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# EXPLAINING MUTUALISM VARIATION: A NEW EVOLUTIONARY PARADOX?

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**The paradox of mutualism is typically framed as the persistence of interspecific cooperation, despite the potential advantages of cheating. Thus, mutualism research has tended to focus on stabilizing mechanisms that prevent the invasion of low-quality partners. These mechanisms alone cannot explain the persistence of variation for partner quality observed in nature, leaving a large gap in our understanding of how mutualisms evolve. Studying partner quality variation is necessary for applying genetically explicit models to predict evolution in natural populations, a necessary step for understanding the origins of mutualisms as well as their ongoing dynamics. An evolutionary genetic approach, which is focused on naturally occurring mutualist variation, can potentially synthesize the currently disconnected fields of mutualism evolution and coevolutionary genetics. We outline explanations for the maintenance of genetic variation for mutualism and suggest approaches necessary to address them.**

**KEY WORDS:** **Coevolution, cooperation, partner choice, partner fidelity feedback, public goods, quantitative genetics, screening.**

The evolutionary paradox of interspecific mutualisms, as typically posed, refers to the apparent disagreement between theoretical predictions that (all else being equal) selection should favor cheaters, and empirical observations of generally positive interactions and long-term cooperation between species. Theoretical and empirical studies alike, motivated by this seeming paradox, have made much progress toward understanding the selective mechanisms that favor interspecific mutualism and thus explaining the long-term persistence of mutualisms despite the potential fitness advantages of cheating (Bshary and Grutter 2002; Kiers et al. 2003; Sachs et al. 2004; Heath and Tiffin 2009; Leigh 2010; Archetti et al. 2011b; Jander et al. 2012). Here we argue, however, that we are missing a fundamental aspect of mutualism evolution that would allow faster progress toward understanding the more general question of *how* mutualisms evolve, in the larger context of species interactions, as opposed to simply what selects for cooperative mutualist partners. We suggest that a critical question in mutualism evolution is *what maintains genetic variation for partner quality in mutualisms*?

Answering this question will require researchers to apply evolutionary genetic methods to the study of genetic variation in mutualisms. Viewing mutualistic interactions through the lens of the maintenance of genetic variation, a classic framework shared by all of evolutionary biology, can (1) bridge the divide between the largely disconnected fields of coevolutionary genetics and mutualism evolution; and (2) provide a more predictive understanding of how mutualisms evolve. Given the importance of these interactions to natural and managed systems, including human health, a predictive understanding of mutualism evolution is an important goal. We first define what we mean by "genetic variation in mutualism," then describe why we believe that it is an important and underexplored evolutionary problem, and finally discuss the mechanisms that might promote this variation and how they can be investigated.






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## What is Mutualism Variation?

The classic "paradox" of mutualism (Box 1) is, at its core, a microevolutionary and coevolutionary predicament, and thus requires that we study intraspecific genetic variation in both interacting partners. Thus, although we note that similar concepts of cheating, cooperating, and coexistence are commonly applied to communities of mutualist species (Box 2), we focus here on intraspecific genetic variation in partner quality within interspecific mutualisms. Partner quality in species A can be measured as the effect of a mutualist genotype on the fitness of its partner, species B. Thus, partner quality represents how cooperation varies continuously within a species (in this case, species A), is the sum fitness effect of any traits that mediate the mutualism, and can be decomposed into both genetic and environmental components using quantitative genetic methods.

Partner quality variation per se is agnostic to the underlying motivation of any partner, for example whether a less-beneficial individual is "cheating" in the strict sense by gaining a fitness benefit (Sachs et al. 2010; Frederickson 2013; Ghoul et al. 2013). It is also agnostic to the nature of mechanisms that might select for it (i.e., partner choice, screening, sanctions, partner fidelity feedback; see Box 1 and Weyl et al. 2010; Kiers et al. 2011; Archetti et al. 2011b; Frederickson 2013). Instead, understanding partner quality variation, and the forces that shape it, constitutes a general framework necessary to address these very issues. In other words, we argue that studying genetic variation in mutualisms is necessary for addressing fully the importance of punishment and partner choice (Bull and Rice 1991) as well as the tragedy of the commons in one-to-many interactions (Hardin 1968; West et al. 2002). Recent economic theory on mutualisms refers to these as the concepts of "hidden characteristics," "hidden actions," and "collective actions" (Archetti et al. 2011b), but the benefits of an evolutionary genetic approach to testing these theories remains. Our view of partner quality complements coevolutionary models of mutualism that include more and less cooperative types (e.g., Trivers 1971; Axelrod and Hamilton 1981; Bull and Rice 1991; Doebeli and Knowlton 1998; West et al. 2002; Foster and Kokko 2006; Weyl et al. 2010). Partner quality as a continuously varying trait is also consistent with current understanding of other ecologically relevant traits and allows predictive evolutionary models to be applied to, and tested with, genetic variation from contemporary natural populations (Lande and Arnold 1983; Falconer and Mackay 1996). Developing a predictive understanding of microevolution, that is, genetic changes within and among contemporary natural populations, is necessary if we aim to unravel the selective agents that promote mutualism and the environments under which beneficial interactions persist versus dissolve, particularly in the face of rapid environmental change.

### Box 1: The Classic Paradox of Mutualism Evolution

Evolutionary theory predicts that stabilizing mechanisms are necessary for promoting interspecific cooperation. All else being equal, natural selection would otherwise favor mutualist genotypes that optimize their own fitness by minimizing the costs of returning benefits to a partner (so-called "cheaters"; see Frederickson 2013; Ghoul et al. 2013). Thus, in the absence of stabilizing mechanisms, cheating would be expected to drive the degeneration of partner quality and thus the benefits of mutualism (Trivers 1971; Axelrod and Hamilton 1981; Bull and Rice 1991; Doebeli and Knowlton 1998; West et al. 2002). We define a "stabilizing mechanism," therefore, very broadly as any biological reality or selective process that can increase the likelihood that high-quality mutualists remain in the population. Sachs (2004), Leigh (2010), and Archetti et al. (2011b) provide in-depth reviews of the large body of empirical and theoretical work on these mechanisms. Briefly, cooperative partners can be favored by selection if mutualists are vertically transmitted, if interactions between individuals are repeated, by positive fitness feedbacks, or if discrimination mechanisms actively favor higher-quality partners over cheaters. Here we use "discrimination mechanism" to encompass partner choice (Bull and Rice 1991), sanctions (Kiers et al. 2003) one-to-many partner fidelity feedback (Bull and Rice 1991; Archetti et al. 2011b), and screening (Archetti et al. 2011b). Empirical studies in diverse mutualist systems have found evidence for these mechanisms. Cooperation can even persist in one-to-many interactions, without discriminating mechanisms, if host benefits are simply a nonlinear function of the number of cooperating symbionts in a host (Archetti and Scheuring 2011, 2013). Importantly, while stabilizing mechanisms can explain how high partner quality might be favored by natural selection, they typically cannot explain why variation in mutualism persists (but see discussion of Archetti and Scheuring 2013 in the text).

## A New Paradox of Mutualism Evolution

Recent theoretical papers have pointed out that many previous models of mutualism are in the ironic position of eroding the very genetic variation that drives them (Foster and Kokko 2006; McNamara and Leimar 2010; Frederickson 2013). For example,

## Box 2: The Community Ecology of Partner Quality

Mutualisms often occur in variable mutualist guilds, in which multiple species interact either simultaneously or through space and time. The question of how multiple competing species coexist in mutualist guilds (reviewed by Jones et al. 2012) is somewhat analogous to the persistence of intraspecific variation in partner quality; here we include a representative sampling of some key literature. Considering how a host interacts with different species throughout ontogeny (Palmer et al. 2010), or how spatial heterogeneity alters the outcome of competition (Yu et al. 2001), can help explain how diverse guilds persist. High species diversity might actually facilitate species coexistence, if mechanisms of choosing the highest-quality partner species are less effective in highly diverse communities (Hart et al. 2012). Stable associations between high-rewards hosts and high-quality mutualists versus low-rewards hosts and low-defense mutualists, in one ant-acacia community suggest that competitive asymmetry might maintain stable coexistence of alternative strategies (Heil et al. 2009).

The interplay between the intraspecific evolution of partner quality and interspecific guild-level dynamics are of increasing interest to mutualism researchers, but are only beginning to be explored. Ferriere et al. (2007) show that pairwise coevolution of partner quality can determine the ability of a third-party species to exploit the interaction and, vice versa, that levels of partner quality are determined by the presence of the third party early in coevolution. Nuismer et al. (2013) show that the coevolution of mutualistic traits that mediate partner–partner interactions alters the interaction structure of the larger mutualist community. Such eco-evolutionary feedbacks are underexplored in mutualisms, but empirical studies on these dynamics will be valuable as part of a larger effort to understand the joint effects of ecology and evolution on natural populations (Johnson and Stinchcombe 2007).

the breakdown of mutualisms due to the fixation of uncooperative genotypes would be the static endpoint of directional selection favoring partners of lower quality, as shown by classic models of cooperation (Box 1; Trivers 1971; Axelrod and Hamilton 1981; Bull and Rice 1991). The fixation of the most beneficial partner genotypes, in contrast, would be predicted by most models of mutualism stability, including both positive frequency dependence in which positive fitness feedbacks favor high investment in one's partner (Law and Koptur 1986; Parker 1999; Weyl et al. 2010), as well as models of discrimination mechanisms (Box 1) in which individuals actively favor the highest quality partners (Bull and Rice 1991; West et al. 2002; Archetti et al. 2011a). Partner quality variation, however, is necessary for the operation of discriminating mechanisms commonly thought to maintain mutualism (e.g., partner choice, sanctions, screening), both as the variation that these mechanisms act upon, and also as a selective force favoring their evolution in the first place (Foster and Kokko 2006; McNamara and Leimar 2010; Frederickson 2013). Nevertheless, few theoretical papers have attempted to explain the maintenance of genetic variation in interspecific mutualisms (but see discussion below; also Friesen and Mathias 2010; Archetti and Scheuring 2013).

Despite theoretical work, few empirical studies have evaluated the evolutionary genetic processes that maintain partner quality variation (Hoeksema 2010), even though researchers working in diverse systems typically observe abundant genetic variation for mutualism benefits (Thompson 1988; Hoeksema 2010; Heath 2010; McNamara and Leimar 2010). Instead, mutualism work has tended to focus on the stabilizing mechanisms that select for high-quality partners (Bshary and Grutter 2002; Kiers et al. 2003; Sachs et al. 2004; Heath and Tiffin 2009; Leigh 2010; Archetti et al. 2011b; Jander et al. 2012). In our opinion, this search for stabilizing mechanisms, sometimes in interactions tens of millions of years old, has distracted us from a more fundamental question: What evolutionary and ecological forces maintain genetic variation in mutualism benefits? Addressing the question of mutualism variation requires that we gain insight into the contemporary coevolutionary dynamics in natural populations. Below we explore potential explanations for the persistence of mutualism variation, using the classic evolutionary framework on the maintenance of genetic variation and incorporating models of mutualism that attempt to explain variation in partner quality. We organize these potential explanations, along with the approaches necessary to address them, in a decision tree (Fig. 1).

## Mutation–Selection Balance and Mutualism Variation

Most forms of natural selection are expected to erode genetic variation, and understanding the presence of abundant genetic variation in selected traits in natural populations is a central, and as yet unresolved, goal in evolutionary biology (Walsh and Blows 2009). For mutualism traits, this challenge is especially acute: mutualisms are by definition beneficial interactions that enhance fitness, and theory predicts that genetic variation for fitness-related traits should be reduced by selection (Charlesworth 1987). In one simplified scenario, directional selection acts to increase partner quality (e.g., positive fitness feedbacks, discriminating mechanisms), whereas mutation at the one or many underlying loci introduces genotypes of lower quality.

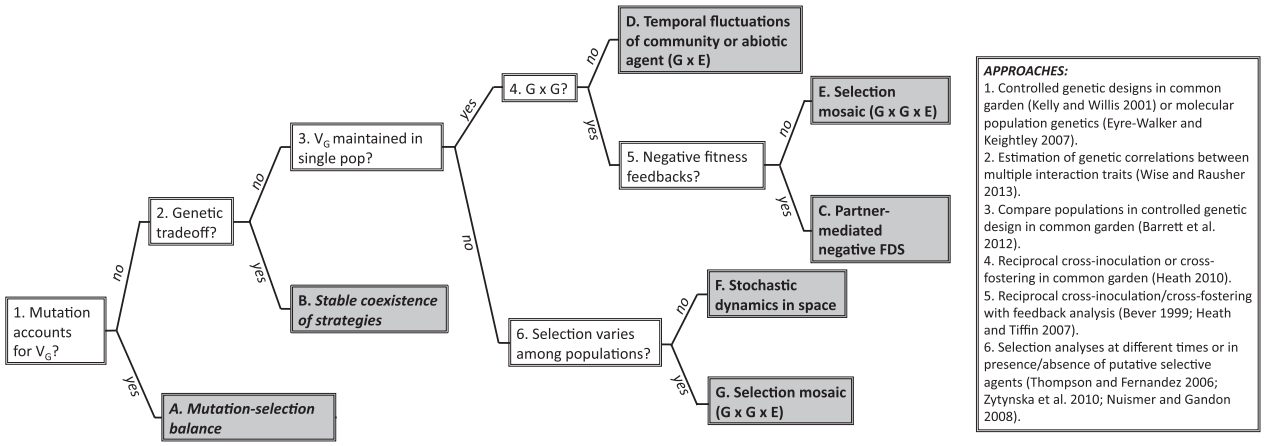


**Figure 1. Decision tree outlining possible explanations for the persistence of partner quality variation in mutualism (A–G in gray boxes, referenced in the text). Each branch point features a numbered question (1–6 in white boxes), with the genetic approaches necessary to answer the question and example studies summarized in the inset box.**

Because most traits mediating mutualisms are quantitative, understanding the role of mutation in maintaining genetic variation in partner quality will be challenging. Mutualism models that study maintenance of variation in partner quality (Foster and Kokko 2006; Weyl et al. 2010; Archetti and Scheuring 2013) frequently compete uncooperative or cooperative "types," and are thus divorced from the continuous distribution of partner quality often observed in nature—highlighting the need to formally confront coevolutionary models with empirical data (K. D. Heath and S. L. Nuismer, unpubl. ms.). The extent to which mutation–selection balance alone can explain the persistence of variation in quantitative traits has been the subject of debate (Lande 1975; Turelli 1985); nevertheless, when traits are polygenic, the power of mutation to generate variation increases (Turelli 1985). Thus, it is possible that variation in many of the lower-level traits underlying partner quality (e.g., secondary metabolite concentrations, carbon allocation) that are targeted by coevolutionary selection could be explained by transient mutations, if they are affected by many genes and thus have broad mutational targets. It is also possible that stabilizing mechanisms such as sanctions that are only partially effective, either because they cannot respond to nonzero benefit levels or cannot punish individual partner genotypes (Kiers et al. 2006; Jander et al. 2012), might impose somewhat weak selection and thus increase the time that less-than-optimum mutations persist in a population. Estimating the strength of selection that stabilizing mechanisms actually impose on partner quality in nature would thus go a long way toward improving our understanding of the evolution of partner quality.

Determining whether genetic variance in quantitative traits exceeds that predicted by mutation–selection balance is exceptionally challenging, even when selection is known (A in Fig. 1). Traits governed by mutation–selection balance should harbor many deleterious, recessive alleles at low frequency, whereas those subject to balancing selection should harbor an excess of intermediate frequency alleles (Mitchell-Olds et al. 2007). One approach for discriminating between these patterns is to manipulate both selection and inbreeding simultaneously and compare the selection response in inbred versus outbred populations (Kelly and Willis 2001). Alternatively, if selected genes or genomic regions are known, molecular population genetics approaches can identify patterns of nucleotide variation and polymorphism consistent with mutation–selection balance (Eyre-Walker and Keightley 2007).

One additional challenge for assessing mutation–selection balance is that most theory assumes that mutations are unconditionally deleterious, whereas we know this is unlikely. In mutualistic interactions, for example, it might be the case that mutations that are deleterious for a host interacting with one partner genotype might be neutral or even beneficial for the host when interacting with other partner genotypes. If the fitness effects of mutations in species A depend on the genotype of species B (Wade 2007; Heath 2010), a qualitative prediction would be that such fluctuating selection could allow greater amounts of quantitative genetic variation to be maintained than under traditional mutation–selection balance models. A better understanding of the genetic architecture of partner quality, including number of loci and prevalence of intergenomic epistasis, is a necessary first step.

## Selection Can Maintain Variation

Although mutation–selection balance is one possible explanation for mutualism variation, more complex explanations involving trade-offs or balancing selection (see below) are typically necessary to explain the amount of genetic variation observed in fitness-related traits (Mitchell-Olds et al. 2007). Indeed,

consistent positive selection for partner quality is likely too simple an explanation for the mutualisms that surround us. Although recognition of this fact is not, in itself, revolutionary (Thompson 1988; Bronstein 1994; McNamara and Leimar 2010), it is our opinion that a research program that systematically focuses on natural variation and investigates how selection acts on mutualist genotypes in a natural environment remains underdeveloped.

Potential explanations for the maintenance of partner quality variation can generally be organized into three categories: (1) stable coexistence of alternative strategies driven by tradeoffs; (2) variable selection within a single population; or (3) spatial variation. In the first scenario, multivariate selection acts on multiple traits that underlie partner quality, and trade-offs between these traits promote coexistence of multiple strategies. In the latter two, selection on partner quality varies in either time or space.

## STABLE COEXISTENCE OF MUTUALIST STRATEGIES

In the absence of fluctuating selection, stable coexistence could occur if genetic trade-offs exist between different mutualism-related traits, generating distinct strategies within a mutualist species, even if selection for increased partner quality is consistent (B in Fig. 1). Addressing this hypothesis requires studying the genetic architecture of multiple traits that underlie partner quality, and across multiple life-history stages. For example, if low-quality genotypes that gain higher fitness in mutualism are necessarily worse at competing for resources, stable coexistence could emerge (Ferriere et al. 2002; Hoeksema and Kummel 2003). Similarly if a genetic trade-off underlies success in different life-history stages, some genotypes might excel at symbiosis, whereas others excel at nonsymbiotic, free-living stages (Denison and Kiers 2004; Sachs et al. 2011b; Archetti et al. 2011b), potentially maintaining variation. Discrimination mechanisms like partner choice or sanctions that are not precise enough to target individual partner genotypes could promote the coexistence of high- and low-quality partners, if trade-offs ensure that low-quality partners who evade punishment enjoy a fitness advantage elsewhere (Friesen and Mathias 2010). Importantly, genetic trade-offs between selected traits must be quite strong (i.e., genetic correlations approaching $\pm 1$) to constrain individuals from having high fitness at all life-history stages and thus offer an explanation for coexistence (Conner 2012).

## BALANCING SELECTION IN MUTUALISMS

A second possibility is that variable selection actively maintains variation in partner quality (Mitchell-Olds et al. 2007). As we outline here, however, numerous mechanisms of balancing selection acting in mutualisms can actively maintain intermediate frequency variants in mutualist populations, and empirical evidence on how various selective mechanisms might contribute to mutualism variation remains scanty. Nonlinear public goods, genotype-by-environment interactions ($G \times E$), negative frequency-dependent selection, and selection mosaics can generate fluctuating selection pressures through time and/or space (Thompson 2005). These mechanisms can maintain variation within a single population, or among populations. Thus, identifying the spatial scale at which genetic variation in mutualism exists is a useful first step forward for understanding the forms of balancing selection that might be responsible (Parker 1995; Hoeksema and Thompson 2007; Anderson and Johnson 2008; Heath 2010; Barrett et al. 2012).

Applying economic game theory to situations in which a host interacts with more than one symbiont simultaneously, Archetti and Scheuring (2011, 2013) show mutualism persistence and the coexistence of high- and low-quality partners in the absence of sanctions or partner choice mechanisms. Their models have two key features: first, selection acts on symbionts at both the within-host and among-host level, similar to evolutionary genetic models of multilevel selection (Frank 1996). Second, coexistence is facilitated by an assumption that the benefits ("common goods") received by all symbionts on a host are a nonlinear (sigmoidal) function of the number of high-quality symbionts. Consequently, if there are enough high-quality symbionts for fitness of the host (and the benefit returned to symbionts) to saturate, there is an advantage to cheating for symbionts. Too high a frequency of cheating, however, results in reduced fitness for the host, and reduced benefits returned to the symbionts. Archetti and Scheuring (2013) propose examining the distribution of partner quality variation to distinguish these dynamics from systems in which discriminating mechanisms like partner choice or sanctions act to favor high-quality partners, because balancing selection should maintain partner quality variants at intermediate frequencies. Experimental evolution approaches could be used to eliminate among-host competition and thus test for multilevel selection directly. Similarly, experimental manipulations of symbiont quality in model systems, such as the legume–rhizobium mutualism, could test the key assumption that host benefits are a sigmoidal response to the proportion of high-quality symbionts.

Coevolution itself, and thus the coevolutionary dynamics that maintain mutualism variation, require intergenomic epistasis ($G \times G$) for fitness (Parker 1995; Thompson 2005). $G \times G$ represents how the fitness impact of mutualist genotypes (i.e., their partner quality) depends on the genetic identity of their partner in mutualism. As discussed earlier, $G \times G$ adds a level of complication to models of selection on mutualist partners (Nuismer et al. 2003) and impedes our ability to easily estimate partner quality, because the quality of a partner might depend on the individual with whom we measure it. Nevertheless $G \times G$ can help us understand the maintenance of genetic variation in mutualism. $G \times G$ underlies the potential for negative frequency-dependent selection in mutualism as well as the selection mosaic—two powerful

explanations for the persistence of variation in partner quality in mutualisms.

Negative FDS, mediated via G × G, could maintain variation in partner quality in even a single population, if the fitness value of high- and low-quality alleles decreases with their frequency in the population (C in Fig. 1). With a preponderance of negative fitness feedbacks, G × G itself might actively maintain variation. In a mutualism between species A and B, negative fitness feedbacks occur when the genotype of A that benefits the most from a given genotype of partner B in turn confers greatest fitness benefits to a different genotype of B (Bever 1999). Thus, as a particular genotype of B increases in frequency, genetic changes in its mutualist partner A feedback to decrease its fitness, leading to cyclical dynamics that maintain multiple genotypes. Because assessing the form of fitness feedbacks in G × G interactions requires manipulating combinations of many partner genotypes under controlled conditions, they have rarely been examined. Heath and Tiffin (2007) found positive feedbacks in a legume–rhizobium mutualism, suggesting that G × G alone would not maintain the observed partner quality variation.

Negative FDS is perhaps best epitomized by the temporal cycles that favor the maintenance of highly diverse pathogen-resistance (R) genes in plant populations (Brown and Tellier 2011). Such negative FDS might potentially operate even in the presence of (imperfect) stabilizing mechanisms, if low-quality mutualists are frequent and detrimental enough to impose strong selection on their partners. For example, with presymbiosis partner choice, in which signals exchanged before benefit exchange might be dishonest signals of partner quality (Heath and Tiffin 2009), some low-quality partners would be expected to interact and thus gain high fitness benefits. Their proliferation would, in turn, be expected to impose selection for host recognition, leading partner genotypes to cycle in ways similar to models of host–pathogen interactions. Such cyclical dynamics can contribute to high levels of genetic variation even within a single location. The extent of coevolutionary dynamics consistent with negative FDS in mutualisms is not well understood, because empirical evidence is indirect at best (Sachs et al. 2011a).

## SPATIAL VARIATION AND MULTITROPHIC INTERACTIONS

In the absence of negative FDS, agents external to the mutualism itself can impose fluctuating selection and maintain variation for partner quality even in a single population (D and E in Fig. 1), if the genotypes favored by selection differ among the alternative environments experienced by that population (G × E; Gillespie and Turelli 1989). One possibility is that G × E maintains variation in one (or both) partners independent of each other (D in Fig. 1); therefore, this explanation is not coevolutionary in the narrow sense. With the coevolutionary extension of G × E, G × G × E, the fluctuating environment alters how genotypes interact to determine partner quality and thus alters selection—generating a selection mosaic for partner quality that can maintain variation (E in Fig. 1; Thompson 2005; Gomulkiewicz et al. 2007). For example, the community context in which an interaction occurs is one aspect of the environment, and the presence of third-party species is well-known to alter selection on traits that mediate mutualism (Thompson and Fernandez 2006; Nuismer and Ridenhour 2008; Zytynska et al. 2010). A fluctuating abiotic environment can also alter selection; for example, variation in soil nitrogen would be predicted to generate variable selection for partner quality in nitrogen-fixing rhizobia (Heath et al. 2010; Akçay and Simms 2011).

Even in the absence of spatially variable selection, and when fitness feedbacks are generally positive, the persistence of ecologically relevant genetic variation becomes much easier to explain when spatial variation is considered. Models show that stochastic variation in the distribution of mutualist genotypes among populations in space, combined with G × G for fitness, can generate different coevolutionary trajectories and thus the fixation of different genotypes among populations (Parker 1999; Nuismer et al. 2000). Thus, studying mutualism evolution in an explicit spatial context is critical for determining whether we even need to invoke complex selective mechanisms to explain the persistence of variation in partner quality.

Finally, spatially variable selection has long been invoked as a potential mechanism for maintaining genetic variation (Brodie et al. 2002; Benkman et al. 2003; Thompson 2005; Yeaman et al. 2010). G × E and its coevolutionary cousin, G × G × E (the selection mosaic), have great power to explain the maintenance of variation, because various ecological factors are expected to vary among natural populations in which mutualisms coevolve (Thompson 2005). G × G × E, suggestive of selection mosaics, has been detected in a number of coevolutionary systems (Piculell et al. 2008; Heath et al. 2010; Zytynska et al. 2010); however, the manipulative experiments necessary to detect these genetic interactions are rarely paired with reciprocal transplants necessary for ecological relevance of the selective agent (Nuismer and Gandon 2008). Using acacias and their rhizobium symbionts, Barrett et al. (2012) performed a large-scale study investigating patterns of local adaptation among populations, because this is the expected pattern if selection mosaics maintain variation. They found little evidence to support selection mosaics, although their design measured the effects of entire symbiont populations on hosts, and thus could not parse individual genetic effects.

Multiplayer interactions introduce the potential for even more complex evolutionary dynamics (including G × G × G), especially when the traits that govern multispecies interactions are mediated by overlapping genetic pathways (Hoeksema 2010; Hersch-Green et al. 2011; Wise and Rausher 2013). Because of the

complex genetic designs and large sample sizes needed to detect such higher-order genetic effects, studies of multispecies genetic interactions are rare, although the burgeoning field of community genetics has made some progress toward this goal, mostly in antagonistic systems to date (Wade 2007; Hersch-Green et al. 2011).

## Outlook

It is time for synthesis between studies on the selective forces promoting mutualism and evolutionary genetic studies focused on the maintenance of genetic variation. It is our opinion that this synthesis will be accomplished by attempting to understand variation for mutualism and the evolutionary forces that maintain it over multiple spatial scales (Questions 1–6 in Fig. 1).

Studies applying quantitative genetic approaches to partner quality variation will be necessary. Exploring how stabilizing mechanisms like sanctions and partner choice operate within ecologically relevant contexts, including estimating the strength of selection on partner quality, will help us understand how these selective agents act on natural populations to shape mutualism trait means and variances. Controlled genetic designs can reveal the genetic architecture of partner quality, including whether it is correlated to any other fitness-related traits that might drive genetic trade-offs. Manipulative studies in the field or in a common garden can resolve the spatial structure of partner quality variation and thus identify whether balancing selection might maintain variation locally or across multiple populations. Reciprocal transplants of genotypes among populations can resolve whether natural selection favors different genetic variants in different locations.

Evolutionary genomic and population genetic approaches have the potential to differentiate between patterns of nucleotide-level genetic variation consistent with competing models for the maintenance of genetic variation, including discriminating between traits governed by mutation–selection balance versus balancing selection. Sequence-based, or bottom-up, evolutionary genomics (Barrett and Hoekstra 2011) can pinpoint mutualism genes that are targeted by natural selection and ultimately help identify lower-level phenotypic traits that underlie higher-order effects such as partner quality. Although evolutionary ecologists typically proceed in the opposite direction (from the top-down), combining these approaches will be useful, particularly in microbial mutualisms, where underlying traits that contribute to partner quality variation are difficult to identify and even harder to measure.

Combining these approaches requires studying naturally occurring genetic variation, and will be fruitful for illuminating the evolutionary dynamics operating within mutualisms on shorter timescales, ultimately helping us understand how mutualisms will evolve into the future as well as how they remain beneficial, yet dynamic, for millions of years.

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### LITERATURE CITED

Akçay, E., and E. L. Simms. 2011. Negotiation, sanctions, and context dependency in the legume-rhizobium mutualism. Am. Nat. 178:1–14.

Anderson, B., and S. D. Johnson. 2008. The geographical mosaic of coevolution in a plant-pollinator mutualism. Evolution 62:220–225.

Archetti, M., and I. Scheuring. 2011. Coexistence of cooperation and defection in public goods games. Evolution 65:1140–1148.

———. 2013. Trading public goods stabilizes interspecific mutualism. J. Theor. Biol. 318:58–67.

Archetti, M., F. Ubeda, D. Fudenberg, J. Green, N. E. Pierce, and D. W. Yu. 2011a. Let the right one in: a microeconomic approach to partner choice in mutualisms. Am. Nat. 177:75–85.

Archetti, M., I. Scheuring, M. Hoffman, M. E. Frederickson, N. E. Pierce, and D. W. Yu. 2011b. Economic game theory for mutualism and cooperation. Ecol. Lett. 14:1300–1312.

Axelrod, R., and W. Hamilton. 1981. The evolution of cooperation. Science 211:1390–1396.

Barrett, L. G., L. M. Broadhurst, and P. H. Thrall. 2012. Geographic adaptation in plant-soil mutualisms: tests using Acacia spp. and rhizobial bacteria. Funct. Ecol. 26:457–468.

Barrett, R. D. H., and H. E. Hoekstra. 2011. Molecular spandrels: tests of adaptation at the genetic level. Nat. Rev. Genet. 12:767–780.

Benkman, C., T. Parchman, A. Favis, and A. Siepielski. 2003. Reciprocal selection causes a coevolutionary arms race between crossbills and lodgepole pine. Am. Nat. 162:182–194.

Bever, J. D. 1999. Dynamics within mutualism and the maintenance of diversity: inference from a model of interguild frequency dependence. Ecol. Lett. 2:52–61.

Brodie, E. D., B. J. Ridenhour, and E. D. Brodie. 2002. The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. Evolution 56:2067–2082.

Bronstein, J. 1994. Conditional outcomes in mutualistic interactions. Trends Ecol. Evol. 9:214–217.

Brown, J. K. M., and A. Tellier. 2011. Plant-parasite coevolution: bridging the gap between genetics and ecology. Annu. Rev. Phytopathol. 49:345–367.

Bshary, R., and A. Grutter. 2002. Experimental evidence that partner choice is a driving force in the payoff distribution among cooperators or mutualists: the cleaner fish case. Ecol. Lett. 5:130–136.

Bull, J., and W. Rice. 1991. Distinguishing mechanisms for the evolution of co-operation. J. Theor. Biol. 149:63–74.

Charlesworth, B. 1987. The heritability of fitness. Pp. 21–40 *in* J. Bradbury and M. B. Anderson, eds. Sexual selection: testing the alternatives. John Wiley & Sons, Lond.

Conner, J. K. 2012. Quantitative genetic approaches to evolutionary constraint: how useful? Evolution 66:3313–3320.

Denison, R. F., and E. T. Kiers. 2004. Lifestyle alternatives for rhizobia: mutualism, parasitism, and forgoing symbiosis. FEMS Microbiol. Lett. 237:187–193.

Doebeli, M., and N. Knowlton. 1998. The evolution of interspecific mutualisms. Proc. Natl. Acad. Sci. 95:8676–8680.

Eyre-Walker, A., and P. D. Keightley. 2007. The distribution of fitness effects of new mutations. Nat. Rev. Genet. 8:610–618.

Falconer, D. S., and T. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Benjamin Cummings, San Francisco, CA.

Ferriere, R., J. Bronstein, S. Rinaldi, R. Law, and M. Gauduchon. 2002. Cheating and the evolutionary stability of mutualisms. Proc. R. Soc. Lond. B Bio. 269:773–780.

Ferrière, R., M. Gauduchon, and J. L. Bronstein. 2007. Evolution and persistence of obligate mutualists and exploiters: competition for partners and evolutionary immunization. Ecol. Lett. 10:115–126.

Foster, K. R., and H. Kokko. 2006. Cheating can stabilize cooperation in mutualisms. Proc. R. Soc. B 273:2233–2239.

Frank, S. A. 1996. Models of parasite virulence. Quat. Rev. Biol. 71:37–78.

Frederickson, M. E. 2013. Rethinking mutualism stability: cheaters and the evolution of sanctions. Quat. Rev. Biol. 88. *In press*.

Friesen, M. L., and A. Mathias. 2010. Mixed infections may promote diversification of mutualistic symbionts: why are there ineffective rhizobia? J. Evol. Biol. 23:323–334.

Ghoul, M., A. S. Griffin, and S. A. West. 2013. Towards an evolutionary definition of cheating. Evolution. *In press*.

Gillespie, J. H., and M. Turelli. 1989. Genotype-environment interactions and the maintenance of polygenic variation. Genetics 121:129–138.

Gomulkiewicz, R., D. M. Drown, M. F. Dybdahl, W. Godsoe, S. L. Nuismer, K. M. Pepin, B. J. Ridenhour, C. I. Smith, and J. B. Yoder. 2007. Dos and don'ts of testing the geographic mosaic theory of coevolution. Heredity 98:249–258.

Hardin, G. 1968. The tragedy of the commons. Science 162:1243–1248.

Hart, M. M., J. Forsythe, B. Oshowski, H. Bücking, J. Jansa, and E. T. Kiers. 2012. Hiding in a crowd—does diversity facilitate persistence of a low-quality fungal partner in the mycorrhizal symbiosis? Symbiosis 59:47–56.

Heath, K. D. 2010. Intergenomic epistasis and coevolutionary constraint in plants and rhizobia. Evolution 64:1446–1458.

Heath, K. D., and P. Tiffin. 2007. Context dependence in the coevolution of plant and rhizobial mutualists. Proc. Biol. Sci. 274:1905–1912.

———. 2009. Stabilizing mechanisms in a legume-rhizobium mutualism. Evolution 63:652–662.

Heath, K. D., A. J. Stock, and J. R. Stinchcombe. 2010. Mutualism variation in the nodulation response to nitrate. J. Evol. Biol. 23:2494–2500.

Heil, M., M. González-Teuber, L. W. Clement, S. Kautz, M. Verhaagh, and J. C. S. Bueno. 2009. Divergent investment strategies of Acacia myrmecophytes and the coexistence of mutualists and exploiters. Proc. Natl. Acad. Sci. 106:18091–18096.

Hersch-Green, E. I., N. E. Turley, and M. T. J. Johnson. 2011. Community genetics: what have we accomplished and where should we be going? Philos. Trans. R Soc. B 366:1453–1460.

Hoeksema, J. D. 2010. Ongoing coevolution in mycorrhizal interactions. New Phytol. 187:286–300.

Hoeksema, J. D., and M. Kummel. 2003. Ecological Persistence of the plant–mycorrhizal mutualism: a hypothesis from species coexistence theory. Am. Nat. 162:S40–S50.

Hoeksema, J. D., and J. N. Thompson. 2007. Geographic structure in a widespread plant–mycorrhizal interaction: pines and false truffles. J. Evol. Biol. 20:1148–1163.

Jander, K. C., E. A. Herre, and E. L. Simms. 2012. Precision of host sanctions in the fig tree-fig wasp mutualism: consequences for uncooperative symbionts. Ecol. Lett. 15:1362–1369.

Johnson, M. T. J., and J. R. Stinchcombe. 2007. An emerging synthesis between community ecology and evolutionary biology. Trends Ecol. Evol. 22:250–257.

Jones, E. I., J. L. Bronstein, and R. Ferrière. 2012. The fundamental role of competition in the ecology and evolution of mutualisms. Annal. NY Acad. Sci. 1256:66–88.

Kelly, J. K., and J. Willis. 2001. Deleterious mutations and genetic variation for flower size in *Mimulus guttatus*. Evolution 55:937–942.

Kiers, E. T., R. A. Rousseau, and R. F. Denison. 2006. Measured sanctions: legume hosts detect quantitative variation in rhizobium cooperation and punish accordingly. Evol. Ecol. Res. 8:1077–1086.

Kiers, E. T., R. F. Denison, A. Kawakita, and E. A. Herre. 2011. The biological reality of host sanctions and partner fidelity. Proc. Natl. Acad. Sci. 108:E7.

Kiers, E., R. Rousseau, S. West, and R. Denison. 2003. Host sanctions and the legume–rhizobium mutualism. Nature 425:78–81.

Lande, R. 1975. The maintenance of genetic variability by mutation in a polygenic character with linked loci. Genet. Res. 26:221–235.

Lande, R., and S. Arnold. 1983. The measurement of selection of correlated characters. Evolution 37:1210–1226.

Law, R., and S. Koptur. 1986. On the evolution of non-specific mutualism. Biol. J. Linn. Soc. 27:251–267.

Leigh, E. G. 2010. The evolution of mutualism. J. Evol. Biol. 23:2507–2528.

McNamara, J. M., and O. Leimar. 2010. Variation and the response to variation as a basis for successful cooperation. Philos. Trans. R. Soc. Lond., B-Biol. Sci. 365:2627–2633.

Mitchell-Olds, T., J. H. Willis, and D. B. Goldstein. 2007. Which evolutionary processes influence natural genetic variation for phenotypic traits? Nat. Rev. Genet. 8:845–856.

Nuismer, S. L., and S. Gandon. 2008. Moving beyond common garden and transplant designs: insight into the causes of local adaptation in species interactions. Am. Nat. 171:658–668.

Nuismer, S. L., and B. J. Ridenhour. 2008. The contribution of parasitism to selection on floral traits in *Heuchera grossulariifolia*. J. Evol. Biol. 21:958–965.

Nuismer, S. L., J. N. Thompson, and R. Gomulkiewicz. 2000. Coevolutionary clines across selection mosaics. Evolution 54:1102–1115.

Nuismer, S. L., P. Jordano, and J. Bascompte. 2013. Coevolution and the architecture of mutualistic networks. Evolution 67:338–354.

Nuismer, S., R. Gomulkiewicz, and M. Morgan. 2003. Coevolution in temporally variable environments. Am. Nat. 162:195–204.

Palmer, T. M., D. F. Doak, M. L. Stanton, J. L. Bronstein, E. T. Kiers, T. P. Young, J. R. Goheen, and R. M. Pringle. 2010. Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. Proc. Natl. Acad. Sci. 107:17234–17239.

Parker, M. 1999. Mutualism in metapopulations of legumes and rhizobia. Am Nat 153:S48–S60.

———. 1995. Plant fitness variation caused by different mutualist genotypes. Ecology 76:1525–1535.

Piculell, B. J., J. D. Hoeksema, and J. N. Thompson. 2008. Interactions of biotic and abiotic environmental factors in an ectomycorrhizal symbiosis, and the potential for selection mosaics. BMC Biol. 6:23.

Sachs, J. L., M. O. Ehinger, and E. L. Simms. 2010. Origins of cheating and loss of symbiosis in wild Bradyrhizobium. J Evolution Biol 23:1075–1089.

Sachs, J. L., C. J. Essenberg, and M. M. Turcotte. 2011a. New paradigms for the evolution of beneficial infections. Trends Ecol. Evol. 26:202–209.

Sachs, J. L., J. E. Russell, and A. C. Hollowell. 2011b. Evolutionary instability of symbiotic function in *Bradyrhizobium japonicum*. PLoS ONE 6:e26370.

Sachs, J., U. Mueller, T. Wilcox, and J. Bull. 2004. The evolution of cooperation. Quat. Rev. Biol. 79:135–160.

Thompson, J. N. 1988. Variation in interspecific interactions. Annu. Rev. Ecol. Syst. 19:65–87.

———. 2005. The geographic mosaic of coevolution (interspecific interactions). 1st ed. University of Chicago Press, Chicago, IL.

Thompson, J. N., and C. C. Fernandez. 2006. Temporal dynamics of antagonism and mutualism in a geographically variable plant-insect interaction. Ecology 87:103–112.

Trivers, R. L. 1971. The evolution of reciprocal altruism. Quat. Rev. Biol. 46:35–57.

Turelli, M. 1985. Effects of pleiotropy on predictions concerning mutation-selection balance for polygenic traits. Genetics 111:165–195.

Wade, M. J. 2007. The co-evolutionary genetics of ecological communities. Nat. Rev. Genet. 8:185–195.

Walsh, B., and M. W. Blows. 2009. Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. Annu. Rev. Ecol. Evol. Sci. 40:41–59.

West, S., E. Kiers, E. Simms, and R. Denison. 2002. Sanctions and mutualism stability: why do rhizobia fix nitrogen? Proc. R. Soc. Lond. B.-Biol. 269:685–694.

Weyl, E. G., M. E. Frederickson, D. W. Yu, and N. E. Pierce. 2010. Economic contract theory tests models of mutualism. Proc. Natl. Acad. Sci. U.S.A. 107:15712–15716.

Wise, M. J., and M. D. Rausher. 2013. Evolution of resistance to a multiple-herbivore community: genetic correlations, diffuse coevolution, and constraints on the plant's response to selection. Evolution 67:1767–1779.

Yeaman, S., Y. Chen, and M. C. Whitlock. 2010. No effect of environmental heterogeneity on the maintenance of genetic variation in wing shape in *Drosophila melanogaster*. Evolution 64:3398–3408.

Yu, D. W., H. B. Wilson, and N. E. Pierce. 2001. An empirical model of species coexistence in a spatially structured environment. Ecology 82:1761–1771.

Zytynska, S. E., S. Fleming, C. Tetard-Jones, M. A. Kertesz, and R. F. Preziosi. 2010. Community genetic interactions mediate indirect ecological effects between a parasitoid wasp and rhizobacteria. Ecology 91:1563–1568.

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