

Negotiation, Sanctions, and Context Dependency in the Legume-Rhizobium Mutualism

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ABSTRACT: Two important questions about mutualisms are how the fitness costs and benefits to the mutualist partners are determined and how these mechanisms affect the evolutionary dynamics of the mutualism. We tackle these questions with a model of the legume-rhizobium symbiosis that regards the mutualism outcome as a result of biochemical negotiations between the plant and its nodules. We explore the fitness consequences of this mechanism to the plant and rhizobia and obtain four main results. First, negotiations permit the plant to differentially reward more-cooperative rhizobia—a phenomenon termed “plant sanctions”—but only when more-cooperative rhizobia also provide the plant with good outside options during negotiations with other nodules. Second, negotiations may result in seemingly paradoxical cases where the plant is worse off when it has a “choice” between two strains of rhizobia than when infected by either strain alone. Third, even when sanctions are effective, they are by themselves not sufficient to maintain cooperative rhizobia in a population: less cooperative strains always have an advantage at the population level. Finally, partner fidelity feedback, together with genetic correlations between a rhizobium strain’s cooperativeness and the outside options it provides, can maintain cooperative rhizobia. Our results show how joint control over the outcome of a mutualism through the proximate mechanism of negotiation can affect the evolutionary dynamics of interspecific cooperation.

Keywords: sanctions, partner choice, partner fidelity feedback, biological markets, partner control, context dependency.

Introduction

Evolutionary biologists have long wondered how it is that costly traits that benefit the fitness of unrelated individuals can be adaptive (Darwin 1859; Herre et al. 1999; Bronstein et al. 2003; Sachs et al. 2004). The puzzle is especially acute for mutualisms in which individuals encounter and interact with multiple partners from the environment, as their fitness interests often conflict (Bull and Rice 1991; Noë and Hammerstein 1995; West et al. 2002b, 2002c;

Sachs and Wilcox 2006). In such mutualisms, selection will favor any individual with a mutation that increases its own fitness by increasing fitness benefits received from partners and/or reducing the fitness cost of benefits provided to partners. In the absence of counterselection, this evolutionary trajectory destabilizes the mutualism.

One proposed counterselection mechanism follows the metaphor of a “biological market” (Noë and Hammerstein 1994, 1995; Hammerstein 2001; Noë 2001; Simms and Taylor 2002). In this extended metaphor, controlling individuals choose “trading partners” from a market of potential “traders” (Noë and Hammerstein 1994). Partners are chosen according to their relative quality, quantified as the fitness benefits they provide minus the costs they impose. For such choice to maintain cooperation and hence mutualism, controlling individuals must accurately target fitness benefits to high-quality partners (Bshary and Grutter 2002; Simms and Taylor 2002; Foster and Kokko 2006). However, biological market theory does not consider how such targeting may be achieved, implicitly assuming that the controlling individuals have complete control over “payments” in their market transactions. In reality, the allocation of rewards is likely to be determined by a biological process that gives both parties some degree of control. In this article, we ask how such an allocation mechanism affects the predictions from biological market theory, using a mathematical model of the symbiosis between legumes and rhizobia.

Rhizobia are soil-dwelling alpha- and beta-proteobacteria that form nitrogen-fixation symbioses with many legumes (Sprent 2007; Masson-Boivin et al. 2009). Rhizobia infect legume roots, stimulate hosts to produce nodules, and differentiate into specialized endosymbiotic cells called bacteroids, which reduce atmospheric dinitrogen in exchange for plant photosynthates (Trainer and Charles 2006; Prell et al. 2009). Several aspects of the symbiosis decouple plant and rhizobial fitness. Rhizobia are transmitted horizontally among hosts and not vertically

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through seeds (Sprent et al. 1987), and symbiotic rhizobia likely derive fitness benefits through release into the soil from senescing nodules (Kiers et al. 2003; Denison and Kiers 2004; Simms et al. 2006; Heath and Tiffin 2009). Further, individual plants usually host multiple rhizobial genotypes (Dowling and Broughton 1986; Hagen and Hamrick 1996; Burdon et al. 1999; Thrall et al. 2000; Sachs et al. 2009), making them vulnerable to free riders, which are endosymbiotic rhizobia that hoard plant resources rather than investing in the energy-intensive nitrogen-fixation reaction (Denison 2000; Trainer and Charles 2006; Ratcliff et al. 2008). In response, host legumes might select against less-mutualistic rhizobia by targeting resources to more-mutualistic partners (Denison 2000; Simms and Taylor 2002). Several empirical studies (Singleton and Stockinger 1983; Singleton and van Kessel 1987; Kiers et al. 2003, 2006; Simms et al. 2006) have supported key components of this hypothesis, which has been termed “plant sanctions” (Denison 2000; West et al. 2002c) and “partner choice” (Simms and Taylor 2002).

The existing conception of plant sanctions implicitly assumes that legume hosts completely control resource allocation. However, selection will favor any rhizobia that can “manipulate” plant resource allocation, which makes it likely that the allocation of resources is ultimately under joint control. It is not clear whether plants lacking absolute control over rhizobial fitness can exert selection in favor of more cooperative rhizobia. Furthermore, although several reports have found support for plant sanctions, others have not (Marco et al. 2009; Gubry-Rangin et al. 2010). This suggests that the mechanisms determining fitness costs and benefits might produce sanctions under some conditions but not under others, which leads to the question of when each case is expected. Finally, plant sanctions have often been conceptualized as all-or-none, where a partner is either cut off or not (Denison 2000; West et al. 2002c), whereas plants are likely to adjust rewards to a nodule much more continuously, for example, by adjusting the carbon allocation to each nodule. Using either naturally occurring strains (Simms et al. 2006) or experimental manipulation of atmospheric N availability to nodules (Kiers et al. 2006), studies have found that nodule growth exhibits continuous variation that correlates with strain performance (or N concentration in the air), suggesting that plant allocation of resources also varies continuously. When sanctions are not binary and partners vary in quality, questions arise regarding how much the plant should reward each partner and how this value is determined.

A recent model by Akçay and Roughgarden (2007) is useful for answering these questions. In keeping with the trade metaphor, this model considers cooperation as the outcome of biochemical “negotiations” between the plant

and the rhizobia. In contrast to the purely plant-controlled sanctions model, the negotiation model affords both partners some degree of control over the outcome and predicts a stable division of benefits to each party. Akçay and Roughgarden (2007) proposed that this kind of negotiation process might underlie differential rewarding of nodules. However, they did not venture beyond the simplest case of a single nodule on one plant and did not model variation in rhizobium traits that would make rhizobia more or less mutualistic. Here, we address these issues by extending their negotiation model to the case of two nodules on a single plant. Our results challenge important aspects of the prevailing theories of partner choice and plant sanctions.

Our negotiation model is meant to represent an underlying biological process during which both partners in a mutualism can exert control over the outcome of an interaction. In “Mechanisms for Control of the Mutualism” in the online edition of the *American Naturalist*, we review the current knowledge of possible mechanisms of control by each party in the legume-rhizobium symbiosis. The plant has a number of possible mechanisms for regulating the allocation of resources to the nodule, including adjusting the oxygen flux into the nodule’s cortex (Denison 2000) and regulating the active transport of C_4 dicarboxylic acids (Benedito et al. 2010). There is much less known about the mechanisms by which rhizobia can control the outcome, but this situation is due partly to methodological difficulties and partly to a tacit assumption that the plant is in complete control. One potential pathway of rhizobium control might involve regulating the cycling of amino acids, which has been implicated in the symbiosis between pea plants and *Rhizobium leguminosarum* (Lodwig and Poole 2003; Prell et al. 2009), but this hypothesis has yet to be tested. Nonetheless, as we show below, several empirical observations regarding legume-rhizobium mutualisms can be better explained under an assumption of joint control via a negotiation-like mechanism than under the assumption of pure plant control. We therefore hope that our results will spark empirical research into mechanisms by which rhizobia can exert control over resource allocation decisions; to that end, we point out potentially fruitful avenues for empirical research in “Discussion.”

The Negotiation Model with Two Nodules

We model the negotiation process between a legume host and two nodules, each occupied by a single rhizobial genotype, on the basis of the dynamics introduced by Akçay and Roughgarden (2007). We label the nodules N1 and N2. The carbon flux into and nitrogen flux out of N1 and N2 are denoted (I_{C1} , I_{A1}) and (I_{C2} , I_{A2}), respectively (C is

for carbon and A for ammonium or amino acid). These fluxes give rise to growth rates r_p , r_{N1} , and r_{N2} for the plant, nodule 1, and nodule 2, respectively. Akçay and Roughgarden (2007) use simple metabolic models to derive the growth of the plant and the nodule as functions of the carbon and nitrogen fluxes; we use slightly modified versions of their growth rates (see “Different Growth Functions” in the online edition of the *American Naturalist*). Our results are qualitatively unchanged when different growth functions are used, as long as these functions exhibit diminishing benefits from the resource received (i.e., nitrogen for the plant and carbon for the nodules) and accelerating costs for the resource given up (carbon for the plant, nitrogen for the nodules). Plant growth rate is a function of the total nitrogen flux received from the nodules and the carbon flux allocated to them, that is, $r_p(I_{Ap}, I_{Cl})$, where $I_{At} = I_{A1} + I_{A2}$ and $I_{Ct} = I_{C1} + I_{C2}$, while the growth rate of each nodule depends only on the carbon flux it receives and the nitrogen it exports.

Like Akçay and Roughgarden (2007), we assume that at each negotiation step there are small, stochastic fluctuations in the carbon flowing into and the nitrogen flowing out of one of the nodules, say N1, which brings the growth rates of the plant and the nodules to r'_p , r'_{N1} , and r'_{N2} . This creates three possible cases: (1) $r'_p > r_p$ and $r'_{N1} > r_{N1}$; (2) $r'_p < r_p$ and $r'_{N1} < r_{N1}$; or (3) one growth rate is higher, and the other is lower. In the first two cases, there is no conflict between the nodule and the plant. In case 1, the new rates are accepted, and in case 2, the rates revert back to the previous values. In the third case, however, one player's growth rate is lowered. This player responds by shutting down the fluxes completely, which represents an effort on this player's part to regain the more advantageous previous fluxes. This move triggers a phase during which both players stop exchanging nutrients and therefore forgo the growth resulting from this exchange. We call this phase the “war-of-attrition game,” because of its similarity to models of animal conflicts where individuals engage in costly behavior (by displaying, fighting, or simply waiting) to wear down their opponent and obtain a resource (Bishop and Cannings 1978).

The duration over which each party stays in the war-of-attrition game is determined by its potential gain from winning this stage of the game and its opportunity cost of staying in the war of attrition. Akçay and Roughgarden (2007) proposed that each party stays in the war of attrition until its potential gain equals its loss. Suppose that $r'_p > r_p$ but $r'_{N1} < r_{N1}$. If the fraction of the period the plant stays in is given by p , we have

$$p(r_p - r_{p,2}) = (1 - p)(r'_p - r_p), \quad (1)$$

where $r_{p,2}$ denotes the growth rate that the plant can achieve with its other nodule (N2) alone. We assume that

a nodule cannot grow when not receiving carbon fluxes from the plant, so we have, for the fraction of time q that nodule N1 stays in the game,

$$qr'_{N1} = (1 - q)(r_{N1} - r'_{N1}). \quad (2)$$

If $p > q$, the plant wins, and the new growth rates of the plant and N1 are r'_p and r'_{N1} . (N2 is unaffected by the negotiation between the plant and N1.) In the one-nodule case, where $r_{p,2} = 0$, these negotiation dynamics lead to the maximum of the product of r_p and r_{N1} , which in bargaining theory is called the “Nash bargaining solution” (Nash 1950, 1953) and has been proposed by Roughgarden et al. (2006) as an alternative to the Nash equilibrium as an outcome of behavioral dynamics between animals. In the two-nodule case, we are interested in whether and how the plant can use the negotiation process to distinguish between rhizobia of differing mutualistic quality and reward them accordingly, a phenomenon that has variously been termed “plant sanctions” (Denison 2000; West et al. 2002c) and “partner choice” (Simms and Taylor 2002).

Variation in Partner Quality and Negotiation

To model variation in partner quality, we introduce a rhizobium trait that modulates the negotiation behavior of a nodule. Suppose that a rhizobium strain occupying N1, instead of staying in the war-of-attrition game for a fraction q of the time, as given by equation (2), stays for a time $\beta_{N1}q$, with $\beta_{N1} > 0$. Thus, strains with higher β will stay in the war-of-attrition stage longer, making them less likely to accept losses in their growth rate and more likely to impose losses on the plant's growth rate. In anthropomorphic terms, these strains are more “stubborn” during negotiations. In a single-nodule setup, the outcome of negotiation with such strains would be skewed toward benefitting the nodule to the detriment of the plant. Thus, strains with high β would appear as low-quality partners (fig. 1).

At the long-term equilibrium of the negotiation dynamics, all new rates will be rejected and the following conditions hold for a focal nodule N1: (1) no alternative pair of rates R' exists that is simultaneously more beneficial to both the nodule and the plant; (2) for $r'_p > r_p$ and $r'_{N1} < r_{N1}$, $\beta q > p$; and (3) for $r'_p < r_p$ and $r'_{N1} > r_{N1}$, $p > \beta q$. For small fluctuations around the equilibrium, these three conditions can be summarized into a first-order condition:

$$\frac{1}{r_p^* - r_{p,2}} \frac{\partial r_p}{\partial I_{A1}} + \beta_{N1} \frac{1}{r_{N1}^*} \frac{\partial r_{N1}}{\partial I_{A1}} = 0, \quad (3)$$

$$\frac{1}{r_p^* - r_{p,2}} \frac{\partial r_p}{\partial I_{C1}} + \beta_{N1} \frac{1}{r_{N1}^*} \frac{\partial r_{N1}}{\partial I_{C1}} = 0, \quad (4)$$

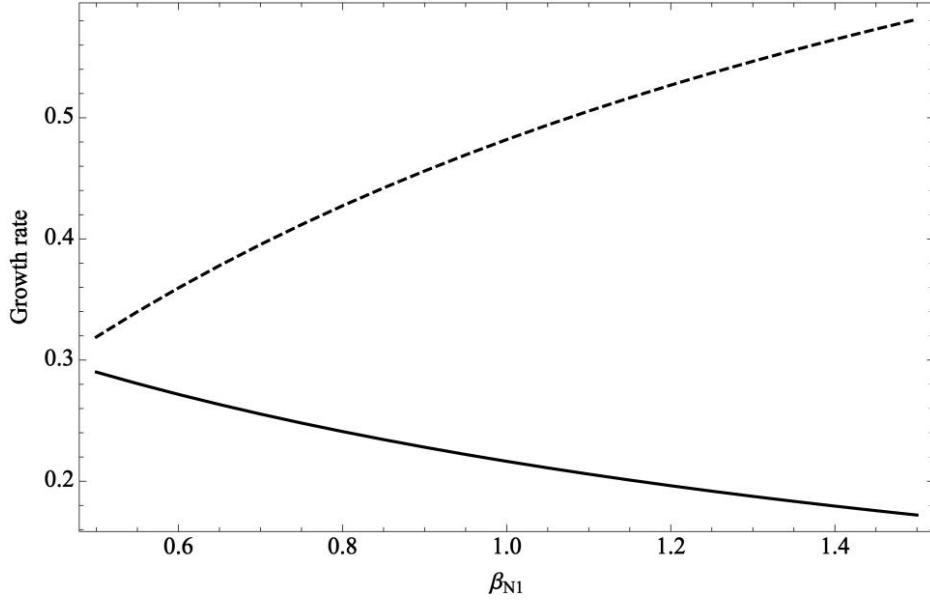


Figure 1: Change in the growth rates of the plant and the nodule in a single-nodule case, where the equilibrium is given by equations (3) and (4), with $r_{p,2} = 0$. The solid line represents the plant's growth rate, r_p^* , and the dashed line represents the nodule's, r_{N1} .

where r_p^* and r_{N1}^* are the equilibrium growth rates of the plant and N1, respectively, and $r_{p,2}$ is the growth rate that the plant can achieve by relying only on N2 while the carbon and nitrogen fluxes with N1 remain shut down.

Similarly, the outcome of the negotiation with N2 is characterized by the following conditions:

$$\frac{1}{r_p^* - r_{p,1}} \frac{\partial r_p}{\partial I_{A2}} + \beta_{N2} \frac{1}{r_{N2}^*} \frac{\partial r_{N2}}{\partial I_{A2}} = 0, \quad (5)$$

$$\frac{1}{r_p^* - r_{p,1}} \frac{\partial r_p}{\partial I_{C2}} + \beta_{N2} \frac{1}{r_{N2}^*} \frac{\partial r_{N2}}{\partial I_{C2}} = 0, \quad (6)$$

where $r_{p,1}$ is the growth rate the plant can achieve by relying only on N1. The quantities $r_{p,1}$ and $r_{p,2}$ represent the “outside options” available to the plant and determine how long the plant can “hold out” during the war-of-attrition stage. Intuitively, the equilibrium outcome balances the gains of the plant and each nodule, given by the partial derivatives of r_p , r_{N1} and r_{N2} , relative to their opportunity costs of holding out during negotiations, given by the denominators in each term. The relative gains of the rhizobia are weighted by their β traits. The opportunity cost for the plant depends on its outside options when negotiating with a given nodule, which in general will be a function of the external environment and all partners' traits.

Outside Options and Plant Sanctions

The Outside Option Equals the Current Rates

The war-of-attrition phase with a focal nodule is the crucial determinant of the long-term negotiation outcome. Its outcome depends on the growth rate the plant can achieve with the other nodule only, that is, the plant's outside option. This outside option determines how much “bargaining power” the plant holds vis-à-vis the negotiating nodule. We first consider the case where, when negotiating with N1, the plant simply maintains the current nitrogen and carbon fluxes with N2. Thus, the plant's growth rate during the war-of-attrition phase with N1 is given by

$$r_{p,2} = r_p(I_{A2}, I_{C2}). \quad (7)$$

Similarly, the outside option when negotiating with N2 is given by $r_{p,1} = r_p(I_{A1}, I_{C1})$. Hence, at equilibrium, the plant's outside options will be $r_{p,1} = r_p(I_{A1}^*, I_{C1}^*)$ and $r_{p,2} = r_p(I_{A2}^*, I_{C2}^*)$. Substituting these terms into equations (3)–(6), we can find the equilibrium nitrogen fixation and carbon allocation rates for particular pairs of strains with β_{N1} and β_{N2} . Furthermore, by the implicit-function theorem, we can express the equilibrium fluxes of carbon to and nitrogen from the nodules as functions of β_{N1} and β_{N2} . To find how the equilibrium fluxes change with changing β s, we take the total derivative of each of the four equations with respect to β_{N1} and β_{N2} and solve the resulting eight equations for the eight partial derivatives

$\partial I_{A1}^*/\partial \beta_{N1}$, $\partial I_{C1}^*/\partial \beta_{N1}$, $\partial I_{A1}^*/\partial \beta_{N2}$, and so on, as a function of the first and second derivatives of the growth rate functions at the equilibrium. We found analytical expressions for these partial derivatives, but they are too cumbersome to reproduce here. Instead, we compared the growth rates of two nodules with different β s by evaluating the equilibrium conditions numerically (fig. 2). The nodule with the higher β always has a higher growth rate. Thus, a negotiation process in which the current rates represent the plant's outside option will not lead to effective sanctions by the plant, since it fails to withhold rewards from the less beneficial strain.

This result can be understood intuitively by considering the plant's negotiation with one of the nodules, say N1. With constant outside options, a nodule with higher β always receives a higher growth rate because it is less likely to accept losses during negotiation. In turn, paying a higher price for nitrogen from a tougher negotiator in N1 diminishes the plant's outside option when negotiating with N2. This shift gives the plant a poorer equilibrium bargain with N2, which further depresses the plant's outside option against N1 (i.e., $r_{p,2}$).

Temporary Redirecting of Fluxes

In the previous subsection, we assumed that the plant does not use the carbon withheld during the attrition phase to improve its outside option. We now suppose that, when locked in a war of attrition with N1, the plant redirects a fraction ρ of the carbon flux I_{C1} (that would have gone to N1) to N2 and in return receives an added nitrogen flux at rate $\kappa_{N2}\rho I_{C1}$, with $\kappa \geq 0$. Here, κ stands for a strain-specific rhizobium trait that quantifies how much the strain (temporarily) ramps up nitrogen fixation when supplied with additional carbohydrates: a strain with high κ supplies the plant with more extra nitrogen than does a strain with lower κ . We assume that the added nitrogen fixation and exchange of fluxes operate at a very short timescale and hence are not themselves subject to negotiation. When the plant redirects its carbon flux, its growth rate while in the war of attrition becomes

$$r_{p,2} = r_p(I_{A2} + \rho\kappa_{N2}I_{C1}, I_{C2} + \rho I_{C1}), \quad (8)$$

and similarly for $r_{p,1}$,

$$r_{p,1} = r_p(I_{A1} + \rho\kappa_{N1}I_{C2}, I_{C1} + \rho I_{C2}). \quad (9)$$

When all else is equal, increasing a strain's κ has little influence on the equilibrium growth rate of its own nodule but decreases the equilibrium growth rate of the other nodule (see Mathematica code, available through the online edition of the *American Naturalist*). When a plant hosts two strains of rhizobia, one with low and the other with high β , the low- β strain can have a higher equilibrium

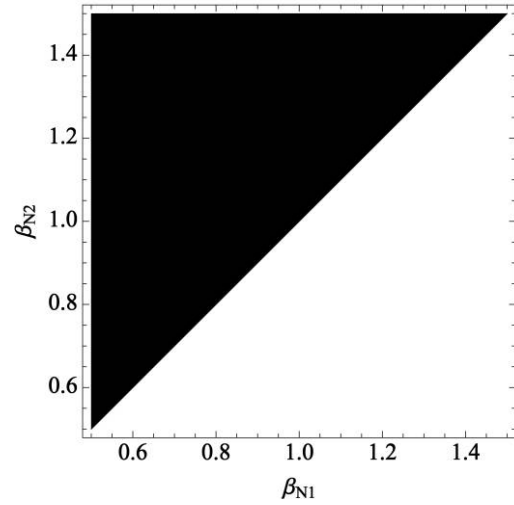


Figure 2: Comparison of growth rates of nodules with different β traits. The black region denotes pairs of β_{N1} and β_{N2} for which N2 has a higher growth rate than N1, $r_{N2} > r_{N1}$; the white region denotes pairs for which N1 has a greater growth rate than N2, $r_{N1} > r_{N2}$. The region above the diagonal is black, which means that the nodule with the higher β always has a higher growth rate than the nodule with the lower β . Thus, under the assumption that the outside option equals current rates, negotiations cannot produce effective sanctions.

growth rate than a strain with higher β , provided that its κ is sufficiently larger than the high- β strain's κ (fig. 3). Thus, for the plant to effectively impose sanctions against high- β rhizobia, there must be a negative correlation between the β and κ traits of the strains.

The intuition underlying this result is simple: the plant benefits by relying on a strain with high κ for temporary nitrogen needs in a way that is similar to a firm manager who hires substitute workers when the union goes on strike. A strain with higher κ improves the outside option for the plant, allowing it to drive a tougher bargain with the other nodule. Thus, a low- β strain with high κ can offset the bargaining advantage of a high- β , low- κ nodule, drive down the latter's growth rate, and make plant sanctions appear effective.

Soil Nitrogen

Another outside option for the plant is to take up mineral nitrogen from the soil. However, increasing nitrogen uptake from the soil requires growing new roots, which is unlikely to happen fast enough to affect nitrogen uptake during the brief war-of-attrition stage. We therefore include the effect of a constant nitrogen uptake from the soil by adding it to the plant's total nitrogen supply. As we show in "Mineral Nitrogen Uptake from the Soil" in the online edition of the *American Naturalist*, allowing for

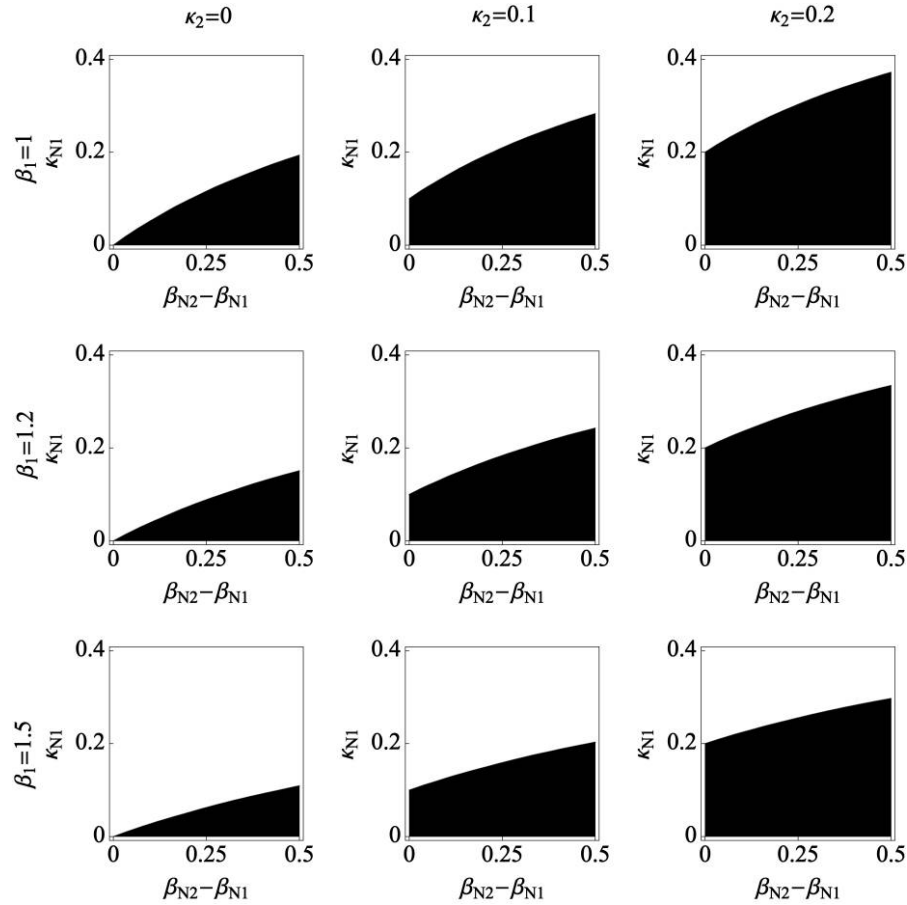


Figure 3: Comparison of the growth rates of nodules with different (β, κ) pairs. As in figure 2, the black regions indicate when N2 has a higher growth rate than N1, and vice versa for white regions. We assume that $\beta_{N2} > \beta_{N1}$; the X-axis in each panel shows the difference in the β traits. Therefore, black regions correspond to cases where negotiations lead to ineffective sanctions (higher growth rate of less cooperative, higher- β nodules), while the white regions correspond to effective sanctions. The figure shows that effective sanctions are possible when κ_{N1} is sufficiently greater than κ_{N2} , with the threshold difference being an increasing function of $\beta_{N2} - \beta_{N1}$. Here, $\rho = 0.5$.

mineral nitrogen uptake does not affect the growth rate relationship between two nodules with different β and κ traits. In other words, the conditions under which plant sanctions appear to be effective are qualitatively unchanged. However, higher mineral uptake rates move the negotiation equilibria in the plant's favor for a given value of rhizobium β and, at the same time, increase the selection pressure for higher β , all else being equal. This happens because increasing the availability of nonnegotiated nitrogen improves the plant's bargaining position, which decreases rhizobium growth rate. As the nodule's growth as a function of β is concave (as can be seen in fig. 1), this increases the marginal benefit of increasing β . In other words, as the nodule's bargaining position deteriorates, the advantage of being a tougher negotiator (i.e., having a higher β) increases. Empirical research on mycorrhizae supports this prediction (Johnson 1993): when big blue-

stem plants were fertilized, less beneficial fungal species took over their roots. Kiers et al. (2002) note that no conclusive data exist for the evolutionary response of rhizobia to nitrogen fertilization and go on to argue that the effect of increased nitrogen fertilization on the evolution of rhizobium effectiveness is ambiguous, as plant sanctions might counteract the effect of reduced plant dependence on nodules. Our model suggests otherwise.

Effect of Negotiations on Plant's Growth Rate

Here, we compare the plant's growth rate when it hosts different strains in the two nodules (i.e., has an opportunity to exert choice) with that when it hosts the same strain in both nodules. Figure 4 shows that in most cases, the plant's growth rate with choice is intermediate between its growth rates with each strain alone. However, for some

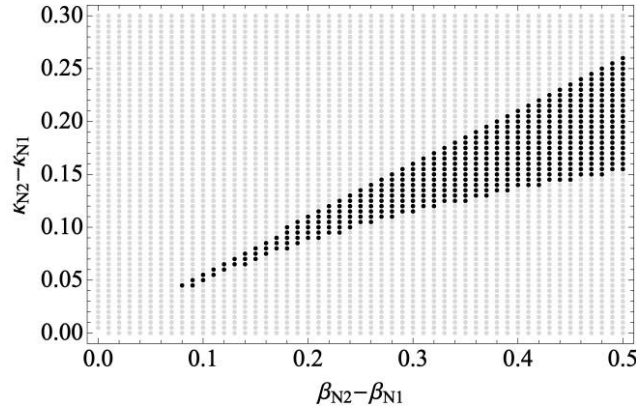


Figure 4: Effect of choice on plant's growth rate. This figure compares the plant's growth rate when it has a choice between two strains with negotiation traits $(\beta_{N1}, \kappa_{N1})$ and $(\beta_{N2}, \kappa_{N2})$ with its growth rate when it is infected with either of the strains alone. Here, $\beta_{N1} = 1$, $\kappa_{N1} = 0$, and $\rho = 0.5$. The gray points correspond to a plant that has an intermediate growth rate when it has a choice, relative to a plant that hosts either of the strains alone. The black points correspond to a plant that has a lower growth rate when it has a choice, relative to a plant that hosts either of the strains alone. This happens when the higher- β strain also has a higher κ .

combinations of β and κ , the plant's growth rate is lower when it has a choice than when it is infected with only the worse strain. This happens when a high- β , high- κ strain is paired with a lower- β , lower- κ one, which is roughly the opposite of the conditions that make plant sanctions effective.

To understand this rather counterintuitive result, consider first what happens when a plant hosts high- β , high- κ strains in both nodules. Both nodules drive a hard bargain, yet during the negotiation phase, each also gives the plant temporarily increased nitrogen fluxes that improve the plant's bargaining position. These two effects counteract each other, giving the plant an intermediate growth rate. Conversely, a low- β , low- κ strain will accept losses readily yet will not grant the plant good outside options when it is negotiating with a competing nodule, again resulting in an intermediate growth rate for the plant. When these two strains are paired on the same plant, however, the high- β strain can take advantage of the plant's weak bargaining position, created by the low κ of the other strain, while at the same time being very unwilling to accept losses during the negotiation. This situation reduces the plant's growth rate below that reached with either strain alone. Furthermore, the plant cannot exercise effective sanctions under these conditions and ends up rewarding the high- β nodule disproportionately.

Negotiation and the Evolution of Cooperative Rhizobia

To ask how the negotiated outcomes affect the evolutionary dynamics of the rhizobium traits β and κ , we assume that the fitness of the rhizobium strain occupying a nodule

is reflected in the growth rate of the nodule. We conduct an invasion fitness analysis in a monomorphic population with resident values β_r and κ_r , by considering the growth rate of a mutant strain when it coinfects a plant with the resident strain. By implicit differentiation of the first-order conditions (eqq. [3]–[6]) and using the expressions (8) and (9) for the outside options, we calculate the partial derivatives of the growth rate of a mutant nodule r_m with respect to its negotiation traits, β_m and κ_m , and evaluate these derivatives at $\beta_m = \beta_r$ and $\kappa_m = \kappa_r$. Under the assumptions of adaptive dynamics, these derivatives give the direction of evolutionary change of the population averages of β and κ in the absence of a covariance between these traits (Dieckmann and Law 1996; Brown et al. 2007). The vector field depicting the gradient of the invasion fitness for a range of resident β and κ values (fig. 5a) shows that selection will uniformly act to increase β . The selection gradient acting on κ is mostly flat, except when the resident has a high β , in which case there is weak selection for intermediate values of κ_r . Selection on κ is mostly flat because a nodule's κ trait affects its own growth rate only indirectly, through its effect on the other nodule's negotiation equilibrium. Therefore, the effect of κ on a strain's own fitness can be thought of as a second-order effect.

To check whether these results carry over to a more realistic population with many rhizobial strains, we conducted simulations of diverse rhizobium populations with different correlations between β and κ . These simulations (see Mathematica code for details) confirm that, regardless of the correlation between the two traits, the highest- β strains almost always win out in the long term.

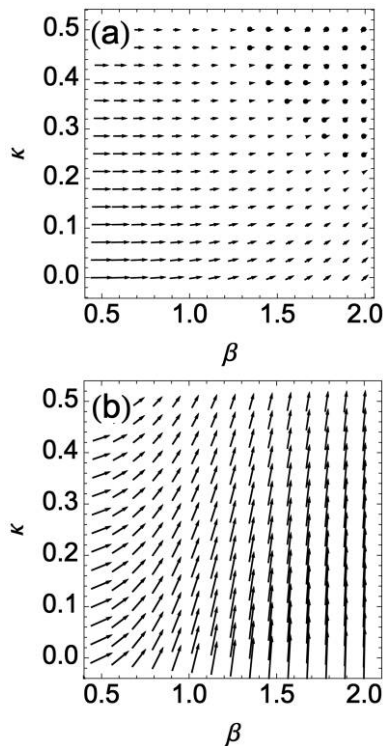


Figure 5: Vector field depicting the selection gradients on the negotiation traits, β and κ . Panel *a* is for the case without partner fidelity feedback (PFF; $s = 0$ in eq. [10]), and *b* is for the case with PFF ($s = 1$). In *a*, there is selection to increase β throughout its range, while selection to increase κ is weak when β is small but becomes stronger as β grows. Selection gets weaker on each trait as it increases in magnitude. In *b*, there is still selection to increase β , but the magnitude of this selection is weaker than that in *a*. In contrast, there is strong selection to increase κ because κ helps the plant's growth rate, which yields a payoff to the rhizobia through PFF while imposing no direct cost.

These results show that plant sanctions through negotiation, even when effective at the level of a single plant, are still unable to maintain beneficial rhizobia in the population. The fundamental reason for this result is that the negotiation process allows a nodule with a high β to “manipulate” the plant into providing it with more carbon. To resist this manipulation, the plant must rely on strains with higher κ . But even if these strains fare better when they cohabit a plant with a high- β , low- κ strain, they do much worse when they are the sole occupant, since the plant can then play two high- κ nodules against each other. Thus, high- β strains are able to invade a population of low- β strains, regardless of their κ values.

Partner Fidelity Feedback

What constrains β from increasing indefinitely? One possibility is that a higher β imposes costs on the rhizobium:

during the transient negotiation dynamics, strains with higher β spend less time growing because they spend more time in the war-of-attrition phase than do strains with lower β . This cost is not accounted for in our analyses above, which consider only the equilibrium of the negotiation dynamics. Akçay and Roughgarden (2007) found in their simulations that the war-of-attrition stage imposed, on average, a roughly 7% cost, relative to the same nitrogen fixation and carbon fluxes reached by a cost-free process. This cost will be higher for high- β strains, because they will stay in the war-of-attrition stage longer, and it might eventually grow so large as to negate the advantage of increasing β .

Partner fidelity feedback (PFF), that is, benefits that rhizobia obtain from increased plant growth rate, might also constrain β . Intuitively, PFF must play some role in the legume-rhizobium symbiosis, since plants that grow more also photosynthesize more and have more resources available for dividing among themselves and their symbionts. To model the effect of PFF, suppose that the lifetime fitness of nodule N1 is given by

$$w_{N1} = r_{N1} r_p^s, \quad (10)$$

where $s \geq 0$ is a parameter denoting the strength of PFF. When $s = 0$, there is no automatic fitness benefit to the nodule from the plant's growth; $s > 0$ implies some benefit, which becomes stronger as s increases. Although this benefit could be derived from a model that accounts for growth and reproduction of both the nodule and the plant, s provides a simple way to account for PFF. Note that the behavior of the parties during the negotiation is still based on the growth rates of the plant (r_p) and the nodule (r_{N1}), as opposed to being determined by the fitness function w_{N1} . To assume otherwise would require that the partners exchange information about their growth rates and anticipate future returns, both capabilities that we are reluctant to ascribe to plants and bacteria.

To find how PFF affects the selection gradients for β and κ , we again calculate the derivatives of rhizobial fitness given by equation (10) with respect to these traits (fig. 5). Adding PFF (e.g., when $s = 1$) favors higher κ and does so more strongly as β increases. This is because hosting strains with high κ increases the growth rate of the plant, which then yields a return on the rhizobium fitness through PFF. Positive selection to increase β is diminished but has not vanished. Nonetheless, the strong selection for increased κ arising from PFF, together with a negative covariance between κ and β , can maintain low- β strains in the population. To illustrate this, we computed the evolutionarily stable (ES) (β, κ) pairs as a function of the PFF exponent s , assuming a negative, linear relationship between β and κ (fig. 6). The evolutionarily stable β decreases with the PFF exponent s , meaning that the ES rhizobia

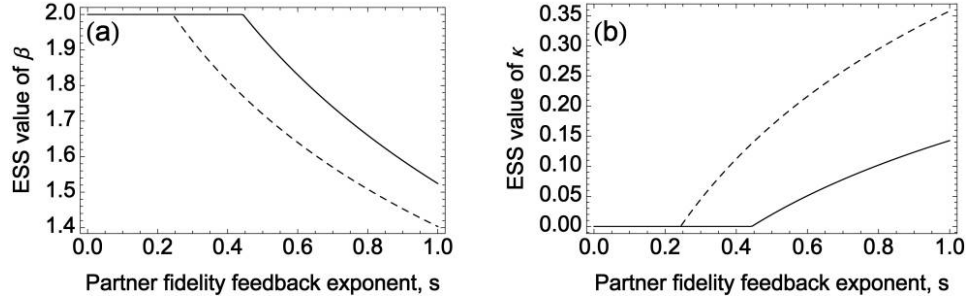


Figure 6: Evolutionarily stable strategy (ESS) values of β (a) and κ (b) as a function of the partner fidelity feedback (PFF) exponent s . Here, we assume a negative, linear relationship between β and κ , with two different values for the slope. For the solid curve, we assume $\kappa = 0.6 - 0.3\beta$, while the dashed curve is drawn under the assumption $\kappa = 1.2 - 0.6\beta$. In each case, the maximum value of β is assumed to be 2 (making κ nonnegative). While the particular value of this upper limit is arbitrarily chosen for our numerical calculations, its empirical value can be interpreted as corresponding to the least cooperative strain in the population. As can be seen, this least cooperative strain will be evolutionarily stable up to a threshold PFF coefficient s , and only for higher s will more-cooperative strains be able to persist in the population. This threshold s is lower when the relationship between β and κ is more negative.

become more cooperative with stronger PFF. Further, for a given value of the PFF coefficient s , a more negative relationship between β and κ will decrease the ES value of β and increase the ES value of κ .

Discussion

Negotiation Can Undermine Partner Choice/Sanctions and Creates Context Dependency

Our model yields four main results. First, the correlation between the rhizobium negotiation traits β and κ affects the plant's ability to reward more-cooperative strains. When β and κ are negatively correlated, strains that demand less carbon per nitrogen fixed also provide the plant with better outside options; moreover, negotiation provides higher rewards for the more cooperative rhizobium strain. This combination enables plant sanctions. However, negotiation disables plant sanctions when these traits are not negatively correlated. These results partially support the conjecture by Akçay and Roughgarden (2007) that the negotiation process might underlie plant sanctions, yet they also illuminate a more complex picture where effective sanctions depend on the combination of strains co-infecting the host plant. These findings also provide a possible explanation for the empirical results that show effective plant sanctions in some cases (Kiers et al. 2003, 2006; Simms et al. 2006) but not in all (Marco et al. 2009; Gubry-Rangin et al. 2010).

Our second result is that negotiation creates context-dependent variation in the plant's fitness costs and benefits. Context dependency here refers to the fact that the negotiation outcome and its effect on the plant's overall growth depend on the combination of strains with which

the plant is interacting. In particular, with certain combinations of strains (low- β , low- κ strains paired with high- β , high- κ strains), a plant might be worse off when it has a "choice" between two strains than when it is infected by either strain alone. This prediction might explain the results of an inoculation experiment by Heath and Tiffin (2007), in which *Medicago truncatula* plants were sometimes less fit when they had a choice (i.e., inoculated with two *Sinorhizobium medicae* strains that offered different levels of benefit) than when inoculated with either strain alone. This result is paradoxical from a naive market-theory perspective but arises naturally from an underlying negotiation model where exerting choice is predicated on having good outside options.

Maintenance of Mutualism Requires Both Partner Fidelity Feedback and Sanctions

Our third result is that, regardless of the correlation between κ and β , selection always favors higher β , even when plants can successfully reward lower- β nodules. This result underscores a general point about sanction models that seems to have been overlooked so far. Consider two symbiont strains that interact with a single host species. Suppose that strain 1 is the more cooperative strain; that is, the host grows better when singly inoculated by strain 1. Denote by w_{ij} the fitness of strain i when it coinfects the host with strain j . Effective host sanctions should result in $w_{12} > w_{21}$; that is, when both strains infect a single host, strain 1 has a higher fitness than does strain 2. On the other hand, the evolutionary stability of strain 1 requires $w_{11} > w_{21}$, and conversely, the evolutionary stability of strain 2 requires $w_{22} > w_{12}$. In general, effective host sanctions do not guarantee or preclude either condition. For

example, our model without PFF generates the following pattern: $w_{22} > w_{12} > w_{21} > w_{11}$. Thus, even effective host sanctions cannot maintain strain 1 in the population.

Our final result is that both sanctions and partner fidelity feedback (PFF; Bull and Rice 1991; Sachs et al. 2004; Foster and Wenseleers 2006) might be necessary to maintain mutualism. West et al. (2002c) showed that PFF alone is not sufficient to maintain cooperative rhizobia, because the feedback benefits that a cooperative strain produces by helping the host are diluted across all nodules, regardless of strain, giving rise to a “tragedy of the commons.” This effect is present in our model as well: even with PFF, selection favors higher β , albeit with diminished strength. On the other hand, our second negotiation trait, κ , does not suffer from the commons tragedy, because while higher κ in a focal nodule improves the plant’s position against the other nodule, it does not directly affect the negotiation outcome between the focal nodule and the plant. Hence, a focal nodule’s κ value affects its own growth rate only indirectly, by changing the plant’s baseline growth rate (this effect is small but positive). On the other hand, with PFF, a higher- κ strain receives feedback benefits from improving the plant’s growth, which means that PFF strongly favors higher κ . Nonetheless, favoring high κ does not by itself maintain low- β strains. For this to occur, κ and β must be negatively correlated, which brings us back to the condition for effective plant sanctions. Note, however, that sanctions here play a rather incidental role; their existence is a side consequence of adequate negative correlation between the negotiation traits.

Control and Outside Options in Biological Markets

The main thrust of our article is that whenever rhizobia have some control over the outcome, the plant must rely on other rhizobia to sanction less cooperative ones, at least in the short term. This basic fact creates evolutionary and ecological patterns that are more complex than those from pure host-control models (e.g., West et al. 2002c). Our model provides possible explanations for empirically observed patterns that had not been anticipated by previous sanctions models, a fact that offers indirect support for our model and for rhizobium control in general. More generally, the issue of joint control of the mutualistic outcome and its effects on evolutionary and ecological dynamics presents a fertile ground for future theoretical and empirical work.

The partner-choice model of Kummel and Salant (2006) in a mycorrhizal mutualism offers an interesting comparison in this regard. Kummel and Salant characterize each fungal strain by an exchange function that describes how much resource the strain yields in exchange for a given amount of carbon. The plant then allocates carbon in a

way that equalizes the marginal costs of obtaining resources from the strains that it chooses. Even though Kummel and Salant did not consider fungal fitness in their paper, their model can also yield cases with ineffective sanctions, that is, where the less beneficial strain is evolutionarily stable despite optimal partner choice by the plant (E. Akçay, unpublished analysis). One possible interpretation is that this outcome arises because plant choices are constrained by the set of available supply curves, which is determined by the fungal population’s composition. Hence, plants in this model lack complete control over the allocation of rewards and can exercise choice only when interacting with the right combination of fungal strains.

Another set of models, by Johnstone and Bshary (2002, 2008), explores how control over the interaction influences the exchange of services in the cleaner-fish mutualism, where there is conflict over the duration of the interaction between an “exploiter” (cleaner fish) and a “victim” (client fish). Johnstone and Bshary (2002) find that as exploiter control increases, so too does the evolutionarily stable level of exploitation, while the duration of each interaction decreases. Johnstone and Bshary (2008) add a market context to this model, where interaction duration influences partner availability for both types of partners, and find that the market cannot prevent the cooperation breakdown that is caused by increased exploiter control. In one sense, the control variable in Johnstone and Bshary (2002, 2008) fulfills a role similar to that of β : it shifts the stable outcome in the exploiter’s favor. A major difference between these models and ours is that the clients in Johnstone and Bshary (2008) have no way of retaliating against the cleaner’s manipulation of interaction duration, either during the interaction itself (e.g., by manipulating the exploitation level) or by exercising outside options, such as visiting less manipulative cleaners (e.g., chosen by reliable indicators of their tendency to manipulate). Furthermore, their model does not consider evolution of the control trait in the cleaner population. Selection in their model would always favor higher control by the exploiters (since there is no immediate cost to higher control) and therefore lead to the breakdown of cooperation, just as selection in our model favors increased β .

We suggest that the next stage of biological market theory should include more-explicit models of the mechanisms determining the exchange rates for services and resources and the effect of outside options within these mechanisms. Such models would require more detailed knowledge of the proximate mechanisms of resource exchange (Bshary and Bronstein 2004), which often is not immediately available. Nonetheless, models such as ours underscore the importance of these mechanisms and provide a theoretical motivation for empirical inquiry into

them. For example, from current knowledge that we review in “Mechanisms for Control of the Mutualism,” we might infer that plants have the upper hand in the legume-rhizobium interaction. However, this view might just as well reflect our relative ignorance about mechanisms of rhizobial control. Methodological difficulties have hampered inquiry into how endosymbiotic bacteroids might exert control during the nitrogen-fixation stage of the interaction. Further, most studies of bacteroid physiology are purely mechanistic and focus on single nodules, rarely if ever considering how bacteroid traits expressed in one nodule might influence the trait expression and performance of strains in other nodules. In contrast, empirical research on the regulation of nitrogen fixation adopts the plant’s perspective and assumes plant control (e.g., Schulze 2004). We suggest that our approach can usefully merge these two perspectives by integrating evolutionary theory with a mechanistic approach to control and regulation of the symbiosis. Such a synthesis produces many new empirical questions, some of which we consider below.

Rhizobium Traits and Empirical Questions

Our model predicts that the effect of a rhizobium strain on the plant’s growth will depend on which other strains occupy the other nodules of the plant. This kind of context dependency poses a problem for defining and measuring cooperativeness of rhizobium strains. In principle, a quantity such as the ratio of nitrogen fixation to carbon allocation, I_A/I_C , might denote how beneficial a nodule is to the plant, but these rates will vary, depending on the other strains being hosted by the same plant. Therefore, a strain’s cooperativeness must be defined and measured within a specified context, which challenges the use of terms such as “cooperators” and “cheaters” to denote genotype-specific properties of rhizobia. Nonetheless, it is possible to define a standard context in which to evaluate strains, such as single inoculation, as is done in most empirical studies (e.g., Simms et al. 2006; Heath and Tiffin 2009; Heath 2010). In that case, our model posits that there is more than one way to be cooperative: a strain can either have a low β and thereby “demand” less during the negotiation process or have a high κ and thereby help the plant drive a harder bargain with another nodule. In a single-strain inoculation, the strain’s β is evaluated in the context of its κ and its κ is evaluated in the context of its β , and the joint action of the two traits determines the strain’s nitrogen-fixation level. Thus, to predict a strain’s cooperativeness in other contexts (e.g., in mixed inoculations or in soil), we must understand what biological mechanisms underlie such traits and determine how best to measure them.

One can identify the rhizobium traits important to the

interaction outcome by investigating in detail the physiological mechanisms by which legumes and rhizobia interact. For example, a recent study by Ratcliff and Denison (2009) showed that an ethylene inhibitor produced by rhizobia shifts the symbiotic outcome in favor of the rhizobia. More research into the mechanistic basis for such effects is needed. An evolutionary perspective suggests that it might be especially profitable to find and study mechanisms underlying natural polymorphisms in functionally relevant genes. Functional genetic studies of nitrogen fixation (Masson-Boivin et al. 2009) and cellular interactions between plant and rhizobium cells (Lodwig et al. 2003; Prell et al. 2009) should be conducted with an eye toward uncovering such variability in rhizobial populations. Once control traits have been identified, our model makes a number of specific predictions about the outcome of the mutualism as a function of different combinations of traits. These predictions should be tested, as they have important implications for the evolutionary dynamics of this interaction.

Another way to test whether bacteroids exercise any control in the interaction is to detect variation in these unidentified traits by observing the fitness associations that they create between mutualism partners. For example, β is predicted to generate antagonistic covariation between plant and rhizobium fitnesses. So variation in β can be detected as a negative correlation between the fitnesses of rhizobium and host plant across genetically identical host plants that have been singly inoculated with different rhizobium genotypes. In turn, κ is predicted to generate antagonistic variation between the fitnesses of strains that coinfect a plant. Hence, variation in κ can be detected by measuring individual fitnesses of pairs of rhizobium genotypes inoculated onto different individual host plants, with the pairs representing “cells” in a factorial array of rhizobium combinations. One caveat is that a negative genetic correlation between β and κ might mask some fitness covariances. Nonetheless, examining fitness covariances among plant genotypes and rhizobial strains and determining whether such covariation maps onto specific genetic loci in rhizobia is an area ripe for study. Few studies have measured genetic variation and covariation between the fitnesses of plants and rhizobia in either natural or managed systems.

Future Directions for Theory

Our extension of the negotiation framework of Akçay and Roughgarden (2007) to interactions with two nodules generates interesting insights about the evolution of the symbiosis but does not yet capture all important aspects of the interaction. We briefly discuss some of these aspects,

both to delineate limitations of our model and to sketch out questions that we intend to tackle in future work.

First, we assume that the plant does not simply cut off a nodule with high β . In the short term, such a move would not be optimal for the plant, as the remaining nodule would have a much better bargaining position and therefore be able to shift the outcome in its own favor. In the longer term, however, the plant can replace the cutoff nodule with one or more new nodules. More generally, the number of nodules on a single plant is highly plastic, and plants continue to “search” for more rhizobia and initiate nodules while negotiating with existing nodules. This means that the plant is not constrained by its current outside options but instead might expand its outside options and gain advantage when dealing with existing nodules. The optimal nodulation strategy of the plant therefore remains an important question to be answered by future work.

Second, we assume that only nodule growth determines rhizobial fitness. Nodule growth might be the appropriate quantity for studying sanctions after nodule formation, but the overall evolutionary dynamics of the rhizobia will also depend on the number of nodules each strain initiates (Heath and Tiffin 2009). Plants that host effective nodules initiate fewer nodules (Gao et al. 2010; Gubry-Rangin et al. 2010), which will have additional selective effects on the soil population of rhizobia. Furthermore, strains differ in their competitiveness for nodulation (e.g., Amarger 1981; Dowling and Broughton 1986), with potential trade-offs between nitrogen fixation and nodulation effectiveness. Future theory, therefore, must address the nodulation dynamics of the interaction.

A related issue is the evolutionary consequence of the spatial population genetic structure of rhizobia (Bever and Simms 2000). In a structured population, a rhizobium strain might co-occur on a plant with others of its own genotype more frequently than with a strain randomly selected from the population. This situation would create indirect fitness effects on the rhizobium traits, which will be largely neutral for β (as β has little effect on the other nodule), but negative for κ (because higher κ harms the other nodule). An indirect cost of high κ could counteract some of the positive selection pressure on this trait. On the other hand, spatial structure also makes it more likely for rhizobia to compete with kin (West et al. 2002a), which might negate some of the indirect costs and benefits of rhizobium traits (depending also on population elasticity; Platt and Bever 2009). Predicting which of these effects will be stronger requires a model that more explicitly specifies the life cycle of rhizobia, including dispersal. Another potential effect of spatial structure is to increase the chance that the same plant and rhizobium genotypes interact repeatedly over generations, adding intergenerational part-

ner fidelity feedback to the “developmental” PFF that we modeled here, with largely similar effects.

Finally, we have yet to consider variation in plant negotiation traits. Here, we have concentrated on the evolution of rhizobium traits. There is some justification to this simplification, as rhizobia might be expected to evolve faster than would plants. However, to fully understand this mutualism, we must also consider plant traits and their evolution. In particular, plants might also evolve to be more or less “stubborn” during negotiations. Variation in plant traits will likely influence the distribution of benefits and generate genotype-by-genotype interactions in natural populations (Heath 2010). This question and the question of coevolution of plant and rhizobial negotiation traits we leave for future study.

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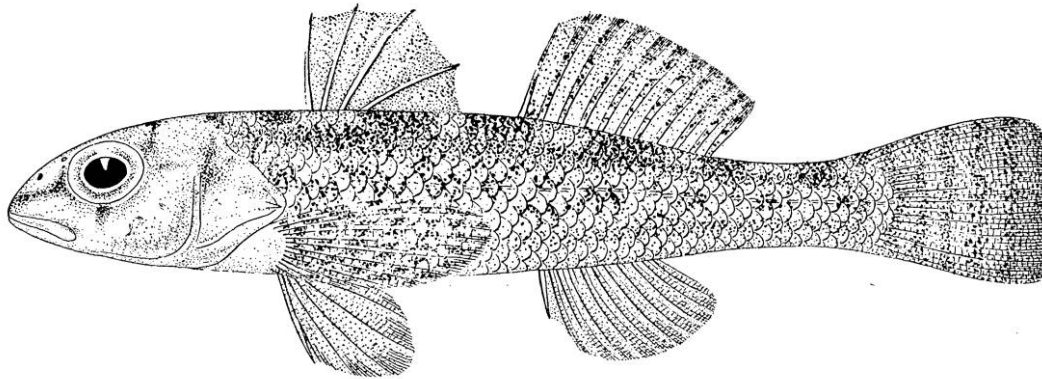
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