Context-dependent outcomes of mutualistic interactions

Jason D. Hoeksema and Emilio M. Bruna

10.1 Introduction

The common conception of mutualisms is of interacting species that experience enhanced performance in the presence of each other. Most mutualisms do not always conform to this stereotype, however. The outcome of interactions can vary over time and space along a continuum from strong mutualism to commensalism, or even change sign from mutualism to parasitism (Bronstein 1994a, Herre et al. 1999, Sachs and Simms 2006, Thompson 1988). This plasticity is often driven by the abiotic and biotic context in which interactions take place, such as the abundance of key nutrients or the identity of species present in the community. It can also be driven by genetic context, i.e., genetic variation in one or both species. Although for some species interactions many studies currently exist addressing context dependency, for others we know next to nothing, and many critical questions remain to be answered across all major types of mutualisms. Here, we aim to articulate some of those questions, assess progress to date on answering them, and highlight areas in particular need of future research.

We define context dependency of a mutualism to be a change in interaction net outcome; i.e., a change in the magnitude of how one species influences the average fitness correlates of another species. This change is in response to variation in the abiotic, biotic, or genetic context in which those two species are interacting, assuming that both species benefit from the interaction under at least some context. This definition includes cases in which a strong mutualism, wherein both species benefit substantially from the interaction, shifts to a weak mutualism or even changes sign to a parasitism from the perspective of at least one of the two species. Both of these cases involve context-dependent changes in the magnitude of an interaction. Thus, a change in sign of the interaction (from mutualism to parasitism) is a special case of the more general definition of context dependency—any change of interaction magnitude in response to context. In a mutualism with multiple potential partner species on both sides of the interaction, we do not consider a change in performance of a focal species when paired with alternate partner species to constitute context dependency. Rather, context dependency in such interactions would be observed as a change in interaction outcome between a particular focal pair of species, when compared between different background communities (potentially including other species participating in the interaction). Our definition of context dependency is broadly consistent with that used in previous reviews. For example, in a meta-analysis of 247 published studies of context dependency across mutualism, predation, and competition, Chamberlain et al. (2014) quantified context dependency in two ways: the coefficient of variation (CV) of interaction magnitude among contexts, and a change in sign of the interaction among contexts.

Chamberlain et al. (2014) showed that context dependency is often substantial in mutualisms, albeit not consistently more than in predator/prey or competitive interactions. The implications of such

context dependency may be far-reaching (Agrawal 2001, Bronstein 1994a, Thompson 1988, 2005, Thrall et al. 2007). If outcomes of mutualisms are context dependent, then interaction strengths estimated in a particular context cannot be extrapolated over time or space. Instead, they may be complex functions of their environment. Moreover, plasticity of outcomes can potentially stabilize mutualisms by ameliorating conflicts of interest between partners and promoting partner fidelity (Agrawal, 2001). If outcomes of a mutualism depend on contextual factors that vary over space and/or time, and if variation in outcomes is genetically based, then natural selection by species on each other's traits may also vary over time and space, potentially generating polymorphisms in populations and driving interpopulation divergence. It is therefore essential to understand the genetic basis of distributions of outcomes within populations, as well as genetically based variation in outcomes of interactions among abiotic or biotic environmental contexts (i.e. interaction norms) to predict how interactions will evolve (Thompson 1988).

The distribution of published studies on context dependency is extremely uneven among the major classes of mutualisms. For example, thousands of field and laboratory experiments have investigated the effects of mycorrhizal fungi, which inhabit the roots of plants and potentially enhance plant nutrient uptake from soils, on plant performance. Many of those experiments have recorded and/or manipulated contextual factors that could influence the outcomes of the interactions. Several reviews and meta-analyses have synthesized the results of these experiments (e.g., Hoeksema et al. 2010, Johnson et al. 1997, Jones and Smith 2004, Karst et al. 2008). Similarly, there is a relatively long history in agricultural ecology of exploring the dependence of the legume–rhizobia symbiosis, in which bacteria inhabit the roots of plants and provide fixed nitrogen (N) in exchange for plant photosynthetic products, on a variety of abiotic contextual factors such as soil N or phosphorus (P) availability, soil salinity, or water availability (see reviews by Graham et al. 2003, Serraj et al. 1999, Streeter and Wong 1988, Zahran 1999).

Plant–pollinator interactions are also among the most studied of mutualisms with regard to context dependency, with much of the attention focusing on how the outcome of these interactions changes as a function of local abiotic and biotic environments. For instance, a large body of experimental and observational work has documented that pollinator effects on plant fruit or seed production can vary with resources, the diversity and structure of the local habitat, and the presence of other herbivores, predators, and other species with which pollinators and plants interact (reviewed in Knight et al. 2005, Garcia-Camacho and Totland 2009, Ashman et al. 2004). The magnitude of pollinators' beneficial effects can also be affected by traits such as floral shape and color, nectar production, volatile emission, and other such cues used to attract pollinators, all of which can also be influenced by the local environmental or genetic context (Bradshaw and Schemske 2003, Ashman et al. 2005, Temeles and Kress 2003).

In contrast, surprisingly few studies have explicitly investigated effects of contextual factors on mutualisms formed by grasses and fungal endophytes, plants and their protective ants, cleaner fish and the clients from which they remove parasites, the nutritional symbiosis between cnidarian animals and dinoflagellate algae, or protective mutualisms between insects and symbiotic bacteria. For example, in the grass–endophyte symbiosis, very few studies have investigated contextual factors besides water and nutrient availability, despite the obvious and important influence that light, fire and other factors could have on the outcome of the symbiosis (Cheplick and Faeth 2009). For ant–plant mutualisms, meta-analyses have found that plant growth form or life-history strategy (annual or perennial) can influence the outcome of interactions, as does the diversity of ants with which plants interact (Chamberlain and Holland 2009, Trager et al. 2010). However, the way in which the abiotic or genetic context influences their outcome remains largely unknown. For instance, few studies have experimentally manipulated soil nutrients to explore how changes in characteristics such as plant quality and reward production influence ant and herbivore behavior; not unexpectedly, those that have suggest local resource availability can have far-reaching effects (e.g., Trager and Bruna 2006, Kersch and Fonseca 2005, Heil et al. 2001). Microclimate and light, which can influence ant community composition, rewards to partners, and plant growth, also appear to be important (Alonso 1998, Kersch and

Fonseca 2005, Folgarait and Davidson 1995). For cleaner–client fish interactions, only recently have experimental studies begun to accumulate showing mutual benefits for both cleaners and hosts, much less elucidating abiotic or biotic contextual factors that may alter interaction outcomes (but see, e.g., Cheney and Cote 2005).

Even in the most heavily studied mutualisms, many questions on context dependency are only in the earliest stages of being answered. Our overall goal for the remainder of this chapter is to suggest a roadmap for fruitful areas of investigation on context dependency in mutualisms. We start by outlining the four major types of research tools that can be used in such investigations. We then identify eight big questions on context dependency in mutualisms, assess progress to date in the application of the four major research tools to answering them, and point out areas in particular need of future research.

10.2 Tools for studying context dependency of mutualisms

We recognize four primary tools that can be used to explore context dependency in mutualisms: models, observational field studies, experiments, and meta-analyses.

10.2.1 Mathematical, graphical, and conceptual models

Models explicitly focusing on mechanisms of context dependency can help formalize and explore testable hypotheses on context dependency, and also allow the modeling of long-term dynamics (see also Chapter 4). In Box 10.1, John Jaenike uses a mathematical model to predict how the benefits conferred by bacterial endosymbionts to their insect hosts may vary in response to contextual factors,

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Box 10.1 Context dependency in defensive endosymbioses

John Jaenike

Many insects harbor bacterial endosymbionts that may defend their insect hosts against pathogens. Although little empirical work has confirmed how contextual factors may alter outcomes of these putative mutualisms, these systems may eventually provide substantial insights into factors driving variation in animal–microbe mutualisms. Outcomes of defensive symbioses are hypothesized to depend on how context-dependent factors affect two key variables controlling the impact of a parasite or pathogen on a host individual or population: the force of infection (the rate at which susceptible individuals become infected) and pathogen virulence (the extent to which host fitness is decreased by the infection). The review and mathematical model below illustrate how these two variables can be influenced by several biotic and abiotic variables, including community diversity and composition, epidemic fluctuations or outbreaks in pathogen populations, and ambient environmental temperatures.

In most models of pathogen dynamics, the force of infection increases with the number of infected hosts, which depends on host population size or density and the prevalence of infection (McCallum et al. 2001). For a single host species with age structure, the force of infection, λ, is $\beta \int_0^\infty y(a,t)\,da$,

where $Y(a,t)$ is the number of infected individuals of age a at time t, and β is the transmission rate, which, in this model, is assumed to be independent of host age (Anderson and May 1991). Now consider a community in which a pathogen can be passed among several host species. In a community of n host species sharing a common pathogen, the above equation can be modified to say that the force of infection for focal host species i is $\lambda_i = \sum_{j=1}^{n} \beta_{ij} y_j$, where β_{ij} is the transmission rate of the pathogen from individuals of species j to species i, including transmission from species i to itself, and Y_j is the number or density of infected individuals of species j. (This formulation ignores age structure within the host populations.) Therefore, if the density of species i remains the same, then the force of infection is greater in a community in which more species share a given pathogen and in which there are more opportunities for interspecific transmission. This would suggest that, in the case of protection against pathogens with a broad host range, there will be greater selection for protection by a defensive endosymbiont in species-rich communities, such as those in tropical regions. In addition, because defensive symbionts are acquired via lateral transfer from other host species, tropical communities, being more diverse, probably have more symbiont-infected host species that can serve as sources of

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Box 10.1 *Continued*

defensive symbionts for other species (Jaenike 2012). Moreover, the net fitness effect on an insect host of carrying a particular symbiont can depend on the host plant species on which the insect is feeding (e.g., Tsuchida et al. 2011). Thus, the species diversity and composition of a community in which a host species resides is likely to influence both selection for and opportunities to utilize defensive symbionts.

The force of infection by many pathogens is temporally variable. Many parasites, especially microparasites of insects, undergo wide fluctuations in prevalence, characterized by periodic outbreaks interspersed with longer periods of low infection prevalence (Briggs et al. 1995). Because these epidemics are associated with large changes in insect population size and the prevalence of parasitism, there is corresponding variation in the force of infection and thus the strength of selection for a defensive symbiont. One might think that the incidence of mutualism with a defensive symbiont would depend on the average prevalence of pathogen infection, but this is not the case. Consider a host-sterilizing parasite that either infects a constant proportion (p) of the host population every generation, or that undergoes periodic epidemics, infecting np of the hosts once every n years. If the host species carries a heritable defensive symbiont, then, for a constant rate of parasitism, the prevalence of symbiont inheritance will reach a stable equilibrium frequency, where the advantage due to selection is balanced by losses due to imperfect maternal transmission (Jaenike 2012). A numerical model shows that pathogen epidemics result in fluctuations in the prevalence of symbiosis with

a defensive symbiont (Box 10.1 Figure 1 A), and, perhaps more importantly, a greater mean frequency of mutualism establishment by the symbiont, even though the mean prevalence of pathogen infection is the same in the different cases (Box 10.1 Figure 1 B). In essence, the epidemic ratchets up the frequency of symbiosis occurrence, which then gradually declines during disease-free periods due to imperfect maternal transmission or the costs of symbiosis in unparasitized hosts, as has been found for pea aphids harboring symbiotic *Hamiltonella defensa* (Oliver et al. 2008). From a practical standpoint, the persistence of a defensive symbiont through long disease-free periods could make it difficult to identify the selective agent responsible for the persistence of a defensive symbiont in natural populations.

The prevalence of a defensive symbiont, and therefore the expected virulence of pathogen infections, can vary geographically due to environmental tolerance limits of a defensive symbiont. For example, parasites and/or defensive symbionts can have narrower temperature ranges than their hosts. The *Drosophila*-parasitic nematode *Howardula aoronymphium* is less tolerant of high temperatures than several of its host species (Jaenike 1995). *Drosophila hydei* carries a strain of *Spiroplasma* that confers protection from wasp parasitism (Xie et al. 2010) and whose transmission fidelity drops from 0.99 at 25 °C to 0 at 15 °C (Osaka et al. 2008). Thus, the level of protection that a defensive symbiont can provide may vary geographically and seasonally, and this could lead to spatial or temporal variation in the prevalence of parasitism and of symbiont infection (Box 10.1 Figure 2).
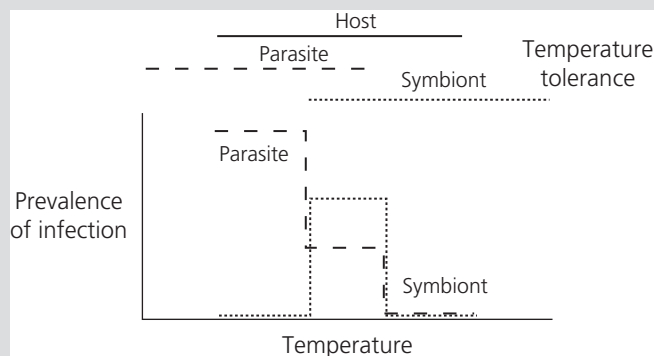

Figure 1 Variation among interacting hosts, parasites, and defensive symbionts in temperature tolerance could lead to geographic variation in the prevalence of parasitism and symbiont infection. It is assumed that, in the absence of parasites, the symbiont will be lost due to imperfect maternal transmission or the cost of infection.
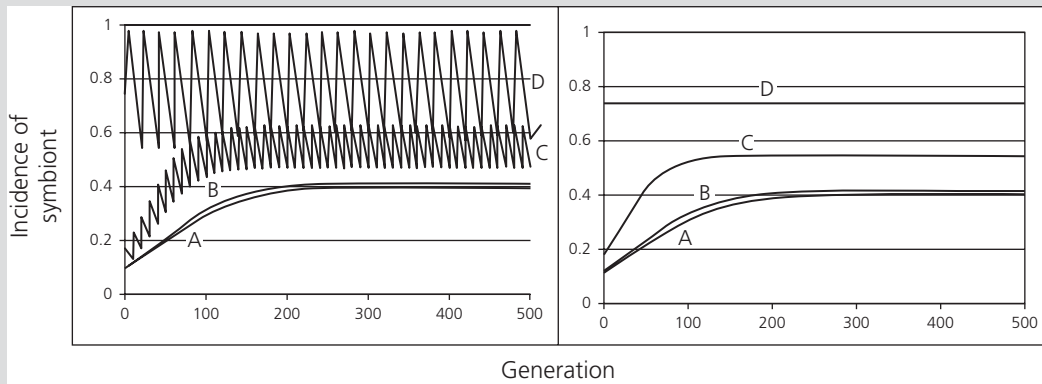
Box 10.1 *Continued*

Figure 2 Incidence of a defensive endosymbiont that protects against a pathogen that either kills or sterilizes its host. The model assumes that the fitness of unparasitized hosts or parasitized hosts carrying the symbiont is 1, while that of parasitized hosts lacking the symbiont is 0. Maternal transmission is set at 0.97, which corresponds to what is often seen in nature. The prevalence of parasitism = 0.05 every generation (A), 0.1 every other generation (B), 0.5 once every 10 generations (C), or 100% once every 20 generations (D). The generation to generation fluctuations in the prevalence of symbiont infection and the 20-generation moving average are shown on the left and right, respectively.

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including the species diversity and composition of the surrounding community, fluctuations in pathogen populations, and abiotic environmental factors. Such theoretical studies have the advantage of being able to explore a range of potential scenarios, which is especially helpful for interactions that have received limited empirical attention. However, it must be kept in mind that simple mathematical models will often make necessarily unrealistic assumptions.

10.2.2 Observational field studies

Detailed observations can measure the outcomes of mutualisms along biotic and abiotic gradients. For example, in the potential mutualism between coral reef fish and cleaner goby fish (Gobiidae), observational studies documented that environments with higher parasite loads were associated with lower rates of cheating (i.e., consumption of material such as mucus and scales) by the cleaning gobies (Cheney and Cote 2005). While such studies have the advantage of showing how mutualistic outcomes vary in a realistic context, a key limitation is that relationships of outcomes to contextual factors are correlative. Such correlations can suggest hypotheses for further testing, but can also be driven by other, unmeasured variables. In Box 10.2, Silvia Lomáscolo

Box 10.2 Elucidating context dependency in seed dispersal interactions

Silvia B. Lomáscolo

The outcome of seed dispersal interactions is difficult to assess because seeds do not have a specific "target" as pollen does in plant–pollinator interactions. Whether a seed lands in a site that is appropriate for germination and establishment depends greatly on the general characteristics of the surrounding environment, which often vary among microsites and with climate stochasticity. Quantity and quality of dispersal (Schupp et al. 2010) will also determine the fate of seeds. Quantity of dispersal is the number of seeds removed by a specific seed disperser agent, which may vary with fruit availability (Perea et al. 2013). Quality includes the treatment of seeds in frugivores' guts and the behavior of the seed disperser, which ultimately determine where the seed is deposited. Seed treatment is probably the least variable of all the factors considered above, while disperser behavior and concomitant seed deposition may greatly vary according, among other things, to overall characteristics of the environment. Variation in one or more of these factors, i.e., the context where the interaction takes place, will determine its outcome. The resulting complexity in quantifying the effect of a seed disperser on plant reproduction has led to the commonly cited statement that "the implied marriage of animal foraging with plant demography is rarely consummated" (Howe 1989).

The quantitative component of seed dispersal, i.e., the number of seeds removed by a specific frugivore, has been shown to vary among populations of a specific plant species (Perea et al. 2013). Seed dispersal effectiveness (SDE) of a frugivore, defined as the number of seedlings resulting from dispersal by a specific seed disperser relative to other seed dispersers (or relative to no dispersal; Schupp 1993, Schupp et al. 2010), includes the quantitative and the qualitative aspects together, and it has also been shown to vary according to the environment in which the population is located, and with overall fruit availability (Calviño-Cancela and Martín-Herrero 2009). As an example, a study done on the fleshy-fruited shrub, *Corema album*, in northwest Spain (Calviño-Cancela and Martín-Herrero 2009) shows that in two out of three populations of this plant, gulls are the most efficient dispersers as they deposit seeds in open habitat, compared to blackbirds, which perch on shrubs under which defecated seeds are not highly successful. In a third population, however, blackbirds were just as efficient dispersers as gulls were. The suggested mechanism is that very few shrubs grow where this third population is located, and hence blackbirds take seeds to an open habitat, just as

gulls do. Also, competition for fruit may decrease the proportion of seeds dispersed by the most efficient disperser (Calviño-Cancela and Martín-Herrero 2009). Therefore, the overall effectiveness of the complete assemblage of seed dispersers, which is included in the SDE concept (Schupp et al. 2010), may vary if fruit availability varies between populations.

Although the above-mentioned examples are first attempts to identify context dependency and the environmental variables associated with it, we are still far from understanding how much context dependency really exists in fruit–frugivore systems (Schupp et al. 2010), and whether the environmental variables found to be associated with context dependency in our examples are really the cause of the variation observed in SDE. Does SDE generally vary with microhabitat availability? Or with fruit limitation? Or with variability in roosting/perching/nesting site of frugivores? To answer these types of questions, we need to identify populations of plants located in habitats that differ in the variable of interest (e.g. microhabitat availability, degree of fruit limitation, roosting/perching/nesting site of frugivores), and measure SDE for those populations, either for a specific seed disperser or for the complete assemblage of dispersers. But populations may share a genetic background that will determine the requirements for germination and establishment. Hence, they may not be treated as independent data points to be compared. This is similar to the problem faced when trying to compare species (Felsenstein 1985), which violates the assumptions of most statistical tests and often leads to inappropriate interpretation of the patterns in the data. Phylogenetic comparative methods (PCMs) have been designed to ensure that taxa to be compared are independent (Harvey and Pagel 1991), and they can help in the identification of the causes of context dependency in the questions proposed for fruit–frugivore systems. Using the example above, say that we are interested in identifying whether perching site availability is causing the variation observed in SDE by blackbirds. We could take the experimental route, which may prove efficient for addressing some questions, and quantify SDE for blackbirds while controlling the number of perches in the habitat. Alternatively, we could identify several populations of *Corema album* where SDE by blackbirds varies and measure perching site availability in the habitat. These two variables can be plotted on the phylogeny of the plant populations, and their correlation can be quantified using different PCMs, depending on whether the variables are quantitative or categorical (Felsenstein 1985,

Box 10.2 *Continued*

Martins and Hansen 1997, Giannini 2003). PCMs may help to identify general causes of context dependency in plant–seed disperser systems, a true challenge for future research on context dependency in interspecific interactions.

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describes how observational field studies can be combined with phylogenetic comparative methods to advance our understanding of context dependency in plant–frugivore seed-dispersal mutualisms.

10.2.3 Manipulative field and laboratory experiments

Manipulating the biotic, abiotic, and genetic context can isolate the relative importance of particular factors. For example, in a study of a potential mutualism between leguminous plants and their root-symbiotic rhizobia bacteria that fix atmospheric nitrogen, Sprent (1976) found that *Phaseolus vulgaris* plants subjected to water stress exhibited a 70% reduction in the number of bacterial root nodules and a 90% reduction in nitrogen fixation rate per gram of nodule when compared to well-watered plants. Because contextual factors are manipulated by the experimenter and randomly assigned to experimental units independent of other potential causal factors, and outcomes of mutualisms can be compared among treatments and appropriate controls, such experimental studies have the advantage of stronger inference with respect to causation, compared to field observational studies. On the other hand, although contextual factors can often be most unambiguously manipulated in laboratory or greenhouse studies, the latter can be limited in realism and in the diversity of contextual factors that are explored.

One novel and potentially powerful experimental technique is the manipulation of genomic regions responsible for key traits governing the establishment or function of a mutualism. Mutant and wild type genomes can then be compared to assess how genetic context influences the outcome of interactions. For example, we now have the ability to manipulate regions of the genome responsible for traits attracting pollinators to plants (Bradshaw and Schemske 2003, Kessler et al. 2008; see also Chapter 5). Using such tools allows a comparison of the magnitude of benefits accrued by individual plant genotypes under different ecological conditions. In so doing, we can begin to evaluate at which level of trait expression these interactions shift from being commensal to mutualistic.

10.2.4 Meta-analyses

Synthesizing variation in the outcomes of mutualisms across accumulated empirical studies using formal meta-analysis can allow for more general conclusions than are possible from any particular empirical study. Typically, ecological meta-analyses are conducted by accumulating measures of

effect size from each of many studies, where effect size is a metric for an outcome of interest. There are two distinct ways that effect sizes can be calculated for meta-analysis on context dependency of mutualism. In the between-studies approach, effect size simply quantifies the outcome of a particular mutualism in each study, for example, the log ratio of mean plant performance with versus without an ant mutualist (e.g., Chamberlain and Holland 2009). In this case, context dependency is explored by modeling variation in effect size among studies as a function of contextual factors that vary among studies. These contextual factors are treated as explanatory variables in separate univariate meta-analysis models, or used together as predictors in a multiple regression meta-analysis model. A benefit of this between-studies approach is that it takes advantage of variation among studies in contextual factors, so that individual studies can be used in the meta-analysis even when they did not manipulate contextual factors. However, a caveat is that in such an analysis, associations of effect sizes with variation in contextual factors among studies are correlative, since this variation in context is observed, not experimentally manipulated.

A key concept in the interpretation of such between-studies meta-analyses is that although they may quantify the overall average outcome of a mutualism across many studies, and this average effect size may be useful for some questions, its average magnitude does not tell us a great deal about the degree of context dependency. Rather, to understand context dependency we must focus on the variation around this average effect size, and ask which contextual factors best explain this variation. For example, Chamberlain and Holland (2009) found that in a meta-analysis across 76 studies, ants on average had significantly positive effects on their host plants, in terms of plant performance or reduced herbivory. In addition, factors such as ant diversity—a form of local biotic environmental context—helped explain significant variation in benefits of ants to plants.

However, meta-analyses often also consider explanatory variables that vary among the species used in the studies being reviewed, such as growth habit and other life-history traits. Because they are fixed within species, variables such as these may not help advance our understanding of context dependency in a particular mutualistic interaction between two species. An alternative approach, the within-studies meta-analysis, utilizes only those studies in which a contextual factor of interest was experimentally manipulated or otherwise varied within the study, and an effect size is calculated for each study that quantifies not only the outcome of the mutualism, but how it was altered by the contextual factor in each study (e.g., Chamberlain et al. 2014). This approach may allow for stronger inference on any particular contextual factor, but fewer studies exist that can be utilized in this kind of meta-analysis.

Below, as we review progress in answering eight major questions about context dependency in mutualism, we highlight how each of the four main tools—models, observational field studies, experiments, and meta-analysis—have so far been applied, or could be applied, to make progress on these questions.

10.3 Eight big questions on context dependency in mutualisms

10.3.1 Question 1: What is the relative importance of multiple contextual factors for outcomes of mutualisms, and when do they interact to alter these outcomes?

Organisms engaged in mutualisms are subject to the influence of numerous contextual factors simultaneously. Some of these factors are likely to be significantly more important than others in influencing outcomes of mutualisms, and interactions among these contextual factors are likely common. Yet, relatively few studies in any mutualism system have explicitly examined both the main and interactive effects of multiple contextual factors.

Models of resource-exchange mutualisms between plants and microbial root symbionts (mycorrhizal fungi and rhizobia bacteria) in which plants receive nutrients such as N or P from the microbes in exchange for fixed carbon (C), have focused on abiotic factors such as ambient light and soil nutrient availability as key contextual factors that may influence ecological outcomes (e.g., Akcay and Simms 2011; see also Chapter 4). Typically, species

are predicted to benefit most from the interactions when the resources they are trading are most limiting to their performance, and when their partner has a relative surplus of that limiting resource. For example, models of legume–rhizobia mutualisms predict that the symbiosis will become less beneficial (or parasitic) for the plant host as soil N availability increases, since it may be less beneficial for the plant to trade C for N when it can obtain N directly from the soil. Furthermore, rhizobia may evolve to fix less atmospheric N under these conditions (West et al. 2002). If the plant lacks control over the interaction, which is often the case, then increased soil N availability can lead to parasitism, wherein the costs of the interaction outweigh the benefits for the plant. Under plant control, soil N fertilization should lead to reduced nodulation. Models also predict that when light is limiting to plant photosynthetic rates, the symbiosis should be less beneficial to both partners, as C becomes a more valuable commodity for the plant (see also Chapter 4). Most experiments on context dependency in plant–microbe mutualism have focused on single abiotic factors, finding general support for many of these predictions, although results vary greatly depending on the plant and microbial taxa involved (e.g., Johnson et al. 1997).

An important subset of conceptual models, however, has highlighted the likely importance of interactive effects of multiple abiotic resources, suggesting situations in which contingency of the mutualism on one resource depends on availability of a second resource. For example, Johnson (2010) developed the stoichiometric trade-balance model, which highlights the fact that N is incorporated in large quantities into the photosynthetic apparatus of plants, and also limits fungal assimilation of C received from hosts (see Figure 1 in Johnson 2010). This model predicts that although the arbuscular mycorrhizal (AM) symbiosis will be somewhat beneficial to both partners when overall soil fertility is low, i.e. when N and P are both relatively scarce, the mutualism will be strongest when soil N availability is high and soil P availability is low. Under these conditions, plant photosynthesis will not be N-limited, and C-for-P trade can be maximized. Experimental results from AM systems tend to support the trade-balance model (Johnson 2010), wherein N

fertilization in P-rich soil results in depressed AM fungal biomass and negative effects of AM fungi on host plants, but N fertilization in P-poor soils increases fungal biomass and enhances positive effects of AM fungi on host plants (reviewed by Johnson 2010). Wallander (1995) developed a related hypothesis suggesting that the degree of benefit ectomycorrhizal (EM) fungi derive from mycorrhizal symbiosis depends on the relative availability of N and P in the soil.

Lau et al. (2012) argued that it is essential to understand interactive effects of N and light on legume-rhizobial symbiosis, since N availability and plant N status may influence photosynthate production by the host (also see Son and Smith 1988). To test their idea, they manipulated light levels and soil fertility in the symbiosis between soybean (*Glycine max*) and *Bradyrhizobium japonicum*. Although they found no evidence for interactive effects of the two resources on plant benefits, they did find an interaction between light and soil nutrients for the number of nodules produced, suggesting the potential for non-additive effects of light and soil nutrients on rhizobial fitness benefits. Kersch and Fonseca (2005) performed a similar experiment on the ant–plant mutualism between the legume *Inga vera* and its ants, and found no evidence for interactions between ambient light levels and available soil nutrients. Clearly, interactions between light and soil nutrients are not universal in their influence on how plants benefit from mutualisms. Theoretical explorations are needed of how interactive effects of abiotic and biotic factors could impact particular mutualisms, based on the natural history of these systems.

The recent meta-analysis by Chamberlain et al. (2014) compared context dependency of mutualisms among different types of contextual gradients, including abiotic gradients versus biotic gradients (what they called "third party" gradients, which were usually the presence of a third species). One measure of context dependency in mutualisms, the CV of interaction magnitude among contexts, did not vary substantially between abiotic and biotic gradients. However, their second measure of context dependency, measured as a change in sign of the interaction outcome, was much larger for abiotic than biotic gradients. This result supports the

general hypothesis that abiotic gradients may have more dramatic effects on outcomes of mutualisms than biotic gradients, often causing a change from mutualism to parasitism or vice versa.

Hoeksema et al. (2010) used a between-studies meta-analysis to estimate the relative importance of different abiotic and biotic contextual factors for plant responses to inoculation with mycorrhizal fungi. In one analysis across 306 laboratory studies of plant responses to AM fungi, they estimated the relative importance of seven contextual factors, and found that plant benefits were more than twice as large when non-mycorrhizal microbes were present (compared to when the background soil was sterile) and that plants responded much more positively when the fungal inoculum contained more than one species (Hoeksema et al. 2010). Those factors were significantly more important than whether or not fertilizer was added to the background soil in which the experiment was conducted, or whether that soil was sterilized at the outset of the experiment. In another analysis across 130 studies of plant responses to AM fungi, they found support for the trade balance model, as plant growth responses were more positive when plants were relatively more limited by soil P than by soil N (Hoeksema et al. 2010). In theory, this approach could be applied to any mutualism, once sufficient studies have accumulated in which the benefits to one or both partners in the mutualism have been measured. An important caveat to this approach, however, is that associations detected between effect size and study-level characteristics are correlational, since variation in predictors among studies was not manipulated experimentally. Thus, it will continue to be important to use experimental approaches in which multiple abiotic and biotic factors are manipulated to test specific hypotheses from theory to application to particular systems (Chamberlain and Holland 2009).

10.3.2 Question 2: Which aspects of biotic context have the most substantial effects on outcomes of mutualisms?

Since John Thompson (1988) wrote that "There are no analyses showing how the distribution of outcomes in a two-species interaction is affected by interactions with other species," significant progress has been made on this question. For all of the major mutualisms we surveyed here, we found at least a few examples in which mutualism outcomes were found to vary among different biotic contexts (see also Chapter 9). For example, well-studied mutualisms involving a plant partner (mycorrhizae, legume–rhizobia, plant–pollinator) seem to be commonly influenced by the presence or density of herbivores (Bethlenfalvay and Dakessian 1984, Heath and Lau 2011, Strauss et al. 1996), and those involving an animal partner (coral-algal, cleaner–client, and insect–bacteria) are influenced by the presence or density of pathogens or parasites (Cheney and Cote 2005, Douglas 2003; Box 10.1). Mutualisms involving microbial symbionts (mycorrhizae, legume–rhizobia, plant–endophyte) have been shown to be influenced by the presence or composition of non-mutualistic microbes (Larimer et al. 2010). For most mutualisms, however, there are still relatively few studies explicitly investigating the effects of local biotic context, much less comparing the magnitude of effects from different components of the biotic environment. For example, in ant–plant mutualisms, only a few studies have explicitly tested how the composition of the local ant community influences plant benefits from particular ants (Bruna et al. 2004, Palmer et al. 2010; see also Chapter 9). While many ant species can occupy or feed from different plant species, the effects of local plant community composition on ant fitness are largely unknown. In coral-algal mutualisms, explicit studies of the influence of biotic context have been even more limited; obvious needs are to understand how the outcomes of interactions between particular coral and algal species are affected by the presence or density of additional coral or algal species, or by the presence of particular coral pathogens or consumers.

Despite the relative paucity of studies on biotic context in some major mutualisms, one meta-analysis has already been published utilizing studies from the most heavily studied mutualisms, and it suggests some general conclusions relevant to Question 2. Morris et al. (2007) compiled 160 studies of plant performance in experiments in which the presence of putative mutualists and other species (either "enemy" species or other mutualists) were crossed factorially. This set of studies allowed

a meta-analysis of whether the effects of particular mutualists on plants differed depending on the type of mutualist, the presence of additional mutualist species, or the presence of enemy species. One key conclusion was that the effects of mutualists on plants were not altered on average by the presence of additional mutualists, but plant response to mutualists was enhanced by the presence of enemy species. This pattern apparently emerged because belowground microbial mutualists (rhizobia and mycorrhizal fungi) commonly protect plants from pathogens. Larimer et al. (2010) performed a similar meta-analysis across 31 studies of plant responses to factorial combinations of at least two different microbial symbionts. Similar to Morris et al. (2007), they found a synergistic effect on plants coinfected by mutualistic mycorrhizal fungi and pathogenic fungal endophytes, wherein plant benefits from mycorrhizal fungi were enhanced by the presence of the endophyte. In contrast, they found that the effects of mycorrhizal fungi, beneficial endophytes, and rhizobia on plant growth were not synergistic with each other. A broad implication of the results from these two meta-analyses is that to accurately assess the importance of a particular mutualism, it should ideally be measured in the presence of a relatively realistic community context, and that omitting natural enemies or pathogens of the host may likely underestimate the host benefit from the mutualism.

10.3.3 Question 3: How do mutualisms shift along gradients of environmental productivity?

Thrall et al. (2007) developed a set of general hypotheses for how symbiotic mutualisms and parasitisms should be expected to shift along gradients of environmental productivity. They predicted that increasing productivity—for which availability of resources such as nutrients, light, and water are typically used as surrogates—should favor antagonism over mutualism in symbioses, and therefore parasitic versus mutualistic individuals, genotypes, or species. Some theoretical and empirical studies on the major symbiotic mutualisms align well with this prediction. In mycorrhizal and legume–rhizobia interactions, for example, manipulative experiments have shown that weak mutualism or even parasitism by symbionts on their host plants is often favored

when overall soil fertility is high (Jones and Smith 2004, Zahran 1999) and when plant or fungal density is maximized (Bever and Schultz 2005), and observational field studies have shown that reef-building corals are especially common in marine waters of low productivity (Thompson 1988).

On the other hand, extreme nutrient limitation (arguably the opposite of high productivity) can lead to the breakdown of the mycorrhizal mutualism (Treseder and Allen 2002). Moreover, more beneficial mycorrhizal mutualisms are favored when plant photosynthesis is enhanced under high light and/or atmospheric CO_2 concentrations (Johnson 2010, Johnson et al. 1997, Jones and Smith 2004, Treseder 2004), which could be considered higher productivity environments. Similarly, water and salinity stress (again, opposite of high productivity) tend to reduce benefits conferred in the legume–rhizobia mutualism (Serraj et al. 1999, Zahran 1999). Other symbiotic mutualisms, including corals (Douglas 2003) and grass–endophyte symbioses (Cheplick and Faeth 2009, Saikkonen et al. 2006), exhibit inconsistent changes in outcomes along manipulated gradients in productivity. Among non-symbiotic mutualisms, plant–pollinator interactions are inconsistent in their responses to gradients of productivity (Burkle and Irwin 2009). Others, e.g., cleaner–client fish interactions and ant–plant mutualisms, have received very little empirical attention on this question (but see Trager and Bruna 2006).

Thus, to date, it does not appear that patterns of how symbiotic (or other) mutualisms respond to variation in abiotic context support the general prediction that increasing environmental quality should favor antagonism over mutualism. However, this question might be best approached using within-study meta-analysis, with multiple studies in which mutualism outcomes were measured along productivity gradients, or between-study meta-analysis, with multiple studies varying in the productivity of the ambient conditions in which mutualism outcomes were measured.

10.3.4 Question 4: How do mutualisms shift along gradients of diversity?

Thrall et al. (2007) also hypothesized that more diverse community contexts may present more

opportunities for conflict, and predicted that the average effectiveness of particular symbiotic mutualisms might generally decline along gradients of increasing diversity. As far as we are aware, this question has not been explicitly tested empirically, although studies of insect–bacteria defensive endosymbiosis seem to support the idea (Box 10.1). We agree with Thrall et al. (2007) that the plant resource-exchange mutualisms (e.g., mycorrhizae, legume–rhizobia) might provide ideal opportunities to test such predictions, since they range from mutualism to antagonism and occur across gradients of host plant and symbiont diversity. In one experimental study, van der Heijden et al. (1998) found that plant diversity and biomass, plant tissue P content, and fungal hyphal length all increased along an experimental gradient of increasing AM fungal species richness. The meta-analysis by Hoeksema et al. (2010) across laboratory studies of plant responses to AM fungi found that plant responses to inoculation with AM fungi were substantially more positive when multiple fungal species were present in the inoculum, compared to single-species inoculations. In support of the prediction by Thrall et al. (2007), a meta-analysis found that plants tended to benefit less from ant mutualists, in terms of decreased herbivory, when the ant mutualist community was more diverse (Chamberlain and Holland 2009; see also Chapter 9). However, none of these studies has explicitly tested whether or how the benefits exchanged between two particular interacting mutualists were altered by the diversity of the community. More experimental studies are needed measuring mutualism outcomes for particular pairs of interacting species, along explicitly manipulated gradients of diversity.

10.3.5 Question 5: Are mutualism outcomes density dependent, and if so, in what manner?

If mutualisms generally decline in strength with increasing density, this form of context dependency could act as a general mechanism by which populations are regulated through negative feedback. If mutualism outcomes also decline in strength at very low densities, they could contribute to Allee effects, whereby rare species decline precipitously. Holland and DeAngelis (2001) developed analytical and simulation models of density-dependent

outcomes for mutualisms between plants and their pollinating seed predators, and found a broad range of conditions under which populations of one mutualist could be regulated by density-dependent outcomes of its interactions with the other species. Holland et al. (2004) found empirical support for these ideas in experimental and observational field studies of the interaction between the senita cactus (*Pachycereus schottii*) and the senita moth (*Upiga virescens*), whose populations are regulated by density-dependent responses of the cactus. Holland and DeAngelis (2010) developed a general consumer–resource model that shows the generality of density-dependent population regulation in mutualisms (see also Chapter 8). Such phenomena are worthy of study across additional mutualisms, as they could prove to be universal principles of population regulation in mutualisms (Dean 1983).

Neighbor density significantly influences outcomes of mycorrhizal interactions, and results have been fairly consistent among studies. Typically, plant benefits from mycorrhizal fungi decrease as plant density increases in manipulative experiments (reviewed by Koide and Dickie 2002). One explanation for this observation is that at high root densities, overlap of nutrient depletion zones around roots is extensive enough to render mycorrhizal hyphae superfluous to plant nutrient absorption. Variation in fungal densities may similarly affect the benefits derived by plants from the mutualism (Bever and Schultz 2005). Such phenomena could provide density-dependent regulation of populations in mycorrhizal interactions.

Perhaps some of the most comprehensive work on the way in which the outcome of mutualisms is density dependent has been conducted on plant–pollinator interactions. The visitation rates to plants can increase with conspecific density, though this can depend on local diversity and pollinator preference in complex ways (Kunin 1993). All of these factors will interact with local landscape structure to influence the rates of fruit production and gene flow (Côrtes et al. 2013). Nevertheless, much remains to be learned about the way in which pollinator benefits vary with local density. As with other mutualisms, most attention has been focused on only one partner, in this case the sessile plants in which it is easier to assess surrogates of fitness and local recruitment.

Meta-analysis could potentially be used to analyze how outcomes of multiple types of mutualisms vary with densities of the partner species. However, a recent literature search (Chamberlain et al. 2014) found insufficient numbers of studies that manipulated the density of one or both mutualists, so currently meta-analysis on this question would require using a between-studies approach, in which outcomes of particular mutualisms are compared across studies differing in the density at which experimental individuals were grown.

10.3.6 Question 6: Are patterns and consequences of context dependency typically similar for both partner species in mutualisms?

Answering this question is especially essential if we wish to understand the consequences of context dependency in mutualisms for coevolutionary dynamics. Friesen (2012) found sufficient studies to conduct a meta-analysis across experiments in which variation in both host plant and rhizobium fitness components were estimated. Across several methods of analysis, results from the meta-analysis point toward positive fitness correlations between hosts and symbionts, suggesting that antagonistic genotypes are rarely observed and that evolutionary mechanisms that align host and symbiont fitness may be prevalent, leading to mutualistic coevolution (Friesen 2012). Similar meta-analyses may be possible for experiments on mycorrhizal mutualisms in which it is relatively common to quantify some aspect of both fungal and plant performance. See Chapter 4 for a discussion of models of evolutionary processes that could lead to alignment of host and symbiont interests.

Consistent with Bronstein's (1994b) observation that studies of mutualisms are rarely reciprocal, two decades later, few studies quantify consequences of mutualisms for both partners. Moreover, studies are usually short term, which limits the degree to which benefits can be assessed for the focal taxon. As a consequence, Question 6 remains largely unanswered for several major classes of mutualisms. For example, while the costs and benefits for plants of participating in ant–plant mutualisms are well documented, virtually nothing is known about the costs and benefits for their ant partners (but see, e.g., Frederickson and Gordon 2009, Nery

and Vasconcelos 2003). While the conclusion that participating in these interactions is generally beneficial will almost certainly hold true for some ant partners—for instance, ants that obligately reside in plant domatia (plant structures apparently evolved for housing ants)—for facultative participants such as those feeding on extrafloral nectaries the benefits derived may be incidental.

One-sided studies also dominate the literature on plant–pollinator interactions. In much the same way we know little about the benefits of participation in plant–ant interactions for ants, almost nothing is known about the fitness benefits of participation in pollination for animal pollinators, or how these benefits vary as a function of local abiotic and biotic conditions (but see Crone (2013) for a rare example of a demographic response observed in bees in response to changes in floral resources). This is hardly surprising. The tracking of individual pollinators and assessing their fitness is logistically difficult, especially in a field setting. However, it is nevertheless surprising to see that with the exception of highly specialized pollinating seed consumers such as fig wasps or yucca moths, where assessing fitness of pollinators is straightforward, there are almost no attempts to measure how pollinator fitness varies as a function of local environmental conditions or partner identity. While challenging, doing so is an essential component in modeling the evolution of pollination, in that fitness is the currency that ultimately drives the maintenance of mutualisms in ways that more commonly recorded data on pollinator behavior or energetic gains does not.

In the coral reef realm, studies on the reciprocal benefits to the mutualists exist for coral reef fish that obligatively inhabit either corals or sea anemones. Given the obligatory nature of the mutualism for the fish, the benefits to the fish from the symbiosis are relatively straightforward. However, since the host is a sedentary organism that potentially cannot get rid of its inhabitants, it is unclear whether its inhabitants affect the fitness of the host. Liberman et al. (1995), both in a seven-month experimental fish removal study and in following naturally inhabited and uninhabited corals, demonstrated that the coral *Stylophora pistillata* grew more in the presence of the coral reef fish *Dascyllus marginatus* than corals without fish. In addition, since

coral reproduction is a function of the coral surface area, the coral's reproductive output also increased in fish-inhabited corals. Similarly, the sea anemone *Entacmaea quadricolor*'s growth was detrimentally affected by the removal of the anemonefish *Amphiprion bicinctus*, due to the host contracting in the absence of the symbionts and to increased predation on the host by butterflyfish (Porat and Chadwick-Furman 2004).

Symbioses between *Symbiodinium* algae and cnidarian animals form the foundation for coral reefs worldwide, and these ecosystems are under threat from the breakdown of this symbiosis via the phenomenon known as coral bleaching. In Box 10.3,

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Box 10.3 The importance of the combined host–symbiont (holobiont) perspective in studies of cnidarian–*Symbiodinium* mutualisms

Tamar L. Goulet

The obligate mutualisms between many tropical cnidarians (e.g. corals, octocorals, sea anemones) and intracellular dinoflagellates (*Symbiodinium* spp.) are the centerpiece of coral reefs. These interactions can be highly context dependent, as changes in environmental conditions such as elevated or reduced seawater temperatures, higher or lower irradiances, ocean acidification, sedimentation, and even pathogenic organisms can lead to a reduction in *Symbiodinium* density and/or photosynthetic pigments (reviewed in Douglas 2003). This mutualism breakdown, commonly called coral bleaching, has been increasing in frequency and intensity on coral reefs worldwide (Hoegh-Guldberg 1999). The extent of coral bleaching, and whether it eventually leads to host death, differs between cnidarian species and even within a species (Goulet et al. 2008, Baird et al. 2009). With the continuation of global climate change, the projections for coral reef ecosystems are dire, although recently there has been an increased awareness that coral reef response is context dependent (Pandolfi et al. 2011). In an attempt to understand, predict, and potentially ameliorate coral bleaching, comprehending the context dependence of perturbations on the host, the endosymbiont, and the host–symbiont combination (holobiont) is imperative.

A hindrance to this goal is that much of the cnidarian–*Symbiodinium* research approaches the symbioses from the host or symbiont point of view, rarely assessing both partners' contribution to the combined entity of the holobiont. Some studies attribute the demise of the symbiosis to the symbiont present (Strychar and Sammarco 2009). Conversely, coral hosts themselves vary in their ability to withstand environmental stressors such as elevated temperatures (Baird et al. 2009), and hosts exposed to a prior environmental perturbation seem to be hardier facing a new perturbation (Pandolfi et al. 2011). But, since the symbiont resides within the host, the potential of the holobiont is a product of a given host–symbiont genotypic combination. Although the holobiont's physiological properties are based on the symbiotic partners involved, the host–symbiont combination yields a physiology unique to that holobiont. The sea anemone *Aiptasia pallida*, for example, hosts either clade A or B *Symbiodinium* in the Florida Keys and clade B *Symbiodinium* in Bermuda and other geographic locations (Goulet et al. 2005). At ambient temperatures, artificial holobionts, composed of *A. pallida* from Bermuda hosting clade A symbionts, produced oxygen fluxes that differed from either natural host–symbiont combinations of Bermuda host–clade B symbionts or Florida host–clade A symbionts. The artificial holobiont's oxygen production at elevated temperatures was in fact higher than either one of the natural holobionts (Goulet et al. 2005). Infecting the coral *Acropora millepora* with different symbionts, including those not naturally found in the symbiosis, yielded differences in thermal tolerance amongst the holobionts (Mieog et al. 2009). Therefore, reaching conclusions about a given holobiont based on data about one of the partners may not represent the holobiont's capabilities.

The holobiont is a composite of the forces working on the symbiotic partners. External forces may vary in their positive or negative effects on the partners, making the holobiont a fluid entity that may be robust or fragile in any given environment (Box 10.3 Figure 1). Consequently, trade-offs may drive the persistence of a symbiosis in a given environment. For example, *A. millepora* coral colonies hosting clade D *Symbiodinium* are more tolerant of elevated seawater temperatures compared to colonies predominantly hosting clade C (Berkelmans and van Oppen 2006). On the other hand, *A. millepora* colonies hosting clade D grow significantly less compared to colonies hosting clade C *Symbiodinium* (Jones and Berkelmans 2010), but this effect is specific to the coral reef site (Mieog et al. 2009). Therefore, a coral holobiont's survival is context dependent, combining the effects of current environmental conditions with prior perturbation history, host and symbiont physiologies, and the unique physiology of the holobiont. Consequently, when assessing the ability of a holobiont to withstand environmental change, multiple parameters need to be concurrently measured in the host, symbiont, and the resulting holobiont since complex trade-offs may occur within the symbiosis in a given environmental context.
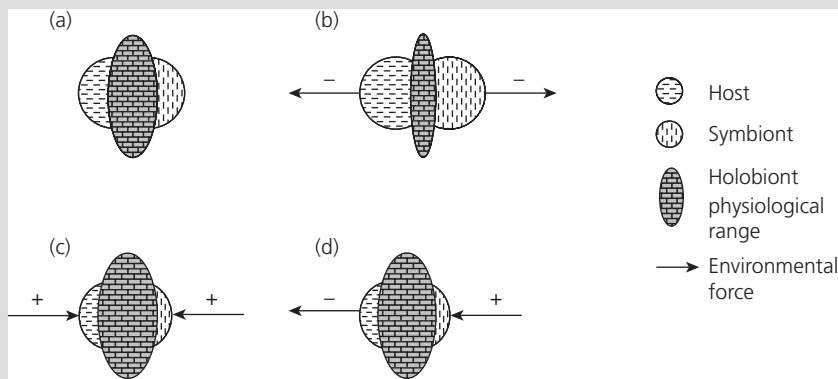
Box 10.3 *Continued*

Figure 1 The holobiont is a unique physiological entity affected by environmental forces acting on the host and symbiont. (a) A host–symbiont combination produces a holobiont with its own unique physiological range. (b) When environmental forces detrimentally (−) affect both the host and symbiont, the holobiont's physiological range may narrow and the demise of the holobiont may ensue. (c) When environmental forces positively (+) affect both host and symbiont, the resulting holobiont may be robust, surviving, and even flourishing, in that environment. (d) When environmental forces negatively affect the host (−) while positively (+) affecting the symbiont, the resulting holobiont may be robust to environmental change. The opposite scenario, a positive effect on the host and a negative effect on the symbiont, may also produce a robust holobiont.

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Tamar Goulet explores how analyzing both sides of the interaction, i.e. understanding context dependency for both the cnidarian and the algae through a "holobiont" approach, is essential for predicting and potentially mitigating the effects of coral bleaching on coral reef ecosystems.

The literature on ant–plant mutualisms illustrates the importance of long-duration studies for answering the question of how species on both sides of an interaction benefit from the mutualism. Most studies of ant–plant mutualisms are of relatively short duration, and as the landscape in which species

are interacting changes, the costs and benefits provided by partners may change as well. Herbivore pressure, rewards offered to partners, the dispersal capability of partner species, and the diversity and abundance of partners can all vary temporally (Diaz-Castelazo et al. 2010, Bruna et al. 2005, 2011, Moraes and Vasconcelos 2009) or as a result of anthropogenic activity (Bruna et al. 2005, Moraes and Vasconcelos 2009). This may be why even well-studied ant–plant systems can show very different dynamics across their range or in different years (e.g., Rudgers and Strauss 2004). While perhaps not as dramatic as the context dependency observed in other systems, the extent to which ant–plant systems exhibit context dependency requires studies evaluating the costs and benefits for both partner species in light of changes to the habitat in which they are found.

10.3.7 Question 7: How important are "cryptic" benefits for outcomes of context-dependent mutualisms?

Studies documenting context dependency in mutualisms necessarily quantify the performance of species on at least one side of the interaction, but do not necessarily measure the actual benefits being exchanged between putative mutualist species. For example, many studies of the mycorrhizal mutualism quantify plant growth with and without mycorrhizal fungi, but do not measure the soil nutrients passing from fungus to plant or the carbohydrates passing from plant to fungus. Despite the lack of quantification, these commodities are often assumed to be the primary mechanisms by which partners benefit from mutualisms. Conversely, cryptic or alternative benefits, such as pathogen protection of host plants by mycorrhizal fungi (Marx 1969, Wehner et al. 2010), are increasingly being discovered in diverse mutualisms. For example, tactile stimulation ("tickling") of client host fish by cleaners may reduce stress in hosts (Bshary et al. 2007, Soares et al. 2011), conveying a cryptic benefit that does not depend on parasite removal. Infection of cleaners by the parasites they have removed from hosts may be a cryptic cost to cleaners; cleaner wrasses have been found to ingest, harbor, and transmit

infective stages of bucephalid trematodes (Jones et al. 2004).

If cryptic benefits are as important as the benefits typically measured, then they have important implications for the design of experiments meant to measure context dependence in mutualisms. Indeed, hypotheses on key contextual factors affecting mutualistic outcomes are usually explicitly grounded in some assumption about the benefits being exchanged. For example, if tactile stimulation is more important than parasite removal as a benefit in cleaner–client mutualisms, then ambient densities of parasites would not be expected to be as significant as a contextual factor affecting the outcome of the mutualism. In studies of the mycorrhizal mutualism, if protection from pathogens is an important cryptic benefit that fungi convey to plant hosts, then pathogen presence is a key contextual factor that should be manipulated or explored. A corollary of this point is that experiments performed in environments from which pathogens have been removed or excluded would be expected to observe smaller benefits to plants from mycorrhizal fungi. This is one possible explanation for why the meta-analysis by Hoeksema et al. (2010) found that plant responses to inoculation with AM fungi were substantially more positive when the background soil for the experiment was not sterile but rather contained a diverse community of non-mycorrhizal microbes (such as pathogens). We suggest that careful quantification and consideration of not only standard but also cryptic benefits in mutualisms will lead to studies that provide a much clearer understanding of the reasons for variation in mutualistic outcomes.

10.3.8 Question 8: How often do outcomes of mutualisms depend on G × E, G × G, and G × G × E interactions?

Outcomes of mycorrhizal interactions (reviewed by Hoeksema 2010, Johnson et al. 1997), legume–rhizobia interactions (e.g., Miller and Sirois 1982), and grass–endophyte symbiosis (Tintjer and Rudgers 2006) can vary substantially across different genotypes of the same microbial or host plant species. The magnitude of such genetic effects is often as large or larger than variation among species

(Johnson 2010), and can span the range from mutualism to parasitism. However, it is the context dependency of this genetic variation that determines the potential for direct selection on mutualism traits from abiotic or biotic factors, coevolution, and geographic mosaics of coevolution.

Within-population genetic variation for how mutualisms respond to abiotic and biotic contextual factors provides the raw material for context-dependent selection on the traits of mutualists. Conversely, context-dependent selection can play a key role in maintaining genetic variation within mutualist populations. Specifically, genotype-by-environment (G × E) interactions for fitness of one or both partners in a mutualism enable spatially or temporally variable selection on a mutualist species, potentially leading to local adaptation, because genotypes have different relative fitnesses in different environments. Similarly, genotype-by-genotype (G × G) interactions for fitness of mutualist partners are a prerequisite for coevolutionary selection, wherein selection on one species differs among genotypes of the other (Heath 2010, Thompson 1988). Finally, genotype-by-genotype-by environment (G × G × E) interactions allow geographic selection mosaics (Thompson 2005) whereby coevolutionary selection varies spatially among different environmental contexts (see also Chapter 7). Thus, quantifying these interactions is an essential prerequisite to understanding whether context-dependent natural selection may generate and maintain trait diversity in mutualisms.

Among all the major mutualisms, mycorrhizal and legume–rhizobia interactions have been the most frequent subjects of studies explicitly manipulating the genotypic composition of both partners. As a consequence, we now have convincing experimental evidence for G × E, G × G, and G × G × E interactions in both of those mutualisms. In some mycorrhizal mutualisms, for example, significant interactions exist between host plant or fungal genotypes and the presence of heavy metal contamination in soils (reviewed by Meharg and Cairney 2000), showing that plant and mycorrhizal fungal populations can adapt rapidly in response to that direct abiotic selection (see Stahl and Smith 1984 for evidence of a similar G × E interaction with respect to drought). In studies of legume–rhizobia mutualisms, salt-tolerant rhizobia strains improve legume host plant performance in the presence of high salinity (e.g., Zou et al. 1995), implying that such G × E interactions would drive local adaptation of the symbiosis in response to salinity gradients. In contrast, other studies have found that salt-tolerant rhizobial strains do not form functional nodules (reviewed by Zahran 1999) or do not improve host performance in saline soils (e.g., Lal and Khanna 1994), or that salt-tolerant host species are less responsive to rhizobial inoculation (Thrall et al. 2008). The latter results imply that salinity gradients could drive evolutionary divergence in dependency on the symbiosis, due to genetic trade-offs in rhizobia or hosts between tolerance of salt stress and symbiotic compatibility.

An experimental study of the interaction between the leguminous plant barrel medic (*Medicago truncatula*) and its *Sinorhizobium* symbionts (Heath 2010) found that both plant and bacterial fitness were substantially altered by an interaction between host and symbiont genotype, i.e. G × G (see also Heath and Tiffin 2007, Parker 1995). If such variation is common, it implies that the outcome of a mutualism involving a particular host or symbiont genotype depends on which partner genotypes it interacts with, and suggests potential for ongoing coevolutionary dynamics in legume–rhizobia interactions. In mycorrhizal interactions, recent studies have tested for three-way interactions between host plant genotype, mycorrhizal fungal genotypes or species, and some aspect of the abiotic or biotic context (Johnson et al. 2010, Piculell et al. 2008), i.e. G × G × E interactions. In an experimental study of interactions between the grass *Andropogon gerardii* and its symbiotic AM fungi, Johnson et al. (2010) estimated plant and fungal performance in all reciprocal combinations of plant populations (G), whole AM fungal guilds of species (G), and sterile soils (E) from three geographic sites differing in climatic and soil characteristics. The authors found a significant G × G × E interaction for the formation of arbuscules, the fungal structures formed inside the plant root through which nutrient exchange occurs. Arbuscule formation was highest in local combinations of plants, fungi, and soils, suggesting the potential for natural selection to drive three-way local adaptation among plants, fungi, and soils. Similarly, experiments have shown that soil N availability

can alter the nature of particular G × G interactions between legume and rhizobia genotypes. In a series of greenhouse experiments on the host plant barrel medic and bacterial symbionts in the genus *Sinorhizobium*, Heath and Tiffin (2007) and Heath et al. (2010) found that nodulation depended on a three-way interaction (G × G × E) between rhizobial strain, host genotype, and soil nitrogen availability, with variation in nodulation across host–symbiont combinations depending on nitrogen environment (see Figure 2 in Heath et al. 2010).

Clearly both mycorrhizal and legume-rhizobial mutualisms harbor enough genetic variation in context dependency that there is strong potential for ongoing direct abiotic and biotic selection, local adaptation, coevolution, and geographic mosaics of coevolution in these mutualisms. We still, however, understand little about the relative importance of abiotic and biotic contextual factors in driving evolution of mutualism traits. Moreover, in most cases the demonstrations of genetic variation affecting fitness outcomes are not tied directly to specific traits, so we do not know what traits are under selection, nor do we know the form of coevolutionary selection (e.g., positive versus negative frequency dependence). For contextual variation in abiotic resources that are traded in these resource-exchange interactions, variation in evolutionary trajectories will likely be driven by how environmental variation in a resource alters the costs and benefits of the trading dynamics for different genotypes. Studies are needed that not only test for interactions between genetic variation and these environmental gradients, but also estimate selection on specific traits from specific sources (Ridenhour 2005).

Among the other major mutualisms (besides mycorrhizae and legume–rhizobia), quantification of G × E, G × G, and G × G × E interactions for fitness of one or both partners has been more limited. In the grass–endophyte symbiosis, for example, a reasonable number of studies provide evidence for significant host genotype by endophyte infection (+/−) interactions on components of host fitness, or interactions among host genotype, endophyte infection, and abiotic factors. For example, Krauss et al. (2007) found that biomass response of *Lolium perenne* host plants to infection with *Neotyphodium lolii* fungal endophytes was significantly influenced by an interaction between host cultivar and fertilization. Our ability to understand the potential for ongoing *coevolutionary* selection in this symbiosis is severely limited by the paucity of studies controlling both host and endophyte genotype and thus exploring G × G or G × G × E interactions. In the coral-algal symbiosis some evidence for G × G interactions exists from studies showing that properties of the holobiont (the host–symbiont combination) are unique to particular combinations of host and algae (Box 10.3). As in mycorrhizal and legume–rhizobia interactions, studies in these systems have not yet taken the step of showing which traits might be under natural selection, and from which abiotic and biotic sources. In bacterial defensive endosymbioses of insects, preliminary evidence demonstrates the potential of G × G × E interactions, whereby variation among environments in pathogen prevalence may cause variation in selection on infection status, which is a trait that may be controlled by the genotypes of both the host and symbiont (Box 10.1; J. Jaenike pers. comm.). Rudgers and Strauss (2004) showed that natural selection on wild cotton plants to allocate resources toward extrafloral nectar for attracting ant protection mutualists varied among wild cotton populations, providing a rare example in which context-dependent outcomes in a mutualism have been shown to lead to altered natural selection on the interaction. Until more studies of diverse mutualisms accumulate, in which genotypes and associated traits of one or both partners are varied experimentally, and their fitness measured across key abiotic and biotic contextual gradients, and the targets and sources of natural selection are estimated, we will have only a rudimentary understanding of how context dependence may be driving trait evolution in mutualisms.

10.4 Concluding thoughts

Most major mutualisms have the potential to dissolve or shift to parasitism under particular conditions. Even interactions with mean outcomes that are consistently mutualistic exhibit substantial variation among contexts (e.g., ant–plant mutualisms; Chamberlain and Holland 2009), much of which

variation remains unexplained. Explaining this variation can aid in answering a wide variety of questions in ecology and evolution, including those outlined here (see also Chapters 4 and 9).

The questions we have highlighted vary in the degree to which they can or have been answered with studies to date. In several cases (Questions 1, 3, 5, 6, and 8), most progress to date has come from studies of mutualisms involving plants. We hope that this review will stimulate further observational and experimental work in other mutualisms. In a few cases (Questions 1, 2, 4, and 6), sufficient studies have accumulated to support relevant meta-analyses, but these analyses are only as informative as the set of studies available. Many more studies could be utilized in new or repeated meta-analyses, especially within-study meta-analyses using studies that have explicitly manipulated contextual factors. In other cases (especially Questions 3, 5, and 6), meta-analyses seem possible with the existing literature, but have not yet been conducted. Overall, however, despite the progress highlighted here, all of the questions we have raised are in need of not only further exploration in the most heavily studied mutualisms, but especially groundbreaking studies in the more empirically challenging systems. Our hope is that by raising these questions, which are applicable across diverse systems, readers will be inspired to forge new ground in search of general principles of context dependency in mutualisms.

Acknowledgments

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