The Legume-Rhizobium Symbiosis

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Introduction

Most plants on earth do not have sufficient soil nitrogen. Yet legumes (plants in the family Fabaceae), unlike most plants, have access to nitrogen from both mineral sources and symbiotic sources. Legumes can become infected with nitrogen-fixing bacteria known as rhizobia. These bacteria live in the soil, and when a legume grows nearby a molecular communication ensues that enables the legume roots to become infected. In a process guided by both the bacteria and the plant, the rhizobia invade plant tissue and ultimately inhabit individual plant cells within the host's roots. Once inside the plant, the rhizobia can become efficient symbionts, breaking down dinitrogen from air pockets in the soil into forms that are useful for the plant, in exchange for sugars from the plant. This legume-rhizobium interaction has become a key model for dissecting the molecular basis for communication among species and represents one of the best-understood mutualistic symbioses. Nonetheless, much remains to be understood about this system. Although the host plant appears to have control over key aspects of rhizobial infection and growth within host tissues, the mechanistic basis of this control is poorly understood, and it is unknown how such control evolved. Also, rhizobia could represent a massive boon to agriculture, since they might allow us to avoid the costly process of industrial nitrogen fixation. But the use of rhizobia in agricultural systems has been relatively unsuccessful, and research has made only modest progress in solving this critical problem. While researchers have characterized hundreds of rhizobial strains that fix nitrogen very effectively under greenhouse conditions, these strains are often unsuccessful at competing against indigenous soil rhizobia under real field conditions. Although some biologists hold great hopes for the use of rhizobia to enhance legume production, this promise still remains mostly unrealized. Understanding this symbiosis is a problem that relates to many fields including biology, biochemistry, ecology, evolution, genetics, physiology, and agronomic sciences. An integrative ecological and evolutionary perspective is useful in the study of the legume-rhizobium symbiosis. This emerging perspective takes into account the immense diversity of the legumes and rhizobia that take part in the symbiosis as well as the diverse ecological conditions under which rhizobia and legumes interact. Moreover, this perspective seeks to understand how natural selection shapes each partner and how the interaction can vary depending on extrinsic conditions. This broad view might ultimately enable us to better manipulate the interaction to optimize agricultural productivity. Here, we begin by inferring the evolutionary history of legume and rhizobial lineages to better understand the diversity of both partners. We then describe the molecular and cellular mechanisms of infection and how the mechanistic basis of the symbiosis can vary among host and symbiont lineages. Finally, we turn to evolutionary and ecological aspects of the interaction. We review the selective forces that maintain cooperation between symbionts and hosts and explore the utility of evolutionary theory for optimizing agricultural productivity.

Introductory Works

Sprent 2001 documents the many species in the legume family that have the ability to form intimate, intracellular associations with diverse nitrogen-fixing bacteria, known as rhizobia. As Sprent, et al. 1987, a seminal review, explains, legumes begin life in a symbiont-free state and they acquire rhizobial symbionts in the soil each generation. Typically, rhizobia first infect legume seedlings soon after germination and form tumors (nodules) on the plant's roots. For a successful infection, a compatible pairing of legume and rhizobial genotypes is required. However, as the key review Denison 2000 describes, the degree of specificity varies widely for both the bacteria and the plant partners. Once a symbiotic nodule is formed, the bacteria fix nitrogen for the plant in exchange for plant-derived photosynthates. Nodules eventually senesce, a process that Denison and Kiers 2004 argues is critical for rhizobia to escape back into the soil; the nodule tissue softens and breaks down, after which a subset of the rhizobia are released from the plant. An ecological meta-analysis, Cleveland, et al. 1999, showed how important legume-rhizobium symbioses are at a global scale, because symbiotic rhizobia convert atmospheric dinitrogen into compounds that are useable to legumes and ultimately to other plants. In terms of agriculture, only a handful of legume species are cultivated on a large scale, but Ferguson, et al. 2010 points out that these crops contribute more than 25 percent of global production, including food staples, fodder for livestock, cover crops, and emerging biofuels. There is a clear need to better understand the legume-rhizobium symbiosis both in the wild and in agriculture. Finally, it is important to note that the legume-rhizobium symbiosis is not unique. Other plants such as alders can also form nitrogen-fixing symbioses with bacteria; moreover, Sturz, et al. 2000 argues that a diversity of bacteria act as nitrogen-fixing bacterial endophytes and can provide growth benefits to many plants.

Cleveland, C. C., A. R. Townsend, D. S. Schimel, et al. 1999. Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global Biogeochemical Cycles* 13:623–645.

This expansive meta-analysis of biological nitrogen fixation discusses the global importance and biome specificity of natural sources of nitrogen fixation.

Denison, R. F. 2000. Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *American Naturalist* 156:567–576.

This seminal paper was the first to broadly review the evolution and ecology of cooperation in the legume-rhizobium symbiosis. Denison offered the first general description of host sanctions in this system and provides key information about the symbiosis in the language of evolutionary biologists and ecologists.

Denison, R. F., and E. T. Kiers. 2004. Lifestyle alternatives for rhizobia: Mutualism, parasitism, and forgoing symbiosis. *FEMS Microbiology Letters* 237:187–193.

This conceptual paper explores rhizobial lifestyles that extend beyond beneficial symbiosis, including exploitation of the legume host (parasitism) and growth in the soil without host infection. Unlike previous work on this topic, this paper explores the different outcomes of symbiosis from the point of view of the bacterium and highlights the diverse ecological contexts that challenge rhizobial bacteria.

Ferguson, B. J., A. Indrasumunar, S. Hayashi, et al. 2010. Molecular analysis of legume nodule development and autoregulation. *Journal of Integrative Plant Biology* 52:61–76.

This paper provides an extremely broad and detailed review of what is known about the molecular bases of host mechanisms that control nodule development. This is one of the key works that attempt to make clear connections with the phenotypes of how hosts control rhizobial infections and the molecular bases of these plant traits. Moreover, the authors offer an excellent description of the global importance of nitrogen fixation in legume nodules.

Sprent, J. I. 2001. *Nodulation in legumes*. Kew, UK: Royal Botanic Gardens.

This book explores the biology and diversity of rhizobial nodulation in legumes.

Sprent, J. I., J. M. Sutherland, and S. M. de Faria. 1987. Some aspects of the biology of nitrogen-fixing organisms. *Philosophical Transactions of the Royal Society of London, B: Biological Sciences* 317:111–129.

This excellent review offers one of the very early introductions to the basic biology of the legume-rhizobium symbiosis. If one wants to learn about this symbiosis in general, this is a great place to start.

Sturz, A. V., B. R. Christie, and J. Nowak. 2000. Bacterial endophytes: Potential role in developing sustainable systems of crop

production. *Critical Reviews in Plant Sciences* 19:1–30.

This review explores the diverse types of beneficial interactions that soil bacteria can have when residing within nonlegume plant roots. It explores multiple mechanisms by which these bacterial lineages can enhance plant growth, especially in agricultural systems.

Evolutionary and Biogeographic History of Legumes

Legumes are one of the most diverse and geographically widespread lineages of plants on earth, with over twenty thousand species distributed nearly worldwide. The biogeographical origin and global spread of legumes is difficult to resolve. One long-standing hypothesis suggests an African origin for the legumes followed by northerly migration in the Paleocene, 50–60 million years ago (Mya). An alternative scenario, suggested in Wolfe 1975 and Doyle and Luckow 2003, is a later, more northern origin and southerly migration in the warm and wet Eocene, about 35–55 Mya. In either case, legumes were initially tropical and later adapted to temperate regions as the planet cooled and became drier. Currently, legumes infected with rhizobia are widespread in both tropical and temperate zones, and Schulman, et al. 1988 showed that these symbioses extend well north of the Arctic Circle. As described in Sprent 2008, traveling from the equator to the poles, tropical legumes often take the form of trees with determinate nodules, whereas temperate legumes are more often herbaceous or perennial forms, usually with indeterminate nodules. Rhizobial symbiosis is thought to have had a large impact on the radiation and success of legumes. For instance, if you remove nodulating species from the legume family, the group becomes half as diverse and mostly restricted to the tropics. Yet nodulation ability itself is not enough to explain the evolutionary success of legumes; as Sprent 2008 shows, nonlegume taxa that nodulate, such as actinorhizal plants, have diversified little compared to legumes. McKey 1994 suggests that the success of symbiotic legumes has been driven by the high-nitrogen lifestyle of legume hosts, characterized by short-lived leaves and many nitrogen-rich leaf compounds. Factors controlling the distribution of legumes and their symbionts have long been debated. As shown in Parker, et al. 2006, symbiont distribution may limit host range expansion in some cases; however, hosts can also potentially acquire different rhizobia when invading novel habitats. Conversely, if hosts import their indigenous symbionts to a new landscape, a sweep of nonnative rhizobia through novel populations is possible (Stępkowski, et al. 2005; Rodríguez-Echeverria 2010). Early nomenclature schemes for rhizobia sought to assign symbiont names according to host association. Recognition that many legumes are “generalist” hosts suggests existing rhizobia populations in newly colonized areas have probably aided in the radiation and spread of legumes.

Doyle, J. J., and M. A. Luckow. 2003. The rest of the iceberg: Legume diversity and evolution in a phylogenetic context. *Plant Physiology* 131:900–910.

This paper provides a nice review of the historical habitats and spatial spread of the legume lineage. A major goal of this paper is to expose the reader to the incredible diversity of plant forms and habits that can be found in the legume family. Doyle and Luckow also review some of the challenges of studying the evolution of this group, in which so many new lineages have been generated over relatively short periods of evolutionary time.

McKey, D. 1994. Legumes and nitrogen: The evolutionary ecology of a nitrogen-demanding lifestyle. In *Advances in legume systematics*. Part 5, *The nitrogen factor*. Edited by J. I. Sprent and D. McKey, 221–228. Kew, UK: Royal Botanic Gardens.

This book chapter describes the hypothesis that legume lifestyle requires a high nitrogen demand.

Parker, M. A., W. Malek, and I. M. Parker. 2006. Growth of an invasive legume is symbiont limited in newly occupied habitats. *Diversity and Distributions* 12:563–571.

This paper describes key experiments that examine the degree to which presence of rhizobial symbionts in the soil can limit the spread of legumes to new habitats. The authors’ experiments showed that colonizing legumes are often limited in their colonization if insufficient rhizobia are present.

Rodríguez-Echeverria, S. 2010. Rhizobial hitchhikers from Down Under: Invasional meltdown in a plant-bacteria mutualism? *Journal of Biogeography* 37:1611–1622.

This molecular survey of rhizobial symbionts reveals that invasive plants can sometimes import their native symbionts with them as the plants colonize novel landscapes.

Schulman, H. M., M. C. Lewis, E. M. Tipping, and L. M. Bordeleau. 1988. Nitrogen-fixation by three species of Leguminosae in the Canadian high arctic tundra. *Plant, Cell and Environment* 11:721–728.

This paper reports some of the northern extreme habitats of functioning legume-rhizobium symbioses.

Sprent, J. I. 2008. Evolution and diversity of legume symbiosis. In *Nitrogen-fixing leguminous symbioses*. Edited by M. J. Dilworth, E. J. James, J. I. Sprent, and W. E. Newton, 1–21. Dordrecht, The Netherlands: Springer.

This paper describes the great divergence of habits and ecology that can be found among legume lineages. The major focus is on how this diversity in growth forms is structured across the globe.

Stępkowski, T., L. Moulin, A. Krzyżańska, A. McInnes, I. J. Law, and J. Howieson. 2005. European origin of *Bradyrhizobium* populations infecting lupins and serradella in soils of Western Australia and South Africa. *Applied and Environmental Microbiology* 71:7041–7052.

This detailed phylogenetic analysis examines the phylogenetic origins of rhizobial symbionts in Australian and South African plants and supports a hypothesis of European origins.

Wolfe, J. A. 1975. Some aspects of plant geography of northern hemisphere during late Cretaceous and Tertiary. *Annals of the Missouri Botanical Garden* 62:264–279.

This paper reviews the historical habitats and spatial spread of the legume lineage.

Evolutionary History of Rhizobia

Rhizobia are defined by their ability to infect legumes, yet studies of multiple rhizobial lineages, such as Sachs, et al. 2010, have revealed that much diversity within these groups is represented by strains that do not exhibit the ability to infect legumes. Typically, symbiotic rhizobia exhibit a life cycle in which they live free in the soil until they infect a legume, subsequently proliferate within host cells, and ultimately can return to the soil when the host nodules senesce. Phylogenetic analysis presented in Sawada, et al. 2003 based on 16S rDNA has revealed at least nine monophyletic lineages of rhizobial bacteria, which are mostly distributed across the alpha- and betaproteobacteria. Research has generally focused on the agronomically useful lineages of rhizobia or those that form symbiosis with model plant study species (*Glycine max*, *Medicago truncatula*, *Lotus japonicus*). These rhizobial lineages include *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* (*Ensifer*). Key rhizobial genes that encode the symbiotic interaction with legumes include *nod*, *nif*, and *fix* genes. *Nod* genes typically identify loci involved in recognition signaling between host and symbiont, host specificity, and initiation of infection. *Nod* genes also function in maintenance of a successful infection, but these functions are less well understood. Both *nif* and *fix* genes are required for nitrogen fixation after infection of a host. *Nif* genes are homologous to one of twenty fixation genes identified in the diazotrophic model bacterium *Klebsiella pneumonia*, but Arnold, et al. 1988 shows through sequence analysis that *fix* genes have no such homologue. Symbiosis-specific loci are generally clustered within rhizobial genomes, either as part of the bacterial chromosome or on plasmids. For instance, the genomes of

Bradyrhizobium japonicum and *Mesorhizobium loti* have all of their *nif* and *nod* genes clustered in genomic islands that mostly encode symbiotic functions (symbiosis islands). As demonstrated in Sullivan, et al. 1995, these islands of symbiosis-specific loci transfer rapidly among unrelated strains of rhizobia. Davila, et al. 2005 shows that *Rhizobium* species, on the other hand, vary in genome architecture but generally have several large plasmids with symbiosis loci. Analysis presented in Finan, et al. 2001 shows that *Sinorhizobium meliloti* has a smaller chromosome with two megaplasmids of 1.3 and 1.6 Mb, one of which contains most of the required symbiosis loci. Such variation in genomic architecture among a homologous set of symbiosis loci is consistent with a complex evolutionary history of horizontal transfer of these genes between rhizobial lineages (Young and Haukka 1996). Although molecular evidence suggests that horizontal transfer of symbiosis loci is evolutionarily rare among distantly related rhizobia, Bailly, et al. 2007 and other studies have revealed that it can be quite common within lineages.

Arnold, W., A. Rump, W. Klipp, U. B. Priefer, and A. Pühler. 1988. Nucleotide sequence of a 24,206-base-pair DNA fragment carrying the entire nitrogen fixation gene cluster of *Klebsiella pneumoniae*. *Journal of Molecular Biology* 203:715–738.

This early DNA sequence analysis characterized a key cassette of genes that are necessary for biological nitrogen fixation.

Bailly, X., I. Olivieri, B. Brunel, J.-C. Cleyet-Marel, and G. Béna. 2007. Horizontal gene transfer and homologous recombination drive the evolution of the nitrogen-fixing symbionts of *Medicago* species. *Journal of Bacteriology* 189:5223–5236.

Bailly and colleagues investigated horizontal gene transfer and recombination among rhizobial strains and revealed extensive transfer of nodulation loci among rhizobia that infect *Medicago*.

Dávila, G., V. González, M. A. Ramírez-Romero, and O. Rodríguez. 2005. *Rhizobium etli* genome biology. In *Genomes and genomics of nitrogen-fixing organisms*. Edited by R. Palacios and W. E. Newton, 133–141. Dordrecht, The Netherlands: Springer.

This book chapter describes the genomic architecture of *Rhizobium etli* and focuses on the taxonomy, evolution, and plasticity of this genome.

Finan, T. M., S. Weidner, K. Wong, et al. 2001. The complete sequence of the 1,683-kb pSymB megaplasmid from the N_2-fixing endosymbiont *Sinorhizobium meliloti*. *Proceedings of the National Academy of Sciences of the United States of America* 98:9889–9894.

This paper describes genomic sequence and important genetic characteristics of the symbiotic megaplasmid in the *Sinorhizobium meliloti* genome.

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

This paper describes a phylogenetic reconstruction of a natural population of *Bradyrhizobium* that reveals the wholesale evolutionary gain and loss of symbiosis loci. The phenotypic and phylogenetic analysis suggests that a broad diversity of rhizobia might have no capacity to form nodule symbioses with legumes. These lineages apparently are lacking in all the key symbiosis-related genes.

Sawada, H., L. D. Kuykendall, and J. M. Young. 2003. Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts. *Journal of Applied Microbiology* 49:155–179.

This review introduces a broad phylogenetic perspective of the different rhizobial lineages, which includes many independent evolutionary origins of symbiotic capacity.

Sullivan, J. T., H. N. Patrick, W. L. Lowther, D. B. Scott, and C. W. Ronson. 1995. Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. *Proceedings of the National Academy of Sciences of the United States of America* 92:8985–8989.

Molecular analysis in this paper showed that a clustered set of symbiosis-specific loci can be transmitted horizontally from one rhizobial genotype to many unrelated strains. This paper shows the incredible potential for a large subset of mobile genes to very rapidly spread through a landscape.

Young, J. P. W., and K. E. Haukka. 1996. Diversity and phylogeny of rhizobia. *New Phytologist* 133:87–94.

The seminal paper reviews the phylogenetics of rhizobial lineages and includes an examination of evidence for horizontal gene transfer of symbiosis loci among divergent rhizobia. This is one of the first papers to lay out the argument that diverse lineages of bacteria have evolved the ability to infect legumes via transfer of novel genes.

Evolutionary Origins of the Legume-Rhizobium Symbiosis

It is difficult to interpret a precise timeline for the origin of the interaction with rhizobia since root tissue does not fossilize well. According to Lavin, et al. 2005, the most recent common ancestor of legumes arose roughly 50–60 million years ago, providing an upper bound for the origin of the legume-rhizobium symbiosis. Nonetheless, Soltis, et al. 2000 points out that the ability to nodulate rhizobia also occurs in several nonlegumes within the order Fabales, and infers that the predisposition to form nodules likely predates the origins of legumes. Within the legume family, phylogenetic analyses in Doyle, et al. 1997 inferred two to three independent origins of the association with rhizobia, but poor phylogenetic resolution makes it difficult to rule out additional origins. Symbiotic capability is not evenly distributed across the legume family. Sprent 2001 points out that approximately 90 percent of legume species in the two largest and best-resolved subfamilies (Papilionoideae, Mimosoideae) form symbiosis with rhizobia, whereas the remaining legume lineages often lack the ability to form nodules. Finally, as suggested in Doyle and Luckow 2003, many nonnodulating legume species appear to be derived from ancestrally nodulating lineages, suggesting that symbiosis has also been lost in some species. Nodule type and mechanism of rhizobial infection are largely determined by the plant and vary greatly across legume lineages. As explained and analyzed in Mergaert, et al. 2006, the two dominant nodule types have been classified as determinate and indeterminate. Determinate nodules lack a continuous meristem, and thus are round and cease growth soon after their development is complete. Indeterminate nodules grow continuously throughout the functional association and consequently are elongated and sometimes branched. Some legumes, such as lupines, do not fit this categorization well and exhibit unique nodule growth and morphology. Host species also exhibit variation in terms of how rhizobia infect plant tissue. Some legumes are only infected through root hairs, whereas other species are infected through cracks and wounds, between epidermal cells, or through other means. Additionally, some legumes (such as *Neptunia*) can switch from root hair infection to crack infection in waterlogged conditions. Sprent, et al. 1989 suggests that the ancestral legume-rhizobium infection formed indeterminate nodules through the root crack infection mode. However, Doyle and Luckow 2003 shows that a more complex scenario of multiple origins of the rhizobial association after diversification of legume lineages may help explain some of the variation in mechanisms of infection and nodule type.

Doyle, J. J., J. L. Doyle, J. A. Ballenger, E. E. Dickson, T. Kajita, and H. Ohashi. 1997. A phylogeny of the chloroplast gene rbcL in the Leguminosae: Taxonomic correlations and insights into the evolution of nodulation. *American Journal of Botany* 84:541–554.

This paper outlines phylogenetic data about the diversification of the legume lineage, one of the most diversifying groups of plants on earth. A key part of the paper provides information about independent origins of nodulation within the legume lineage.

Doyle, J. J., and M. A. Luckow. 2003. The rest of the iceberg: Legume diversity and evolution in a phylogenetic context. *Plant Physiology* 131:900–910.

This paper provides an enlightening review on progress in the field of legume diversity and phylogenetics.

Lavin, M., P. S. Herendeen, and M. F. Wojciechowski. 2005. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. *Systematic Biology* 54:575–594.

Lavin and colleagues provide a detailed description of the early evolutionary history of legumes using a combination of fossil evidence and inference using molecular clock data.

Mergaert, P., T. Uchiumi, B. Alunni, et al. 2006. Eukaryotic control on bacterial cell cycle and differentiation in the *Rhizobium*-legume symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* 103:5230–5235.

This paper investigates the mechanistic bases of legume control over rhizobia in legumes with indeterminate nodules. In particular, it clarifies that hosts with determinate and indeterminate nodule types exhibit divergent mechanisms to control rhizobial differentiation.

Soltis, D. E., P. S. Soltis, M. W. Chase, et al. 2000. Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. *Botanical Journal of the Linnean Society* 133:381–461.

This seminal paper revealed that plant nodulation traits arose before the origins of legumes (since some closely related plants also exhibit a similar capacity for nodulation).

Sprent, J. I. 2001. *Nodulation in legumes*. Kew, UK: Royal Botanic Gardens.

This book offers an encyclopedic description of the ecology and evolution of legume nodulation. In particular, it shows the challenge in defining particular legume lineages as failing to exhibit nodulation capacity (or not) because detailed field surveys must be done to make sure that nodules are absent in multiple ecological contexts.

Sprent, J. I., J. M. Sutherland, and S. M. de Faria. 1989. Structure and function of root nodules from woody legumes. In *Advances in systematic botany*. Edited by C. H. Stirton and J. L. Zarucchi, 559–578. Saint Louis: Missouri Botanical Gardens.

This book chapter provides an excellent description of the basic biology of legume nodulation.

Mechanistic Bases of the Legume-Rhizobium Symbiosis

Research on rhizobia has defined a classic model of molecular cross talk that is necessary for nodulation and initiation of symbiotic nitrogen fixation by rhizobia. As shown in Perret, et al. 2000, legumes emit flavonoids (ketone-containing organic compounds) into the soil to attract rhizobia. Soil rhizobia receive this signal and then emit a nod factor in response, which is a complex oligosaccharide encoded by the *nod* genes (discussed in Evolutionary History of Rhizobia). Receipt of the nod factor in the legume root tip triggers root hairs to curl around the rhizobia, which then forms an infection thread that transports the rhizobia through the legume root. Upon reaching their destination within a root cell, rhizobia are encased in a plant-derived membrane known as a symbiosome. Within the symbiosome, rhizobia ultimately differentiate into a specialized cell type called bacteroids and fix atmospheric nitrogen for the plant in exchange for sugars. Yet this classical model is mostly based upon the detailed study of only a handful of model legumes. As described in this section, the full diversity of both partners makes it clear that no single model is sufficient to fully understand the legume-rhizobium mutualism. For instance, the legume signals that initiate the symbiosis are not simple. The flavonoids are not solely used for the attraction of rhizobia. They are also studied for their antioxidant properties and, more recently, in Cesco, et al. 2010 for their role in affecting nutrient availability in the near-root zone. Moreover, legumes can also release other signaling molecules. Hirsch 1999 presents the hypothesis that lectins, carbohydrate-binding proteins, are also emitted by the plant and may play a role in the specificity observed in the symbiosis. Downie and Walker 1999 shows that rhizobia emit nod factors that can induce root hairs to curl around the bacteria and can also drive other steps necessary for nodulation. The type of nod factor produced can correlate with host specificity, but imperfectly. Perret, et al. 2000 shows that some diverged rhizobial species produce different nod factors but infect the same plant, whereas other diverged species produce identical nod factors but nonetheless nodulate different plants. Moreover, nod factors also induce responses in nonlegume tissues, suggesting that they have other functions. Likewise, it is important to note that a nod-factor-independent mechanism occurs in some rhizobia. Giraud, et al. 2007 analyzes genomes of the symbiotic strains *Bradyrhiozbium* ORS278 and BTAi1 and reveals an absence of nod loci. Mutational analyses on these strains indicated that nodule formation occurs via an alternative pathway. Rhizobia also produce other signaling molecules that can modulate host specificity and infection. Spaink 2000 reviews the diversity of recognition factors that rhizobia can produce during initial infection stages, some of which include lipopolysaccharides, polysaccharides, and glucans. Interestingly, even the fundamental process of nitrogen fixation itself varies among rhizobial lineages, Waters, et al. 1998 demonstrates that in some instances alanine and not ammonia is excreted by soybean bacteroids.

Cesco, S., G. Neumann, N. Tomasi, R. Pinton, and L. Weisskopf. 2010. Release of plant-borne flavonoids into the rhizosphere and their role in plant nutrition. *Plant and Soil* 329:1–25.

This paper offers a clear and detailed description of the varied roles of plant-produced flavonoids.

Downie, J. A., and S. A. Walker. 1999. Plant responses to nodulation factors. *Current Opinion in Plant Biology* 2:483–489.

This paper offers an excellent review of rhizobial-produced nod factors and how plants respond to them.

Giraud, E., L. Moulin, D. Vallenet, et al. 2007. Legumes symbioses: Absence of *nod* genes in photosynthetic *Bradyrhizobia*. *Science* 316:1307–1312.

This genome sequencing analysis describes the full genome sequence of a photosynthetic *Bradyrhizobium* that lacks *nod* genes and must infect legumes via a different mechanism.

Hirsch, A. M. 1999. Role of lectins (and rhizobial exopolysaccharides) in legume nodulation. *Current Opinion in Plant Biology* 2:320–326.

In this key paper, Hirsch explains the varied and important roles of plant-produced lectins.

Perret, X., C. Staehelin, and W. J. Broughton. 2000. Molecular basis of symbiotic promiscuity. *Microbiology and Molecular Biology Reviews* 64:180–201.

This paper offers an excellent review on molecular interactions between legumes and rhizobia and explains that rhizobial specificity to hosts is not as common as initially thought.

Spaink, H. P. 2000. Root nodulation and infection factors produced by rhizobial bacteria. *Annual Review of Microbiology* 54:257–288.

This paper reviews and analyzes the diversity of secreted and cell-surface molecules that rhizobia use to interact with legume hosts.

Waters, J. K., B. L. Hughes II, L. C. Purcell, K. O. Gerhardt, T. P. Mawhinney, and D. W. Emerich. 1998. Alanine, not ammonia, is excreted from N_2-fixing soybean nodule bacteroids. *Proceedings of the National Academy of Sciences of the United States of America* 95:12038–12042.

These authors studied variation in the molecular basis of nutrient exchange between legumes and rhizobia.

Mechanistic Variation in Host Control over Rhizobia

Key features of infection are thought to be completely host controlled. Sen and Weaver 1984 shows that in some cases identical rhizobial strains can form distinct nodule morphologies and exhibit divergent rates of nitrogen fixation when infecting different host species. Experiments presented in Mergaert, et al. 2006 showed that while legumes that form determinate nodules allow nitrogen-fixing rhizobial cells to escape back into the soil after senescence, hosts with indeterminate nodules can force the rhizobial bacteroids to terminally differentiate such that these cells cannot escape. Nonetheless, Paau, et al. 1980 suggests that undifferentiated rhizobia are invariably released upon senescence. Nodule senescence typically begins to occur during flowering or at the end of the growing season, but Gogorcena, et al. 1997 shows that senescence can be induced by plant stressors such as prolonged darkness, whereas González, et al. 1998 shows that it could be induced by water deficiency, and Banba, et al. 2001 demonstrates that it could be caused by failure of bacterial recognition by the plant. Kuykendall 1989 argues that, regardless of the process by which viable rhizobia are reintroduced into the environment during senescence, nodulation itself must aid persistence of nodulating strains in the soil or else the genes that encode these traits would be rapidly lost from rhizobial populations. Host-driven sources of variation such as nodule type and the fate and life cycle of rhizobia released into the soil have received scant attention despite the fact that these traits could shape host-symbiont coevolution, as explained in Denison 2000. The degree to which different legume hosts can control the fate of rhizobial symbionts remains a key problem for future research.

Banba, M., A.-B. M. Siddique, H. Kouchi, K. Izui, and S. Hata. 2001. *Lotus japonicus* forms early senescent root nodules with *Rhizobium etli*. *Molecular Plant-Microbe Interactions* 14:173–180.

Banba and colleagues showed that infections can sometimes occur between rhizobia and hosts that do not match but, in this case, that the nodules rapidly senesce soon after formation.

Denison, R. F. 2000. Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *American Naturalist* 156:567–576.

The lucid discussion offered in this much-cited review paper explains how divergent lineages of legumes might interact with their rhizobial symbionts in fundamentally different ways. Specifically, Denison describes how the type of development a nodule undergoes (e.g., determinate versus indeterminate) might affect legume-rhizobium coevolution.

Gogorcena, Y., A. J. Gordon, P. R. Escuredo, et al. 1997. N_2 fixation, carbon metabolism, and oxidative damage in nodules of dark-stressed common bean plants. *Plant Physiology* 113:1193–1201.

This fascinating experiment places plants in the dark to track the mechanisms of nitrogen-fixation shutdown and the ultimate senescence of the symbiotic nodules.

González, E. M., P. M. Aparicio-Tejo, A. J. Gordon, F. R. Minchin, M. Royuela, and C. Arrese-Igor. 1998. Water-deficit effects on carbon and nitrogen metabolism of pea nodules. *Journal of Experimental Botany* 49:1705–1714.

These authors investigated the importance of a legume's water budget in terms of carbon and nitrogen metabolism in symbiotic nodules.

Kuykendall, L. D. 1989. Influence of *Glycine max* nodulation on the persistence in soil of a genetically marked *Bradyrhizobium japonicum* strain. *Plant and Soil* 116:275–277.

This paper describes a seminal experiment that revealed the marked positive effect that nodulation has on the population density of a *Bradyrhizobium* strain.

Mergaert, P., T. Uchiumi, B. Alunni, et al. 2006. Eukaryotic control on bacterial cell cycle and differentiation in the *Rhizobium*-legume symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* 103:5230–5235.

Mergaert and colleagues investigated the mechanistic basis of host control over rhizobial differentiation in legumes with indeterminate nodules.

Paau, A. S., C. B. Bloch, and W. J. Brill. 1980. Developmental fate of *Rhizobium meliloti* bacteroids in alfalfa nodules. *Journal of Bacteriology* 143:1480–1490.

This paper describes the developmental fate and release of rhizobia within legume nodules.

Sen, D., and R. W. Weaver. 1984. A basis for different rates of N_2-fixation by the same strains of *Rhizobium* in peanut and cowpea root nodules. *Plant Science Letters* 34:239–246.

These authors investigated host-driven differences in symbiotic nitrogen fixation by infecting the same rhizobial strain into different plant species.

The Evolution and Ecology of Rhizobial Cooperation

In symbiotic interactions it is important to resolve both how and why symbionts cooperate with hosts. A previous section of this article, Mechanistic Bases of the Legume-Rhizobium Symbiosis, addresses how rhizobia provide benefits to their hosts by examining the molecular and physiological means by which the symbiosis is initiated and how resources are exchanged between species. To address why rhizobia benefit legumes, one must investigate the selective forces that favor cooperative strains over less beneficial mutants and also the varied ecological conditions that can modulate the net benefits that plants reap from rhizobial infection. Trainer and Charles 2006 shows that nitrogen fixation is costly for rhizobia. Models of mutualism (interspecific cooperation) reviewed in Sachs, et al. 2004 predict that when a symbiont provides a costly service to hosts, selection can strongly favor mutants that exhibit reduced (or zero) benefit. Given the potential benefits of exploiting hosts by failing to fix nitrogen, a key challenge in the field has been to resolve the specific forces that prevent such strains from taking over rhizobial populations, thus causing the symbiotic interaction to break down. Two evolutionary theories of mutualism have been used to model the maintenance of cooperative traits in symbionts: partner fidelity feedback and partner choice/sanctions. However, a limitation in both of these model types is the lack of ecological context. Given that rhizobia provide a nutrient that plants can also get from the soil (nitrogen), variation in ecological factors that affect soil nitrogen can have an important impact on the net benefits that plants receive from the symbiosis. The sections Rhizobial Cooperation in Agricultural Landscapes: Fertilization versus Inoculation and Legume Growth Optimization in Agriculture discuss this challenge, in particular in the context of agricultural settings, in which soil conditions vary in dramatic ways.

Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull. 2004. The evolution of cooperation. *Quarterly Review of Biology* 79:135–160.

This paper reviews theory for the evolution of cooperation between species and makes predictions about the evolution of cheaters. The different models of cooperation that are highlighted here clarify how and why some symbiotic interactions might be more ecologically and evolutionarily stable than others.

Trainer, M. A., and T. C. Charles. 2006. The role of PHB metabolism in the symbiosis of rhizobia with legumes. *Applied Microbiology and Biotechnology* 71:377–386.

These authors conducted a key investigation into rhizobial metabolism during symbiosis with legumes. The focus is on the carbon storage molecule polybetahydroxybutyrate (PHB), which is often produced and stored by rhizobia during the symbiosis with legumes.

PARTNER FIDELITY FEEDBACK

Partner fidelity feedback can be defined as positive fitness correlations between interacting partners. For instance, as described in Simms and Taylor 2002, fitness benefits delivered from symbionts to hosts can feed back automatically and provide returned benefits to the cooperative symbionts. Under this scenario, beneficial rhizobia experience significant fitness rewards as an automatic product of the vigorous plant growth that symbiotic nitrogen fixation enables. Conversely, nonfixing rhizobia experience reduced fitness as a direct effect of their host having fewer resources to invest in the symbiosis. Yet Simms and Taylor 2002 also points out that such fitness feedbacks can only maintain cooperation under restrictive conditions, in particular when individual symbiont and host genotypes interact repeatedly over time. As noted in the section Introductory Works, Sprent, et al. 1987 describes how legumes encounter rhizobia anew each generation from the soil. Moreover, Dowling and Broughton 1986 explains that individual host plants become infected by multiple rhizobial genotypes. Both these points clarify that the legume-rhizobium mutualism does not fit the conditions required for partner fidelity feedback to maintain rhizobial cooperation.

Dowling, D. N., and W. J. Broughton. 1986. Competition for nodulation of legumes. *Annual Review of Microbiology* 40:131–157.

This paper first explained that legume plants most often become infected by multiple rhizobial genotypes. This aspect is key because it reveals that legumes must deal with variation in rhizobial quality and competitiveness.

Simms, E. L., and D. L. Taylor. 2002. Partner choice in nitrogen-fixation mutualisms of legumes and rhizobia. *Integrative and Comparative Biology* 42:369–380.

This theory paper describes the partner fidelity and partner choice models of cooperation in relation to the legume-rhizobium symbiosis. In particular, these authors show that partner fidelity models of cooperation are not likely to be useful in making predictions about the legume-rhizobium symbiosis.

Sprent, J. I., J. M. Sutherland, and S. M. de Faria. 1987. Some aspects of the biology of nitrogen-fixing organisms. *Philosophical Transactions of the Royal Society of London, B: Biological Sciences* 317:111–129.

Sprent and colleagues provide an excellent review of the basic biology of the nitrogen-fixing rhizobia. This paper points out the key fact that legumes must encounter rhizobia anew each generation from the soil.

PARTNER CHOICE AND SANCTIONS

Partner choice and sanctions models, explained in Denison 2000; West, et al. 2002; and Sachs, et al. 2004, predict that hosts preferentially reward beneficial symbiont genotypes and/or punish strains that provide reduced benefits. As pointed out in Heath and Tiffin 2009, a distinction is often made in which partner choice refers to host selection for cooperative strains at the point of infection, whereas sanctions refer to host selection that occurs after nodule formation. Interestingly, legumes do not appear to detect differences in symbiotic effectiveness at the point of nodule initiation. Hahn and Studer 1986 shows that when hosts are coinfected with different strains, they cannot differentiate between symbiotically effective rhizobia and isogenic strains that lack nitrogen-fixation function. Only a few experiments have shown any infection bias for cooperative rhizobial genotypes. For instance, both Heath and Tiffin 2009 and Sachs, et al. 2010 present experiments that created coinfections of genetically divergent rhizobial strains from natural landscapes. These data suggest that hosts can only detect and react to divergent rhizobial strains during nodule initiation, which is more consistent with host-symbiont specificity rather than efficient partner choice. In contrast to the lack of evidence for partner choice, sanctions models are supported by a wealth of empirical evidence. A common experimental test of legume sanctions has been to coinfect legumes with rhizobia that vary in symbiotic quality. Most often, these experiments reveal that legumes can bias their resource investment into nodules with cooperative strains. Kiers, et al. 2003 presents the first critical experiment to show that legume hosts can directly sanction nonfixing rhizobia. Specifically, Kiers, et al. 2003 shows that nodules with cooperative rhizobia grow (and the rhizobia within them proliferate rapidly), whereas nodules with nonfixing rhizobia tend to stay small (and the rhizobia within them exhibit smaller population sizes). However, West, et al. 2002 argues that such control over ineffective rhizobial strains will only be efficient when individual nodules each carry single strains of rhizobia. Empirical work presented in Gage 2002 has shown that coinfected nodules can easily be generated by inoculating plants with high concentrations of two rhizobial genotypes, but Gage’s work suggests that variation in bacterial growth will make coinfection unlikely. While theory predicts that sanctions should break down if individual nodules contain more than one rhizobial genotype, experiments discussed in Sachs, et al. 2010 have found effective sanctions even when many nodules were co-infected. Currently, research is needed to investigate the mechanistic basis of sanctions. Data from Kiers, et al. 2003, whose authors measured oxygenation rate within fixing and nonfixing nodules, suggest that hosts might control oxygen flux into nodules and thus sanction uncooperative rhizobia by starving them for oxygen, but more work is needed to test this hypothesis.

Denison, R. F. 2000. Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *American Naturalist* 156:567–576.

This key review was the first to lay out the sanctions hypotheses for legume control over exploitative rhizobia.

Gage, D. J. 2002. Analysis of infection thread development using Gfp- and DsRed-expressing *Sinorhizobium meliloti*. *Journal of Bacteriology* 184:7042–7046.

This paper offers a dramatic demonstration of how multiple genotypes of rhizobia can coinfect a single nodule. Gage includes a model of bacterial growth within coinfections threads that suggests bacterial growth patterns make such coinfections unlikely in nature.

Hahn, M., and D. Studer. 1986. Competitiveness of a *nif-Bradyrhizobium japonicum* mutant against the wild-type strain. *FEMS Microbiology Letters* 33:143–148.

This seminal paper offered one of the first and most important experiments on how legume-rhizobium interactions are altered by nonfixing rhizobial mutants. Wild-type rhizobia were coinfected into plants with genetically similar nonfixing mutants, showing that legumes are apparently lacking in a mechanism to detect nitrogen-fixation ability at the point of infection.

Heath, K. D., and P. Tiffin. 2009. Stabilizing mechanisms in a legume-rhizobium mutualism. *Evolution* 63:652–662.

These authors examined legume control over rhizobial cooperation by using cross-inoculation among natural populations of hosts.

Specifically, legume control over infection was analyzed at the initial infection stage, in which the plant can potentially regulate infection by more or less beneficial partners, and postinfection, in which the plant can potentially control resource exchange with symbiotic partners that vary in quality.

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

This important experiment first demonstrated that legume sanctions can efficiently control nonfixing rhizobial strains by inhibiting rhizobial cell growth within individual nodules.

Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull. 2004. The evolution of cooperation. *Quarterly Review of Biology* 79:135–160.

This review paper explains how sanctions models relate to other types of host control over symbionts.

Sachs, J. L., J. E. Russell, Y. E. Lii, K. C. Black, G. Lopez, and A. S. Patil. 2010. Host control over infection and proliferation of a cheater symbiont. *Journal of Evolutionary Biology* 23:1919–1927.

This paper offers an experimental demonstration that legumes can efficiently control the infection and proliferation of a cheater rhizobium strain.

West, S. A., E. T. Kiers, E. L. Simms, and R. F. Denison. 2002. Sanctions and mutualism stability: Why do rhizobia fix nitrogen? *Proceedings of the Royal Society of London, B* 269:685–694.

This key theory paper presents a detailed mathematical model of legumes sanctions.

Rhizobial Cooperation in Natural Landscapes

Sanctions mechanisms appear efficient, with few exceptions. For instance, unlike most experimental work on this topic, Heath and Tiffin 2009 found no evidence that legumes could control poorly fixing rhizobia after nodulation. Nonetheless, modeling of sanctions leads West, et al. 2002 to predict that nonfixing strains should be purified from rhizobial populations. But as discussed in Burdon, et al. 1999, nonfixing and poorly fixing rhizobia are commonly uncovered. Sachs, et al. 2010a points out that such “ineffective” strains are most frequently isolated from agricultural and pastoral sites and to a lesser degree in natural landscapes. Only a few ineffective rhizobia from wild plants have been studied in detail. Interestingly, these wild strains, such as those discussed in Sachs, et al. 2010b, have been shown to be rare genotypes within their population, which suggests that cheaters are not very successful. Burdon, et al. 1999 argues that ineffective strains are mismatched with their cultivated hosts, as opposed to exploiting them. This argument makes sense, since rhizobia often fail to fix nitrogen when they encounter atypical hosts. For instance, when rhizobia isolated from one host species are inoculated onto different legumes, invariably only a subset of such cross-inoculated strains can nodulate the novel host, and only a smaller subset efficiently fix nitrogen on the novel host. This pattern suggests that many rhizobia that have been identified as ineffective (on one host) might be highly effective if inoculated onto a more suitable host. Host-symbiont mismatches might be widespread in agricultural settings where plants, bacteria, and even soils are transported among sites, whereas such mismatches might be rare or absent in natural landscapes, where hosts and symbionts have coevolved. One hypothesis that has been posed in many studies including Burdon, et al. 1999; Sachs, et al. 2010 (cited under Partner Choice and Sanctions); and Friesen 2012 is that the appearance of rhizobial exploitation (e.g., ineffective infections) is most often is the result of mismatched host-symbiont pairs. Several sources of data are consistent with this hypothesis. Burdon, et al. 1999 uncovered significant variance among host-symbiont combinations in their ability to form an effective (nitrogen-fixing) symbiosis. Nonetheless, a few of the tested rhizobial strains were ineffective on almost all hosts, suggesting the possibility that these genotypes are exploiting hosts as opposed to being mismatched with them. Sachs, et al. 2010a presents the results of single-strain inoculation tests that showed that nonfixing strains could exhibit a fitness advantage within host tissue, again suggesting that some rhizobia can exploit hosts during infection. But these nonfixing genotypes were shown in Sachs, et al. 2010b to get sanctioned by hosts when the plant is infected by other, more beneficial rhizobia. More recently, Friesen 2012 found that rhizobial mutations that lowered host fitness also lead to poor performance by the infecting rhizobia. As a whole, it appears that rhizobia do have capacity to exploit hosts, but that most ineffective infections are caused by host-symbiont mismatch as opposed to the rhizobia taking advantage of the host.

Burdon, J. J., A. H. Gibson, S. D. Searle, M. J. Woods, and J. Brockwell. 1999. Variation in the effectiveness of symbiotic associations between native rhizobia and temperate Australian *Acacia*: Within-species interactions. *Journal of Applied Ecology* 36:398–408.

This critical set of experiments provided some of the earliest and best evidence of naturally occurring nonfixing rhizobial strains. Specifically, rhizobia collected from multiple plant populations were infected into seed sets from those same populations in a factorial design. This experiment revealed that the benefit that any rhizobial strain gives to a host can depend on host genotype, but also that some rhizobia are apparently nonbeneficial on almost all plant hosts.

Friesen, M. L. 2012. Widespread fitness alignment in the legume-rhizobium symbiosis. *New Phytologist* 194:1096–1111.

This elegant meta-analysis investigated the correlation between rhizobial and legume fitness based on mutations in rhizobia that affect symbiotic quality, as well as variation among wild strains. The key result was that most rhizobia that fail to provide substantial benefit to hosts also suffer from degraded fitness.

Heath, K. D., and P. Tiffin. 2009. Stabilizing mechanisms in a legume-rhizobium mutualism. *Evolution* 63:652–662.

This paper tested the dominant hypotheses about how host legumes control infection by substandard rhizobia. The authors found evidence that legume sanctions can be relatively inefficient in some populations.

Sachs, J. L., M. O. Ehinger, and E. L. Simms. 2010a. Origins of cheating and loss of symbiosis in wild *Bradyrhizobium*. *Journal of Evolutionary Biology* 23:1075–1089.

This paper provided evidence of nonfixing rhizobial strains in natural landscapes. Sachs and colleagues found that a nonfixing strain exhibited superior fitness to fixing strains in single-strain inoculations. They also pointed out that nonfixing rhizobia have been most often isolated from agricultural landscapes.

Sachs, J. L., J. E. Russell, Y. E. Lii, K. C. Black, G. Lopez, and A. S. Patil. 2010b. Host control over infection and proliferation of a cheater symbiont. *Journal of Evolutionary Biology* 23:1919–1927.

This paper showed how a naturally occurring nonfixing rhizobial strain can be sanctioned by the host plant, as long as the host has access to other, more beneficial rhizobia.

West, S. A., E. T. Kiers, E. L. Simms, and R. F. Denison. 2002. Sanctions and mutualism stability: Why do rhizobia fix nitrogen? *Proceedings of the Royal Society of London, B* 269:685–694.

This key theory paper provided detailed models that predict conditions in which beneficial versus nonfixing rhizobia will spread in populations.

Rhizobial Cooperation in Agricultural Landscapes: Fertilization versus Inoculation

Chemical nitrogen fertilization can substantially improve legume growth. But rhizobial inoculation represents an economical and environmentally friendly alternative. An emerging paradigm, spearheaded in Kiers, et al. 2002 and Denison, et al. 2003, suggests that agronomists must factor in natural selection on both crop plants and their symbionts to optimize crop production. Thus, the benefits and costs of each fertilization approach must be considered with this “Darwinian” perspective in mind. As analyzed in detail in Tilman 1999, industrial nitrogen fixation is costly both in terms of the fossil fuel needed to fix nitrogen and in terms of the widespread environmental damage caused by nitrogen runoff. Moreover, nitrogen fertilization is predicted to have negative effects on the legume-rhizobium symbiosis in terms of both the ecology and the evolution of the interaction. In terms of ecology, studies in Vargas, et al. 2000 have shown a reduction in the effectiveness of rhizobial infection under some nitrogen fertilization regimes, as nitrogen fertilization reduces the plant’s need for symbiotically derived nitrogen. In terms of evolution, nitrogen fertilization might relax selection on the host to maintain the relationship with rhizobia. Experiments published in Kiers, et al. 2007 have suggested that agricultural soybean cultivars—which experience repeated cultivation in high nitrogen soils—rapidly degrade in their ability to sanction ineffective rhizobia. These studies suggest that nitrogen fertilizers could lead to a breakdown in the beneficial interaction over time. Rhizobial inoculation of legumes also comes with serious challenges. Triplett and Sadowsky 1992 explains that attempts to employ rhizobial inocula in agricultural settings have not been successful, likely because natural selection among rhizobia has been largely ignored in these attempts. Successful rhizobial inoculation must overcome several hurdles. Santos, et al. 1999 explains that when rhizobia are applied in agricultural settings, the inoculum strains must be able to thrive in soil under the varied conditions of the field. Yet, as explained in Atkins 1984, even if soil ecological conditions are ideal, the inocula must still be able to compete with indigenous rhizobia for nodulation, efficiently escape from senescent nodules, and persist in the soil in order to infect the next season of cultivated hosts. Since inocula are rarely adapted to field soils or agricultural cultivars, they are frequently outcompeted by indigenous rhizobia for infection of legume crops. Triplett and Sadowsky 1992 calls this failure in nodulation success the “rhizobial competition problem.” This problem is particularly harmful since indigenous rhizobia invariably fix nitrogen at mediocre levels compared to strains that have been selected as inocula. Moreover, Kiers, et al. 2002 explains that indigenous rhizobial populations can evolve and improve their ability to compete for infection with each crop season, whereas rhizobial inocula are used year after year from the same cloned stocks. Although, Vargas, et al. 2000 shows that rhizobial inoculation can sometimes cause a short-term benefit for legume growth, this effect often disappears in subsequent years as the legumes tend to increase population sizes of the indigenous rhizobia rather than the inoculated strains. As is discussed in the section Legume Growth Optimization in Agriculture, much more work needs to be done in order to optimize rhizobial inoculants for the varied conditions of agricultural fields. Nonetheless, there is promise that the use of rhizobial inocula will become more efficient and displace nitrogen fertilization on some crops.

Atkins, C. A. 1984. Efficiencies and inefficiencies in the legume/Rhizobium symbiosis: A review. *Plant and Soil* 82:273–284.

This paper provides a detailed account of the various sources of inefficiencies in the legume-rhizobium symbiosis.

Denison, R. F., E. T. Kiers, and S. A. West. 2003. Darwinian agriculture: When can humans find solutions beyond the reach of natural selection? *Quarterly Review of Biology* 78:145–168.

This paper introduces the idea of Darwinian agriculture, a set of practices that uses the theory of natural selection to optimize agricultural production.

Kiers, E. T., M. G. Hutton, and R. F. Denison. 2007. Human selection and the relaxation of legume defences against ineffective rhizobia. *Proceedings of the Royal Society B: Biological Sciences* 274:3119–3126.

This paper showed evidence of how legumes can evolve rapidly and negatively in an agricultural setting. The key theory tested is that constant exposure to nitrogen fertilizer causes mechanisms of legume sanctions to degrade over evolutionary time.

Kiers, E. T., S. A. West, and R. F. Denison. 2002. Mediating mutualisms: Farm management practices and evolutionary changes in symbiont co-operation. *Journal of Applied Ecology* 39:745–754.

This key review laid out a framework of how human practices can alter efficiency of crop production, including legume-rhizobium systems.

Santos, M. A., M. A. T. Vargas, and M. Hungria. 1999. Characterization of soybean *Bradyrhizobium* strains adapted to the Brazilian savannas. *FEMS Microbiology Ecology* 30:261–272.

This paper outlines the varied challenges of using rhizobial inoculants.

Tilman, D. 1999. Global environmental impacts of agricultural expansion: The need for sustainable and efficient practices. *Proceedings of the National Academy of Sciences of the United States of America* 96:5995–6000.

This paper analyzes the relationship between human practices in agriculture and downstream pollution.

Triplett, E. W., and M. J. Sadowsky. 1992. Genetics of competition for nodulation of legumes. *Annual Review of Microbiology* 46:399–422.

This important paper outlined mechanisms of competition among rhizobia for legume infection and explained how this competition can work against inoculation programs.

Vargas, M. A. T., I. C. Mendes, and M. Hungria. 2000. Response of field-grown bean (*Phaseolus vulgaris* L.) to *Rhizobium* inoculation and nitrogen fertilization in two Cerrados soils. *Biology and Fertility of Soils* 32:228–233.

Vargas and colleagues examined short-term effects of fertilization and rhizobial inoculation on bean production. A key focus of the authors is to optimize the response of bean crops in a habitat-specific way. This approach is useful since specific inocula might often vary in their success depending on soil ecology.

Legume Growth Optimization in Agriculture

To solve the rhizobial competition problem, researchers must better understand the ecological and genetic determinants of rhizobial competitiveness and resolve why inocula nodulate hosts at such a low rate compared to native rhizobia. For instance, competition implies ecological interaction between competing strains, which might also be affected by the nutrient content of soils. Bhuvaneswari, et al. 1980 and Graham 2007 suggest that the timing of the infection and the proximity of a strain to the rhizosphere can also affect the odds of a strain nodulating a legume and hence increase competiveness. Moreover, the genomic architecture of competing indigenous rhizobia might modulate competition for nodulation. Due to the clustering of symbiotic genes on plasmids or in genomic islands, horizontal gene transfer can result in the transfer of symbiosis genes from indigenous rhizobia to inoculum and vice versa. These hybrid strains thus represent new competition for the inoculum that in some cases can completely take over symbiont populations, as shown in Sullivan, et al. 1995. Moreover, as shown in Nandasena, et al. 2007, horizontal transfer of symbiosis island loci is linked with the evolution of nonbeneficial or poorly beneficial rhizobia under agricultural conditions. Currently, strain selection programs are trying to address these problems. Programs like EMBRAPA in Brazil evaluate isolates for a variety of competitive qualities and have attained some success in terms of choosing habitat-specific inocula. Both Santos, et al. 1999 and Mostasso, et al. 2002 describe some relative successes in these strain-selection programs. These programs demonstrate that successful inoculation programs must consider the specific ecological conditions of any particular soil habitat before choosing rhizobial strains to use in those settings. Similar programs exist in the United States, Australia, and elsewhere and have also made some progress in studying inoculum success. Denison, et al. 2003 writes that the new field of Darwinian agriculture seeks to apply evolutionary theory toward optimizing agricultural development and sustainability. In the legume-rhizobium symbiosis, we must consider practices that can optimize both plant and rhizobial inoculum success. Support for the success of Darwinian agriculture can be found in improving crop yield as a result of genetic modifications in rhizobial nodulation factors. Denison, et al. 2003 highlights the important point that natural selection does not exist to serve human interests in agriculture. For instance, when humans value specific traits, such as enzymes effectiveness, competitiveness, stress tolerance, and broad-spectrum insect defenses, that coincide with natural selection, we cannot further improve upon those traits. However, when humans value traits that natural selection has not necessarily optimized, improvements by human management can be made.

Bhuvaneswari, T. V., B. G. Turgeon, and W. D. Bauer. 1980. Early events in the infection of soybean (*Glycine max* L. Merr) by *Rhizobium japonicum*: 1. Localization of infectible root-cells. *Plant Physiology* 66:1027–1031.

This paper shows how rhizobial infection of legumes' roots can be highly localized to an area near the growing root tip.

Denison, R. F., E. T. Kiers, and S. A. West. 2003. Darwinian agriculture: When can humans find solutions beyond the reach of natural selection? *Quarterly Review of Biology* 78:145–168.

This paper introduced the Darwinian perspective on how to maximize legume productivity in symbiosis.

Graham, P. H. 2007. Ecology of the root-nodule bacteria of legumes. In *Nitrogen-fixing leguminous symbioses*. Edited by M. J. Dilworth, E. K. James, J. I. Sprent, and W. E. Newton, 23–58. Dordrecht, The Netherlands: Springer.

This book chapter explains the importance of early events during bacterial-plant contact in terms of successful rhizobial infection.

Mostasso, L., F. L. Mostasso, B. G. Dias, M. A. T. Vargas, and M. Hungria. 2002. Selection of bean (*Phaseolus vulgaris* L.) rhizobial strains for the Brazilian Cerrados. *Field Crops Research* 73:121–132.

This paper describes some relatively successful efforts to optimize the legume-rhizobium symbiosis under agriculture in the *cerrado* regions of South America.

Nandasena, K. G., G. W. O'Hara, R. P. Tiwari, E. Sezmiş, and J. G. Howieson. 2007. *In situ* lateral transfer of symbiosis islands results in rapid evolution of diverse competitive strains of mesorhizobia suboptimal in symbiotic nitrogen fixation on the pasture legume *Biserrula pelecinus* L. *Environmental Microbiology* 9:2496–2511.

This is a seminal paper showing the effects of horizontal gene transfer of symbiosis loci under real agricultural conditions. The main conclusion is that horizontal transfer is common and tends to favor the evolution of symbiotically poor rhizobia.

Santos, M. A., M. A. T. Vargas, and M. Hungria. 1999. Characterization of soybean *Bradyrhizobium* strains adapted to the Brazilian savannas. *FEMS Microbiology Ecology* 30:261–272.

This paper by Santos and colleagues describes efforts to choose optimal rhizobial strains for agriculture based on efficiency of nitrogen fixation and competitive ability with other rhizobia in the soil.

Sullivan, J. T., H. N. Patrick, W. L. Lowther, D. B. Scott, and C. W. Ronson. 1995. Nodulating strains of *Rhizobium loti* arise through

chromosomal symbiotic gene transfer in the environment. *Proceedings of the National Academy of Sciences of the United States of America* 92:8985–8989.

Molecular analysis in this paper discovered that a cassette of symbiosis genes from a single rhizobial inoculum strain swept through a diverse population of native soil rhizobia that could not previously infect hosts.

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