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

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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.

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From the algae that help power reef-building corals, to the diverse array of pollinators that mediate sexual reproduction in many plant species, to the myriad nutritional symbionts that fix nitrogen and aid digestion, and even down to the mitochondria found in nearly all eukaryotes, mutualisms are ubiquitous, often ecologically dominant, and profoundly influential at all levels of biological organization[1–6]. Although mutualisms can be simply defined as reciprocally beneficial relationships between organisms, they range from diffuse and indirect interactions to highly integrated and coevolved associations between pairs of species. Such mutualisms usually involve the direct exchange of goods and services (e.g. food defense and transport) and typically result in the acquisition of novel capabilities by at least one partner[2,3].

Current theory[5–8] suggests that mutualisms are best viewed as reciprocal exploitations that nonetheless provide net benefits to each partner. This view stresses the disruptive potential of conflicts of interests among the erstwhile partners. Consequently, identifying factors that influence the costs and benefits to each partner and quantifying their influence constitute primary research objectives[9]. In particular, inquiry centers on the description of conflicts of interest between partners and the attempt to understand what mediates them[10]. This requires a clear appreciation of the spatial, temporal and taxonomic context in which these systems operate. Breakthroughs in understanding have, and will, come precisely because of the increased attention paid to the different ecological and evolutionary scales within which the mutualisms function.

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[11–15]. Similar approaches can also reveal the phylogenetic patterns of relationships both between and within taxa of mutualists, and thus the extent to which speciation in hosts is tracked by speciation in symbionts[16–19], as well as the number of origins of particular types of relationship[11,16]. Results from these studies have a direct bearing on one of the central evolutionary questions concerning mutualism: what factors align the interests of partners so that the relationships remain mutually beneficial and evolutionarily stable?

Current theory of conflict, cooperation and constraint

The potential for conflicts of interest to shape or destabilize mutualistic associations will depend on the extent to which the survival and reproductive interests of the symbiont align with those of the host. Given that conflicts of interest can occur even within the genomes of single individuals[5,6,20], 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 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 them[5–8,21–23]. This framework is logically appealing, and many cases appear to conform well with its predictions[24,25].

However, it is worth scrutinizing why these factors are thought to reduce the potential for conflict among would-be mutualists and noting that those factors are often not independent. First, in the case of vertical transmission, both symbiont and host benefit from successful reproduction by the host. Second, vertical transmission over many generations will tend to reduce the genetic diversity of symbionts by eliminating novel inputs to the symbiont community and by providing a potential bottleneck at each generation[11]. 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 wellbeing and reproductive success[5,6,23,25]. Finally, 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.

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

Throughout the shallow tropical oceans, sessile animals often have symbiotic associations with photosynthetic, single-celled algae. Among the most spectacular and ecologically important are the associations formed between reef-building corals and dinoflagellates of the genus *Symbiodinium*. For many years, these symbionts were considered to be a single species, but physiological and genetic studies[11,28] 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 even single colonies are capable of hosting two or more types of symbiont[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 alga's perspective, the host is more like a landscape composed of more and less suitable conditions than a uniformly hospitable environment[28].

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 outweighs the possible costs of evolutionary conflicts among symbionts[28]. Many of the themes emerging from these studies of corals characterize other symbiotic systems as well[11,26,27,29,35].

Box 2. Figs and yuccas: model systems for understanding evolutionary conflicts

There are over 700 species of figs (*Ficus*) described worldwide. The figs depend on minute pollinator wasps (Agaonidae) for continued sexual reproduction, and the wasps depend on the figs to complete their life cycle. Fossil evidence indicates that this relationship dates back at least 40 million years. In most cases, the relationship is overwhelmingly species-specific. In addition, recent molecular work suggests that the long evolutionary history of figs and their pollinators has been dominated by cospeciation between the two taxa[19].

Although in the long term the two mutualists depend completely upon one another, their reproductive interests are not identical. The fig benefits both from the production of viable seeds and from the production of female pollinator wasps that will potentially transfer the tree's pollen to produce seeds in other trees. The wasps benefit only from the production of offspring (that necessarily come at the expense of approximately 50% of the potentially viable seeds). What prevents the shorter lived and much more numerous wasps from exploiting an ever greater number of seeds is still unanswered[9,45]. However, for most aspects that have been studied, the tree appears to be largely in control of the system[9,45].

It is interesting to compare the fig-wasp system with the yucca-moth system. Although there is the general dependence in both cases, there are instructive differences. The reproductive interests of individual female wasps are much more closely linked to their host than is the case with the moths, because the wasps tend to be trapped within the inflorescence they pollinate. Moreover, the female wasp offspring will carry pollen from the inflorescence in which they developed. In contrast, moths can pollinate and lay eggs in several different flowers, and their offspring are unlikely to provide the additional pollination service because they drop to the ground and emerge as adults much later[10,38,39]. The difference between the figs and yuccas in the degree to which their interests coincide with their partners is probably reflected in the much higher proportion of the fig seeds that support development of wasp offspring compared with the proportion of yucca seeds that support the development of the moth offspring.

Nonetheless, not all mutualisms follow this pattern of vertical transmission with its proposed benefits. 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

Determining 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[11,26–28]. 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[26,27,29], 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 distinctive 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[12,26–28]. 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[23,25]. The ecological flexibility provided by symbiont diversity[28,30] might play an important counterbalancing role.

Patterns of ecological transmission and evolutionary association

For patterns of transmission, it is useful to distinguish between transmission over ecological (generation to generation) and longer evolutionary (lineage to lineage) timescales. For example, systems dominated by strict vertical ecological transmission might be expected to produce concordant phylogenies between host and symbiont at all taxonomic scales, whereas in systems dominated by horizontal transmission, this outcome might be thought to be less likely.

The explosion of systematic analyses using molecular techniques has generated phylogenetic reconstructions for one or both members of several speciose groups of mutualists. These studies show that patterns of transmission over ecological timescales do not necessarily translate into similar patterns at evolutionary timescales; available evidence suggests that all combinations of different patterns of ecological transmission and different degrees of phylogenetic concordance are found. Specifically, there are cases in which both evolutionary and ecological transmission appear to be predominantly vertical[18]. However, vertical evolutionary transmission (between lineages) is also found in cases in which ecological transmission is predominately horizontal (e.g. fig-pollinating wasps[19], luminescent bacteria associated with deep-sea fish[31] and sulfur oxidizing bacteria and some of their bivalve hosts[14,32]), apparently because vertical transmission is not the only mechanism that promotes cospeciation. Moreover, many intracellular bacteria (e.g. *Wolbachia*, Box 3) show predominantly vertical transmission patterns at an ecological level, but this does not necessarily translate into phylogenetic patterns that are concordant with their hosts[33]. Presumably, this is because of sporadic cases of horizontal transfer between distantly related species.

In an additional complexity, determining the extent to which co-cladogenesis is occurring will frequently depend on the taxonomic scale at which the question is asked[14]. For example, the phylogenetic relationships between some lineages of leaf-cutter ants show nearly perfect concordance with the relationships of their associated fungi. However, in some entire lineages the host phylogenetic relationships show essentially no correspondence with those of the fungi. In fact, there appear to be many lineages in which nonspecificity and noncongruence are the rule[16,17] (Box 4). Unfortunately, for most mutualisms, we do not have adequate spatial and taxonomic sampling to determine the extent of concordance between host and symbiont lineages.

Trajectories of costs and benefits

Molecular data can provide a window on the taxonomic identities of mutualists, the structuring of their extant populations (e.g. patterns of spatial distribution and ecological transmission), their histories of phylogenetic associations and their evolutionary origins[14,28,29,34], but provide relatively little information about the often rapid, and sometimes convoluted, evolutionary trajectories of costs and benefits received[4,8].

From studies that compare outcomes across several populations of mutualistic interactions between two species, we know that outcomes can vary among extant populations[4,35,36]. Several studies have documented that net costs and benefits can vary over relatively short timescales[4,36] resulting from: (1) changes in the presence or abundance of influential third parties[36,37]; (2) variation in host densities that results in shifts in patterns of transmission[24]; (3) variation in resource availablility[3,36]; or (4) variation in physical conditions (Box 1). Furthermore, such studies raise questions concerning the degree of local adaptation in host and symbiont populations, such as whether hosts generally benefit most from local, presumably more highly coadapted symbionts.

Moreover, in evolutionary time, comparisons across related taxa (particularly in cospeciating systems) can show different evolutionary outcomes that represent variations on a single theme of mutualistic interaction (e.g. leafcutters, figs, yuccas, ants, plants and lycaenid butterflies). Specifically, phylogenetic analyses reveal that parasitic lineages can be embedded in largely mutualistic groups and/or vice versa[19,38,39]. However, theory suggests that the species that parasitize mutualisms should not be the closest relatives to either partner[38,39]. Available evidence collected from figs (*Ficus*) and fig wasps (Agaonidae), and the yuccas (*Yucca*) and yucca moths (*Tegeticula*), supports this prediction[19,38,39]. Nonetheless, this proposition requires further testing.

Mutualisms as model systems

Mutualisms and rates of molecular evolution

In those instances in which the host and mutualist cospeciate, the absolute times of divergence between pairs of cospeciating mutualists are effectively held constant. 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). Second, comparisons can be made between the rates of accumulation of base changes between the symbionts and their free-living relatives.

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. There is accumulating experimental and comparative support for this proposition. A classic example is the association found between aphids and their bacteria (*Buchnera*) that synthesize necessary amino acids for their hosts[3,11,18,40].

However, *Wolbachia* appears to be a maternally inherited endosymbiont that frequently has large negative effects on its host's reproductive interests. 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*[33]. Superficially, these observations contradict the theoretical predictions.

However, to assess the relevance of these observations, the timescales over which maternal transmission occurs and the magnitude of the negative effects of *Wolbachia* 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 dominated by vertical transmission depends on temporal scale. In addition, *Wolbachia* can often have complex or little, if any, negative effect on its direct individual host[44]. 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[16,17,46]. Ants in the leaf-cutter genera *Acromyrmex* and *Atta* are ecologically and economically important because of the vast quantities of foliage and flowers that they cut to culture the fungi in their often immense nests. Together with three additional genera, leaf-cutter ants are grouped into the monophyletic higher attines, which comprise about one-half of the species diversity of the tribe. Ants in the remaining seven genera of lower attines 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 vegetable 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 ant-associated fungi are members of the family Lepiotaceae (Agaricales; Basidiomycotina), phylogenetic analyses based on ribosomal DNA indicate that the fungus cultivated by several ant species in the lower attine genus *Apterostigma* is distantly related to all other attine fungi, and has been secondarily acquired long after the mutualism originated in the Amazon Basin approximately 50 million years ago[16,17,46]. Second, molecular analyses indicate that several distinct lepiotoid fungal lineages associated with lower attines are essentially identical to current free-living forms.

Together with the apparent lack of morphological modification of many lower attine symbionts, these observations suggest the recent acquisition of novel symbionts from free-living stock[46]. Thus, as can be observed on both ecological and evolutionary scales, the presumably ancestral condition of repeatedly acquiring free-living fungi has been retained in some of the lower attines but appears to have been lost in the higher attines, which have developed longer-term associations with their generally more specialized symbionts.

Depending on the attributes of the taxa available, these comparisons permit the evaluation of several factors that have been suggested to be important in influencing the rates of molecular evolution. Cospeciating mutualists often exhibit different generation times, different body sizes and metabolic rates, different effective population sizes and different degrees of sexual reproduction. Different taxa might also possess very different systems of DNA repair. These contrasts can be productively exploited. For example, Moran and colleagues have found that the aphid-associated *Buchnera* shows much faster rates of molecular evolution than do its free living relatives, an observation that appears to oppose the idea that rates of evolution in mutualists should slow down[18,40]. A similar pattern has been found in lichens[41].

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 changing and potentially 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 dinoflagellates associated with marine invertebrates, fungi associated with attine ants, 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[42], rather than the more typical reverse pattern[43].

There are several possible explanations for these patterns. For example, one school of thought suggests that 'well integrated' (e.g. intracelluar) symbionts are protected by their hosts from a menacing organic world of constantly evolving predators and parasites, and consequently do not 'need' sex[43]. 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 realistic currencies (i.e costs and benefits) at biologically relevant scales of organization. Ideally, all of these should be measurable and capable of being employed across radically different systems. For example, can the 'conflict of interest' and 'costs and benefits' within and between leafcutters that do or do not have vertically transmitted fungi be estimated and then compared with those values for corals that do or do not have vertically transmitted algae? We have implied that factors constraining 'cheating' or 'defection' are increasingly required because the interests of interacting species are not aligned. But can it be shown that increasingly stringent constraints (e.g. no options outside the relationship and/or increased host investment in symbiont control) operate in systems in which there are increasingly incongruent interests?

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. If there is nonalignment, 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.

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The cost of helping

Robert Heinsohn and Sarah Legge

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.

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The study of cooperative breeding in vertebrates aims to understand why some animals forgo independent reproduction and help others to breed instead. Over the past 30 years, the field has developed a rich set of theory[1–3] and has been wracked by some major debates[4,5]. However, enough cooperative species have been studied in detail to establish common ground and to test theory. Indeed, in a recent review of the field, Emlen[6] states that 'the original paradox of cooperative breeding largely disappeared with the widespread confirmation that (1) helpers 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 float or dispersing to breed[3]. Evaluation of the final reproductive rewards for each strategy leads to an ultimate understanding of why a particular decision was made[7]. Implicit in this approach is that the outcome reflects all the costs and benefits of dispersal versus nondispersal, and helping versus nonhelping, but it does not lead to an appreciation of the nature of each cost and benefit. Although we have a large list of benefits to helping[8], we still lack a cohesive framework that explains when they apply in various taxa or ecological circumstances. Less attention has been paid to the costs of helping.

Consider the cooperatively breeding Seychelles warbler, *Acrocephalus sechellensis*. In an elegant study, Komdeur[9] showed that helpers much prefer to feed nestlings that are more closely related to themselves; an important result that emphasized the lability and adaptive nature of helping

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