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PHYTOPHAGOUS INSECT–MICROBE MUTUALISMS AND ADAPTIVE EVOLUTIONARY DIVERSIFICATION

Eric M. Janson,[1,2] John O. Stireman III,[3,4] Michael S. Singer,[5,6] and Patrick Abbot[1,7]
[1] *Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee 37235*
[2] *E-mail: eric.janson@vanderbilt.edu*
[3] *Department of Biological Sciences, Wright State University, Dayton, Ohio 45435*
[4] *E-mail: john.stireman@wright.edu*
[5] *Department of Biology, Wesleyan University, Middletown, Connecticut 06459*
[6] *E-mail: msinger@wesleyan.edu*
[7] *E-mail: patrick.abbot@vanderbilt.edu*

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Adaptive diversification is a process intrinsically tied to species interactions. Yet, the influence of most types of interspecific interactions on adaptive evolutionary diversification remains poorly understood. In particular, the role of mutualistic interactions in shaping adaptive radiations has been largely unexplored, despite the ubiquity of mutualisms and increasing evidence of their ecological and evolutionary importance. Our aim here is to encourage empirical inquiry into the relationship between mutualism and evolutionary diversification, using herbivorous insects and their microbial mutualists as exemplars. Phytophagous insects have long been used to test theories of evolutionary diversification; moreover, the diversification of a number of phytophagous insect lineages has been linked to mutualisms with microbes. In this perspective, we examine microbial mutualist mediation of ecological opportunity and ecologically based divergent natural selection for their insect hosts. We also explore the conditions and mechanisms by which microbial mutualists may either facilitate or impede adaptive evolutionary diversification. These include effects on the availability of novel host plants or adaptive zones, modifying host-associated fitness trade-offs during host shifts, creating or reducing enemy-free space, and, overall, shaping the evolution of ecological (host plant) specialization. Although the conceptual framework presented here is built on phytophagous insect–microbe mutualisms, many of the processes and predictions are broadly applicable to other mutualisms in which host ecology is altered by mutualistic interactions.

KEY WORDS: Adaptive radiation, ecological speciation, natural enemies, plant–insect interactions, symbiosis.

Adaptive radiation is the rapid, selection-driven diversification of one ancestral lineage into many descendent lineages (Schluter 2000). This process may create much of life's ecological and phenotypic diversity; consequently, adaptive radiation has long provided the most comprehensive model for evolutionary diversification (Lack 1947; Simpson 1953; Schluter 1996b, 2000). Indeed, case studies of adaptive radiation provided the biological touchstones for the Modern Synthesis, and modern evolutionary theory that now links the Cambrian explosion to the radiation of Darwin's finches.

Interspecific interactions, mostly in the form of resource-based competition, are central to adaptive diversification, because they act in concert with environmental variation to promote or maintain species diversity (Schluter 2000; Rundle and Nosil 2005). Exemplary studies, however, have rarely considered the impact of interactions other than competition, particularly those involving multiple trophic levels (e.g., predator–prey interactions, symbiosis). Moreover, when noncompetitive species interactions have been explicitly considered, they are often restricted to specific contexts in which the ecological and evolutionary processes described do not necessarily extrapolate across taxa (e.g., adaptive radiation in plant–pollinator mutualisms in which floral traits subject to natural selection are directly involved in reproductive isolation; Johnson et al. 1998; Schluter 2000; Levin 2006 and references therein). Thus, there remains little understanding of the relationship between the process of adaptive evolutionary diversification and the diversity of other community interactions—an omission of sufficient magnitude to lead one biologist to describe this area as "one big, vacant adaptive zone in evolutionary-ecological research" (Futuyma 2003).

Interspecific mutualisms, especially those involving prokaryotic and eukaryotic microbes, are ripe for exploration in this regard, because they are integral to the radiation of many of life's major lineages (Margulis 1981; Smith and Read 1997; Blackwell 2000; Moran 2002). All the more unusual then that studies of precisely how microbial mutualism promote innovation and spur diversification on smaller (ecological) time scales are practically nonexistent. Do microbial mutualisms facilitate the evolution of reproductive isolation and speciation through their influence on host ecology? Do microbial mutualisms shape the path of diversification after facilitating the invasion of novel ecological zones? If so, how? What are the ecological mechanisms underlying diversification for genomes bound by a history of coevolution? How can those mechanisms be discovered empirically?

Here we use the growing body of literature addressing the impact of microbial mutualism on host ecology to address several important implications for adaptive radiation, evolutionary diversification, and species interactions. By linking the growing body of research on the evolutionary ecology of mutualisms (Bronstein et al. 2006) to the evolutionary ecology of diversification (Schluter 2000; Funk et al. 2002; Rundle and Nosil 2005), we hope to provide a path toward a broader understanding of the process of adaptive diversification and its dependence on mutualistic interactions. To do so, we draw from two fields: evolutionary and ecological studies of plant-feeding insects, and the ecology of phytophagous insect–microbe mutualisms. We outline proposed mechanisms of adaptive evolutionary diversification for phytophagous insects, and investigate how explicit consideration of microbial mutualists may facilitate or inhibit these processes: for example, by promoting or inhibiting the colonization of novel host plants. In doing so, we also hope to foster the idea of adaptive diversification as a process that may be frequently tied to the joint host plant exploitation phenotypes generated by insects and their microbial mutualists.

This perspective is divided into three main sections. First, we present an overview of microbial mutualisms in phytophagous insects, underscoring their prevalence and the diversity of benefits that such mutualists confer to their insect hosts. Next, we outline some of the most significant ways in which microbial mutualists can facilitate or impede host evolutionary diversification of their hosts by focusing on how the mutualists may affect two core aspects of adaptive evolutionary diversification: ecological opportunity and divergent selection. Finally, we outline key gaps in current knowledge that must be addressed to achieve more comprehensive understanding of the impact of mutualisms on adaptive evolutionary diversification.

Prevalence of Phytophagous Insect–Microbe Mutualisms and the Benefits Conferred by Microbial Mutualists to their Insect Hosts

Virtually every multicellular organism hosts beneficial microbes. For example, the digestive tracts of animals are rich with a diverse assemblage of bacterial species that may outnumber their own cells (Dillon and Dillon 2004). Plants host an equally impressive diversity of beneficial or potentially beneficial endophytic fungi (Marks and Clay 1990; Varma et al. 1999; Wagner and Lewis 2000; Redman et al. 2002; Arnold et al. 2003; Schardl et al. 2004; Arnold and Lutzoni 2007) and bacteria (Chanway 1998), in addition to their well-known mycorrhizal (Smith and Read 1997) and nitrogen-fixing bacterial (Gresshoff 1990) mutualists. Here, we focus on insects in part because many taxa have independently formed mutualistic associations with a variety of microbial taxa, ranging from bacteria to fungi to protozoans (Buchner 1965; Breznak 1982; Campbell 1990; Gullan and Cranston 1994; Moran 2002; Bourtzis and Miller 2003; Baumann 2005; Moran and Degnan 2006). Species in several of the most diverse phytophagous insect families have been shown to maintain indigenous facultative or obligate mutualistic associations with microbes (Fig. 1). Some of the most prominent examples come from the plant-feeding Chrysomelidae (Coleoptera) (Peterson and Schalk 1994; Jolivet and Verma 2002), Curculionidae (Coleoptera) (Six 2003; Heddi and Nardon 2005), plant-galling Cecidomyiidae (Diptera) (Bissett and Borkent 1988; Gagné 1989), and virtually all plant-feeding hemipteran families (Baumann 2005; Moran et al. 2005c,d; Hosokawa et al. 2006; Takiya et al. 2006). The prevalence of microbial mutualism in certain phytophagous insect taxa, such as Lepidoptera, is more limited, but this perception may be due to the absence of evidence more than evidence of absence (Fermaud and Le Menn 1989, 1992; Roehrich and Boller 1991; McKillip et al. 1997; Mondy et al. 1998a,b; Mondy and Corio-Costet 2000; Broderick et al. 2004; Genta et al. 2006). For phytophagous insects, bacterial mutualisms predominate, but fungal mutualisms are also relatively common (Bisset and Borkent 1988; Fermaud and Le Menn 1989; Gagné 1989; Six

2003). To our knowledge, protistal mutualisms, although common in certain insect groups (e.g., xylophages; Yamin 1979; Breznak 1982), have not been described in insect species that feed on nonwoody plant tissue.

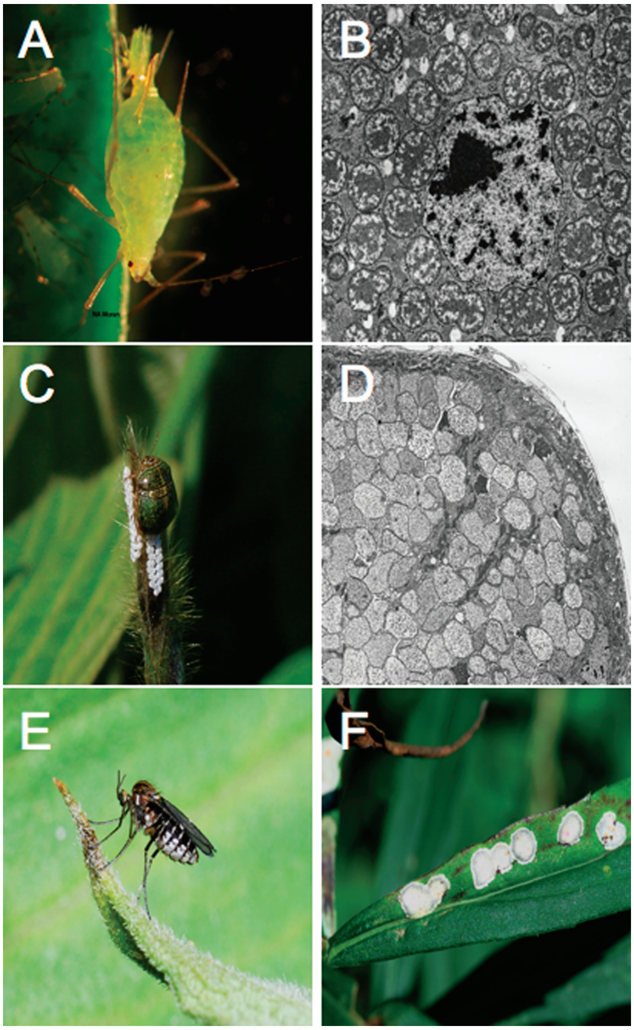


Figure 1. Examples of the diversity of insect–microbe mutualistic interactions. (A) A female *Acyrthosiphon pisum* (Hemiptera: Aphididae) and her offspring. (N. Moran, University of Arizona) (B) Cells of *Buchnera aphidicola* from *A. pisum* localized in a specialized cell known as a mycetocyte. (N. Moran, University of Arizona) (C) A female *Megacopta punctatissima* (Hemiptera: Plataspidae) depositing eggs and symbiont capsules in the field. (T. Hosokawa, National Institute of Advanced Industrial Science and Technology, Japan) (D) Candidtatus *Ishikawaella capsulata* cells in midgut of a female *M. punctatissima*. (T. Hosokawa, National Institute of Advanced Industrial Science and Technology, Japan) (E) *Asteromyia carbonifera* (Diptera: Cecidomyiidae) resting on a leaf of its host plant, *Solidago* sp. (J. Stireman, Wright State University) (F) Examples of *A. carbonifera* galls; gall structure can be partially attributed to the fungal symbiont *Botryosphaeria* sp. (P. Abbot, Vanderbilt University).

Microbes provide a vast array of services that mediate the interactions between phytophagous insects and their host plants and natural enemies. Indeed, the services rendered by microbial mutualists to their insect hosts can be remarkably varied (Smith and Douglas 1987; Saffo 1992; Moran and Telang 1998; Moran 2001; Bourtzis and Miller 2003; Dillon and Dillon 2004; Wernegreen 2005; Moran 2007). Many insect-microbial mutualisms involve nutritional provisioning by the microbe in return for the protected environment provided by the host insect's body (Ollerton 2006). Mutualistic bacteria synthesize limiting metabolites for growth and nutrition (Douglas 1998; Moran et al. 2003; Dillon and Dillon 2004), assist in the breakdown or assimilation of recalcitrant plant compounds (Jones 1984; Genta et al. 2006), provision essential vitamins (Nakabachi and Ishikawa 1999), or recycle nitrogenous wastes (Whitehead et al. 1992; Gauderman et al. 2006). Fungal mutualists can provide a similar spectrum of services. For example, the yeast-like fungal endosymbionts of some hemipterans provide amino acids and other metabolic services, but can also synthesize sterols and enzymes for the degradation of plant material or allelochemical detoxification (Noda et al. 1979; Koyama 1985; Martin 1987; Dowd 1991; Shen and Dowd 1991; Sasaki et al. 1996; Mondy and Corio-Costet 2000; Wilkinson and Ishikawa 2001). Mutualistic microbes also provide nonnutritional services, such as preventing the colonization of pathogenic microbes by either mass action or active involvement in immune reactions (Dillon and Charnley 1988; Berg 1996; Six 2003; Ferrari et al. 2004; Loker et al. 2004; Scarborough et al. 2005); synthesizing various compounds and small molecules used by insects in social interactions (Dillon and Charnley 2002); increasing fitness in extreme abiotic environments (Chen et al. 2000; Montllor et al. 2002; Dunbar et al. 2007); overwhelming plant defenses (Paine et al. 1997); and providing protection from natural enemies (i.e., predators and parasitoids; Weis 1982; Oliver et al. 2003, 2005, 2006).

Impact of Microbial Mutualists on Adaptive Evolutionary Diversification in Phytophagous Insects

Most phytophagous insects tend to be host plant specialists, feeding and carrying out virtually all key life-history activities (e.g., mate-acquisition and reproduction) on one or a relatively small subset of closely related plant species (Futuyma 1991; Bernays and Chapman 1994; Thompson 1994; Novotny and Basset 2005). This host plant specialization is thought to stem from adaptation to variation among potential host plants in such factors as nutritional quality, defensive chemistry, phenology, natural enemies, and competition, each of which can give rise to host plant associated fitness trade-offs that oppose the evolution of general diets (Bernays and Graham 1988; Futuyma and Moreno 1988;

Jaenike 1990; Schluter 2000; Funk et al. 2002; Singer and Stireman 2005). Thus, adaptive diversification in phytophagous insects depends upon exploitation of ecological opportunity provided by novel host plants (i.e., plants that are generally free of enemies or competition, and which the insect has the genetic, morphological, and physiological capacity to exploit), allowing divergent natural selection between populations on alternative host plants to promote host plant (ecological) specialization and ultimately reproductive isolation (Schluter 2000; Funk et al. 2002; Kirkpatrick and Ravigné 2002; Rundle and Nosil 2005). Finally, host plant use is often conservative, in that shifts to new hosts frequently reflect phylogenetic or chemical proximity to ancestral hosts (Ehrlich and Raven 1964; Futuyma et al. 1993, 1994, 1995; Becerra 1997; Janz and Nylin 1998; Winkler and Mitter 2007). By mediating the interactions between plants and insects, microbes can potentially influence both host plant-associated ecological opportunity and divergent natural selection, and thus the evolution of host plant specialization and reproductive isolation (Fig. 2).

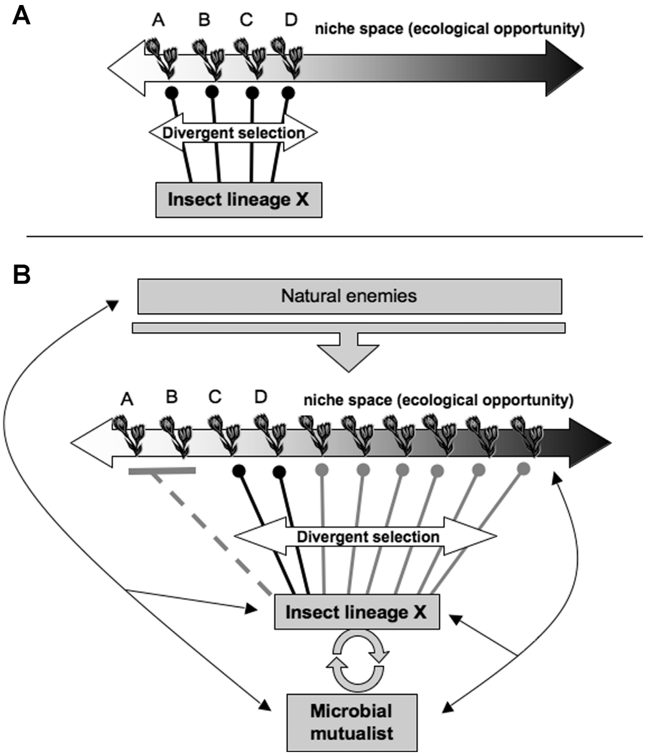


Figure 2. (A) The traditional model of an adaptive evolutionary radiation of an herbivore (lineage X) onto host plants or host plant resources. A fraction of the potential niche space is partitioned by groups (A-D) due to divergent selection arising out of the combination of ecological opportunity, environmental variation, competition, and fitness trade-offs on alternative resources/environments. (B) A multitrophic community perspective of an adaptive evolutionary radiation modulated by divergent selection among habitats/hosts and direct or indirect interactions with mutualists and natural enemies. Solid black arrows demonstrate that natural enemies and host plants interact with both insects and their microbial mutualists, resulting in a "multigenomic" basis to host plant exploitation phenotypes. Mutualists can either facilitate ecological opportunities (solid gray lines) or inhibit them (dashed line), depending on the nature of the interactions, the mode of microbial inheritance, and ultimately the process of host plant specialization (ecological specialization).

MODIFICATION OF ECOLOGICAL OPPORTUNITY BY MICROBIAL MUTUALISTS: FACILITATIVE AND INHIBITORY EFFECTS ON DIVERSIFICATION

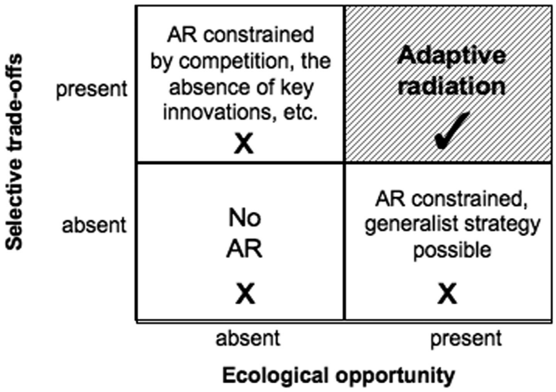
Traditionally, ecological opportunity is loosely defined as evolutionary accessible resources little used by competing taxa (Schluter 2000). Because this definition is awkward to apply directly to the microbial partner in many insect–microbe mutualisms (e.g., bacterial endosymbioses), we will employ a modified definition. We define ecological opportunity as the capacity for an insect symbiotic with a microbial mutualist to establish and maintain a population on a novel host plant (niche). This definition makes the role of mutualistic interactions explicit by accounting for the fact that host plant exploitation phenotypes may require both the insect and its microbial mutualist(s). Thus, not only could enemies, competitors, and/or genetic constraints of either the insect or microbe restrict ecological opportunity, but the context dependency of microbe-insect interactions could also affect novel host plant invasions.

Facilitative effects: environmental buffering through microbial mutualists

Mutualistic symbioses may principally affect insect diversification by providing new ecological opportunity, via "environmental buffering." In essence, microbes can act as key innovations (Simpson 1953; Schulter 2000) for their insect hosts by opening ecological opportunity that would have been otherwise unexploitable (Fig. 3). For example, plants (and plant tissues) can vary widely in primary nutrient concentrations and compositions (Slansky and Rodriguez 1987; Simpson and Simpson 1990; cf. Berenbaum 1995; Behmer and Nes 2003; cf. Zangerl and Berenbaum 2004), impeding host plant shifts due to dietary inadequacies that may exist for maladapted herbivore genotypes (Simpson and Raubenheimer 1993; Bernays and Chapman 1994; Raubenheimer and Simpson 1997). However, for nutritional mutualisms, the dietary sufficiency of a novel host plant is determined by both insect and microbial partners. Thus, microbes may furnish a dietary "buffer" that nurtures colonizing populations during the initial stages of niche or host plant shifts.

For example, plant-feeding insects vary widely in their ability to metabolize the numerous structural variants of phytosterols (Svoboda 1999; Behmer and Nes 2003), or may have dietary strategies that include feeding on plant tissue that lacks free sterols (Behmer and Nes 2003). Certain insect species appear to have

A Insect alone



B Microbe-insect mutualism

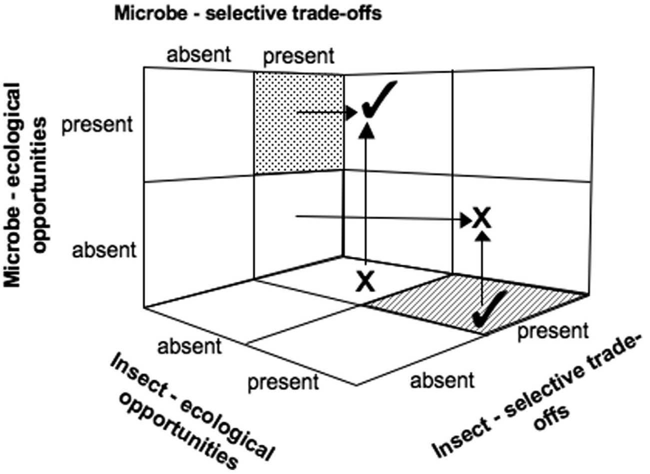


Figure 3. **(A)** ***Hatched box*: radiations of phytophagous insects occur via shifts onto novel host plant species on which they are competitively and evolutionarily viable (ecological opportunity is present) and between which divergent selection occurs (selective trade-offs are present). (B) The ecological opportunity and selective trade-offs experienced by microbial symbionts must be taken into account when considering the adaptive radiations of insect–microbe mutualisms. *Stippled box:* Conditions favorable for the radiation of microbial partners can promote (√) or hinder (X) the radiation of insect hosts otherwise possessing or lacking the appropriate ecological opportunities. Other configurations for the axes and/or new axes are easily imaginable (e.g., an axis for other trophic interactions, such as natural enemies, could be added).**

overcome this difficulty by engaging in fungal mutualisms on their host plants, and metabolizing fungal sterols, rather than phytosterols. For example, the grape berry moth, *Lobesia botrana*, has a mutualistic association with *Botrytis cinerea*, a host plant generalist filamentous ascomycete fungus. *Lobesia botrana* larvae vector *B. cinerea* on their bodies (Fermaud and Le Menn 1992) and directly facilitate rapid infection and development on grape tissue through mechanical wounding of grape berries (Fermaud and Le Menn 1989; Mondy et al. 1998a,b). Fungal material is consumed in the process of grape consumption, and is a significant source of precursor sterols that the moth larvae use to synthesize hormones involved in growth and development (Mondy and Corio-Costet 2000). Thus, *Botrytis* could facilitate successful shifts by *Lobesia* onto novel plants or tissues by reducing at least one facet of the mismatch (nutritionally derived substrates for hormone metabolism) that occurs between maladapted insect genotypes and alternative host plants or tissues (Fogleman and Danielson 2001; Behmer and Nes 2003). Other potential examples of sterol-based, microbially mediated nutritional "buffering" come from the phloem-feeding bark beetles in the genus *Dendroctonus* (Bentz and Six 2006), and the fungus-associated gall midges in the genus *Asteromyia* (E. M. Janson and R. J. Grebenok, unpubl. data)

However, insects need not consume their microbial mutualist to derive some nutritive benefit. For example, fungal infection can induce the accumulation of free amino acids, nonstructural carbohydrates, and organic nitrogen-containing compounds in plant leaves (e.g., Holligan et al. 1973; Farrar and Lewis 1987; Potter 1987; Paul and Ayres 1988 Johnson et al. 2003), which may increase performance of folivorous herbivores by balancing carbohydrate–protein ratios (Bernays and Chapman 1994). Insect-vectored microbial mutualists that directly infect the plant immediately before or during insect attack have the potential to favorably modify plant nutritional quality (e.g., Ayres et al. 2000), which in turn can enable prolonged population persistence and opportunity for adaptive divergence on alternative host plants.

Similarly, microbes may buffer insects against novel plant secondary metabolites, or against increased concentrations of secondary metabolites during host plant shifts (Starmer and Aberdeen 1990; Douglas 1992; Fogleman and Danielson 2001). Despite the capacity for microbes to degrade organic molecules used by plants as defensive allelochemicals (e.g., Hemingway et al. 1977; Douglas 1994; Bhat et al. 1998; van der Vlugt-Bergmans and van der Werf 2001), concrete examples of this phenomenon in plant–herbivore–microbe interactions are rare. One potential example comes from the gypsy moth (*Lymantria dispar*), whose larvae are known to harbor at least 23 different phylotypes of midgut bacteria (Broderick et al. 2004). One phylotype (*Rhodococcus* sp.) is closely related to a species known to degrade monoterpenes (van der Vlugt-Bergmans and van der Werf 2001), a common plant allelochemical that is toxic to insect larvae and adults (Langheim 1994), and may in part explain the relatively high tolerance of monoterpenes in gypsy moth larvae (Powell and Raffa 1999; Broderick et al. 2004; see also Genta et al. 2006). Moreover, recent studies have revealed that induced defenses used by plants to ward off different enemies (e.g., microorganisms versus insects) are subject to a significant degree of antagonistic "crosstalk" (Stout et al.

2006). Accordingly, a symbiotic microbe can induce plant resistance against other free-living microbes, but place susceptibility to it insect host. Thus, instead of detoxifying secondary metabolites, specific microbial mutualists (e.g., fungal ectosymbionts like *Boytryosphaeria* mutualistic with *Asteromyia*) may directly interfere with the induction of insect herbivore-specific host plant defenses.

Microbial mutualists may be especially likely to provide such buffering when they are derived from phytopathogenic ancestors, and thus retain traits useful in host plant exploitation that can be co-opted by insects. This is the case for many fungal mutualists of plant-feeding insects (Bisset and Borkent 1988; Fermaud and Le Menn 1989; Krokene and Solheim 1998; Kluth et al. 2001, 2002; Six 2003; Vega and Dowd 2005; for bacterial examples see de Vries et al. 2001a,b, 2004; Kikuchi et al. 2007), which have wide host plant ranges and the capacity to colonize even distantly related plants relatively easily (Farr et al. 1989; Slippers et al. 2005; Gilbert and Webb 2007; Farr et al. 2008). For example, the gall midge *Asteromyia carbonifera* forms blister galls on over 65 species of *Solidago* in North America (Gagné 1968, 1989; T. Carr, unpubl. data). *Asteromyia carbonifera* galls are unusual in that they are composed primarily of fungal tissue and lack typical plant derived nutritive tissue (Bisset and Borkent 1988); in this case, a highly specific lineage of the filamentous ascomycete *Botryosphaeria dothidea*, which the midges vector in specialized pockets (mycangia) on their abdomens (Bissett and Borkent 1988; E. M. Janson, unpubl. data). Most known noninsect associated *Botryosphaeria* species are plant pathogens/endophytes described from a broad array of higher plants, and found in nearly every region of the world (Smith 1934; Hepting 1971; Farr et al. 1989). Thus, like some intercellular and intracellular microbial mutualists (Dale et al. 2001), mutualisms such as those between gall midges and fungi may owe their evolutionary success to the microbial pathogenic trait precursors that facilitate host plant exploitation.

A general conceptual model of the buffering effects of microbial mutualists on herbivores can be described as follows. Host plant shifts and, ultimately, host plant specialization, require phenotypic trait values in herbivores that allow efficient exploitation of different plant resources (Schluter 2000), resulting in fitness landscapes with local optima that correspond to different trait combinations (Simpson 1944; Arnold et al. 2001). Thus, host plant shifts are akin to crossing valleys of character space that correspond to low population mean fitness. As with phenotypic plasticity and learning (Fear and Price 1998; Price et al. 2003; Paenke et al. 2007), mutualistic microbes may influence adaptive diversification by reshaping the fitness landscape around the peak occupied by an insect herbivore, determining in part the range of peaks in the local character space that are “within the realm of attraction” of the insect host (Price et al. 2003). Specifically, microbial mutualists may raise population mean fitness for a given range of variation in insect herbivorous traits, or move insect host trait values closer to the base of an unoccupied host plant determined adaptive peak, thus promoting peak shifts and population persistence on novel hosts. However, host shifts by herbivores may involve character evolution in the microbe as well, depending on aspects of the mutualism (e.g., the ability of the microbe to provision services across a wide range of plants). In essence, microbe-insect herbivore mutualisms may involve the coevolution of insect and microbe on two fitness surfaces. Ultimately, this process may promote diversification via host range expansion, especially if mutualisms facilitate more distant host shifts outside of conserved sets of hosts frequently observed among plant-feeding insects (e.g., Ehrlich and Raven 1964; Futuyma et al. 1993, 1994, 1995; Becerra 1997; Janz and Nylin 1998; Winkler and Mitter 2007).

Facilitative effects: reduction of insect genetic constraints by microbial mutualists

Microbial mutualists may provide ecological opportunity through alleviation of genetic constraints on host plant use. Theory suggests that lack of genetic variation for traits related to novel host plant exploitation limits adaptive evolution and host plant shifts in herbivorous insects (Lande 1979; Kirkpatrick and Lofsvold 1992; Futuyma et al. 1993, 1994, 1995; Janz et al. 2001; Blows and Hoffmann 2005). The limitations on an insect’s genome will in part determine the adaptive response (both speed and phenotypic direction) to novel host plant-associated selection, and may often preclude the successful colonization of particular host plants or increase the likelihood of extinction upon shifts to novel hosts (Schluter 2000; Arnold et al. 2001). Given that phenotypes expressed by insects involved in mutualisms are the product of at least two genomes with some degree of historical independence, genetic constraints may be relaxed due to the existence of a potentially greater pool of ecologically significant genetic variation (Fig. 3). For example, a microbial population may exhibit substantial genetic variation for traits involved in plant allelochemical detoxification, although an insect population possesses little. By increasing the pool of independent genetic variation from which an insect population can draw, a microbial mutualist may facilitate colonization and population persistence on novel host plants. This is especially likely if host plant-associated adaptations are divided among insects and microbial mutualists along genetic lines of least resistance (i.e., greatest genetic variation; Schluter 1996a, 2000). Furthermore, because of the ability of asexual microbes to acquire genetic material through lateral gene transfer (in bacteria) or parasexual recombination (in fungi), insect–microbe mutualisms may express more genetic variation for certain host-use traits than insect herbivores without mutualists. Such recombination may be more important in microbial mutualists that are primarily facultative or horizontally transmitted (Dillon and Dillon 2004);

evidence from bacterial symbionts suggests that obligate, vertically transmitted mutualists may frequently lose such capabilities (e.g., *Buchnera*, Wernegreen and Moran 2001; Tamas et al. 2002; but see van Ham et al. 2000).

Inhibitory effects: microbial mutualist imposed limits on ecological opportunity

Although microbial mutualists may often possess the potential to open or expand ecological opportunity for their insect hosts, they may also limit ecological opportunity (Fig. 3). Microbes may render host plants more toxic, not less (Starmer and Aberdeen 1990), induce plant defenses synergistically, rather than antagonistically (Stout et al. 2006), or may be susceptible to species-specific plant allelochemicals (Jones 1981; Vega et al. 2003). Lack of genetic variation or adaptively plastic responses in microbial mutualists for traits coopted by insect hosts for host plant exploitation could retard insect adaptation to novel host plants, insect population growth, and, ultimately, population persistence.

A general theme in the modern study of mutualisms is that how these interactions function can depend upon the habitats or communities in which they occur (known as “conditionality” or “context dependency”; Thompson 1988; Bronstein 1994a,b; Chen et al. 2000; Agrawal 2001; Wilkinson et al. 2001; Montllor et al. 2002; Klepzig and Six 2004; Tsuchida et al. 2004; Thompson 2005; Bensadia et al. 2006). For example, in the western flower thrips, *Frankliniella occidentalis*, the gut microbe *Erwinia* sp. can be mutualistic or antagonistic, depending on which host plant the thrips is feeding (de Vries et al. 2004). In such cases, reduction in the level of mutualistic benefit across different host plants may preclude insects associated with mutualistic microbes from successfully exploiting specific host plants, despite the insect’s capacity to recognize the plant species as a potential host. Context dependency may be especially important in mutualisms in which the microbe interfaces directly with the living host plant (e.g., fungal ectosymbioses as seen in *Asteromyia* spp., *L. botrana*, *Dendroctonus* spp.), when the association is obligate and thus cannot be eliminated (e.g., some bacterial endosymbionts), or when novel microbial mutualist species or genotypes are difficult to acquire.

MODIFICATION OF HOST PLANT-ASSOCIATED NATURAL SELECTION BY MICROBIAL MUTUALISTS: FACILITATIVE AND INHIBITORY EFFECTS ON DIVERSIFICATION

Fitness trade-offs among host plants form the basis for divergent selection and ultimately ecological specialization in phytophagous insects, from which reproductive isolation arises as a byproduct (Rice and Hostert 1993; Funk 1998; Feder and Filchak 1999; Schluter 2000; Nosil et al. 2002). Microbial mutualists that interact with the host plant or provide nutrients that are lacking in their insect host’s diet may also exhibit host-associated fitness trade-offs. In such cases, microbial mutualists may favor or hinder host plant-associated genetic differentiation in the insect, depending on whether fitness effects of a host plant on each mutualistic partner are positively or negatively correlated.

Facilitative effects: microbial mutualist promotion of host plant specialization

Vertical transmission coupled with the capacity for rapid adaptation of microbial mutualists may promote divergent selection on herbivores. In aphids, for example, host plants can possess marked variation in nutritional quality among and within species (e.g., Sandström and Pettersson 1994; Bernays and Klein 2002). Nutritional variation may be particularly important for phloem-feeders like aphids that rely on a nutritionally imbalanced diet of marginal quality (Douglas 1998). Virtually all aphids depend on the bacterial endosymbiont *Buchnera aphidicola* to synthesize amino acids missing from their diet (Douglas and Prosser 1992; Douglas 1998). In several aphid lineages, genes involved in the rate-limiting step of tryptophan and leucine production are located on exclusively vertically transmitted plasmids contained within their vertically transmitted primary endosymbionts (Lai et al. 1994; Bracho et al. 1995; Baumann et al. 1997; Rouhbakhsh et al. 1997; Silva et al. 1998; Wernegreen and Moran 2001). These plasmids show inter- and intraspecific variation in functional gene copy number and the number of amino acid biosynthesis pseudogenes (Baumann et al. 1995; Thao et al. 1998; Plague et al. 2003; Birkle et al. 2004; Moran and Degnan 2006). The observed variation in plasmid borne gene copy number among aphid lineages may be adaptive, resulting from selection favoring the functional inactivation of amino acid biosynthesis genes due to costs associated with overproduction of amino acids readily obtained from the diet (Atkinson 1977; Lai et al. 1996; Wernegreen and Moran 2000). Recent studies have also linked the inactivation of chromosomal endosymbiont amino acid biosynthesis genes in various aphid lineages to variation in the nutritional content of their host plant’s phloem (Tamas et al. 2002; van Ham et al. 2003; Moran and Degnan 2006). Given the relatively rapid rate of molecular evolution in *Buchnera* nutrient provisioning genes (Moran et al. 1995; Moran 1996; Wernegreen et al. 2001), aphids may experience fitness trade-offs among host plants due to differential selection for reduction in functional amino acid synthesis genes. In this case, microbial adaptation to host plants with high levels of particular amino acids or their precursors could lower aphid fitness on host plants with lower levels of essential amino acids or their precursors (but see Birkle et al. 2002, 2004). This, in turn, could promote host plant specialization and adaptive diversification.

Direct empirical evidence for microbial mutualists contributing to insect host plant performance, and potentially host plant specialization, has been recently observed in the stinkbug species *Megacopta punctatissima* and *M. cribraria*. These species are closely related, exhibiting over 99% sequence identity in the mitochondrial 16S *rRNA* gene (Hosokawa et al. 2006). Yet, they have distinct host plant preferences—*M. punctatissima* is a pest species that frequently feeds on leguminous crop plants in addition to wild legumes, whereas *M. cribraria* rarely feeds on domesticated plants. Hosokawa et al. (2007) discovered that this distinct host preference difference is reflected in host performance differences—field-collected *M. cribraria* performed poorly on soy (crop) plants relative to *M. punctatissima*. These two species are also known to harbor closely related, obligate, vertically transmitted γ-proteobacterial gut bacteria in specialized midgut structures (Hosokawa et al. 2006). Hosokawa et al. (2007) discovered that when the mutualistic bacterial strain of *M. punctatissima* was transferred to *M. cribraria* individuals, their fitness on soy increased significantly, specifically due to increased egg hatch rate. Conversely, *M. punctatissima* individuals that received the mutualist strain of *M. cribraria* had significantly reduced egg hatch rates on soy. With this study, Hosokawa et al. (2007) demonstrated that host plant-associated performance in an insect can be directly tied to the genotype of its microbial mutualist. More such manipulative studies involving microbial mutualists (e.g., Koga et al. 2007) are crucial to understanding microbial contributions to insect host plant performance and ecological (host plant) specialization.

Microbial mutualists that cannot regulate their mutualistic services in the face of host plant-associated environmental variation may also generate long-term host plant-associated fitness trade-offs for their insect hosts. In such cases, successful exploitation of host plants by phytophagous insect hosts may come about only through genetically based adaptations in the microbes/insects, and not through the ability of microbes to plastically regulate their mutualistic contributions to their insect host in the face of host plant-associated variation. Again, this is illustrated by the primary endosymbiont of aphids, which has generally lost the ability to fine-tune regulation of its amino acid biosynthetic genes because of the genetic decay associated with the endosymbiotic lifestyle (Moran et al. 2003, 2005b; Moran and Degnan 2006). Thus, any adaptive response to variation in host plant nutritional quality is necessarily accomplished through either amplification or inactivation of nutrient provisioning genes, rather than through up- or down-regulation of those same genes. If such host plant-associated adaptation in the microbial mutualist results in fitness variation across host plants for the insect host, host plant specialization following host plant shifts may be favored in the insect host and host plant-specific adaptations may ensue.

Fitness trade-offs and the evolution of reproductive isolation may also arise when host plant-adapted phenotypes arise through genotype × genotype × environment interactions (Thompson 1987, 1988; Agrawal 2001; Wade 2007). Insect–microbe genotypic combinations will vary in their capacity to successfully exploit host plants, and, consequently, the pairing of a particular mutualist genotype with a particular insect genotype may result in high fitness on some host plants, but low fitness on others. Thus, if particular microbial mutualist genotypes are typically transmitted among genetically similar individuals (e.g., individuals occupying the same host plant), outcrossing between host plant-associated populations could result in the frequent generation of ecologically unfit insect–microbe genotypic combinations, which in turn could lead to reinforcement of premating isolation. Insects involved in such mutualisms would also be subject to other causes of host plant-associated reproductive isolation, such as immigrant inviability (Nosil et al. 2005), further reducing gene flow among host plant associated populations.

The most informative studies on this topic have been performed on the pea aphid *Acyrthosiphon pisum*. *Acyrthosiphon pisum* comprises a group of genetically and ecologically distinct host races that feed on leguminous hosts (e.g., Via 1999; Via et al. 2000). Surveys of natural *A. pisum* populations have shown that certain host race populations harbor specific secondary bacterial species at unusually high frequencies (Tsuchida et al. 2002; Leonardo and Muiru 2003; Simon et al. 2003; Ferrari et al. 2004). Fitness variation across different host plants and environmental conditions is dependent on the presence or abundance of specific secondary symbionts (Chen et al. 2000; Koga et al. 2003; Leonardo and Muiru 2003; Tsuchida et al. 2004; Oliver et al. 2005; Russell and Moran 2006; but see Leonardo 2004). Here, the interaction between specific aphid and symbiont genotypes may have facilitated shifts onto novel host plants, allowing subsequent host plant-associated selection to promote host plant specialization and reproductive isolation. This hypothesis is supported by recent evidence that *Regiella insecticola* (pea aphid U-type symbiont [PAUS]) is causally involved in increasing host plant-associated fitness in some pea aphid genotypes, but not others (Leonardo 2004; Tsuchida et al. 2004; Ferrari et al. 2007; see also Chen et al. 2000). Similarly, certain pea aphid × secondary symbiont genotype combinations have been shown to reduce the number of winged dispersal morphs and alter the timing of sexual offspring production (Leonardo and Mondor 2006). Thus, host-mutualist genotypic interactions can affect a variety of traits that may increase positive assortative mating, reduce gene flow among populations, and enhance selection for ecological (host plant) specialization.

Many phytophagous insects possess mutualistic associations with multiple microbial taxa (Six 2003; Broderick et al. 2004; Dillon and Dillon 2004; Moran et al. 2005a; Wu et al. 2006). Consequently, successful exploitation of host plants may be a function of the full combination of microbial and insect genotypes. Microbial mutualist communities that interact intimately within their insect host through the complex sharing of metabolic resources (e.g., Wu et al. 2006; McCutcheon and Moran 2007), or through complementary adaptations to host plants are particularly likely to facilitate host-associated reproductive isolation due to the challenge of coordinating multiple genomes for optimal host plant performance and the many opportunities for intergenomic mismatching.

Inhibitory effects: reduction in host plant (ecological) specialization through microbial mutualists

Rather than fostering diversification, microbial mutualists also have the potential to inhibit divergent selection and subsequent diversification of herbivore populations. Mutualists may attenuate some selective pressures imposed on insects by host plants and preclude divergent adaptation to host plant-associated environmental variation.

For example, as described above, a microbial mutualist lineage may have a broader "host range" and express less phylogenetic conservatism in host plant "use" than its insect partner (i.e., it is able to perform mutualistic services, such as host plant allelochemical detoxification, across a relatively broad range of plants). The microbe may then act as an environmental buffer, with little influence on the variation in host-use patterns and diversification of phytophagous insects. Microbe-mediated buffering that inhibits evolutionary radiation is also likely when microbes that facilitate exploitation of particular host plants can be free-living or horizontally transmitted (e.g., gut microbiota; Dillon and Charnley 2002; Broderick et al. 2004; Kikuchi et al. 2007; secondary endosymbionts of aphids; Darby and Douglas 2003; Russell et al. 2003; Moran et al. 2005c; Russell and Moran 2005) and/or when the insect host is able to recognize many plants as suitable hosts. In this regard, microbial mutualists could provide a form of phenotypic plasticity for host use, favoring general diets over specialization, and hindering the process of host-associated diversification (Price et al. 2003; Moran 2007). Some generalist insect species may even take advantage of microbial amelioration of host-associated fitness trade-offs by managing the consortium of microbial mutualists best suited to each host plant (although we know of no examples; Broderick et al. 2004; Dillon and Dillon 2004). Such management could be achieved through the induction of antibacterial genes that favor specific microbes when challenged with particular host plants or environmental conditions (e.g., Mittapalli et al. 2006).

INSECT–MICROBE MUTUALISMS IN A TRI-TROPHIC CONTEXT: FACILITATIVE AND INHIBITORY EFFECTS ON DIVERSIFICATION

Pressure from natural enemies can facilitate or maintain host shifts in phytophagous insects, and thus can play an integral role in the evolution of host plant specialization and reproductive isolation (e.g., Brown et al. 1995; Murphy 2004; Nosil 2004; Singer and Stireman 2005; Nosil and Crespi 2006). In cases in which microbial mutualists mediate interactions between phytophagous insects and natural enemies, microbes may also play a significant role in adaptive diversification. For example, it was suggested by Weis (1982) that fungal mutualists of *Asteromyia* gall midges, which play a major role in gall formation, might also protect developing midge larvae from parasitoids by forming an impenetrable stroma (hyphal crust; see also Skuhravá and Skuhravy 1992). More recently, experimental evidence has mounted that the presence of specific secondary bacterial symbionts in pea aphids confers at least partial resistance to parasitoid attack (Oliver et al. 2003, 2005, 2006; Ferrari et al. 2004). However, there are also conditions under which a microbial mutualist may increase the vulnerability of its host to enemies, such as when a microbe produces apparent cues that can be used by enemies to locate host insects, or when microbial mutualists induce the release of natural enemy-attracting plant volatiles (Dillon and Dillon 2004; Sullivan and Berisford 2004).

If microbial mutualists frequently provide protection against generalist enemies, then the tri-trophic fitness advantages of host specialization in phytophagous insects (e.g., Bernays and Graham 1988; Bernays and Cornelius 1989; Dyer 1995, 1997) may be attenuated and broad host ranges may be favored. Generalist host-use patterns in ant-tended lycaenid butterflies that maintain obligate mutualisms with ants (Fiedler 1994) support this contention (Jaenike 1990). Conversely, microbial defenses may allow expansion into new niches formerly inaccessible to herbivores due to intense pressure from enemies, which could increase opportunities for divergent selection and ecological speciation (as argued for a marine isopod-bacterial association; Lindquist et al. 2005). In either case, explicit consideration of the role of mutualists in providing enemy-free space for herbivores (sensu Jeffries and Lawton 1984) will likely provide greater insight into the mechanisms by which herbivore diet breadth evolves and its consequences for adaptive diversification. However, it may be difficult to distinguish the effects of nutritional versus defensive benefits of microbial mutualists if in both cases one predicted outcome is broader host ranges and fewer opportunities for host plant related genetic differentiation.

Predictions concerning the effect of enemy–herbivore–mutualist interactions on diversification depend on the prey- or habitat-specificity of the natural enemies exerting selective pressure. Interactions between herbivore–microbe mutualisms and specialized enemies could serve to inhibit adaptive diversification, but under some circumstances, such enemy pressure may favor host plant shifts and host plant associated genetic differentiation. If an herbivore's dominant enemies are specialized to a particular herbivore–plant association (e.g., many parasitoids), shifts onto novel host plants may garner some degree of enemy-free space for herbivores (Lawton 1986; Gratton and

Welter 1999; Singer and Stireman 2005). If microbial mutualists increase the frequency of viable host-shifts onto novel plant taxa by ameliorating nutritional or defensive barriers, and the shift is favored by enemy-free space, these two factors may act synergistically, thus ensuring that alleles associated with the novel host preference increase in frequency. Under conditions in which the enemy or suite of enemies a herbivore faces is dependent upon host plant identity, selection by these enemies is likely to amplify preexisting trade-offs in host plant use and further encourage genetic differentiation of host plant associated populations.

Interactions between microbial mutualists and specialized enemies may also encourage diversification on a single host plant. For example, in the *Asteromyia–Botryosphaeria* gall midge-fungal mutualism, at least four gall morphotypes can be found on a single *Solidago* species (*S. altissima*) (Gagné 1968; Crego et al. 1990). The gall morphs differ primarily in the thickness of the fungal hyphal mass and fungus-derived stroma, which in turn influences parasitism rates by wasps (Weis 1982; Crego et al. 1990; T. Carr and J. O. Stireman, unpubl. data). Furthermore, allozyme (Crego et al. 1990), mtDNA (T. Carr, J. O. Stireman, unpubl. data), and AFLP (J. O. Stireman, unpubl. data) markers reveal that the midges exhibit significant genetic differentiation with respect to gall morphotype. This coincidence of gall morph, parasitism frequency, and genetic differentiation suggests that the diversification of these midges has been driven by interactions between parasitoids and gall morphology mediated through the fungal mutualist.

Microbial mutualists may also provide protection from antagonistic or competitive microbes. For example, Scarborough et al. (2005) demonstrated that the presence of the secondary symbiont *R. insecticola* reduced post-attack sporulation of the aphid fungal entomopathogen *Pandora* (*Erynia*) *neoaphidis* (see also Ferrari et al. 2004). Similarly, specific fungal and bacterial associates of bark beetles provide protection against antagonistic fungi that frequently colonize their galleries (Six 2003; Cardoza et al. 2006). If the antimicrobial benefits conferred by a mutualist are independent of host plant genotype or species, microbial mutualists may facilitate host shifts or expansions by providing some degree of protection from antagonistic microbes that may be associated with novel host plants. However, if the degree of microbial-mediated defense against antagonistic microbes is strongly associated with particular host plant species, host plant genotypes, or environmental conditions (e.g., Klepzig and Six 2004; Hofstetter et al. 2005), shifting hosts may reduce or eliminate the mutualist's defensive capabilities and render the insect host susceptible to antagonistic microbe attack. In the former case, the increased potential for shifting hosts may favor the long-term adaptive diversification of an insect population host by allowing colonization of novel host plants, but divergence may be discouraged by the ability to feed on many hosts. In the latter case, selection for greater specialization creates evolutionary trade-offs in host plant use, again potentially facilitating adaptive divergence of populations, but the reduced ability to explore novel host plants may inhibit diversification.

Conclusions and Future Directions

Many of the most prominent evolutionary radiations of organisms in the history of life appear to coincide with the origin of intimate mutualistic and antagonistic associations. Tightly coupled mutualistic associations have repeatedly resulted in key innovations allowing the invasion of novel adaptive zones (Margulis and Fester 1991; Maynard Smith and Szathmáry 1995; Moran and Telang 1998). The present perspective was motivated by the disparity between the implications of this hypothesis for understanding organismal evolution, and the dearth of empirical investigation into the mechanistic and ecological basis for precisely how mutualisms might influence diversification. Modern evolutionary biology is poised to reveal these ecological mechanisms because the tools to characterize microbes in seemingly inaccessible communities are themselves becoming accessible (e.g., Venter et al. 2004). No longer must the ecologically significant variation that microbes introduce in plant–insect interactions be averaged into the biotic backdrop framing herbivory. In addition, molecular and genomic surveys have given evolutionary biologists a widespread appreciation of the often surprising capacities of microbes for creative meddling in the evolution of eukaryotes (e.g., *Wolbachia*; Charlat et al. 2003). These new insights into microbial symbiont biology suggest new mechanisms in the evolutionary ecology of insect-plant interactions (e.g., Broderick et al. 2003, 2006; Wade 2007).

A particularly productive area for evolutionary ecological studies would focus on the trade-offs between ecological opportunity and ecological specialization mediated by microbial mutualists. We have argued that horizontally transmitted/facultative mutualists may expand ecological opportunity for insect lineages due to their potential to move among insect genotypes and exchange genes with other microbes in host and nonhost microbial communities. However, horizontally transmitted/facultative mutualists might limit an insect host lineage's ability to specialize ecologically due to reduced heritability of these phenotypic benefits and the difficulty of building linkage disequilibrium between insect host genes and microbial mutualist genes involved in plant exploitation (Wade 2007). In this respect, the central tension regarding the role of microbes in evolutionary diversification seems analogous to the evolutionary consequences of learning and plasticity (Paenke et al. 2007). In both, the issue is the introduction of flexibility in the relation between phenotype and genotype. What we currently do not know empirically—in both cases as it turns out—is the effect of that flexibility on the response to selection under different ecological scenarios.

In contrast to horizontally transmitted and facultative mutualists, vertically transmitted, obligate mutualists may promote a rigid phenotype-to-genotype map and ultimately ecological specialization. The consequence is that the reduction in genetic exchange with other microbial genotypes, coupled with coevolutionary adaptation and irreversible processes such as genome reduction, may ultimately limit the ecological opportunity they can provide for their hosts. It is uncertain how common such strict inheritance is for microbes at the plant–insect interface. Many insect lineages may engage in coevolutionary interactions with microbes that are neither strictly vertical nor horizontally transmitted, but rather exhibit characteristics of both depending on the spatial and temporal time scale (Thompson 2005; Mikheyev et al. 2006). There may be a tension between ecological specialization and opportunity even within organismal lineages coevolving with mutualist microbes, and the trade-off between the two might ultimately shape the patterns of adaptive radiation. Moreover, maintaining associations with both obligate/vertically transmitted and facultative/horizontally transmitted mutualists may strike a balance between these trade-offs, allowing for some degree of ecological specialization while maintaining the potential to facilitate exploration of the adaptive landscape.

All of this offers a great opportunity for evolutionary biologists to elucidate mechanisms that generate biodiversity. The genomic revolution has provided enormous new information about the astounding diversity of microbial species and their metabolic capacities, many of which are symbiotic with higher eukaryotes (Moran 2007). But evolutionary ecologists need not become microbiologists nor genomic biologists. Rather, the uniqueness of herbivorous insects—their sheer diversity, borne of their struggle to exploit plants and avoid enemies, and their consistent tendencies to recruit microbial partners-in-aid—means evolutionary ecologists are uniquely positioned to put the genomic revolution to work in pursuit of broad themes in ecological and evolutionary research. If one big, vacant adaptive zone in evolutionary ecological research is the role of diverse community interactions in the adaptive radiation of eukaryotes (Futuyma 2003), then the study of herbivorous insects and their microbial mutualists is rich with unexploited opportunities.

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