefover-Ring, K., Mapes, A.C., Adams, A.S., Bohlmann, J., Raffa, K.F. 2013. Bacteria associated with a tree-killing insect reduce concentrations of plant defense compounds. *Journal of Chemical Ecology* 39: 1003–1006. doi: 10.1007/s10886-013-0313-0.
- Broderick, N.A., Raffa, K.F., Goodman, R.M., Handelsman, J. 2004. Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. *Applied and Environmental Microbiology* 70: 293-300. doi: 10.1128/AEM.70.1.293-300.2004
- Brune, A. 2014. Symbiotic digestion of lignocellulose in termite guts. *Natural Reviews Microbiology* 12(3):168-180. doi: 0.1038/nrmicro3182.
- Brune, A., Dietrich C. 2015. The gut microbiota of termites: digesting the diversity in the light of ecology and evolution. *Annual Reviews Microbiology* 69:145-166. doi: 10.1146/annurev-micro-092412-155715

- Burton, R. A., Gidley, M. J., and Fincher, G. B. 2010. Heterogeneity in the chemistry, structure and function of plant cell walls. *Nature Chemical Biology* 6(10): 724–32. doi:10.1038/nchembio.439.
- Calderón-Cortés, N., Quesada, M., Watanabe, H., Cano-Camacho, H. and Oyama, K. 2012. Endogenous Plant Cell Wall Digestion: A key mechanism in insect evolution. *Annual Review of Ecology, Evolution, and Systematics* 43(1): 45–71. doi:10.1146/annurev-ecolsys-110411-160312.
- Carlini, C. R., and Grossi-de-Sá, M. F. 2002. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. *Toxicon: Official Journal of the International Society on Toxinology* 40(11): 1515–39. doi:10.1016/s0041-0101(02)00240-4.
- Ceja-Navarro, J. A., Vega, F. E., Karaoz, U., Hao, Z., Jenkins, S., Lim, H. C., Kosina P., Infante, F., Northen, T. R., and Brodie, E. L. 2015. Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. *Nature Communications* 6(July): 7618. doi:10.1038/ncomms8618.
- Chaboo, C. S. 2007. Biology and phylogeny of the *Cassidinae gyllenhal* sensu lato (tortoise and leaf-mining beetles) (Coleoptera, Chrysomelidae). *Bulletin of the American Museum of Natural History* 305(June): 1–250.
- Chivers, D. J. 1989. Adaptations of digestive systems in non-ruminant herbivores. *Proceedings of the Nutrition Society* 48(1): 59–67. doi:10.1079/PNS19890010.
- Chomicki, G., Weber, M., Antonelli, A., Bascompte, J., and Kiers, E. T. 2019. The Impact of Mutualisms on Species Richness. *Trends in Ecology & Evolution* 34(8): 698–711. doi:10.1016/j.tree.2019.03.003
- Chung, S. H., Rosa C., Scully, E. D., Peiffer, M., Tooker, J. F., Hoover, K., Luthe, D. S., and Felton, G. W. 2013. Herbivore exploits orally secreted bacteria to suppress plant defenses. *Proceedings of the National Academy of Sciences of the United States of America* 110(39): 15728–33. doi:10.1073/pnas.1308867110.
- Currano, E. D., Labandeira, C. C., and Wilf, P. 2010. Fossil Insect folivory tracks paleotemperature for six million years. *Ecological Monographs* 80(4): 547–67. doi:10.1890/09-2138.1.
- Currie, C. R., and Stuart, A. E. 2001. Weeding and grooming of pathogens in agriculture by ants. *Proceedings of the Royal Society B: Biological Sciences* 268(1471): 1033–39. doi:10.1098/rspb.2001.1605.
- Dai, X., Tian, Y., Li, J., Luo, Y., Liu, D., Zheng, H., Wang, J., Dong, Z., Hu, S., and Huang, Li. 2015. Metatranscriptomic analyses of plant cell wall polysaccharide degradation by microorganisms in the cow rumen. *Applied and Environmental Microbiology* 81(4): 1375–86. doi:10.1128/AEM.03682-14.
- De Fine Licht, H. H., Schiøtt, M., Rogowska-Wrzesinska, A., Nygaard, S., Roepstorff, P., and Boomsma, J. J. 2013. Laccase detoxification mediates the nutritional alliance between leaf-cutting ants and fungus-garden symbionts. *Proceedings of the National Academy of Sciences of the United States of America* 110(2): 583–87. doi:10.1073/pnas.1212709110.
- Després, L., David, J.-P., and Gallet, C. 2007. The Evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology & Evolution* 22(6): 298–307. doi:10.1016/j.tree.2007.02.010.
- Douglas, A. E. 2015. Multiorganismal insects: Diversity and function of resident microorganisms. *Annual Review of Entomology* 60(1): 17–34. doi:10.1146/annurev-ento-010814-020822.
- Duron, O., Morel, O., Noël, V., Buysse, M., Binetruy, F., Lancelot, R., Loire, E. et al. 2018. Tick-bacteria mutualism depends on B vitamin synthesis pathways. *Current Biology: CB* 28(12): 1896–1902.e5. doi:10.1016/j.cub.2018.04.038.
- Engel, P., Martinson, V. G., and Moran, N. A. 2012. Functional diversity within the simple gut microbiota of the honey bee. *Proceedings of the National Academy of Sciences* 109(27): 11002–7. doi:10.1073/pnas.1202970109.

- Fürstenberg-Hägg, J., Zagrobelny, M., and Bak, S. 2013. Plant Defense against insect herbivores. *International Journal of Molecular Sciences* 14(5): 10242–97. doi:10.3390/ijms140510242.
- García-Amado, M. A., Michelangeli, F., Gueneau, P., Perez, M. E., and Domínguez-Bello, M. E. 2007. Bacterial detoxification of saponins in the crop of the avian foregut fermenter *Opisthocomus hoazin*. *Journal of Animal and Feed Sciences* 16(Suppl 2): 82–5. doi:10.22358/jafs/74460/2007.
- Giron, D., Kaiser, W., Imbault, N., and Casas, J. 2007. Cytokinin-mediated leaf manipulation by a leafminer caterpillar. *Biology Letters* 3(3): 340–43. doi:10.1098/rsbl.2007.0051.
- Grell, M. N., Linde, T., Nygaard, S., Nielsen, K. L., Boomsma, J. J., and Lange, L. 2013. The fungal symbiont of *Acromyrmex* leaf-cutting ants expresses the full spectrum of genes to degrade cellulose and other plant cell wall polysaccharides. *BMC Genomics* 14(1): 928. doi:10.1186/1471-2164-14-928.
- Gutzwiller, F., Dedeine, F., Kaiser, W., Giron, D., and Lopez-Vaamonde, C. 2015. Correlation between the Green-Island phenotype and *Wolbachia* infections during the evolutionary diversification of Gracillariidae leaf-mining moths. *Ecology and Evolution* 5(18): 4049–62. doi:10.1002/ece3.1580.
- Hammerbacher, A., Schmidt, A., Wadke, N., Wright, L. P., Schneider, B., Bohlmann, J., Brand, W. A., Fenning, T. M., Gershenson, J., and Paetz, C. 2013. A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce. *Plant Physiology* 162(3): 1324–36. doi:10.1104/pp.113.218610.
- Hansen, A. K., and Moran, N. A. 2014. The impact of microbial symbionts on host plant utilization by herbivorous insects. *Molecular Ecology* 23(6): 1473–96. doi:10.1111/mec.12421.
- Hemming, J. D. C., and Lindroth, R. L. 1995. Intraspecific variation in aspen phytochemistry: Effects on performance of gypsy moths and forest tent caterpillars. *Oecologia* 103(1): 79–88. doi:10.1007/BF00328428.
- Hosokawa, T., Koga, R., Kikuchi, R., Meng, X.-Y., and Fukatsu, T. 2010. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proceedings of the National Academy of Sciences* 107(2): 769–74. doi:10.1073/pnas.0911476107.
- Howard, J. J., Cazin, J., and Wiemer, D. F. 1988. Toxicity of terpenoid deterrents to the leaf-cutting ant *Atta cephalotes* and its mutualistic fungus. *Journal of Chemical Ecology* 14(1): 59–69. doi:10.1007/BF01022531.
- Jackson, L. W. R. 1967. Effect of shade on leaf structure of deciduous tree species. *Ecology* 48(3): 498–99. doi:10.2307/1932686.
- Kaiser, W., Huguët, E., Casas, J., Commin, C., and Giron, D. 2010. Plant green-island phenotype induced by leaf miners is mediated by bacterial symbionts. *Proceedings. Biological Sciences* 277(1692): 2311–19. doi:10.1098/rspb.2010.0214
- Kohl, K. D., Connelly, J. W., Dearing, M. D., and Forbey, J. S. 2016. Microbial detoxification in the gut of a specialist avian herbivore, the Greater Sage-Grouse. *FEMS Microbiology Letters* 363(14). doi:10.1093/femsle/fnw144.
- Kohl, K. D., and Dearing, M. D. 2012. Experience matters: Prior exposure to plant toxins enhances diversity of gut microbes in herbivores. *Ecology Letters* 15(9): 1008–15. doi:10.1111/j.1461-0248.2012.01822.x.
- Kroth, P. G. 2015. The biodiversity of carbon assimilation. *Journal of Plant Physiology* 172(January): 76–81. doi:10.1016/j.jplph.2014.07.021.
- Kubicek, C. P., Starr, T. L., and Glass, N. L. 2014. Plant cell wall-degrading enzymes and their secretion in plant-pathogenic fungi. *Annual Review of Phytopathology* 52: 427–51. doi:10.1146/annurev-phyto-102313-045831.
- Levin, D. A. 1976. The chemical defenses of plants to pathogens and herbivores. *Annual Review of Ecology and Systematics* 7: 121–59.

- Liebhold, A. M., Elmes, G. A., Halverson, J. A., and Quimby, J. 1994. Landscape characterization of forest susceptibility to gypsy moth defoliation. *Forest Science* 40(1): 18–29. doi:10.1093/forestsience/40.1.18.
- Lindroth, R. L., and St. Clair, S. B. 2013. Adaptations of quaking aspen (*Populus tremuloides* Michx.) for defense against herbivores. *Forest Ecology and Management* 299: 14–21. doi:10.1016/j.foreco.2012.11.018.
- Martens, E. C., Lowe, E. C., Chiang, H., Pudlo, N. A., Wu, M., McNulty, N. P., Abbott, D. W. et al. 2011. Recognition and degradation of plant cell wall polysaccharides by two human gut symbionts. *PLoS Biology* 9(12): e1001221. doi:10.1371/journal.pbio.1001221.
- Mason, C. J., Couture, J. J., and Raffa, K. F. 2014. Plant-associated bacteria degrade defense chemicals and reduce their adverse effects on an insect defoliator. *Oecologia* 175(3): 901–10. doi:10.1007/s00442-014-2950-6.
- Mason, C. J., and Raffa, K. F. 2014. Acquisition and structuring of midgut bacterial communities in gypsy moth (Lepidoptera: Erebidae) larvae. *Environmental Entomology* 43(3): 595–604. doi:10.1603/EN14031.
- McCutcheon, J. P., and Moran, N. A. 2012. Extreme genome reduction in symbiotic bacteria. *Nature Reviews Microbiology* 10(1): 13–26. doi:10.1038/nrmicro2670.
- McFall-Ngai, M., Hadfield, M.G., Bosch, T. C. G., Carey, H. V., Domazet-Lošo, T., Douglas, E. E., Dubilier, N. et al. 2013. Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences* 110(9): 3229–36. doi:10.1073/pnas.1218525110.
- McKenna, D. D., Shin, S., Ahrens, D., Balke, M., Beza-Beza, C., Clarke, D. J., Donath, A. et al. 2019. The evolution and genomic basis of beetle diversity. *Proceedings of the National Academy of Sciences* 116(49): 24729–24737. doi:10.1073/pnas.1909655116
- McNab, B. K. 1988. Food habits and the basal rate of metabolism in birds. *Oecologia* 77(3): 343–49. doi:10.1007/BF00378040.
- Mithöfer, A., and Boland, W. 2012. Plant defense against Herbivores: Chemical aspects. *Annual Review of Plant Biology* 63: 431–50. doi:10.1146/annurev-arplant-042110-103854.
- Mohnen, D. 2008. Pectin structure and biosynthesis. *Current Opinion in Plant Biology, Physiology and Metabolism* - Edited by Markus P. and Kenneth K., 11(3): 266–77. doi:10.1016/j.pbi.2008.03.006.
- Moran, N. A. 2007. Symbiosis as an adaptive process and source of phenotypic complexity. *Proceedings of the National Academy of Sciences* 104(suppl 1): 8627–33. doi:10.1073/pnas.0611659104.
- Morrison, C. R., and Windsor, D. M. 2018. The Life history of *Chelymorpha alternans* (Coleoptera: Chrysomelidae: Cassidinae) in Panamá. *Annals of the Entomological Society of America* 111(1): 31–41. doi:10.1093/aesa/sax075.
- Mueller, U. G., Gerardo, N. M., Aanen, D. K., Six, D. L., and Schultz, T. R. 2005. The evolution of agriculture in insects. *Annual Review of Ecology, Evolution, and Systematics* 36: 563–95.
- Mueller, U. G., Schultz, T. R., Currie, C. R., Adams, R. M., and Malloch, D. 2001. The origin of the attine ant-fungus mutualism. *The Quarterly Review of Biology* 76(2): 169–97.
- Murakami, T., and Higashi, S. 1997. Social organization in two primitive attine ants, *Cyphomyrmex rimosus* and *Myrmicocrypta ednaella*, with reference to their fungus substrates and food sources. *Journal of Ethology* 15(1): 17–25. doi:10.1007/BF02767322.
- Patel, D. D., Patel A. K., Parmar, N. R., Shah, T. M, Patel, J. B, Pandya, P. R., and Joshi, C. G. 2014. Microbial and carbohydrate active enzyme profile of buffalo rumen metagenome and their alteration in response to variation in the diet. *Gene* 545(1): 88–94. doi:10.1016/j.gene.2014.05.003.

- Pilon, F. M., Visôto, L. E., Guedes, R. N. C., and Oliveira, M. G. A. 2013. Proteolytic activity of gut bacteria Isolated from the Velvet Bean Caterpillar *Anticarsia gemmatilis*. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology* 183(6): 735–47. doi:10.1007/s00360-013-0744-5.
- Pope, P. B., Mackenzie, A. K., Gregor, I., Smith, WQ., Sundset, M. A., McHardy, A. C., Morrison, M., and Eijsink, V. G. H. 2012. Metagenomics of the Svalbard Reindeer rumen microbiome reveals abundance of polysaccharide utilization Loci. *PLOS ONE* 7(6): e38571. doi:10.1371/journal.pone.0038571.
- Quinlan, R. J., and Cherrett, J. M. 1978. Aspects of the symbiosis of the leaf-cutting ant *Acromyrmex Octospinosus* (Reich) and Its Food Fungus. *Ecological Entomology* 3(3): 221–30. doi:10.1111/j.1365-2311.1978.tb00922.x.
- Rand, A. S., Dugan, B. A., Monteza, H., and Vianda D. 1990. The Diet of a Generalized Folivore: Iguana Iguana in Panama”. *Journal of Herpetology* 24(2): 211–14. doi:10.2307/1564235.
- Rio, R. V. M., Attardo, G. M., and Weiss, B. L. 2016. Grandeur Alliances: Symbiont Metabolic Integration and Obligate Arthropod Hematophagy”. *Trends in Parasitology* 32(9): 739–49. doi:10.1016/j.pt.2016.05.002.
- Rose, J. K. C. 2003. *The Plant Cell Wall*. CRC Press.
- Salem, H., Bauer, E., Kirsch, R., Berasategui, A., Cripps, M., Weiss, B., Koga, R. et al. 2017. Drastic Genome reduction in an herbivore’s pectinolytic symbiont”. *Cell* 171(7): 1520–1531.e13. doi:10.1016/j.cell.2017.10.029.
- Salem, H., Kirsch, R., Pauchet, Y., Berasategui, A., Fukumori, K., Cripps, M., Windsor, D. M., Fukatsu, T., and Gerardo, N. M. Symbiont digestive range reflects host plant breadth in herbivorous beetles. *Current Biology*, In press.
- Schiøtt, M., Rogowska-Wrzesinska, A., Roepstorff, P. and Boomsma, J. J. 2010. Leaf-cutting ant fungi produce cell wall degrading pectinase complexes reminiscent of phytopathogenic fungi”. *BMC Biology* 8(1): 156. doi:10.1186/1741-7007-8-156.
- Seaman, F. C. 1984. The effects of tannic acid and other phenolics on the growth of the fungus cultivated by the leaf-cutting ant, *Myrmicocrypta buenzlii*. *Biochemical Systematics and Ecology* 12(2): 155–58. doi:10.1016/0305-1978(84)90028-0.
- Shen, S. K. and Dowd, P. F. 1990. Insect Symbionts: A Promising Source of Detoxifying Enzymes. *ACS Symposium Series - American Chemical Society (USA)*. <http://agris.fao.org/agris-search/search.do?recordID=US9305809>.
- Shiffman, M. E., Soo, R. M., Dennis, P. G., Morrison, M., Tyson, G. W., and Hugenholtz, P. 2017. Gene and genome-centric analyses of koala and wombat fecal microbiomes point to metabolic specialization for eucalyptus digestion. *Peer Journal* 5(November): e4075. doi:10.7717/peerj.4075.
- Stotz, H. U., Pittendrigh, B. R., Kroymann, J., Weniger, K., Fritsche, J., Bauke, A. and Mitchell-Olds, T. 2000. Induced plant defense responses against chewing insects. Ethylene signaling reduces resistance of Arabidopsis against Egyptian Cotton Worm but not Diamondback Moth. *Plant Physiology* 124(3): 1007–18. doi:10.1104/pp.124.3.1007.
- Suen, G., Scott, J. J., Aylward, F. O., Adams, S. M., Tringe, S. G., Pinto-Tomás, A. A., Foster, C. E. et al. 2010. An insect herbivore microbiome with high plant biomass-degrading capacity. *PLOS Genetics* 6(9): e1001129. doi:10.1371/journal.pgen.1001129
- Sugio, A., Kingdom, H. N., MacLean, A. M., Grieve, V. M., and Hogenhout, S. A. 2011. Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. *Proceedings of the National Academy of Sciences* 108(48): E1254–63. doi:10.1073/pnas.1105664108.
- Underwood, W. 2012. The plant cell wall: A dynamic barrier against pathogen invasion. *Frontiers in Plant Science* 3: 85. doi:10.3389/fpls.2012.00085.
- Visôto, L. E., Oliveira, M. G. A., Guedes, R. N. C., Ribon, A. O. B., and Good-God, P. I. V. 2009. Contribution of gut bacteria to digestion and development of the Velvetbean Caterpillar, *Anticarsia gemmatilis*. *Journal of Insect Physiology* 55(3): 185–91. doi:10.1016/j.jinsphys.2008.10.017.

- van der Vlugt-Bergmans, C. J., and van der Werf, M. J. 2001. Genetic and biochemical characterization of a novel monoterpene epsilon-lactone hydrolase from *Rhodococcus erythropolis* DCL14. *Applied and Environmental Microbiology* 67(2): 733–41. doi:10.1128/AEM.67.2.733-741.2001.
- Vogelman, T. C., Nishio, J. N., and Smith, W. K. 1996. Leaves and light capture: Light propagation and gradients of carbon fixation within leaves. *Trends in Plant Science* 1(2): 65–70. doi:10.1016/S1360-1385(96)80031-8.
- Walton, J. D. 1994. Deconstructing the cell wall. *Plant Physiology* 104(4): 1113–18.
- Weber, N. A. 1966. Fungus-growing ants. *Science* 153(3736): 587–604. doi:10.1126/science.153.3736.587.
- Welte, C. U., de Graaf, R. M., van den Bosch, T. J. M., Op den Camp, H. J. M., van Dam, N. M., and Jetten, M. S. M. 2016. Plasmids from the gut microbiome of cabbage root fly larvae encode SaxA that catalyses the conversion of the plant toxin 2-phenylethyl isothiocyanate. *Environmental Microbiology* 18(5): 1379–90. doi:10.1111/1462-2920.12997
- Windsor, D. M. 1987. Natural history of a subsocial tortoise beetle, *Acromis sparsa* Boheman (Chrysomelidae, Cassidinae) in Panama. *Psyche: A Journal of Entomology*. 94(1-2), 127–50.
- Windsor, D. M., Riley, E. G., and Stockwell, H. P. 1992. An introduction to the biology and systematics of Panamanian tortoise beetles (Coleoptera: Chrysomelidae: Cassidinae). In *Insects of Panama and Mesoamerica. Selected Studies*. (Ed.) by Quintero, D., Aiello, A. Selected studies. Oxford University Press, New York. 372–91.
- Wirth, R., Herz, H., Ryel, R. J., Beyschlag, W., and Hölldobler, B. 2003. The natural history of leaf-cutting ants. In *Herbivory of Leaf-Cutting Ants: A Case Study on Atta colombica in the Tropical Rainforest of Panama*, (Ed.) by R. Wirth, H. Herz, R. J. Ryel, W. Beyschlag and B. Hölldobler, 5–48. Ecological Studies. Berlin, Heidelberg: Springer Berlin Heidelberg. doi:10.1007/978-3-662-05259-4_2.
- Wittstock, U., and Gershenzon, J. 2002. Constitutive plant toxins and their role in defense against herbivores and pathogens. *Current Opinion in Plant Biology* 5(4): 300–7.
- Zelitch, I. 1975. Pathways of carbon fixation in green plants. *Annual Review of Biochemistry* 44: 123–45. doi:10.1146/annurev.bi.44.070175.001011.
- Zhang, H., Guiguet, A., Dubreuil, G., Kisiala, A., Andreas, P., Emery, P. J. R., Huguet, E., Body, M., and Giron, D. 2017. Dynamics and origin of cytokinins involved in plant manipulation by a leaf-mining insect. *Insect Science* 24(6): 1065–78. doi:10.1111/1744-7917.12500.
- Zhu-Salzman, K., Bi, J.-L., and Liu, T.-X. 2005. Molecular strategies of plant defense and Insect counter-defense. *Insect Science* 12(1): 3–15. doi:10.1111/j.1672-9609.2005.00002.x.