

Evolutionary origins and diversification of mutualism

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3.1 Webs in the tree of life

In this chapter, we describe how investigating the past may contribute to our understanding of the evolutionary and ecological processes that shape mutualism. Species interactions are considered to have the potential to generate new ecological opportunities that might have fostered the diversification of lineages in the past (Farrell et al. 1992), and mutually beneficial partnerships are occasionally cited to account for the rise of particular lineages to ecological dominance. Ancient mutualisms, for example the associations of land plants with ectomycorrhizal fungi (Remy et al. 1994) or animal pollinators, seem like plausible candidates for diversification in partnership. Although insect pollination mutualisms have long been implicated in flowering plant diversification (Darwin 1862), insect lineages comprising the contemporary pollinators of flowering plants were already highly diverse prior to the origin of angiosperms (Labandeira and Sepkoski 1993). This example illustrates how a phylogenetic perspective is needed to understand whether or how past mutualisms might have shaped contemporary patterns of species diversity. In this chapter, we aim to interpret the kinds of evidence that suggest when mutualisms appeared and how beneficial partnerships might have tipped the balance of speciation and extinction to promote diversification.

Figure 3.1 illustrates an extreme case of diversification in partnership (Cruaud et al. 2012).

Molecular phylogenies and ecological associations for approximately two hundred closely related plant species and their specialized, obligate pollinators are depicted without taxonomic names so that we might focus our attention instead on general problems of interpretation. A glance at this web in the tree of life suggests the parallel diversification of plant and pollinator clades, as well as instances in which ancient pollinators might have diversified after switching from one host plant lineage to another. For example, a lineage of seven plant species at the upper left is pollinated exclusively by a particular insect lineage, and congruent branching patterns in the two phylogenies suggests diversification in partnership (see arrows in Figure 3.1). However, the plant lineage at the upper left descends from the base of the tree and appears to be more ancient than the associated pollinator lineage. Regardless of whether more recent diversification catalyzed by host shifts or ancient codiversification (parallel cladogenesis) might have dominated the history of this mutualism, the multiplicity of lineages engaged in partnership is impressive. In fact, most evidence for extensive patterns of codiversification involves exceptionally specialized and obligate mutualisms. Might this reflect a coincidence, a bias in sampling, or a biological reality?

This chapter examines this question by comparing evidence for origins and diversification across the spectrum of species interactions, from mutualism to antagonism. We regard mutualisms as reciprocal exploitations that nonetheless provide benefits

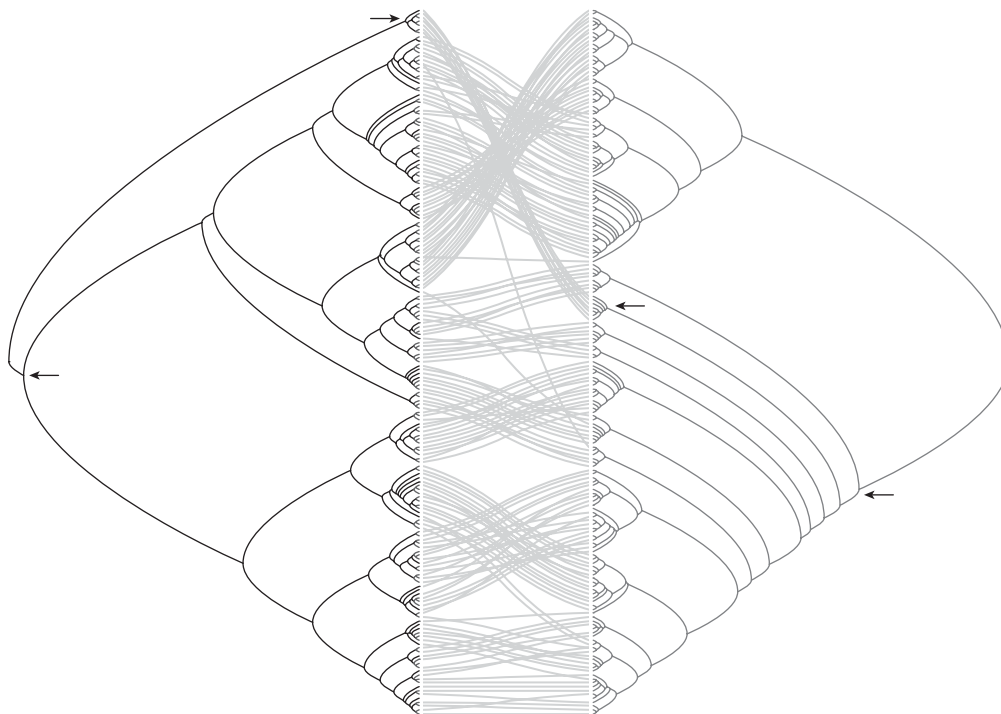


Figure 3.1 Cophylogeny of figs and fig wasps redrawn from the supplementary information in Cruaud et al. (2012). A molecular phylogeny estimate for 186 *Ficus* species (Moraceae) based on five genes (at left) is paired with an independent phylogeny estimate for fig-pollinating wasps (Agaonidae) based on six genes (at right) with observed species-specific associations (middle). Arrows point to a pair of congruent figs and pollinator clades that appear to have originated at different times.

to each partner, and we distinguish mutualism from cooperation, where the former is reserved for positive interactions among different species while the latter refers to positive interactions among individuals of the same species (Boucher 1992; see Chapter 1). The exact nature of associations among species may vary at the level of populations, over time, and according to ecological context, so that classifying particular species interactions only as mutualistic, antagonistic, commensal, etc. is not always possible (Thompson 1988). However, analyzing such variation in light of genetic change within species populations, phylogenetic patterns among lineages, and fossil evidence may shed light on evolutionary conditions that supported the origin and diversification of mutualism. The objective of this chapter is to review approaches, limitations, and opportunities for identifying such conditions from a macroevolutionary perspective.

3.2 Investigating origin and diversification

3.2.1 The fossil record

Paleontology provides a compelling, but largely incomplete, record of mutualism that serves to illustrate the kinds of insights that can be gained about species interactions from the history of life. Wilf and Labandeira (Box 3.1) describe how fossil evidence is compared with contemporary ecology to shed light on the early history and persistence of mutualisms over time. For example, fossils point to ancient origins of mutualism at least 1.5 bya in the case of mitochondria, 450 mya for mycorrhizal symbiosis, and 250 mya for pollination of seed plants by insects. The range of mutualisms for which we have a paleontological record remains rather narrow, and simultaneous preservation of partners is so rare that interactions are typically inferred from other sources

Box 3.1 The fossil record of mutualisms

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Interspecies interactions are well represented in the fossil record, especially those involving plants and insects. Most of the known associations were antagonistic, as the voluminous record of insect herbivory over the past ~410 million years notably shows (Labandeira 2002). Several interactions show remarkable levels of early behavioral specialization by insects. These include Middle Jurassic leaf mimicry (Wang et al. 2012) and rolled-leaf hispine beetle feeding on gingers, an extant association, from the late Cretaceous (Wilf et al. 2000).

Well-supported evidence for mutually beneficial associations is rare but extremely informative. Endosymbiosis in macroorganisms is ancient. Many species in the oldest diverse animal faunas, from the Ediacaran Period (631–541 Ma), were large, flattened, and frondose, implicating photosynthetic endosymbiosis that was perhaps analogous to the mid-Mesozoic origin of zooxanthellae symbioses in corals. Arbuscular mycorrhizae are found through the entire record of land plants when preservation is sufficient, starting with the ~410 Ma Rhynie Chert (Remy et al. 1994). This deposit

also contains anatomically preserved lichens that represent cyanobacterial-algal symbioses (Taylor et al. 1997).

Pollination is one of the most important mutualisms and probably accounts for the evolution of a large share of today's plant and insect diversity. Pollination probably evolved through the consumption of spores, pollen, and exudates from plant reproductive structures such as pollen drops (Labandeira 2002). The large prepollen of many late Paleozoic seed plants exceeded the size range typically transported by wind and indicates a likely role for entomophily (Taylor and Millay 1979). Insect guts containing prepollen and pollen date to the Early Permian (Krassilov and Rasnitsyn 1997) and are well documented from the mid-Mesozoic (Labandeira 2002). Pollination of cycads and bennettites ("cycadeoids") by beetles was presumably widespread during the Jurassic, based on beetle pollination of many living cycads and extensive borings found in fossil bennettite cones (Crepet 1974). Mid-Mesozoic true flies, scorpionflies (Box 3.1, Figure 1), and lacewings bore prominent siphonate probosces that in all likelihood functioned to access fluid rewards. Mesozoic gymnosperms offered these rewards through angiosperm-like reproductive structures with recessed ovules, which

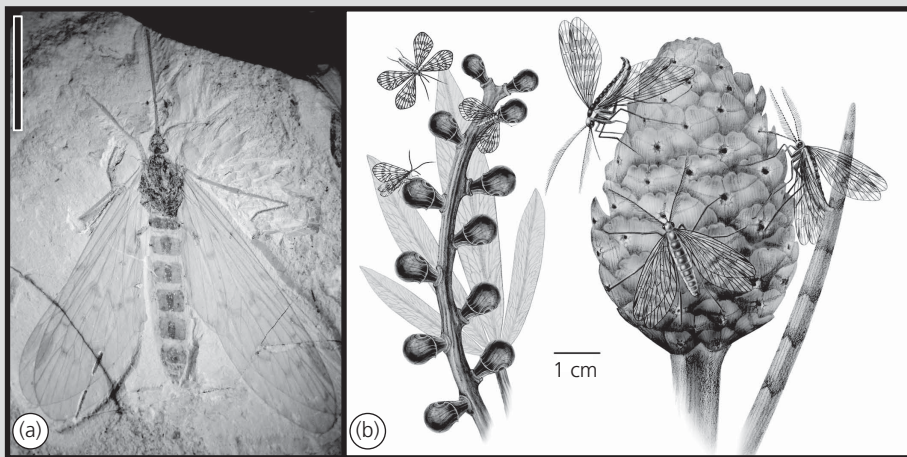


Figure 1 Selected paleontological reconstructions of mutualism (Labandeira 2010). (a) The long-proboscate mesopsychid scorpionfly *Lichnomesopsyche glorioe* from the Middle Jurassic of Inner Mongolia, China, a probable pollinator of some gymnospermous plants. (b) Reconstructions of two mid-Mesozoic pollinator associations between mesopsychid scorpionflies and their inferred host plants. At left, *L. glorioe* feeds on the pollen drops of *Caytonia sewardi* via an integumental tube under the lower lip of the cupule. At right is another mesopsychid, *Vitimopsyche kozlovi*, from the mid Early Cretaceous of Liaoning, China, feeding on catchment funnel nectaries from the ovulate cone of a cheirolepidiaceus conifer and achieving pollination in the process. Original art by Mary Parrish (See Plate 1 for color version).

continued

Box 3.1 *Continued*

were often connected to the outer female organ surfaces by tubular or channeled structures. These lineages, representing three unrelated orders of insects, constituted a long-proboscid, mid-Mesozoic guild of likely pollinating insects prior to the significant ecological presence of angiosperms (Labandeira 2010).

From Early Cretaceous amber, female thrips are known with specialized collecting structures (ring setae) that amassed large loads of gymnosperm pollen, strongly indicating pollination (Peñalver et al. 2006), as well as an ancestral bee species that harbored another distinctive specialization (branched hairs) for accessing pollen (Danforth and Poinar 2011). By the early Late Cretaceous, there is significant evidence for specialized pollination of angiosperms by diverse insects, inferred primarily from fossil flowers that show nectaries, resin ducts, bilateral symmetry, and pollen often presented as multiple attached grains and bearing sticky surfaces (Crepet 1996). Other anthophilic Cretaceous insects included basal moths, several lineages of small, pollinating beetles, and the earliest occurrence

of a modern bee, a stingless honey bee worker from one of the deposits that yielded much of the floral evidence for pollination (Michener and Grimaldi 1988). During the Paleogene, several groups of pollinating flies are known, such as long-proboscate, hovering tanglevein and flower flies, and many other specialized groups including diverse bees and butterflies. One of the most celebrated pollination mutualisms as emphasized in this chapter, figs and fig wasps, has a fossil record starting from 34 Ma, consisting of spectacularly preserved female wasps with attached fig pollen (Box 3.1, Figure 2; Compton et al. 2010, Peñalver et al. 2006).

Seed dispersal is perhaps the most significant mutualism involving vertebrates, in all likelihood accounting for significant diversifications in frugivores and plants. Some Paleozoic wetland plants had large, robust seeds, up to ca. 11 cm long. These seeds had large nucelli that probably were nutritious, although no likely tetrapod dispersers are known. However, fish were well diversified and had reached large body sizes by this time; speculatively, fish may have played a dispersal role, especially in flooded forests (see also Tiffney 1986). There is a similar lack of direct evidence for dispersers of Mesozoic seed plants, but it seems quite likely that many groups, especially those with large seeds such as cycads, ginkgophytes, and some conifers, provided forage to the diverse large-bodied reptiles of this interval. Large angiosperm fruits and seeds do not become significant in the record until after the end-Cretaceous extinction (66 Ma), along with modern birds and characteristically frugivorous, arboreal mammals (Wing and Tiffney 1987). Rare Eocene conifers also exhibited large, fleshy, specialized cone receptacles identical to those of living podocarps with bird-dispersed seeds (Wilf 2012). Eocene mammalian frugivory is spectacularly displayed by body fossils with gut contents and coprolites from the Messel oil shales of Germany (Schaal and Ziegler 1992). More recently, grasslands represent a mutualism with vertebrate herbivores at a global scale, to the extent that herbivores participate in maintaining biome structure and composition through grazing, disturbance, and consequent selective pressures that favor particular plant lineages and adaptations. Widespread grasslands and characteristic vertebrate adaptations were present by the late Oligocene to Miocene on many continents, but the existence of older grassland biomes is contentious. It is of considerable interest that in Patagonia, the presence of high-crowned teeth (hypsodonty) in diverse mammal

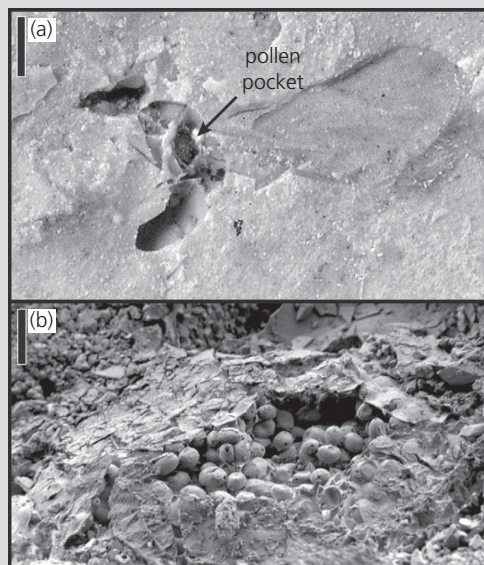


Figure 2 Fossil evidence of mutualism (Compton et al. 2010). (a) Holotype of the 34-million-year-old agaonid wasp, "*Ponera*" *minuta*, from the late Eocene of the Isle of Wight, United Kingdom (scale bar, 500 μ m). (b) The pollen pocket, located on the anterior thorax, is enlarged under SEM, showing lodged *Ficus* pollen (scale bar, 20 μ m). Used with permission from Royal Society Publishing and The Natural History Museum, London.

Box 3.1 *Continued*

clades by 26 Ma and earlier has long been taken as evidence for precocious South American grasslands, but recent pollen data show that grasses were not abundant there before about 2 Ma (Palazzesi and Barreda 2012).

The fossil record of interactions is, of course, incomplete, and inferences about the nature of past associations, whether mutualistic or antagonistic, require multiple lines of evidence. It is even more challenging to gain insight from the record as to whether or how past associations might have influenced the diversification of lineages in partnership. Although many extant associations are preserved, extinct associations become more prevalent with geologic age, especially before the Cenozoic. Beyond establishing minimum ages, the value of particular fossil occurrences for broader evolutionary studies, such as their widespread use in anchoring phylogenetic analyses of cladogenesis through time, depends on many factors and is dramatically improved with well-understood geologic context and absolute dating of the fossils, temporally and spatially dense sampling, and rigorous use of morphological characters to support identifications and behavioral interpretations.

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of evidence. When morphological, anatomical, or chemical features resembling those of a contemporary mutualism are observed in the fossil record, it is postulated that ancestors may have interacted in a similar manner. For example, evidence that insects pollinated early seed plants includes the simultaneous occurrence of elongate insect proboscides and tubular channels in gymnosperm cones during the Mesozoic (Figure 1 in Box 3.1). Although the coexistence of potential partners can sometimes be firmly established by paleontological records, fossils are for the most part silent about past ecology such as whether ancient mutualisms were facultative or obligate. At the same time, fossils are indispensable for calibrating molecular phylogenies based on DNA sequence divergence to estimate dates of origin (Arbogast et al. 2002) and to infer the evolution of morphological traits (Donoghue et al. 1989).

3.2.2 Phylogeny estimation

The earliest phylogenetic studies of mutualism did not consider the evolutionary history of both partners simultaneously, as in Figure 3.1, but instead examined the phylogeny of one partner in relation to the mutualistic traits or associations of the other (Remy et al. 1994, Pellmyr and Thompson 1992, Hibbett et al. 2000). We begin by considering the challenges of estimating phylogeny and inferring ancestral conditions in the first place, before examining what further insight might be gained by comparing the phylogenies of interacting lineages. The tendency for descendants to carry on as their ancestors once did (i.e., phylogenetic conservatism) offers hope that we might be able to identify the origin of a mutualism in the tree of life by mapping the distribution of traits and species associations on a phylogeny. In the case of land plants, for example, the similar anatomy of fossilized roots and contemporary plants exhibiting ectomycorrhizal associations (Remy et al. 1994) suggests that this mutualism dates from the Ordovician period. Not so long ago, simple parsimony was the primary vehicle for inferring origins of mutualism (Pellmyr et al. 1996). More recent advances in phylogenetic theory and computation have fueled an explosion of new statistical and analytical methods

that expand the potential to reconstruct histories of mutualism. For example, maximum likelihood estimation can be used to evaluate the probability of ancestral mutualism given a phylogeny and models of evolutionary transition among alternative conditions such as parasitism or commensalism. Hibbett et al. (2000) applied such a model to suggest that ectomycorrhizal symbioses involving basidiomycete fungi have been unstable and dynamic since the Ordovician, considering that the ectomycorrhizal condition appears to have arisen repeatedly from ancestors that derived their nutrition from dead, decaying wood (saprophytes). One advantage of this approach over parsimony has to do with how uncertainty about ancestral states is handled. A maximum likelihood estimate explicitly models the probabilities of any and all possible ancestral scenarios, however improbable some scenarios might appear to be. Baum and Smith (2013) provide an accessible introduction to these methods while their practical application and theoretical development are treated by Lemey et al. (2009) and Felsenstein (2004) respectively.

Bayesian approaches have become especially popular, wherein an evolutionary model, DNA sequences, dates from the fossil record, and other prior information are used simultaneously to estimate phylogeny, divergence times, and model parameters. The posterior probability distributions that result from Bayesian inference can be used to statistically test alternative hypotheses about the evolution of interactions. For example, a recent analysis of diverse fungal life histories found it significantly more probable that fungi feeding exclusively on dead wood (saprophytes) evolved from pathogens that infect and destroy living plant tissues or from the commensal associates of foliage (endophytes) than vice versa (Arnold et al. 2009). Uncertainty surrounding such inferences is often sufficiently large that alternative scenarios cannot be rejected. Much of the explanatory zeal that characterized the early literature on coevolution of mutualism (Chapter 7) is likely to be tempered as more sophisticated modeling grapples with the complexity and uncertainty surrounding past events.

When interactions among species are highly specialized and pairwise, it may be possible to

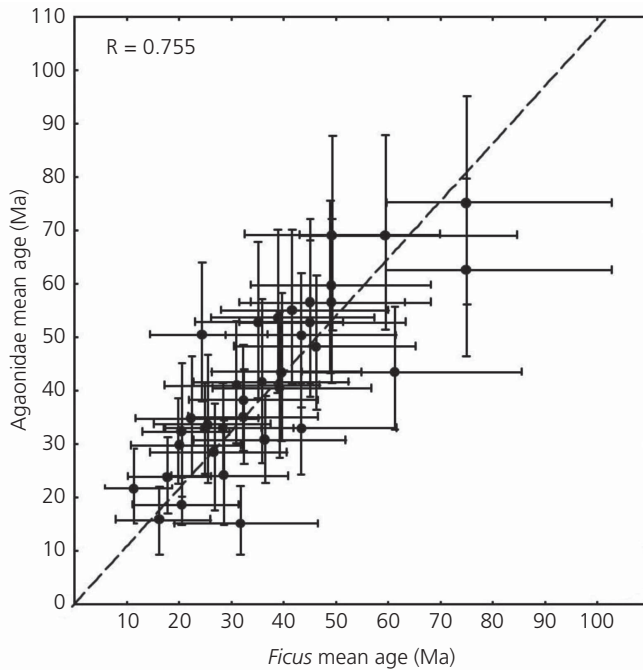


Figure 3.2 Correlated divergence times of figs and pollinating fig wasps (Cruaud et al. 2012). Error bars indicate 95% confidence intervals for the minimum ages of plant lineages that are uniquely associated with particular pollinator lineages. Used with permission from Oxford University Press.

compare independent fossil-calibrated estimates of molecular divergence times for lineages in partnership (Figure 3.2). Such “double-dating” evaluates the alternative hypotheses of codiversification and asynchronous speciation. For example, Silvieus et al. (2008) compared divergence times among fig trees, obligate pollinators, and non-pollinating seed-eating parasites, which can be nearly as host-specific as the pollinators of certain lineages of figs. Age estimates for clades of fig species and their wasp pollinators supported codiversification, while the timing of speciation in parasites appeared to be asynchronous with respect to host plant speciation. In this case, unique insight on diversification was obtained through the comparison of contrasting life histories in a similar ecological context (e.g., pollinators and parasites associated with the same host). However, this approach is not applicable to a wide array of mutualisms that are either facultative or generalized on at least one side of the interaction. It is possible that partnerships often arise, persist, or dissolve with little or no impact on the diversification of either lineage. A number of other problems with “double-dating”

are noteworthy. First, as illustrated by the large error bars in Figure 3.2, uncertainty associated with age estimates reduces our power to reject the null hypothesis of asynchronous divergence. Second, relatively weak permutation tests are employed instead of linear models because age estimates for hierarchically nested clades are autocorrelated. Lastly, accurate and independent fossil evidence or geological dates for partner lineages are often unavailable (see Box 3.1).

Phylogenetic approaches to identifying shifts in diversification rate are also popular (Paradis 2011). Maximum likelihood models of speciation and extinction (Stadler 2010) have been employed to suggest that mutualism promotes diversification across a broad spectrum of interactions (Litsios et al. 2012, Gomez and Verdu 2012, Stireman et al. 2010). Examples of symbiotic dinoflagellates, mycorrhizal fungi, gut endosymbionts, cleaner fish, pollinators, and seed dispersers will serve to illustrate this central point in Section 3.4. We will also aim to describe how models that estimate diversification rates and patterns of character evolution simultaneously (Maddison et al. 2007) provide further opportunity

to test the hypothesis of mutualism as a driver of diversity. For example, are gains or losses of mutualistic traits associated with shifts in rates of speciation or extinction? In rare circumstances, it may even be possible to identify the genetic changes responsible for such shifts (Chapter 5). Wolfe et al. (2014) associated the origin and expansion of ectomycorrhizal symbiosis in agaric mushrooms with the loss of the particular metabolic pathway that is required to derive nutrition from decaying wood.

Beyond phylogeny and the fossil record, the ecology of contemporary interactions and actual observations of evolution in species populations must also inform our interpretation of the past (Boucher et al. 1982). A synthetic understanding of the causes and consequences of mutualism will require a view of history that is consistent with microevolutionary processes (Chapter 5) and experimental evidence. In the remainder of this chapter, we aim to explore the extent of macroevolutionary patterns with this broader goal in mind.

3.3 Origins of mutualism

We have outlined how the fossil record and phylogeny can establish minimum ages for lineages in partnership and trigger speculation on the origin of mutualism. For example, the origin of land plants has been hypothesized to involve a fungal symbiosis that enabled plants to contend with the terrestrial challenge of efficiently acquiring mineral nutrients while avoiding desiccation (Pirozynski and Malloch 1975). The symbiotic functions implied by this hypothesis are based on the similar appearance of fungal hyphae in fossilized early land plants when compared to those found in contemporary ectomycorrhizae. That some reference to modern ecology is nearly always invoked in such historical interpretations raises the question of whether we could ever recognize the origin of a mutualism with no modern analog. Nonetheless, phylogenetic evidence that potential partners were at least likely to have been contemporaneous is valuable and, in rare cases of fossil copreservation, it may even be possible to establish the antiquity of particular partnerships. In this section, we examine in greater depth the phylogenetic evidence for opportunism

and antagonism as alternate paths to the origin of mutualism.

3.3.1 Chance and opportunity

When partner phenotypes appear to be coupled, as in the example of elongate insect proboscides matching the tubular channels of gymnosperm cones (Figure 3.2), we are tempted to regard the exchange of benefits among mutualists as necessarily the result of adaptation where an interaction has precipitated some kind of evolutionary genetic change in one or more partners. (Chapter 5 provides examples of this phenomenon and how to recognize it.) However, the origin of a mutualism need not coincide with genetic change in either partner, and mutually beneficial associations can originate from unexpected encounters among species. The expansion of a species' geographic range, for example, could immediately engage partners in the exchange of benefits with no prior history of interaction. Janzen (1980) described the likely scenario in which a vertebrate frugivore newly colonizes the habitat of a tree species already possessing fruit traits associated with vertebrate seed dispersal. Mutualism is observed among partners with little or no history of association in the case of exotic invasive plant species dispersed by native birds (Gleditsch and Carlos 2011, Deckers et al. 2007, Foster and Robinson 2007), where interactions happen to confer mutual benefit by chance. Such opportunities for mutualism are likely to be influenced by particular traits regardless of how the organisms came to possess them. In other words, mutualism may arise from coincidental combinations of preexisting adaptations and not necessarily by the operation of selective processes optimizing interactions around mutual benefit.

It may be especially challenging to identify evolutionary origins of mutualism among generalists. Fleshy-fruited plants that enclose their propagules in colorful, nutritious pulp often simultaneously attract and reward a broad range of vertebrates, including bats, birds, and primates, any of which may be able to deposit viable seeds in favorable environments (Herrera and Pellmyr 2002). Vertebrate foraging and perching behavior, functional morphologies that swallow intact seeds, and gut

metabolism favoring rapid passage further appear to facilitate seed dispersal. Whether such traits evolved in the context of interactions among particular species or clades is likely to remain obscure because the very adaptations that appear to foster opportunities for mutualism among generalists are often resilient to changes in partner affiliation (see also Box 10.2 on the context dependency of seed dispersal interactions).

3.3.2 From antagonism to mutualism and back

Mutualisms may originate from antagonistic interactions such as parasitism and predation (Thompson 1982, Thompson 1994) and the collapse of mutualism also has the potential to give way to parasitic or predatory interactions (Kiers et al. 2010). In this section, we consider the kinds of evidence and theory that support such evolutionary transitions. We begin with examples of bacteria in which diverse life histories and a multiplicity of associations with eukaryotic hosts provide an opportunity to observe general patterns. Molecular phylogeny suggests that mutualistic bacteria have evolved more often from parasitic than from free-living ancestors (Sachs et al. 2014). There appear to have been numerous independent origins of mutualism in bacteria with little or no ecological genomic predisposition for particular hosts, and horizontal transfer of genes may have often accompanied the evolution of mutualistic associations, especially involving nitrogen fixation (Sachs et al. 2011). Contrary to theoretical models predicting that mutualists could be vulnerable to extinction and reversal to parasitism (Chapter 4), empirical data suggest that once bacterial lineages evolve to be mutualists, they rarely return to parasitic or free-living states (Sachs et al. 2011).

The phylogenetic hypothesis that mutualists are descended from antagonistic ancestors is best evaluated in simple cases, where uncertainty about ancestral states is low. Parrots, for example, comprise a large clade of seed predators with beaks capable of cracking most seeds. Vulturine parrots endemic to the island of New Guinea, however, have unusually soft beaks and pass intact seeds through the gut while maintaining a diet of fruit pulp instead of

seeds (Mack and Wright 1998). The derived phylogenetic position of vulturines suggests that changes in behavior, diet, and morphology were associated with the loss of a seed predatory habit and the origin of a seed dispersal mutualism. This particular transition from predator to disperser almost certainly involved prior associations with the same or similar plant lineages, whereas other transitions from antagonism to mutualism appear to be accompanied by radical host shifts. For example, the cleaner wrasse belongs to a family of reef fish in which the habit of cleaning other fish species appears to have evolved only once from a coral-feeding habit (Cowman et al. 2009). In none of the cases of cleaner wrasse, vulturine parrots, or symbiotic bacteria does phylogeny suggest evolutionary reversals to antagonism.

Obligate pollination mutualisms involving seed predators are among the most striking cases in which phylogeny and ecology point to antagonistic origins of mutualism. Examples include wasps (Agaonidae: Chalcidoidea) that pollinate the flowers of figs (*Ficus*), and various seed-eating moths that pollinate *Yucca* plants, *Senita* cacti, and *Glochidion* trees (Moe et al. 2012). The insects lay eggs in a fraction of the flowers they pollinate where their offspring prey on developing seeds. They appear to have retained the highly specialized, seed-predatory life histories of their ancestors while having also acquired adaptations for pollen delivery that serve the reproductive interests of both partners (Pellmyr and Thompson 1992). The extreme species specificity of obligate mutualisms, in which neither partner persists without the other, provides an opportunity to infer ancient host associations with a degree of confidence that is rarely the case in generalized or facultative mutualisms. Identifying the origins of mutualism is therefore limited not only by the extent of systematic knowledge and the power of phylogenetic methods but also by our incapacity to recognize likely participants in ancient associations and the precise nature of their interactions.

Theory on the evolution of virulence predicts the evolution of reduced antagonism (and perhaps shifts to mutualism) in cases of parasitism where host fidelity is high and the availability of alternate hosts is low (Chapter 4). A tendency toward

mutualism is thought to arise when partners are maternally inherited, or are otherwise vertically transmitted to offspring. Alternatively, parasites are predicted to be more virulent when horizontal modes of infecting new hosts are prevalent. Ecological or genetic change altering the predominant mode of parasite transmission could trigger evolutionary transitions to or from mutualism. An experimental test of this theory manipulated the transmission mode of a jellyfish photosymbiont (Sachs and Wilcox 2006). Horizontally transmitted algae reduced the growth rate of the host and were more prolific compared to vertically transmitted algae in accord with the prediction of increased virulence. It remains unclear whether altering the mode of transmission alone would be sufficient to trigger the breakdown of mutualism and an evolutionary shift to parasitism.

Interactions among fig wasps and their nematode parasites illustrate how the evolution of less virulent genotypes may be favored in scenarios of vertical transmission when potential hosts are few (Herre et al. 1999). Nematodes are transported among figs by female wasps, and opportunities for colonizing new hosts depend on the number of females that pollinate and lay eggs in a given fig. Nematode reproductive success is closely tied to that of a particular wasp in figs pollinated by a single female where nematodes have no choice other than to infect her offspring. In agreement with theory on the evolution of virulence, Herre (1993) demonstrated that nematodes of singly pollinated fig species were less virulent than those of multiply pollinated fig species. However, a transition to mutualism in which vertically transmitted nematodes increase the fitness of their host wasps has yet to be observed. The fact that horizontal transmission of mutualistic partners is common in nature is also a challenge for virulence theory (Sachs and Wilcox 2006).

Microbes may provide the most compelling evidence for the rapid evolution of mutualism from antagonism. For example, *Wolbachia* (see Box 2.1) are maternally inherited bacteria known to confer cytoplasmic incompatibility between infected and uninfected invertebrates such that reduced fecundity is observed among infected females who mate with uninfected males. As a *Wolbachia* infection spread among populations of the fruit fly *Drosophila*

simulans, increases in fecundity among infected individuals were observed over time (Weeks et al. 2007). The evolution of *Wolbachia* from a parasitic, host fecundity-decreasing strain to a fecundity-increasing strain supports the prediction from virulence theory that transitions to mutualism may be favored under conditions of vertical transmission in which opportunities for parasites to colonize new hosts are curtailed.

Few mutualisms lend themselves to such elegant tests of virulence theory, and evidence of origins, whether antagonistic or otherwise, is unavailable for some of the most obvious partnerships on earth. Endosymbiosis among prokaryotes, for example, is thought to have produced eukaryotic life when mitochondria and plastids evolved from interactions among free-living cyanobacteria and proteobacteria, respectively (Kutschera and Niklas 2005). We do not know whether ancestral prokaryotic associations might have shifted from parasitism to mutualism prior to or concurrent with the evolutionary events that caused mitochondria to develop an integral metabolic role in the eukaryotic cell. Perhaps experimental evolution, molecular genetics, and cell biology will provide mechanistic insights in the future.

Context dependency of species interactions is a further source of frustration in attempting to embed the origin of mutualism in antagonistic interactions. Costs and benefits for partners are likely to vary among ecological conditions as influenced by third parties and the abiotic environment (Chapter 10). For example, toxic alkaloids produced by fungal plant pathogens (Clavicipitaceae) are thought to defend infected grasses against herbivores (Faeth 2002, Rodriguez et al. 2009), but the nature of this interaction could range from mutualism to antagonism depending on the extent of herbivory. Associations of ectomycorrhizal fungi with host plants also vary from parasitic to mutualistic according to the ecological context (Hoeksema et al. 2010), and experimental evidence from the nitrogen-fixing legume-*Rhizobium* symbiosis suggests that non-mutualistic rhizobia may be selected when nitrogen is not limiting. Progress in ecological genetics has highlighted the importance of context in shaping the dynamics of interactions, and how even some very ancient partnerships continue to traverse a

continuum between antagonism and mutualism (Heath and Stinchcombe 2013). It might be worthwhile to consider the additional possibility that some mutualisms might trace their origins to commensal situations in which one partner benefits without affecting the other, or amensalism, in which a partner is harmed without benefiting the other.

3.4 Diversification of mutualism

The possibility that mutually beneficial interactions might promote species diversification has attracted the interest of evolutionary biologists since Darwin (Dodd et al. 1999, Vamosi and Vamosi 2010). Empirical examples of the phenomenon are for the most part limited to cases in which mutualism either catalyzes a new ecological opportunity or enforces the evolution of reproductive isolation (Hembry et al. 2014). Increased diversification is generally attributed to either an elevated rate of speciation or a decreased extinction rate. Whether and how species associations affect extinction rates are likely to depend on the nature, frequency, specificity, and persistence of partnerships through evolutionary time. For example, defensive mutualisms could contribute to the persistence of species populations by reducing rates of predation whereas mutualists that are wholly dependent on their partners for survival might share an increased risk of extinction. Mutualism could also elevate rates of speciation either indirectly by expanding the range of ecological opportunities available to lineages in partnership or directly by influencing the reproductive isolation and divergence of partnered populations.

The alternative possibility, that mutualism could hinder species diversification, is also worthy of consideration. Interdependence among species is commonly expected to increase the risk of extinction despite relatively little evidence to support the idea (Dunn et al. 2009). Perhaps coextinction is pervasive but so rapid that it leaves no trace, or mutualisms might be more resilient than is assumed by models of coexistence. Efforts to develop theoretical models of divergence in mutualisms along these lines have been relatively modest compared to the extensive theory on mutualism origin and maintenance (see Chapter 4). An alternative

approach involves empirical comparisons of relative diversity. Considering that lineages diverging from a common ancestor have had equal time to diversify, evidence for a shift in the diversification rate can be gleaned by comparing the relative size of a mutualistic lineage to a non-mutualistic sister group. Insight may also be gained by comparing the relative diversity of lineages engaged in particular mutualisms. A review of patterns of diversity among relatives and partners aims to examine whether mutualism has special or predictable consequences for diversification.

3.4.1 Comparing relatives

The comparison of closely related clades either engaged in mutualism or not may identify differences in relative diversity that could be attributed to mutualism. Mutualisms involving symbiotic fungi, dinoflagellates, gut endosymbionts, cleaners, pollinators, and seed dispersers suggest conditions and circumstances for speculation. Our consideration of these examples represents but the first step toward understanding potential influences of beneficial interactions on divergence and speciation. A recent study of ambrosia gall midges, for example, examined how the acquisition of fungal symbionts might catalyze lineage diversification. These insects induce galls in their host plants by laying eggs and by inoculating their hosts with pathogenic fungal spores where midge larvae feed on fungal hyphae inside of the galls. Joy (2013) compared lineages of ambrosia gall midges that form a mutualism with fungi to non-mutualistic lineages and the number of host plant taxa in the diet of each. These comparisons suggest not only that the mutualism between gall midges and fungi catalyzed diversification, but that the mutualistic lineage also had a sevenfold increase in the range of host plants used, consistent with the idea of mutualism facilitating niche expansion.

The fig pollination mutualism provides an example of how a shift in diversification may result from the rapid evolution of reproductive isolation (Moe et al. 2012). *Ficus* is ten times more numerous in species than its closest relatives in the tribe Castilleae of the mulberry family (Moraceae) that participate in a similarly specialized insect

brood-site pollination mutualism. The accelerated rate of speciation in *Ficus* compared to Castilleae can be attributed to both the nature and accessibility of floral rewards. The life history of thrips pollinators of Castilleae involves pollen-feeding throughout life and multiple visits to inflorescences per generation. In comparison, the fig ovules that serve as brood sites for fig wasps are enclosed by inflorescences in a manner that limits each pollinator to a single visit per lifetime, which carries a severe fitness consequence for choosing an unfavorable fig (Moe and Weiblen 2012). Fig wasps might, therefore, be highly inclined to pollinate figs resembling their birth fig, with the further consequence of restricted gene flow among figs exhibiting divergent chemosensory attractants (Moe et al. 2012). In this scenario, accelerated speciation in figs need not necessarily be the result of mutualism facilitating niche expansion; rather, diversification could be a byproduct of extreme specialization in which the reproductive interests of partners are inextricably aligned.

Mutualism with sea anemones has been implicated in the diversification of clownfish (Litsios et al. 2012). Clownfish secrete protective mucus that permits their close association with anemones despite the presence of poisonous nematocysts that aid anemones in preying upon other small fish. Molecular phylogeny supported the monophyly of clownfish, with inferred rates of speciation significantly higher than in the recently identified sister group of damselfish. However, there are comparable cases in which mutualistic clades are no more species rich than their non-mutualistic relatives. For example, specialized cleaner fish (*Labroides*) are no more diverse than their antagonistic relatives (Labrichthyines) after ~10 My of mutualism (Cowman et al. 2009). That only five *Labroides* species are known, compared to ten species of coral-feeding Labrichthyines (Parenti and Randall 2000), indicates that the acquisition of a mutualistic habit need not necessarily result in diversification.

Molecular genetic analysis has revealed extensive species diversity among *Symbiodinium* dinoflagellates (Sampayo et al. 2009), the photosynthetic symbionts (“zooxanthellae”) of marine invertebrates including anemones, corals, jellyfish, sponges, flatworms, and

mollusks, in which products of photosynthesis and inorganic nutrients are exchanged (see Box 10.3 for examples). Although fossil-calibrated molecular dating suggests that *Symbiodinium* is ancient, at least of early Eocene origin (Pochon et al. 2006), it remains unclear whether *Symbiodinium* is more or less diverse than other free-living or endoparasitic dinoflagellates. Relationships among major dinoflagellate clades are poorly resolved, and taxonomic knowledge of most groups is inadequate to either estimate or compare species diversity. Conversely, we might compare the diversity of marine invertebrate lineages with “zooxanthellae” to closely related lineages without such symbionts. For example, are anthozoans (corals and sea anemones) hosting *Symbiodinium* more diverse than their non-zooxanthellate relatives? Thousands of mutualistic soft coral species appear to be closely related to a few hundred species of plankton-feeding sea pens (Pennatulacea). However, once again, appropriate sister group comparisons are hindered by poor phylogenetic resolution and clade support (McFadden et al. 2006). Efforts to compare relatives are likely to be aided by phylogenomic approaches that hold promise for a more highly resolved tree of life.

3.4.2 Comparing partners

In Box 3.2, Jiri Hulcr describes methods for comparing the relative diversity of partners. He emphasizes examples in which phylogenetic patterns are suggestive of codiversification. Although such patterns can be impressive, the accumulation or loss of species in partnered lineages is also likely to be affected by intrinsic and extrinsic factors such that the diversification of partners need not be coupled. For example, comparable numbers of fig species and fig wasp species suggests parallel diversification (Figure 3.2), but the pattern is less striking in other brood-site pollination mutualisms such as yuccas and yucca moths (Althoff et al. 2012, Smith et al. 2008). Many mutualisms, in fact, exhibit highly asymmetrical patterns of diversity among partner lineages. In the lichen symbiosis, numerous lineages of ascomycete fungi engage a relatively small set of algae and cyanobacteria as photobionts (Arnold et al. 2009). If we assume that species concepts are biologically meaningful, then it follows that rates of diversification are

substantially higher for the lichenized fungi than for their algal photobionts. Mutualism has been implicated in the diversification of ascomycetes by enabling these organisms to extend the range of environments suitable for growth and survival. In other partnerships, the diversification of one lineage or the other may have occurred long prior to the origin of the mutualism. For example, the oxpecker bird cleans ticks and ectoparasites from dozens of ungulate species (Nunney 1993) that evidently radiated in Africa long before the oxpecker evolved its highly specialized diet.

Asymmetrical diversity among mutualistic partners (see Chapter 11) more often involves a large number of associates with a small number of hosts. For example, in the case of fungal symbionts and vascular plants, approximately thirty species of alder trees (*Alnus*) are facultatively associated with hundreds of ectomycorrhizal fungi (Walker et al. 2014). Sampling ectomycorrhizal communities across the geographic distribution of a given alder species suggests a degree of phylogeographic concordance between hosts and symbionts that is better explained by comigration than by codiversification (Kennedy et al. 2011). The whistling thorn (*Acacia drepanolobium*), a long-lived East African tree hosting four different ant symbionts, illustrates how unequal numbers of partners could be selected and maintained in a mutualism. Each ant symbiont provides a different quality of service during host plant development and whistling thorns achieve the greatest overall benefit when all four are present (Palmer et al. 2010). Codiversification, therefore, appears to be but one of several possibilities.

Fungus-farming ants (see Box 5.1) were once thought to have codiversified with their symbiotic fungi (Currie et al. 2003). However, more recent phylogenetic evidence suggests that multiple lineages of fungi were domesticated independently (Dentinger et al. 2009). The discovery of an unexpected branch in the fungal tree of life that is also engaged in the mutualism with fungus-farming ants reminds us to exercise caution in interpreting patterns of codiversification when sampling is incomplete. In another example, Hongoh et al. (2005) sampled termite gut symbionts from multiple host species and found that congeneric

termites harbored similar bacteria regardless of the individual, colony, location, or host species. The authors suggest that the gut microbiome may have diversified in parallel with termite lineages, but, as in early studies of fungus gardening ants, sampling was rather limited.

Returning to the extreme case of figs and their pollinating wasps, Weiblen and Bush (2002) reported the first phylogenetic comparison of obligate mutualists and antagonists interacting with the same set of hosts. Although the extent of species specificity in fig pollination has been questioned (Machado et al. 2005), most subsequent comparisons of pollinating and non-pollinating wasps have shown that speciation in figs appears to be more closely coupled with pollinator diversification than with speciation in the non-pollinating parasites (Marussich and Machado 2007, Silvieus et al. 2008; but see also Jousset et al. 2008). This pattern might be best explained by the impact of selection acting on pollinator host recognition (see Section 3.2.1) for the evolution of plant reproductive isolation (Moe and Weiblen 2012). However, in such highly specialized mutualisms, where partners are often codistributed, it is difficult to disentangle the relative influence of geographic isolation and selection on speciation (Hembry et al. 2014). It seems likely that these factors interact to produce phylogenetic patterns.

Mechanistic explanations for patterns of species diversity can be confusing if the distinction between trait and lineage diversification is not made very explicit (see Chapter 7). For example, cophylogeny has little to do with coevolution (meaning correlated trait evolution in the strict sense), although the two have often been conflated (see examples in Box 7.1). However tempting it is to interpret congruent phylogenetic patterns among obligate mutualists as evidence of coevolution, such patterns need not be explained by reciprocal selection. This is why we have limited the scope of our chapter thus far to questions of when and where mutualisms arose, how often they may have been lost, and whether rates of partner speciation or extinction correlate through time. We conclude our treatment by considering how to evaluate hypotheses that particular traits have influenced the origin and diversification of mutualism.

Box 3.2 Phylogenetics in symbiology

Jiri Hulcr

Detecting a symbiotic relationship between two organisms or two groups of organisms can be carried out by experiments, such as the removal or manipulation of one partner to measure effects on the other partner. Where experiments are not feasible, or where complex symbiotic systems are studied, the inference of pattern may provide some insight. One such approach is, of course, phylogenetics. Many phylogenetic methods are available to measure the extent and even statistical significance of co-occurrence between putative symbionts across populations, between species, or in evolutionary time.

The phylogenetic approach with the longest tradition in symbiology includes tests of congruence between phylogenies of two groups of putatively symbiotic organisms (species, or populations within species). This approach is based on the assumption that a stable symbiosis may lead to shared evolutionary paths such that phylogenies of the groups are predicted to be congruent. A number of methods exist to test for congruence of evolutionary histories; for example, congruence between two cladograms using programs such as TreeMap (Charleston 2011) and Jane (Conow et al. 2010) or between data matrices underlying phylogeny estimates as with the incongruence length difference test (Farris et al. 1994), likelihood ratio tests (Huelsenbeck and Bull 1996), and the Shimodaira–Hasegawa test (Huelsenbeck et al. 1997), or the relative timing of cladogenesis. Examples of the latter are the studies of Rønsted et al. (2005) and Cruaud et al. (2012), which compared phylogenies of figs and fig wasps, the specialized fig pollinators. These analyses supported the prediction of parallel phylogenies of symbionts both in terms of the pattern and timing of cladogenesis.

Increasingly, many symbiotic systems are shown to be not bipartite but multipartite. For example, communities of microbial symbionts of animal intestines often include thousands of operational taxonomic units (OTUs), a product of diffuse community coevolution rather than stepwise cocladogenesis. From the perspective of phylogenetic analysis, symbiotic communities differ from one-on-one symbioses in two main respects. First, it is not possible, or at least not informative, to test for cophylogeny between the macrosymbiont and each of the thousands of suspected microsymbionts. Second, symbionts differ widely in the specificity of their association. In such complex communities, the test statistic is no longer the congruence of two phylogenies, but whether the distribution of the symbiotic communities on the macrosymbiont phylogeny (or population network) is non-random. Symbiont

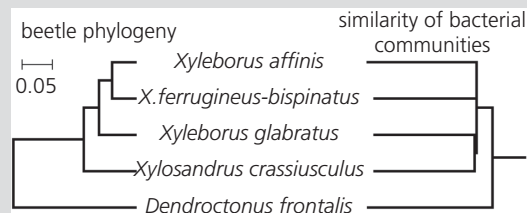


Figure 1 Microsymbiotic communities are often highly similar among closely related macrosymbionts. For example, bacteria are shared among *Xyleborus* and *Xylosandrus* ambrosia beetles to a greater extent than with a more distant relative (Hulcr et al. 2012). Such analyses of complex symbiotic systems are increasingly possible due to the integration of phylogenetics, statistics, and high-throughput DNA sequencing. The scale refers to patristic phylogenetic distances between beetle species, derived from concatenated sequences of 28S ribosomal and cytochrome oxidase I mitochondrial DNA. Reproduced under the Creative Commons Attribution license.

community similarity can be measured by indices that are entirely non-phylogenetic (e.g., the Bray–Curtis index), or they can take into account the phylogenetic relatedness of the microsymbionts involved (e.g., UNIFRAC; Lozupone and Knight 2005). Correlation between community similarities and phylogenetic distances may be tested by methods that correct for non-independence among phylogenetic distances (for example the Mantel test, or permutational multivariate analysis of variance; Oksanen et al. 2011).

High-throughput DNA sequence data and multiplexing of samples allows for the integration of phylogenies, community similarity measures, and environmental factors in statistically robust sampling designs enabling analyses of complex symbiotic systems (Box 3.2, Figure 1). Hulcr et al. (2012) tested for signatures of symbiosis between ambrosia beetles and diverse communities of bacteria without prior taxonomic knowledge of these microbes. By comparing the effect of the host beetle phylogeny to that of beetle provenance using the similarity among the respective bacterial communities, it was shown that the dominant bacterial taxa are vertically transmitted, rather than acquired from the environment. However, no bacterial taxa appeared to be obligate symbionts.

Species-level and population-level phylogenetic methods serve to detect patterns of association and have limited capacity to identify mechanisms of symbiosis. However, phylogenetics may be used to illuminate mechanisms of a symbiosis when focused on genes that encode or regulate symbiotic functions. For example, Moulin et al. (2004)

Box 3.2 *Continued*

examined the phylogeny of *Bradyrhizobium*, nitrogen-fixing bacteria that live in symbiosis with plants. According to housekeeping and ribosomal genes with no particular symbiotic function, the symbiotic habit evolved several times independently among these bacteria. On the other hand, genes involved in the signaling pathways of symbiosis appear to be monophyletic and only loosely congruent with the organismal phylogeny. This suggests that mutualistic function may not have evolved independently but rather might have arisen by lateral transfer of genes facilitating the spread of symbiotic capacity among mutualistic *Bradyrhizobium*.

Despite the power of phylogenetics as an inferential tool in symbiology, there remain essential questions that analyses of pattern are unable to answer. For example, measuring the fitness costs and benefits of associations to understand whether associates are mutualists, commensals, or parasites generally will require experimental study of biological processes.

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3.5 Innovation and adaptation

It is popular to invoke adaptive trait evolution when interpreting repeated origins of mutualism. New mutualistic phenotypes have the potential to increase fitness, affect reproductive isolation, or enable partners to occupy new niches. For example, lipid-rich appendages attached to seeds (elaiosomes) are regarded as adaptations for seed dispersal by ants (myrmecochory) that have arisen independently in many different plant lineages (Lengyel et al. 2009). Lengyel et al. (2009) compared diversification rates among elaiosome-bearing plant lineages to non-myrmecochorous sister groups and attributed the greater diversification of ant-dispersed lineages to either a reduced extinction rate or to ant-mediated barriers to gene flow affecting plant speciation. A similar example involves elaborate presentations of floral rewards to animal pollinators

that incidentally might have accelerated the evolution of reproductive isolation among plant populations. Hodges and Arnold (1995) proposed that the evolution of “nectar spurs” (specialized tubular structures often varying among relatives in coloration, form, and the localization of nectar) may have triggered the diversification of floral form and the rapid evolution of reproductive isolation by limiting floral attractiveness to particular kinds of visitors such as hummingbirds, bees, or butterflies. Such hypotheses are both appealing and difficult to test.

At the very least, we would like to know whether the gain or loss of mutualistic traits is significantly correlated with increasing or decreasing rates of diversification. Maddison et al. (2007) introduced a binary-state speciation and extinction model (BiSSE) to specifically address such questions. Unlike methods that indirectly compare diversification rates among sister clades of mutualists and

non-mutualists, BiSSE directly examines whether changes in character states along branches of a phylogenetic tree are correlated with shifts in rates of diversification among clades. A further improvement to this model includes the potential for trait evolution to coincide with speciation as well as to occur along branches of the tree (Goldberg and Igic 2012). Although statistical power is low when taxon samples are fewer than several hundred or when the frequency of alternative character states is uneven (Davis et al. 2013), these models have the potential to identify mutualistic traits associated with diversification. They have supported key innovation hypotheses involving mutualism between gall midges and fungi (Stireman et al. 2010), primates and plants (Gomez and Verdu 2012), and sea anemones and clownfish (Litsios et al. 2012).

If adaptive traits are associated with the origin of mutualism, then perhaps the coevolution of mutualistic traits could play a role in maintaining partnerships over time. Theory predicts the collapse of mutualism in which partners might either defect or assume antagonistic relations under conditions in which the cost of participating in the mutualism exceeds the benefit (Chapters 4, 6 and 13). It has been popular to measure correlations among interacting traits and to attribute the matching of mutualistic traits to “coevolutionary complementarity” through which partnerships are maintained by reciprocal selection (Chapter 7). Phylogenetic methods cannot easily disentangle correlation and causation, but we might yet gain some insight by comparing the strength of trait correlations within lineages (e.g., allometric relationships) to mutualistic trait correlations among partnered lineages (i.e., phenotype matching). When the latter correlations are stronger than simple allometry, reciprocal selection is a potential explanation that may be tested by experiment. For example, lengths of fig wasp ovipositors that interact with fig flowers in the exchange of pollination services for seed provisioning to offspring are more highly correlated with flower size than with wasp body size (Weiblen 2004). Patterns of phenotypic variation in the obligate moth pollinators of the Joshua tree (*Yucca brevifolia*) also match geographic variation in floral forms despite substantial gene flow between varieties (Yoder et al. 2013). Intriguingly, a theoretical model of trait

coevolution suggested that mutualism might, in fact, favor the conservation of phenotypes, whereas antagonism favors trait diversification (Yoder and Nuismer 2010). Empirical evidence of fitness differences influencing oviposition preference according to the larval performance of pollinating seed predators (Moe and Weiblen 2012) is consistent with this model in suggesting the conservation of matching phenotypes in such mutualisms (Godsoe et al. 2010).

Insect herbivores and their microbial associates hold promise as systems in which to evaluate the possibility of mutualism as a catalyst for adaptive diversification (Janson et al. 2008). Dietary specialization is common among phytophagous insects such that opportunities for diversification may arise through the colonization and exploitation of novel host plants. Microbial mutualists that confer benefits to their insect hosts by assimilating toxic or recalcitrant compounds in the diet or by protecting hosts from natural enemies could affect the balance of speciation and extinction. Microbes might either promote or prevent diversification by influencing the palatability of host plants to herbivores, or by modifying fitness trade-offs associated with the colonization of new host plants, or by altering tri-trophic interactions (Janson et al. 2008). Insect life histories seem especially amenable to experimental studies that manipulate host plants in conjunction with the gut microbiome to measure fitness and gauge the extent of adaptive evolution.

3.6 Conclusion

Mutualism is often regarded as an engine for ecological opportunity, a source of new adaptive landscapes, and a stage for coevolutionary dynamics. Patterns emerging from investigations of the past suggest processes affecting the origin and diversification of mutualism. Paleontology provides essential but incomplete information. Phylogenetic models and comparative methods have potential to bridge gaps in knowledge insofar as inferences are robust to uncertainty. Mutualism can arise through coincidental combinations of preexisting adaptations, from antagonistic interactions such as parasitism or predation, or perhaps even from amensalism. Comparisons of relative species diversity between

mutualistic and non-mutualistic sister groups indicate that mutualism is sometimes associated with diversification, but lineages in partnership need not diversify in parallel. The repeated evolution of mutualism is often interpreted in terms of adaptive traits. Although such hypotheses are difficult to test, a promising approach may be to apply phylogenetic models that simultaneously estimate character change within lineages and shifts in rates of speciation and extinction among lineages. Another intriguing avenue for comparative study in the context of mutualism is to examine the strength of trait correlations within lineages to that of interacting traits among lineages in partnership.

Future directions and opportunities for understanding the evolutionary history of mutualism will doubtless be advanced by the availability of genomic data. Key questions remain unanswered. Do mutualisms yield unique patterns of diversification over time or have other forms of species interaction such as parasitism diversified similarly? Perhaps it is not the beneficial nature of mutualism, but rather the extent of species specialization or reproductive isolating mechanisms that account for patterns of diversity. Ecological context, population density, generation time, and the geographic distribution of genetic variation in relevant traits are also worthy of consideration. When mutualisms are not maintained, should we expect extinction or antagonism? There appears to be relatively little evidence to support the idea that species interdependence increases the risk of extinction. Habitat loss and climate change are likely to impact mutualism in the future, but it remains unclear whether mutualists are more or less at risk than parasites or other non-mutualists in changing environments (see Chapter 12). The potential for decline of nitrogen-fixing mutualisms in response to anthropogenic nitrogen deposition is already a serious concern (Kiers et al. 2010). If we are to measure risks of extinction, we need to understand the interplay of specialization, partnership lability, and trophic cascades on the survival of species. Opportunities are emerging to investigate these questions and to better understand responses of mutualisms to global change, including the potential for extinction, ecosystem collapse, or unprecedented kinds of interaction.

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