

## Multiple mutualist effects: conflict and synergy in multispecies mutualisms

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**Abstract.** Most organisms interact with multiple mutualistic species that confer different functional benefits, yet current conceptual frameworks do not fully address this complexity. A network approach considers multiple mutualistic interactions within a functional type and has been largely nonmechanistic, with little attention to the fitness consequences of specific interactions. Alternatively, consumer–resource approaches have explicitly characterized the mechanisms and fitness consequences of resource exchange, but have not been extended to functionally divergent partners. First, we merge these approaches using graphical models to define the multiple mutualist effects (MMEs) that occur when a focal species has multiple partner mutualists. This approach mirrors food web research that has been advanced by studies of multiple predator effects as well as by detailed investigations of modules nested within larger networks. Second, we define the pathways through which a focal mutualist and two or more partner species could interact, reviewing examples of MMEs that span a range from positive to negative fitness effects. Third, given the potential for nonadditivity demonstrated by the existing literature, we pose new hypotheses for species–interaction outcomes by examining factors such as the extent of overlap in rewards exchanged among partners and their resulting network topologies. Our synthesis illustrates how the consideration of MMEs can improve the ability to predict the outcomes of losses or gains of mutualisms from ecosystems.

**Key words:** *antagonism; community ecology; complementarity; connectance webs; diversity; energy-flow webs; functional group; functional webs; multiple predator effects; mutualism; mutualistic network; species interaction.*

### INTRODUCTION

Organisms often interact with more than one mutualist in nature. Predicting the effects of these multiple-partner mutualisms may benefit from a marriage of consumer–resource and network approaches to the study of multispecies mutualisms. Consumer–resource dynamics explicitly link the mechanisms of resource exchanges to the fitness of partner species (Holland et al. 2005). When these approaches include more than two species, they have focused on pairwise interactions involving functionally similar exchanges (Jones et al.

2012), whereas most organisms associate with mutualists that provide functionally diverse rewards (e.g., reproductive, nutritional, or defensive; Janzen 1985). Network approaches have significantly expanded the community-level perspective on mutualisms (Jordano et al. 2003, Encinas-Viso et al. 2012, Martín González et al. 2012). However, networks largely omit mechanisms and measures of individual fitness (Blüthgen 2010). Furthermore, networks have focused on bipartite interactions within a single functional type (e.g., plant–pollinator networks). Bipartite studies will underestimate the importance of mutualism in community dynamics if synergism or complementarity among functionally different partners occurs (Gustafson and Casper 2006, Palmer et al. 2010, McKeon et al. 2012), and overestimate it if different partners are in conflict (Levin and Anderson 1970, Pearson et al. 1994, Stanton et al. 2002). The existence of several examples of conflict

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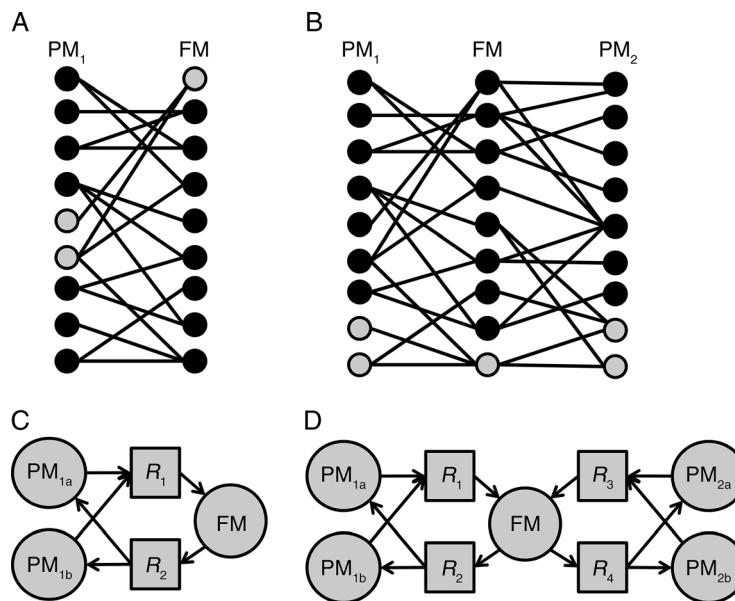


FIG. 1. Graphical models of mutualistic interactions. (A) Mutualistic networks are connectance webs between focal mutualists (FM) and a group of partner species that confer a similar type of benefit ( $PM_1$ ; e.g., pollinator–plant network). They depict which species interact but do not show resource exchange (rewards) or the fitness consequences for the interactors (benefits). (B) The connectance approach can be expanded to include partners that provide functionally different types of rewards, shown as functionally different partner groups ( $PM_1$  and  $PM_2$ ; e.g.,  $PM_1$ , pollinator species; FM, plant species;  $PM_2$ , mycorrhizal fungi). (C) The consumer–resource framework applied to functionally similar partner species ( $PM_{1a}$  and  $PM_{1b}$  both conferring the same reward,  $R_1$ ; e.g., two species of pollinators) by Jones et al. (2012) and Holland et al. (2005) is similar to the energy-flow food webs of Paine (1980). (D) Our expansion of the consumer–resource framework to encompass functionally different partner mutualists. Functional groups are represented by numerical subscripts and species as alphabetical subscripts.

or synergy among mutualistic partners (e.g., Morris et al. 2007) illustrates that embracing the complexity of multispecies mutualisms is critical to understanding the role of mutualisms in ecology.

Consider advances in the study of predator–prey interactions that have resulted from embedding these interactions within complex food webs (Menge 1995, Morin 1995, Polis and Winemiller 1996, Chalcraft et al. 2007, Bruno and Cardinale 2008). A variety of approaches to constructing food webs has been used, including connectance webs, energy flow webs, and functional webs (Paine 1980). Each approach conveys different information about the same set of species, and each has an analog in mutualistic networks. Connectance webs are constructed from observations of which species interact (Fig. 1A, B), and network connections can be weighted by the number of times an interaction is observed (quantitative network). For mutualisms, these are the bipartite networks. Energy-flow webs trace back to Elton (1927) and Lindeman (1942) and represent the movement of resources among organisms. These webs can be constructed from observations of which species interact combined with some measurement of energy transfer. Analogs are found in the consumer–resource approach to the study of mutualism (Holland et al. 2005, Jones et al. 2012; see Fig. 1C). Finally, functional webs require experimental manipulation of the abun-

dances of individual species; these show which interactions have the largest influence on community structure (Paine 1980). The functional-web approach is critical for capturing nonadditive effects of species interactions. While functional webs could have analogs for mutualisms, to date these have not been widely used because most experimental studies of mutualism manipulate a single partner species, rather than taking a comparative approach (but see Stachowicz and Whitlatch 2005, Mack and Rudgers 2008). Fortunately, an expanding number of studies have manipulated multiple partners (Tables A1–2 in Appendix A), factorially or in parallel, thereby increasing the information available to construct functional mutualist webs. Borrowing from the approaches used in food-web ecology can expand the conceptual framework for research on mutualisms.

Studies of modules within complex predator–prey webs have led to advances in our understanding of community structure (e.g., Menge 1995) including the detection of synergistic and antagonistic effects of predators on prey abundance, known as multiple predator effects (MPE; Sih et al. 1998). MPEs occur when the individual influences of different predator species on prey behavior, ecology, or evolution are nonadditive (Sih et al. 1998). Thus, effects of predators on the abundance of a prey species cannot be predicted from even the best connectance or energy-flow web (e.g.,

Paine 1980). Functional study of MPEs has improved our ability to forecast the outcomes of losses of predators from ecosystems (Hixon and Carr 1997, Cardinale et al. 2003, Ives et al. 2005, Bruno and Cardinale 2008). For example, in salt marshes the roles of predatory arthropods in trophic cascades depended on whether predators were complementary in their effects on prey or antagonistic toward one another (Finke and Denno 2005). Mutualisms involving multiple species can similarly include nonadditive effects, such as complementarities (Stachowicz and Whitlatch 2005, McKeon et al. 2012) or antagonisms among subsets of partners (Addicott 1978, Stanton et al. 2002).

In contrast with predator–prey interactions, however, multiple mutualisms have the potential for far greater complexity than multiple predator effects because very different guilds or functional groups can be involved. We use *functional group* to define subsets of partner species by the functional services they confer to a focal species, rather than using *guild*, which more typically refers to species exploiting the same resources in a similar way (Simberloff and Dayan 1991, but see Stanton 2003). In multiple mutualisms, nonadditive effects are likely to occur in the form of complementarities when the effect of one partner (e.g., a protective mutualist) on a focal species is enhanced by the presence of a partner from another functional group (e.g., a nutritional mutualist). A new framework for multiple *mutualist* effects, which captures the complexity of functionally diverse mutualisms, could generate insights that parallel the advances made in predator–prey ecology by considering multiple *predator* effects. Of particular interest is delineating the factors that may generate nonadditivities in multiple mutualisms.

Here, we construct a framework for studying multiple mutualist effects (MMEs). First, we use models and functional webs to merge network and consumer–resource approaches and to introduce greater functional diversity than has been captured by prior models. Second, we synthesize the mutualism literature to characterize the pathways through which multiple partner species can affect a focal mutualist (and each other), with an eye toward identifying causes of nonadditive effects and the conditions under which different MMEs are most likely to occur.

#### MERGING NETWORK AND CONSUMER–RESOURCE APPROACHES TO STUDY MULTIPLE MUTUALIST EFFECTS

We define *multiple mutualist effects* (MMEs) as the effects of two (or more) mutualist partners on a single individual of a focal species. While in some cases there is a clear distinction between “hosts” and “associates” based on size or life habit (e.g., plant = host; associates = pollinators and mycorrhizal fungi), such terminology is not generalizable to mutualisms involving species of similar size or life history. Thus, we define a *focal mutualist* (FM) as the species associated with a number of other mutualists, and *partner mutualists* (PM) as the

two or more species that exchange rewards with the focal species (Fig. 1). The identities of the focal mutualist and partners are a matter of perspective because most species interact with multiple mutualistic partners, and it is often the case that a single species could be either a focal or partner species depending on how the interaction is defined. Associations with multiple partner species can occur simultaneously or at different stages during the life cycle of the focal mutualist and can be spatially overlapping or physically separated (e.g., mycorrhizal fungi in plant roots and fungal endophytes in leaves). Here, we restrict our definition to interactions in which a single individual of the focal species can associate with at least two other partner species. Thus, we do not consider cases in which individuals within a focal species specialize on different partners (e.g., a plant species that encounters different pollinator species in different parts of its range) because, in such cases, a single individual of the focal species does not face potentially conflicting (or synergistic) ecological pressures. In addition, MMEs require that interactions between pairs of focal–partner mutualists are bidirectional and mutualistic (+,+) under at least some contexts, and thus, for simplicity, we do not consider unidirectional chains of positive interactions among multiple species, such as facilitation cascades (Altieri et al. 2007). However, it may be possible to extend this framework to a greater diversity of species interaction types (e.g., Menge 1995).

Traditional network approaches (Fig. 1A) can be expanded to represent diverse functional groups of mutualists simultaneously (Fig. 1B). Partner mutualists are placed into the same or different *functional groups* based on the nature of the rewards they exchange with the focal mutualist (see Plate 1). Mutualistic partners that provide qualitatively the same reward to the focal species are said to be “functionally similar” (e.g., multiple insect species that pollinate the same plant). Our network notation represents species as individual circles and functional groups as numerical subscripts. Typical networks are bipartite with all partners in the same functional group (e.g., PM<sub>1</sub> in Fig. 1A). In contrast, two (or more) partners that provide functionally different rewards to the focal mutualist are represented in separate columns (PM<sub>1</sub> and PM<sub>2</sub> in Fig. 1B). For example, a single plant species (focal) can derive rewards from a functionally diverse partner assemblage that includes pollinators (dispersal) and mycorrhizal fungi (nutrition) as well as ants (protection), rhizobia (nutrition), and others.

We represent resource exchange in mutualisms explicitly, by using consumer–resource diagrams developed by Jones et al. (2012) and Holland et al. (2005) for functionally similar partner species (Fig. 1C; identified by the same numerical subscripts, e.g., PM<sub>1a</sub> and PM<sub>1b</sub>). Here, we use the term *reward* (R) broadly to include resources and services exchanged between species. Following Jones et al. (2012), the partner mutualists

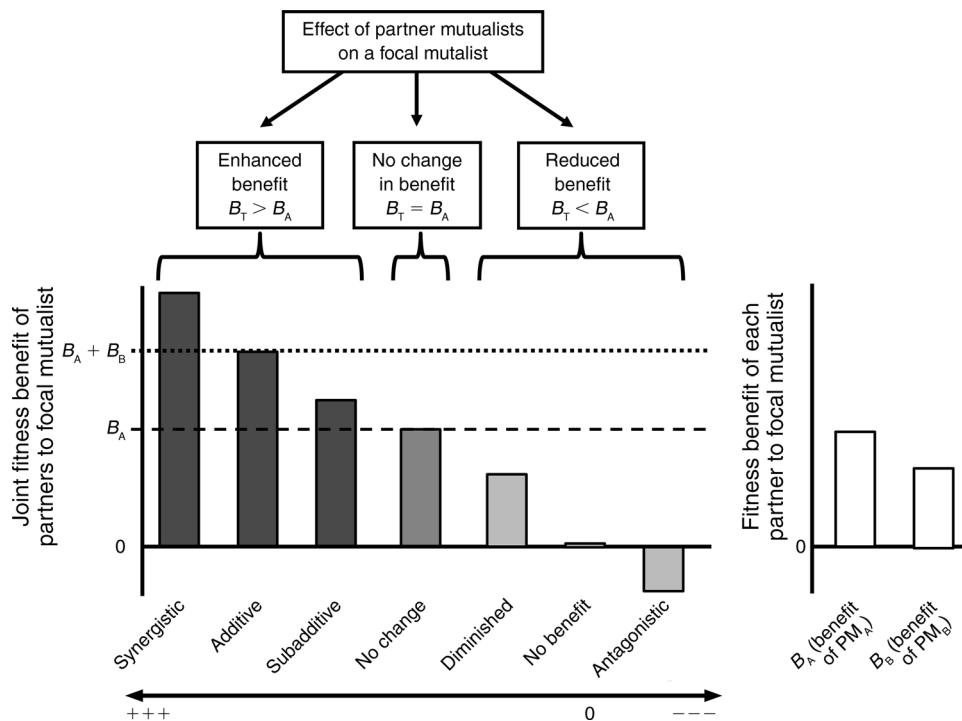


FIG. 2. Multiple mutualist effects (MMEs) on fitness of a focal mutualist. Association with multiple mutualistic partners can enhance, reduce, or have no effect on the fitness benefits received by the focal mutualist.  $B_T$  = total benefits conferred by all partners,  $B_A$  = benefit conferred by the best mutualist partner species  $PM_A$ , and  $B_B$  = benefit conferred by another partner  $PM_B$ . The value of 0 on the y-axis (solid line) indicates the threshold at which the net effect of associating with all partners is zero for the focal mutualist. The dashed line indicates threshold at which fitness consequences of associating with all partners equals the benefit of associating with the single best mutualist (i.e., no change in benefits;  $B_T = B_A$ ), and the dotted line indicates when fitness consequences of all partners is additive. Additional explanations of each MME class and subclass (e.g., synergistic) are presented in Table A3 of the Appendix.

provide a reward to the focal mutualist ( $R_1$ , e.g., pollination) and the focal species produces a reward for the partners ( $R_2$ , e.g., nectar). Benefits ( $B$ ) are defined as the increases in fitness gained as a function of the rewards exchanged.

What has been lacking in prior work on mutualism is a framework that explicitly considers both the mechanisms and connectance patterns associated with *functionally diverse partner mutualists*. We address this gap by combining network and consumer–resource approaches through the consideration of intermediate-sized modules in functional interaction webs. In Fig. 1D, we build the consumer–resource approach into a network-based model that encompasses mutualist functional groups, where functional groups are represented by numerical subscripts and species within each functional group by alphabetical subscripts. The complexity of resource exchange potentially doubles when partners both provide and receive different kinds of rewards (compare Fig. 1D to Fig. 1C). In the next section we explore the pathways through which this complexity can lead to the nonadditivity that is characteristic of MMEs.

#### MULTIPLE MUTUALIST EFFECTS (MMEs)

##### *How do multiple partner species affect a focal mutualist?*

It is well established that changing the abiotic environment or the presence of other species (predators, competitors) can alter the magnitude or direction of mutualistic interactions—the phenomenon of context dependency (Bronstein 1994, Stachowicz 2001, Bruno et al. 2003, Kiers et al. 2011). We explore how multiple mutualisms can generate context dependency by altering the strength and/or outcome of interactions for both the focal mutualist and the partners. Association with multiple partners can enhance, reduce, or have no effect on the total fitness benefit obtained by a focal mutualist when compared to the benefit conferred by the single “best” partner (Fig. 2; Appendix: Table A3). Examples of such outcomes have been documented in the literature (for additional examples see Table A1 in Appendix), and here we synthesize them under a unifying framework (Fig. 2; Appendix: Table A3).

Focal mutualists receive *enhanced benefits* when the total fitness benefit conferred by two or more partners is greater than that of associating with the single best partner. These enhanced benefits can be synergistic, additive, or subadditive (Fig. 2; Appendix: Table A3).

For example, Gustafson and Casper (2006) observed a synergistic effect on the growth of big bluestem grass, *Andropogon gerardii*, from two species of arbuscular mycorrhizal (AM) fungi. When both AM fungi species were present, plants not only grew larger than with either fungus alone but also surpassed the additive expectation for combined benefits from each partner. Similar synergistic benefits have been documented for two species of mutualistic gastropods living on the seaweed *Chondrus crispus* (Stachowicz and Whitlatch 2005).

In contrast, *reduced benefits* of multiple partners occur when the total fitness increase conferred by all partners together is smaller than the benefit from the single best partner (subclasses: diminished, no benefit, and antagonistic; Fig. 2; Appendix: Table A3). For example, seed production of tall bellflower (*Campanula americana*) was diminished when more pollinator species were present relative to when the high-efficiency bumblebees dominated, because the diverse assemblage included low-efficiency pollinators (halictid bees) (Lau and Galloway 2004). In some cases, MMEs may even cause the interaction to grade into parasitism for the focal species. Finally, association with multiple partners can produce *no change in benefits* when the benefit of having two or more partners is equivalent to interacting with the single best partner (Fig. 2).

#### *How do multiple partner species affect each other?*

While research exploring the effects of multiple partners on a focal mutualist has been limited, even fewer studies have tested the effects of partner species on each other, which may be negative, positive, or neutral and occur via direct and/or indirect pathways (examples in Appendix: Table A2). Most research has focused on direct competition or interference among partners that both receive and provide functionally similar rewards to the focal mutualist as in Fig. 1C (Levin and Anderson 1970, Pearson et al. 1994, Stanton et al. 2002). For example, in the presence of an additional plant species, a native loosestrife experienced reduced pollinator visitation and seed set than when grown alone, due to increased competition for focal mutualist pollinators (Brown et al. 2002). However, partner mutualists can also interact indirectly via the shared, focal mutualist (Bascompte 2009) and can even have positive effects on each other (Crowley and Cox 2011). For example, nitrogen-fixing rhizobia increased colonization and sporulation of AM fungi on plant roots, and AM fungi increased the number and biomass of rhizobia nodules on roots (Bagyara et al. 1979, Champawat 1990, Geneva et al. 2006). Anemone fish indirectly benefited damselfish by increasing the size of their shared mutualists (anemones) sufficiently that they could be used for protection by the damselfish (Schmitt and Holbrook 2003); however, the net effect of the anemone fish on the damselfish may still be negative due to interference

competition (Schmitt and Holbrook 2003, Holbrook and Schmitt 2004, Holbrook and Schmitt 2005).

#### *What factors promote multiple mutualist effects?*

Clearly, as the number of participants in a set of interactions increases, the ability to predict the outcomes becomes increasingly difficult. The different approaches in Fig. 1 generally represent a trade-off between including more participants (Fig. 1A, B) vs. more mechanistic detail (Fig. 1C, D). Functional-web approaches that experimentally manipulate key players can help to identify the optimal balance. Further, our synthesis of empirical studies (Appendix: Tables A1, A2) suggests that baseline expectations can be set by examining the extent of overlap in rewards exchanged among the partners and the resulting network topologies (Fig. 3). We next explore how additional factors may modify initial expectations based on the overlap in rewards (Fig. 4)

##### *1) Functional overlap in rewards.—*

Hypothesis 1.A. *Minimal functional overlap in rewards provided to the focal mutualist will result in enhanced benefits for the focal mutualist and positive interactions among partners* (Fig. 3A, C, Fig. 4).—For example, fungal endophytes in the genus *Epichloë* gain shelter and nutrition from their grass hosts and obtain gamete dispersal from a fly “pollinator,” with no overlap in the rewards provided by each partner (Clay and Schardl 2002). While the net fitness consequences of supporting multiple partners has not been directly tested for *Epichloë*, we expect benefits to the fungus to be enhanced by multiple partners, rather than reduced. Functional differences in rewards may also be less discrete. Rhizobia and AM fungi can both increase plant nutrition, but differences in the nutrition they provide—e.g., nitrogen fixation from the bacteria vs. enhanced phosphorus uptake from the fungi—increase the likelihood of an enhanced benefit (Cluett and Boucher 1983). Even superficially similar partners can offer rewards that differ functionally, making delineation of functional groups tricky. For example, two marine snail species had synergistic effects on a focal seaweed because they were complementary in their feeding preferences for different species of epibionts that encrusted the seaweed (Stachowicz and Whitlatch 2005, described in Fig. 3A). Unless both snails were present, the seaweed was overgrown and lost biomass. Partners that supply non-overlapping rewards to the focal mutualist are additionally likely to have indirect positive effects on one another. Not only is coexistence among the partners more probable (intra-specific competition > interspecific competition; Chesson 2000), but also the increase in quality or quantity of the focal mutualist may, in turn, indirectly increase the rewards that partner species receive from the focal species. In the snail–seaweed example, without both snail species consuming different epibionts to allow seaweed growth, neither partner would gain shelter (Stachowicz and Whitlatch 2005).

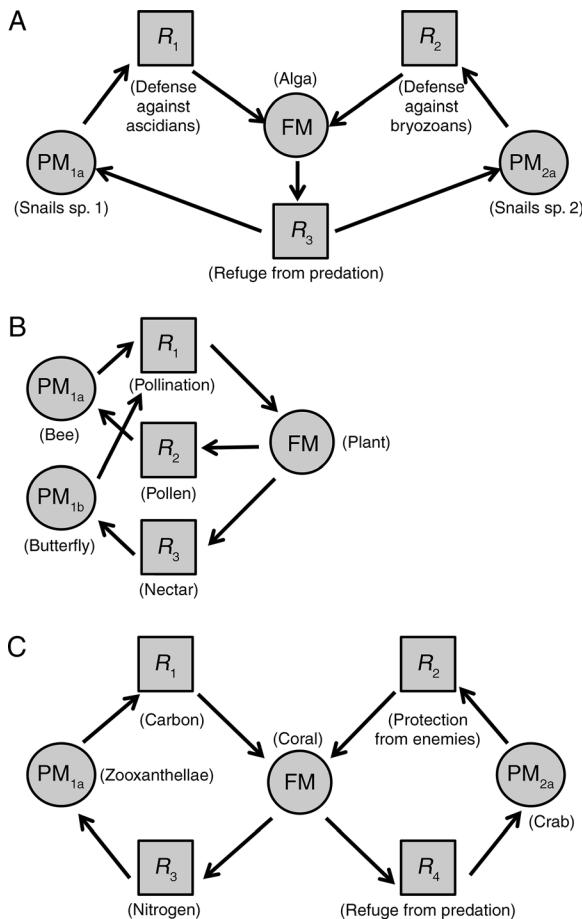


FIG. 3. Interaction webs depicting functional overlap in rewards; specific examples are given in parentheses. (A) Mutualists from functionally different partner groups ( $PM_{1a}$  and  $PM_{2a}$ ) confer unique rewards ( $R_1$  and  $R_2$ ) to the focal mutualist (FM) in exchange for the same reward ( $R_3$ ) (e.g., Stachowicz and Whitlatch 2005). (B) Mutualists from the same functional group ( $PM_{1a}$  and  $PM_{1b}$ ) confer the same reward ( $R_1$ ) to the FM but receive unique rewards ( $R_2$  and  $R_3$ ). (C) Mutualist partners both confer and receive unique rewards (e.g., Miller and Hay 1996, Stachowicz and Hay 1999). Functional groups are represented by numerical subscripts, species within groups as alphabetical subscripts.

Hypothesis 1.B. *High overlap in rewards provided to the focal mutualist will result in reduced benefit for the focal mutualist and negative interactions among partners (Figs. 1C, 3B, and 4).*—For example, both ants and fungal endophytes can protect a focal plant from herbivory; these partners could deter the same suite of herbivores, and the rewards to the focal mutualist would be functionally redundant. However, when strong intraspecific density dependence limits the abundance of any one partner species, additional partners of even redundant species can lead to additive effects. This appears to occur for crustaceans that remove sediment from the coral in which they live but are limited to a

single mated pair per species per host coral (Stier et al. 2012). When partners provide very similar rewards, they may compete for the focal mutualist, which could result in direct (interference) or indirect (exploitative) negative interactions among partner species. For example, competition among species of ant bodyguards represents an additional cost to associating with a focal plant (Palmer et al. 2000).

Hypothesis 1.C. *When multiple partners receive functionally identical rewards, the partners will be more likely to compete than when rewards are divergent, generating relatively more negative effects of partners on each other and increasing the potential for negative feedback to the focal mutualist (Figs. 1C, 3A, and 4).*—For example, Mack and Rudgers (2008) tested the effect of two fungal symbionts—arbuscular mycorrhizal fungi (AMF) and endophytic fungi—on a shared grass host, tall fescue (*Lolium arundinaceum*). Both symbiont species received photosynthetic carbon. The fungal endophyte suppressed AMF colonization, suggesting that it was the better competitor for the shared plant reward. However, despite negative effects of one partner on another, there was no net fitness reduction for focal plants with both partners relative to plants with only the fungal endophyte (no change in benefits, Fig. 2).

Hypothesis 1.D. *Alternatively, when different partner species receive non-overlapping rewards from the focal mutualist (Fig. 3B, C), competition among partners will be reduced. This will decrease the potential for partners to have negative effects on each other and reduce the likelihood that, together, they will provide reduced benefits to the focal mutualist (Fig. 4).*—For example, corals provide distinct rewards to zooxanthellae (nitrogen) and crabs (shelter from predators and defense against competitors), and each partner species provides a distinct reward to the coral (carbon or protection from enemies, respectively) (Fig. 3C; see Plate 1). Although the simultaneous effects of both partner mutualists have not been directly tested, our framework predicts that an enhanced benefit to the focal coral (e.g., synergism) would be most likely (see Glynn 1976, Miller and Hay 1996, Stachowicz and Hay 1999, Stewart et al. 2006, Gates and Ainsworth 2011).

We envision that tests of these hypotheses may include factorial manipulations of mutualist presence in systems where species vary in the degree of overlap in rewards. In addition, as more empirical data accumulate, meta-analyses of studies that have manipulated functionally different vs. similar partners should become informative.

## 2) Reliability of partner mutualists in time and space.—

Hypothesis 2.A. *When there is spatial or temporal heterogeneity in the presence, abundance, or quality of a partner species, focal mutualists that support multiple partners will have higher fitness than will specialists (Fig. 4).*—We expect such a result even when rewards from some partners are redundant or of lower quality than



subsidized by a few abundant generalist species (Bascompte 2009).

3) *Cost of focal mutualist-conferred rewards.*—

Hypothesis 3.A. *Enhanced benefits to the focal mutualist will be more likely if rewards produced by the focal mutualist are cheap rather than costly, because the benefits of multiple partners should outweigh the low cost of association (Fig. 4).*—If the reward provided by the focal mutualist is free to produce, such a reward is classified as a by-product (Connor 1995), and may be especially likely to favor association with multiple partners. For example, Bracken et al. (2007) showed that the green filamentous seaweed *Cladophora columbiana* provided a structurally complex habitat for aquatic invertebrates that excreted nitrogen. Habitat provision was a simple by-product of the algal growth form, and thus the reward provided to interstitial invertebrates had no additional cost to the alga. Each invertebrate species alone did not reach sufficient abundance to produce enough nitrogen to exclusively support the growth of *C. columbiana*, but the nitrogen produced by the consortium of several partner species exceeded the seaweed's requirement. Thus, despite overlap in the rewards provided and received, there is unlikely to be a cost to the focal mutualist, and we would predict no change or enhanced benefits for the focal mutualist (Fig. 2). It is not yet clear in this example whether a single partner could reach high enough density in the absence of other partners to compensate for the loss of partner diversity or whether recruitment limitation or intraspecific competition constrains the density of each partner (as in Stier et al. [2012]). Further, if there is a trade-off between the cost of a reward and the amount produced (i.e., inexpensive rewards are more plentiful), then by-product and low-cost rewards may reduce the likelihood of runaway exploitation of the focal mutualist resulting from competition among partner species for scarce rewards (see Hypothesis 3.B). Mutualist removals combined with estimates of the costs of rewards could be used to test this hypothesis. Physiological manipulations of rewards (e.g., gluing extrafloral nectaries, Rudgers and Strauss 2004), and mutant isogenic lines that lack rewards (e.g., nectariless plant varieties) would help to estimate the direct and indirect costs of rewards to focal mutualists (e.g., Strauss et al. 2002). Meta-analyses of mutualisms that vary in the costliness of rewards could also prove informative.

Hypothesis 3.B. *Receipt of by-product (or low cost) rewards from the focal species should increase the likelihood of positive effects of partners on each other.*—In cases where conferring by-product rewards has a positive effect on the focal mutualist (as described above), the resulting increase in the quantity or quality of the shared focal species may in turn indirectly yield more resources for all or other partners. For example, two fish partners received refuge (a by-product reward) from anemones. One species, anemone fish, increased

the size of their focal anemone via protection, but intraspecific interactions limited this species to a maximum of two individuals per host (a mated pair). The larger anemones increased the abundance of a damselfish species that also used the anemone as a refuge (Schmitt and Holbrook 2003, Holbrook and Schmitt 2004, 2005). This indirect benefit was a consequence of strong intraspecific competition in one of the partners that allowed focal resources to fall to another species (see also Stier et al. 2012). Alternatively, if the cost of focal mutualist-conferred rewards is high, then rewards can become limiting, leading to conflict among partner species (i.e., negative effects). For example, ant mutualists can receive expensive rewards from acacia, e.g., shelter in the swollen thorns and nutrition from extrafloral nectaries, in exchange for protection against herbivores. The ants compete for association with desirable acacia and weaker competitors can be displaced by more dominant ant species, indicating clear negative effects among partners (Palmer et al. 2000). More generally, aggressive “cheaters” that usurp high value rewards might commonly produce this sort of negative interaction among partners via their detrimental effects on the focal mutualist.

4) *Direct interactions among mutualistic partner species.*—

Hypothesis 4.A. *Direct antagonistic interactions among partner mutualists will lead to reduced benefits for the focal mutualist (Fig. 4).*—Antagonisms among partners could result from predation by a partner species, competition for the focal mutualist, competition for a shared resource external to the focal mutualist, or as a by-product of the partner's natural history (e.g., a behavioral syndrome of aggressiveness). As discussed above, direct antagonism among partners should be more likely when partners have a high degree of overlap in the rewards received from the focal mutualist because partners will have increased likelihood of physical contact and direct competition for rewards. Additionally, predatory interactions between functionally different partners can occur as a by-product of the rewards provided by one partner species. For example, Ness (2006) found that *Solenopsis xyloni*, a particularly aggressive ant species, was the most effective anti-herbivore protector of fishhook barrel cactus, *Ferocactus wislizeni*. However, the aggressive nature of this ant species also generated a substantial indirect cost: the ants attacked pollinators that visited cactus flowers, reducing seeds per fruit and mass per seed. Another ant species, *Crematogaster opuntiae*, was a less effective defender of the cactus than *S. xyloni* but did not attack pollinators, and thus it may confer a higher net fitness benefit than *S. xyloni*, particularly when plant fitness is pollinator limited. Such direct antagonisms resulting in reduced benefits to the focal mutualist might be common in protective mutualisms where aggressive defenders remove other partner species as well as pests. Exceptions will occur when the predator is a superior mutualist, and



PLATE 1. Many organisms, including important foundation species such as coral, associate with multiple partner mutualists from both similar and distinct functional groups. Functionally similar partners are those that confer the same reward to the focal mutualist. For example, several species of crabs, such as pictured here, provide corals with protection from enemies. Alternatively, partner mutualists may provide functionally unique rewards to the focal species. Here, the coral *Oculina arbuscula* gains protection from algal competitors by harboring the herbivorous crab *Mithrax forceps* and gains an energy source from photosynthate provided by symbiotic zooxanthellae that live within coral tissues. Photo credit: J. J. Stachowicz.

there is no synergy gained from associating with multiple partners. Focal mutualists could also experience reduced benefits by associating with multiple partners if the reward produced by the focal species is limiting to the partner species and the competitively superior partner produces an inferior benefit. While such a scenario seems possible, we are not aware of any examples.

**Hypothesis 4.B.** *Direct or indirect mutualisms among partner species will result in enhanced benefits to the focal mutualist.*—For example, frugivorous birds that consume and disperse the seeds of wild cherries (*Prunus avium*) in Spain sometimes had handling failure; cherries fell to the ground where non-climbing, seed-dispersing mammals could consume them (Hernandez 2008). As a by-product of this behavior, mutualism between the bird and mammals enhanced net dispersal rewards to the tree. Similarly, microbial consortia in which multiple taxa perform distinct but coordinated metabolic functions that together benefit their host may also provide a rich source of examples of this phenomenon (Hussa and Goodrich-Blair 2013). In the human colon, for example, various bacteria sequentially metabolize complex polysaccharides into simpler forms that ultimately can be used by human gut epithelial cells (Flint et al. 2007). Each bacterial species in the metabolic chain produces,

as a by-product, the primary resource used by the next species in the chain, with the human at the chain's end. Multiple bacteria species likely benefit from this collective action because they all depend on the persistence of their shared host. Looking for these direct antagonisms or mutualisms among partner species will require experiments that manipulate the presence of at least one partner and assess fitness-related responses of other partner species in the system.

5) *Focal mutualist control.*—

**Hypothesis 5.A.** *Mechanisms that allow the focal mutualist to regulate the multiple mutualist effects (MMEs) will minimize negative interactions among partner species reducing the potential for runaway exploitation of the focal mutualist (antagonistic MMEs; Figs. 2, 4).*—These mechanisms could be traits that evolved in response to association with multiple mutualists (e.g., partner choice) or exaptations that served other functions, but also make control possible (Frederickson 2013). For example, in plants, spatial or temporal separation of floral rewards to pollinators from extrafloral rewards to bodyguards may reduce the costs associated with antagonistic interactions between these two functional groups (Willmer and Stone 1997). In a more specialized control mechanism, plants may even send volatile signals that deter ants when attracting

pollinators is a priority (Raine et al. 2002). Similarly, spatial partitioning of ectomycorrhizal fungal species in roots has been hypothesized as a mechanism that allows a focal plant to regulate resources to functionally different partner species (Hoeksema and Kummel 2003). Fine-tuned control has been demonstrated in a laboratory setting for plants and arbuscular mycorrhizal fungi; in both species, individuals can alter the amount of rewards provided based on the amount of rewards received (Kiers et al. 2011). Conversely, focal mutualists may gain enhanced benefits from clustering partner species in close proximity, if this facilitates beneficial direct interactions among partners.

**Hypothesis 5.B.** *Focal mutualist control will produce a positive effect of the partners on each other when this control yields an increase in the quality or quantity of the rewards received by partner species. Alternatively, lack of control by the focal mutualist will open opportunities for competition or other negative effects among partner mutualists.*—For example, in contrast to some acacias, which have mechanisms that spatially or temporally separate bodyguards and pollinators (Willmer and Stone 1997), the fishhook barrel cactus enables antagonisms between aggressive defender ants and pollinators (Ness 2006). Ultimately, tests of Hypotheses 5.A and 5.B will require manipulations of the degree of control, which could prove difficult because the mechanisms allowing for control remain unidentified in many systems.

#### DISCUSSION: CONCLUSIONS AND FUTURE DIRECTIONS

Multiple mutualistic partners can affect a focal mutualist in emergent ways that cannot be predicted from the independent effect of each mutualist alone. Here, we have shown that these multiple mutualist effects (MMEs) are common in nature and can arise through a number of different pathways. By merging network and consumer–resource models of mutualism, we introduce a new framework to classify and understand such complex, multispecies mutualisms.

Our unified approach shows that predicting when MMEs will occur and what their outcomes may be (Fig. 2) requires explicit consideration of the overlap in rewards exchanged among partners (Fig. 3). In addition, the costs of rewards, the degree of spatial/temporal overlap between mutualists, direct interactions among partners, and the degree to which focal mutualists directly control partners can modify these expectations in predictable ways (Fig. 4). Despite a collection of examples demonstrating MMEs for a wide range of ecological systems (Appendix: Tables A1–A2), the number of empirical studies measuring fitness consequences of MMEs remains small. Too few studies are available to quantitatively test the relative strength and potential interactions among different factors (e.g., overlap and control) in influencing the emergence and extent of MMEs. This remains a key challenge for future research.

Our synthesis suggested several approaches that would advance the understanding of MMEs and their ecological consequences. (1) Manipulate the presence of all partners in a factorial design to assess the net fitness effects of each species on the others singly and in combination. Studies of multiple predator effects provide experimental guidance (e.g., Griffen 2006, Byrnes and Stachowicz 2009), but care must be taken in interpreting additive, replacement, and response surface designs (McCoy et al. 2012). (2) Manipulate the rewards provided by and to the focal mutualist to characterize the degree of overlap in (and cost of) rewards. (3) Use structural equation models (Grace 2006) with field observations on the abundances of focal and partner mutualists to evaluate alternative models of direct and indirect pathways of interaction. (4) Observe temporal and spatial patterns of association between focal and partner mutualists to characterize the reliability of the interactions, and make use of demographic models for spatiotemporally variable MMEs (Palmer et al. 2010, Rudgers et al. 2010).

In summary, we illustrate that complex interaction outcomes can arise when a focal species supports multiple mutualistic partners that provide or receive functionally different rewards. Given the central role that mutualisms play in a wide variety of ecosystems by maintaining dominant and foundation species (e.g., corals, figs, grasses, legumes), MMEs are likely to have important consequences for local diversity, community composition, and possibly ecosystem functioning. In the same way that the effects of predator diversity on ecosystem functioning can depend on the degree of complementarity in predators' resource use, or on the frequency of intraguild predation (Finke and Denno 2005), the effects of mutualist diversity on ecosystem functioning may depend on the degree of overlap in the benefits exchanged. Many partner mutualists—from symbiotic algae to pollinators—are now threatened by climate change, disease, and anthropogenic disturbances, while other mutualists have been newly introduced to novel habitats. The altered diversity and structure of partner communities will likely have far-reaching ecological consequences, and we hope this synthesis will help to refine understanding of those outcomes.

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## SUPPLEMENTAL MATERIAL

### Appendix

Examples from the literature of multiple mutualists effects on the focal (Table A1) and partner (Table A2) mutualists, and the range of multiple mutualist effects on focal mutualist fitness (Table A3) ([Ecological Archives E095-070-A1](#)).