The evolution of mutualisms: exploring the paths between conflict and cooperation


Mutualisms are of fundamental importance in all ecosystems but their very existence poses a series of challenging evolutionary questions. Recently, the application of molecular analyses combined with theoretical advances have transformed our understanding of many specific systems, thereby contributing to the possibility of a more general understanding of the factors that influence mutualisms.

The passage of symbionts from parent to offspring (vertical transmission), genotypic uniformity of symbionts within individual hosts, spatial structure of populations leading to repeated interactions between would-be mutualists, and restricted options outside the relationship for both partners are thought to align interests and promote long-term stability. Conversely, movement of symbionts between unrelated hosts (horizontal transmission), multiple symbiont genotypes and varied options are thought to unravel the host-symbiont align with those of the host. Given that conflicts of interest can occur even within the genomes of single individuals, it seems unlikely that the interests of mutualists will ever be completely concordant. Although there is no general theory of mutualism, several factors that can help align mutualists' interests have been identified.

The expanding availability of a wide range of molecular data has produced qualitative leaps in the types of information available to researchers. This information can be usefully combined with the results from field and laboratory studies. For example, genetic characterization of mutualists has facilitated the unambiguous determination of the number and identity of interactants (e.g. genotypes and species), the degree and scale of their specificities and their patterns of ecological transmission. Similar approaches can also reveal the phylogenetic patterns of relationships both between and within taxa of mutualists, and thus the evolutionary history of organisms. Further, molecular analyses have shown that microbial communities can be highly complex and diverse, suggesting that the interactions between hosts and symbionts may be dynamic and continuously evolving. The resulting genetic homogeneity of symbionts within a host reduces selection for traits that increase between-symbiont competitive ability to the detriment of the host's growth and survival. A vertical transmission implies a continual interaction between host and symbiont lineages. The absence of an independent phase in a symbiont's life cycle facilitates the evolution of complete dependence, which reduces the evolutionary viability of nonsymbiotic alternatives over the long term.
Being dominated by cospeciation between the two taxa\textsuperscript{19}. This work suggests that the long evolutionary history of figs and their pollinators has made the relationship overwhelmingly species-specific. In addition, recent molecular data indicate that the wasps depend on the figs to complete their life cycle. Fossil evidence indicates that support development of wasp offspring compared with the proportion of the fig seeds that are produced by the wasps. What prevents the shorter lived and much more numerous wasps from exploiting the figs?

There are over 700 species of figs (\textit{Ficus}) described worldwide. The figs depend on minute pollinator wasps (Agaonidae) for continued sexual reproduction, and the wasps benefit only from the production of offspring (that necessarily support development of wasp offspring). However, in several ecologically dominant corals, it is now known that a single coral species and the single colony are capable of hosting two or more types of symbiont\textsuperscript{12,26–28}. Zonation of symbionts across the reef and within colonies appears to be related to levels of light. During adverse conditions, such as unusually high temperature, the mutualism between corals and algae can break down ("coral bleaching") in complex patterns that reflect this zonation. Thus, from the coral's perspective, the host is more like a landscape composed of more and less suitable conditions than a uniformly hospitable environment\textsuperscript{26}.

Patterns of ecological transmission and evolutionary association

Nonetheless, not all mutualisms follow this pattern of vertical transmission, and there are many exceptions. For example, many marine symbionts (Box 1) and mutualist associates of plants (e.g. pollinators (Box 2) and mycorrhizae) are horizontally transmitted, yet they are usually clearly beneficial. Moreover, vertical transmission does not guarantee benevolence (Box 3). Given these exceptions, it is important to determine the extent to which real systems conform to these patterns, and what factors are most responsible for determining conformity where it exists.

Identifying the players

Determing the number and identities of the participants in mutualistic associations is a necessary first step for any evolutionary analysis, but it can be a surprisingly nontrivial task. Hosts and symbionts often lose characters found in their closest free-living relatives, or gain novel characters, making them difficult to distinguish and characterize taxonomically. The traditional solution for bacterial and fungal symbionts has been culturing. However, in some symbioses, what is successfully cultured does not necessarily reflect the actual community present in intact associations; and in other systems, symbionts cannot presently be cultured\textsuperscript{21,25,26}. For these reasons, molecular analyses have played a critical role both in genetically characterizing isolated mutualists and in screening assemblages directly to assess the nature of symbiont communities. The resulting discoveries of stunning and unexpected diversity have transformed our understanding of mutualisms involving corals (Box 1), leaf-cutter ants (Box 4), and root symbionts\textsuperscript{27,28}, among others. It is important to appreciate that symbiont diversity, cryptic and otherwise, can occur at different levels. At the level of different host species, different hosts can contain morphologically indistinguishable symbionts that are nevertheless quite distinct both genetically and functionally. At the level of different individual hosts within a species, genetically different symbionts can be found in association with different host individuals (or populations). Even within individual host organisms, several distinct symbionts can be found\textsuperscript{21,25,26}.

The recognition that individual hosts can act as landscapes for communities of potentially competing symbionts (Box 1) raises the question of why competition among symbionts does not destabilize the mutualism, much as competition among parasites is believed to result in selection for increased virulence\textsuperscript{21,25,26}. The ecological flexibility provided by symbiont diversity\textsuperscript{27,28} might play an important counterbalancing role.

Box 1. Marine invertebrates and photosynthetic algae: the ecological significance of cryptic symbiont diversity

Throughout the shallow tropical oceans, sessile animals often have symbiotic associations with photosynthetic, single-celled algae, previously the most spectacular example being the reefs. These associations are typically formed between reef-building corals and dinoflagellates of the genus \textit{Symbiodinium}. For many years, these symbionts were considered to be a single species, but physiological and genetic studies\textsuperscript{12,26–28} have revealed enormous, previously unsuspected, diversity. What was once considered a single species is now recognized as a group with at least three clades that, by extrapolation to free-living forms, are distinct at the family or ordinal level. These studies also revealed that there was no obvious concordance between host and symbiont phylogenies.

Despite the growing appreciation of this cryptic diversity, it remained widely assumed that any single host formed an association with only one type of symbiont. However, in several ecologically dominant corals, it is now known that a single coral species and the single colony are capable of hosting two or more types of symbiont\textsuperscript{12,26–28}. Zonation of symbionts across the reef and within colonies appears to be related to levels of light. During adverse conditions, such as unusually high temperature, the mutualism between corals and algae can break down ("coral bleaching") in complex patterns that reflect this zonation. Thus, from the coral's perspective, the host is more like a landscape composed of more and less suitable conditions than a uniformly hospitable environment\textsuperscript{26}.

From the coral's perspective, horizontal transmission and complex mixtures of symbionts might provide short-term ecological flexibility to cope with fluctuating physical conditions that outweigh the possible costs of evolutionary conflicts among symbionts\textsuperscript{25}. Many of the themes emerging from these studies of corals characterize other symbiotic systems as well\textsuperscript{12,26–28}.
Box 3. Wolbachia and Buchnera: the implications of horizontal versus vertical transmission for the evolution of mutualism

Theory suggests that vertical transmission selects for more benign relationships, and that symbionts transmitted vertically should generally have benign or even positive effects on their hosts. It is accumulating experimental and comparative support for this proposition. A classic example is the association found between aphids and their bacterium (Buchnera) that synthesizes necessary amino acids for their hosts[15-18]. However, Wolbachia appears to be in a maternally inherited endosymbiont that frequently has large negative effects on its host's reproductive success. At times, the bacteria distort the host's sex ratio, often leading to all female broods, or produce reproductive incompatibility with other host individuals that do not carry the same strain of Wolbachia[19]. Superficially, these observations contrast the theoretical predictions. However, to assess the relevance of these observations, the timescales over which maternal transmission occurs[20, 21] must be considered. Although most cases show that at an ecological timescale Wolbachia is transmitted vertically, there is clear phylogenetic evidence that Wolbachia jumps from lineage to lineage; that is, whether its propagation is considered to be dominant vertically transmission depends on temporal scales. In addition, Wolbachia can often have complex life histories, and any negative effect on its distal individual host[22]. Critical questions involve determining the actual routes and frequencies of horizontal transmission, as well as the magnitude of negative effects under real ecological situations, and then determining if there is a correspondence between "how bad the bugs are" and "how much evolutionary jumping they can do".

Box 4. Fungus-growing ants and their fungi: phylogenetic transitions in patterns of symbiont acquisition

The exclusively New World fungus-gardening ants in the tribe Attini (Formicidae) comprise over 200 described species, all obligately dependent upon the cultivation of fungus for food[23-27]. Ants in the leaf cutter genus Acromyrmex and Atta are ecologically and economically important because of the vast quantities of foliage and flowers that they cultivate and consume in their immense nests. Together with a few additional genera, leaf cutter ants are grouped into the monophyletic higher attine ant lineages, which comprise about one-half of the species diversity of the tribe. Ants in the remaining seven genera of lower attine ants are less conspicuous, frequently cryptic, and do not attack plants. The symbiotic associations of lower attine ants and their fungi are diverse: some species grow their fungi entirely on dead vegetation, matter, some entirely on caterpillar frass and others on a mixed substrate that can even include seeds. Molecular data have been decisive in identifying the evolutionary origins and phylogenetic relationships of attine fungal symbionts. First, although most anti-associated fungi are members of the family Leotiomycetaceae (Agaricales), Bdelloipsidyn, phylogenetic analyses based on rRNA (RNA) indicate that the fungi cultivated by several ant species in the lower attine genus Acronicta is closely related to other attine fungi, and has been secondarily acquired long after the mutualism originated in the Amazon Basin approximately 50 million years ago[24, 25]. Second, phylogenetic analyses indicate that several distinct lepiotoid fungal lineages are associated with lower attines and have been transmitted across the phylogenetic transitions in patterns of symbiont acquisition over evolutionary time, comparisons across related taxa (particularly in co-occurring systems) can show different evolutionary outcomes that represent variations on a single theme of mutualistic interaction (e.g. leaf cutters, lice, yuccas, ants, plants and cyanobacteria). Specifically, phylogenetic analyses reveal that parasitic lineages can be embedded in largely mutualistic groups and/or vice versa[26]. However, theory suggests that the species that parasitize mutualisms should not be the closest relatives to either partner[27]. Available evidence collected from lice (Eucnemidae) and fungwaps (Agaricales), and the yuccas (Yucca) and yucca moths (Tegeticula) supports this prediction[15, 18, 25]. Nonetheless, this proposition requires further testing.

Mutualisms as model systems

Molecular data are providing new insights into the evolution of mutualism. In those instances in which the host and mutualist co-occur, the absolute times of divergence between pairs of co-occurring mutualisms and their free-living relatives can be measured. This allows a series of potentially instructive comparisons to be made in the accumulation of substitutions in homologous DNA sequences. First, comparisons can be made between the accumulation of substitutions at a given gene or set of genes in the "host" and in the "symbiont" (or parasite).

Box 3. Fungus-growing ants and their fungi: phylogenetic transitions in patterns of symbiont acquisition

The exclusively New World fungus-gardening ants in the tribe Attini (Formicidae) comprise over 200 described species, all obligately dependent upon the cultivation of fungus for food[23-27]. Ants in the leaf-cutter genus Acromyrmex and Atta are ecologically and economically important because of the vast quantities of foliage and flowers that they cultivate and consume in their immense nests. Together with a few additional genera, leaf-cutter ants are grouped into the monophyletic higher attine ant lineages, which comprise about one-half of the species diversity of the tribe. Ants in the remaining seven genera of lower attine ants are less conspicuous, frequently cryptic, and do not attack plants. The symbiotic associations of lower attine ants and their fungi are diverse: some species grow their fungi entirely on dead vegetation, matter, some entirely on caterpillar frass and others on a mixed substrate that can even include seeds. Molecular data have been decisive in identifying the evolutionary origins and phylogenetic relationships of attine fungal symbionts. First, although most anti-associated fungi are members of the family Leotiomycetaceae (Agaricales), Bdelloipsidyn, phylogenetic analyses based on rRNA (RNA) indicate that the fungi cultivated by several ant species in the lower attine genus Acronicta is closely related to other attine fungi, and has been secondarily acquired long after the mutualism originated in the Amazon Basin approximately 50 million years ago[24, 25]. Second, molecular analyses indicate that several distinct lepiotoid fungal lineages are associated with lower attines and have been transmitted across the phylogenetic transitions in patterns of symbiont acquisition over evolutionary time, comparisons across related taxa (particularly in co-occurring systems) can show different evolutionary outcomes that represent variations on a single theme of mutualistic interaction (e.g. leaf cutters, lice, yuccas, ants, plants and cyanobacteria). Specifically, phylogenetic analyses reveal that parasitic lineages can be embedded in largely mutualistic groups and/or vice versa[26]. However, theory suggests that the species that parasitize mutualisms should not be the closest relatives to either partner[27]. Available evidence collected from lice (Eucnemidae) and fungwaps (Agaricales), and the yuccas (Yucca) and yucca moths (Tegeticula) supports this prediction[15, 18, 25]. Nonetheless, this proposition requires further testing.

Mutualisms as model systems

Molecular data are providing new insights into the evolution of mutualism. In those instances in which the host and mutualist co-occur, the absolute times of divergence between pairs of co-occurring mutualisms and their free-living relatives can be measured. This allows a series of potentially instructive comparisons to be made in the accumulation of substitutions in homologous DNA sequences. First, comparisons can be made between the accumulation of substitutions at a given gene or set of genes in the "host" and in the "symbiont" (or parasite).
free living relatives, an observation that appears to oppose the idea that rates of evolution in mutualists should slow down18,40. A similar pattern has been found in lichen41.

**Mutualisms and the adaptive significance of sex**

Current theory regarding the adaptive value of sexual reproduction revolves around the ideas that sexual reproduction serves to: (1) maintain adaptation in the face of a constantly evolving and threatening biotic world and (2) remove deleterious mutations. Potentially, comparisons between groups of related species characterized either with or without sexual reproduction could be useful to assess the relative importance of these two proposed functions. For example, some groups of mutualists, such as endophytic fungi that associate with marine invertebrates, fungi associated with ant species, perhaps algae in some lichens, clavicipitaceous (i.e. smut-like) grass endophytes, and the fungal cultivars of fungus-gardening termites, are derived from free-living groups capable of both sexual and asexual reproduction. In each case, it appears that the balance between sexuality and asexuality has been shifted towards the latter. Interestingly, in the case of the endophytic fungi associated with grasses, the fungi appear to reduce the host’s tendency to reproduce sexually, rather than the more typical reverse pattern10.

There are several possible explanations for these patterns. For example, one school of thought suggests that "well integrated" (e.g. intracellular) symbionts are protected by their hosts from a menacing organic world of constantly evolving predators and parasites, and consequently do not 'need' sex19. An alternative, less benign, view of mutualisms suggests that mutualistic relationships are better characterized as a series of ongoing arms races. In this scenario, sex might be the critical element that allows one member to "keep up", or if suppressed in one member has allowed the other to "get ahead". Further progress in this area will depend on knowing the extent to which sex is actually absent; determining whether symbionts are represented by a single clone or are genetically heterogeneous, and estimating the phylogenies of the partners over various spatial and taxonomic scales. Ultimately, molecular data will play a crucial role in distinguishing among various possible interpretations.

**Conclusions**

Most organisms are involved either directly or indirectly in mutualistic interactions. However, there is no general theory of mutualism that approaches the explanatory power that 'Hamilton's Rule' appears to hold for the understanding of within-species interactions. Underlying problems revolve around explicitly defining vague terms, such as 'alignment of interest', and employing biologically relevant scales of organization. Ideally, all of these should be characterized as a series of ongoing arms races. In this scenario, sex might be the critical element that allows one member to "keep up", or if suppressed in one member has allowed the other to "get ahead". Further progress in this area will depend on knowing the extent to which sex is actually absent; determining whether symbionts are represented by a single clone or are genetically heterogeneous, and estimating the phylogenies of the partners over various spatial and taxonomic scales. Ultimately, molecular data will play a crucial role in distinguishing among various possible interpretations.

Ultimately, we cannot begin to determine whether there are any general principles or consistent patterns that characterize mutualisms if we misunderstand individual case studies. Ideally, for a number of cases, we need to identify and quantify the costs and benefits to each party, and to understand what factors influence variation in those costs and benefits. Importantly, we need to understand conflicts of interest and attempt to identify what factors maintain the alignment of interests between mutualists. What prevents the system from breaking down? To do this, it is crucial that we identify the mutualists, and understand their diversity, patterns of transmission and structuring at several spatial, temporal and evolutionary scales. This poses the monumental task of documenting basic, descriptive natural history for many distinct systems and coupling it with the often indispensable information that can increasingly be obtained from molecular approaches.

**Acknowledgements**

We thank Koos Boomsma and Jack Werren for stimulating discussions. We thank Betsy Arnold, Jenny Apple, Eighbert Leigh, Elisabeth Kalko, Sadie Jane Ryan, Andy Dobson, Jon Howe, Penny Barnes, Andrew Baker, Rob Rowan, DeWayne Shoemaker and Rod Page for help and useful comments during the evolution of this article. STER Post Doctoral Fellowships supported SAR and UAM and made this collaboration possible.

**References**

The cost of helping

Robert Heinsohn and Sarah Legge

The study of cooperative breeding in vertebrates aims to understand why some animals forgo independent reproduction and help others to rear their offspring. Over the past 30 years, the field has developed a rich set of theory and has been wracked by some major debates. However, enough cooperative species have been studied to start to establish common ground and to test theory. Indeed, in a recent review of the field, Emery3 stated that “the original paradox of cooperative breeding largely disappeared with the widespread confirmation that (1) help- ers frequently do improve their chances of becoming breeders and, (2) they frequently do obtain large indirect genetic benefits by helping to rear collateral kin.” With identification of these direct and indirect benefits to helpers, the original questions asked by researchers would appear to be “largely answered”. Despite this claim, some important questions remain unanswered. In particular, our understanding of the varying level of helper contributions within and between species remains poor. The approach to cooperative breeding has often been to compare the outcomes of philopatry and helping with the other options of dispersing to find a new territory or non-helping. It does not lead to an appreciation of the nature of each cost and benefit. Although we have a large list of benefits to helping9, we still lack a cohesive framework that explains when they apply in various taxa or ecological contexts. Less attention has been paid to the costs of helping.

Cooperative breeding in mammals, birds and fish has provided evolutionary biologists with a rich framework for studying the causes and consequences of group-based reproduction. Helping behaviour is especially enigmatic because it often entails an individual sacrificing personal reproduction while assisting others in their breeding attempts. The decision to help others to reproduce is affected by immediate and future costs analogous to those of direct reproduction, but these components of the equation have usually been neglected. Recent research suggests that the type of benefit sought could determine the extent of help given.

Robert Heinsohn and Sarah Legge are in the Division of Botany and Zoology, Australian National University, Canberra, ACT 0200, Australia (robert.heinsohn@anu.edu.au; sarah.legge@anu.edu.au).

Consider the cooperatively breeding Seychelles warbler, Acrocephalus seychellensis. In an elegant study, Kondev10 showed that helpers much prefer to feed nestlings that are more closely related to themselves; an important result that emphasized the liability and adaptive nature of helping.

34 Gargas, A. et al. (1995) Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny, Science 268, 1492–1495