

MiniReview

Lifestyle alternatives for rhizobia: mutualism, parasitism, and forgoing symbiosis

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Abstract

Strains of rhizobia within a single species can have three different genetically determined strategies. Mutualistic rhizobia provide their legume hosts with nitrogen. Parasitic rhizobia infect legumes, but fix little or no nitrogen. Nonsymbiotic strains are unable to infect legumes at all. Why have rhizobium strains with one of these three strategies not displaced the others? A symbiotic (mutualistic or parasitic) rhizobium that succeeds in founding a nodule may produce many millions of descendants. The chances of success can be so low, however, that nonsymbiotic rhizobia can have greater reproductive success. Legume sanctions against nodules that fix little or no nitrogen favor more mutualistic strains, but parasitic strains that use plant resources only for their own reproduction may do well when they share nodules with mutualistic strains.

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1. Introduction

Rhizobia are soil bacteria best known for their symbiosis with legume plants. They are classified in various genera, including *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Sinorhizobium*. Rhizobia typically receive carbon fixed by the plant, while the plant receives nitrogen fixed by the rhizobia. Molecular aspects of infection of plant roots by rhizobia, and subsequent N₂ fixation by the bacteroid form of rhizobia inside root nodules, have received considerable attention. Our focus, however, is on why rhizobia that lack genes for infecting legumes are so common in the rhizosphere of some suitable host legumes [1]. Rhizobia that infect legumes but then fix little or no N₂ are also common in some soils [2]. Why have rhizobium strains that do fix N₂ in symbiosis not consistently

outcompeted and displaced those of the same species that do not?

Infecting legumes and fixing N₂ in symbiosis is not the only viable life-history strategy for rhizobia, as proved by the numerical abundance of strains with alternative strategies. The strategies of bacteria and plants, however sophisticated, are genetically programmed, rather than learned or chosen. Therefore, the strategy of an individual rhizobium changes only through mutation. For example, an *R. leguminosarum* bv. *phaseoli* strain with the genetic capacity to fix N₂ in symbiosis is still considered a symbiotic strain when it is between hosts, in contrast to nonsymbiotic *R. leguminosarum* bv. *phaseoli* strains, which are unable to infect legumes [3].

Rhizobia genetically programmed to infect legume plants but then to fix little or no N₂ (perhaps due to a mutation in a nitrogenase gene) are less familiar than mutualistic N₂ fixers, but these “ineffective” strains are widespread [2,4,5]. Different strains of rhizobia infecting

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the same legume species can vary 10-fold, at a given location, in the net benefits they provide their hosts [6], as measured by plant growth. Strains that fix only a small amount of N_2 , as opposed to none, might be considered either mutualistic or parasitic, depending on the relative value of carbon and nitrogen to a plant in particular circumstances.

Rhizobium strains that follow a third rhizobial strategy, forgoing symbiosis altogether, sometimes outnumber related symbiotic strains [1,3,7]. For example, *R. leguminosarum* bv. *phaseoli* lacking the key symbiotic gene *nifH* and unable to nodulate bean plants were more than 40 times as abundant as symbiotic members of their species in the rhizosphere of bean plants [3].

Why has not one of these three different rhizobial strategies displaced the others? All rhizobia spend some time in the soil, where they presumably compete for various resources. Symbiotic rhizobia, both mutualistic N_2 -fixers and parasitic nonfixers, compete for host plants in addition to soil resources. We might expect those rhizobium strains genetically programmed to fix N_2 in symbiosis to displace the nonfixing or nonsymbiotic strains. But N_2 fixation is an energetically expensive process, consuming resources that rhizobia could otherwise use for their own growth and reproduction. Perhaps, therefore, we should expect nonfixing or nonsymbiotic rhizobia to displace N_2 -fixing mutualistic strains. Instead, all three strategies persist, although their relative abundance varies among locations [1,5,7,8].

Does the present diversity of rhizobial strategies simply represent a balance between mutation and natural selection, with selection consistently favoring the one “best” strategy for a given location? Or does the relative fitness (survival and reproduction) of rhizobia following these three different strategies vary over time, allowing all three strategies to coexist?

Before attempting to answer these questions, we will consider the merits of all three strategies, from the viewpoint of a single rhizobium cell. A key question is whether a symbiotic, N_2 -fixing rhizobium that lost one or more symbiotic genes (due to mutation or loss of a symbiotic plasmid) would thereby increase or decrease the expected number of descendants it would have in the soil a year later.

2. Benefits and risks of different strategies for rhizobia

Participating in symbiosis by founding a nodule can dramatically enhance the reproductive success of rhizobia. A single rhizobium cell that infects a soybean root may produce up to 10^{10} descendants inside a large nodule [9,10]. Even when some rhizobia inside some nodules lose the ability to reproduce, especially in nodules with indeterminate growth (Fig. 1), it appears that every nodule contains large numbers of reproductively viable rhiz-

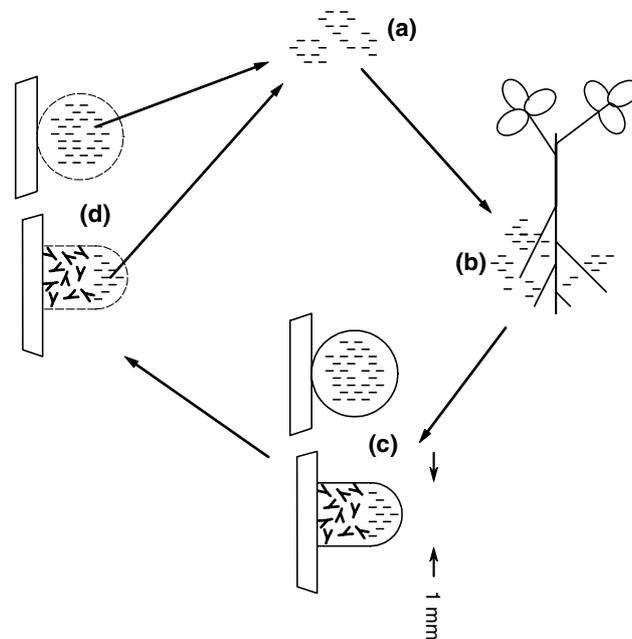


Fig. 1. Life cycle of symbiotic rhizobia: (a) rhizobia in the soil; (b) rhizobia competing for opportunities to nodulate a legume root; (c) determinate and indeterminate nodules, showing increased size and altered shape of bacteroids in indeterminate nodules; (d) senescing nodules with release of rhizobia (bacteroids from determinate nodules and undifferentiated cells from indeterminate nodules). Rhizobium numbers in nodules are much greater than shown, whereas their size is much less.

obia [11]. If even a fraction of the rhizobia inside a nodule successfully escape into the soil, a rhizobium could produce many more descendants in the soil by founding a nodule than by remaining in the soil.

Attempting to infect a legume root may carry significant risks for rhizobia, however. First, the chances of success may be quite low. A soil in which a compatible host was last grown five years before can still contain 2.5×10^4 rhizobia per g of soil [12]. The roughly 2×10^9 g ha⁻¹ of soil in the plow layer would then contain 5×10^{13} rhizobia. A soybean field with 4×10^5 plants, each forming 100 nodules, would offer only 4×10^7 opportunities to found a nodule. Thus, the chances that a given symbiotic rhizobium cell would successfully found a nodule would be about one in a million (10^{-6}). Furthermore, we suggest that aggregations of rhizobia around a root might attract high populations of predatory protozoa, which are a significant threat to rhizobia even in bulk soil [13]. In addition, many rhizobia produce various antibiotics active against other rhizobia [14–16] and exposure to these antibiotics could be greater for rhizobia in the throng attempting to infect a legume root than it would be for those that remain in the bulk soil.

So is infecting plants a good strategy for rhizobia? A 10^{-6} chance of producing 10^9 reproductively viable descendants seems like a good strategy, even under the

unlikely, worst-case scenario that all unsuccessful attempts to nodulate end with death due to predation or antibiotics. Spawning salmon would jump at those odds. But not all 10^9 rhizobia inside a nodule escape alive into the soil. In nodules with indeterminate growth, as in alfalfa, reproductive viability is apparently limited mainly to those rhizobia that have not differentiated into the bacteroid form [11]. Some rhizobia may be digested by the plant during nodule senescence [17]. Although there is very little field-credible data on the microbiology of nodule senescence [18], dense populations of rhizobia escaping from senescing nodules might also be especially attractive to predators.

Considering all the risks and potential benefits of symbiosis for rhizobia, the number of descendants produced by the average symbiotic rhizobium cell over the course of a year could be less than that of a nonsymbiotic rhizobium, at least when populations of competing symbiotic rhizobia are high. For simplicity, assume an annual legume, with one opportunity per year for rhizobia to nodulate. The number of descendants produced by a symbiotic rhizobium (averaging those near and far from the root) would be the chance that it will succeed in founding a nodule, times the number of rhizobia released from an average nodule, times the fraction of those rhizobia that become established in the soil after nodule senescence (Table 1). If this product is greater than 1.0, then the population of symbiotic rhizobia will increase, until increasing competition for opportunities to nodulate brings it down below 1.0. Depending on conditions, population size might be more likely to overshoot and oscillate than to approach the equilibrium value of 1.0. Meanwhile, a nonsymbiotic rhizobium that divided every two months would produce 64 descendants in a year, assuming none died. Actual generation times and survival rates for nonsymbiotic rhizobia in soil are not known. Root exudates would increase opportunities to reproduce in the rhizosphere, perhaps increasing the relative fitness of nonsymbiotic rhizobia. However, we assume that nonsymbiotic rhizobia would

also be subject to some sort of density dependent population regulation.

Under what conditions would losing symbiotic genes decrease the expected reproductive success of a rhizobium? When a compatible legume crop is first grown after a prolonged absence, symbiotic rhizobia would be less common. With fewer competitors, each individual would have a greater chance of founding a nodule, so symbiotic rhizobia would have greater average reproductive success than nonsymbiotic strains. As the population of symbiotic rhizobia increases, however, competition for hosts would decrease the reproductive success of the average symbiotic rhizobium. Thereafter, nonsymbiotic rhizobia could have higher fitness than symbiotic strains, especially if soil resources are abundant. The chance of founding a nodule in a given year would also depend on the abundance of host plants and on nodule number per plant, which decreases as plants respond to increasing nitrogen availability (Table 1).

Because symbiotic rhizobia spend so much time in the soil between hosts, we doubt that ecological specialization for the soil vs. nodule environment is a significant factor in coexistence of indigenous symbiotic and nonsymbiotic strains. Rhizobia do not fix N_2 in the soil, so there would be little metabolic cost to maintaining symbiotic genes, other than the direct cost of replicating a little more DNA. Inoculum strains grown under laboratory conditions are a different story; they could be poorly adapted to conditions prevalent in a particular soil, despite their superior symbiotic performance.

We also need to explain the variability in N_2 fixation among those rhizobia that are symbiotic. Again, our focus is not on the genetic or physiological basis for these differences, but rather on why those rhizobia genetically programmed to fix the most N_2 have not displaced, or perhaps been displaced by, those that fix the least. If there were no metabolic costs to rhizobia from fixing N_2 , then the increased host plant photosynthesis that results from higher rates of N_2 fixation [19] might increase photosynthate supply to rhizobia in nodules.

Table 1

The fitness benefits to a symbiotic rhizobium from attempting to nodulate a legume is the product of (1) the chance of success, (2) the average number of viable rhizobia released from a senescing nodule, and (3) the probability that a given rhizobium will survive long enough after nodule senescence to become established in the soil

	Chance of founding a nodule > if:	Rhizobia released per nodule > if:	Survival after leaving nodule > if:
Symbiotic rhizobia have greater fitness than those that never infect legumes if:	Symbiotic rhizobia are rare, there are more host plants, or less soil nitrogen	Fewer symbiotic rhizobia and less soil N may favor nodule growth	Rhizobia hoard PHB in nodule – but nodule may attract predators
Among symbiotic rhizobia, those that fix N_2 have greater fitness if:	Some nonfixing rhizobia may be recognized and excluded	Host sanctions favor N_2 -fixers, but maybe less if mixed nodules common	Lingering effects of sanctions exceed benefit from maybe saving more PHB

The table summarizes factors affecting these three parameters and how they, in turn, affect the relative fitness of symbiotic vs. nonsymbiotic rhizobia and (among symbiotic rhizobia) more- vs. less-mutualistic strains.

But rhizobia have other priorities that can conflict with N_2 fixation, such as hoarding resources like polyhydroxybutyrate (PHB) to support their own subsequent growth in the soil. Various roles have been suggested for PHB [20,21]. But the fact that it accumulates only in those rhizobia that will have descendants in the soil (bacteroids in determinate nodules, but only undifferentiated rhizobia in indeterminate nodules [21], Fig. 1) suggests that rhizobia accumulate PHB mainly for their own use, not for the benefit of their plant host [11]. PHB has been shown to enhance bacterial survival under starvation [22], although we have not seen similar studies specific to rhizobia. A clear tradeoff between N_2 fixation and PHB accumulation is seen in the higher N_2 fixation rate of a mutant unable to make PHB [23] and in the higher PHB accumulation of a nonfixing mutant, relative to an N_2 -fixing strain [24].

Each plant is typically infected by several different strains of the same rhizobium species [25,26], which creates a potential tragedy of the commons [27] – why pay an individual cost if benefits are shared? Rhizobia that support greater host plant photosynthesis by fixing more N_2 may receive some additional photosynthate, but do they also increase photosynthate supply to other rhizobia infecting the same host? If so, they are helping rhizobia that will compete with them in the future for soil resources and host plants. Less mutualistic rhizobia, which use available resources mainly to support their own growth and reproduction, rather than to meet the N needs of their host, might therefore be expected to leave more descendants, displacing more cooperative strains from the soil [11]. A mathematical analysis confirmed that, if legumes treated fixing and nonfixing rhizobia the same, nonfixing rhizobia would outcompete N_2 fixers [9].

Symbiotic N_2 fixation by rhizobia has persisted for millions of years, however. We have therefore concluded that legume plants must not treat all rhizobia the same. Ideally, legumes would recognize and exclude less-beneficial rhizobia when they first approach their roots. This would be an example of “partner choice,” in which less cooperative partners can be identified “in advance of any possible exploitation” [28]. Some legumes have been shown to exclude some less-beneficial or harmful strains of rhizobia [29]. Unfortunately, legumes cannot consistently recognize and exclude nonfixing rhizobia, especially those that are closely related to their usual symbiotic partners [24,30].

3. Host sanctions select for mutualistic rhizobia

The evolutionary persistence of symbiotic N_2 fixation by rhizobia may therefore depend on legumes cutting off resources to less-productive nodules. Such postinfection sanctions against rhizobia, based on their actual symbiotic performance, would be more reliable than identity-

based exclusion [11]. If rhizobia that fix less N_2 receive less of one or more fitness-limiting resources, that would tend to counteract the benefits (e.g., greater growth or PHB accumulation) that would otherwise accrue to nonfixing rhizobia. Legumes that impose such sanctions would waste less photosynthate on nonfixing rhizobia, so they should displace otherwise similar legumes that do not impose sanctions [31]. The effects of host sanctions on rhizobium evolution would be an important side effect of a plant strategy that enhances competitiveness against other plants.

But can legumes actually respond to the N_2 fixation rate of individual nodules? Earlier research showed that nodules containing nonfixing strains grow less [32]. However, it is at least conceivable that this could reflect plants allocating resources preferentially to rhizobia with the right “recognition signals” – signals that might sometimes be retained by nonfixing mutants. Complications arising from metabolic or signaling differences among strains can be avoided by using an N_2 -free atmosphere to prevent N_2 fixation.

When an entire root system was thus prevented from fixing N_2 , the nodule interior O_2 concentration (which limits rhizobium respiration and perhaps growth), decreased [33]. Legumes control nodule interior O_2 by adjusting nodule gas permeability [34]. Cutting off the O_2 supply to all rhizobia infecting a given plant would not have much effect on the composition of the next rhizobium generation in the soil, however, because growth of both fixing and nonfixing rhizobia would be affected. We therefore extended this earlier work by supplying N_2 -free air to only one nodule per soybean plant. We did this by surrounding attached nodules with tiny chambers that allowed control of gas composition. One nodule on each plant was exposed to an N_2 -free ($Ar:O_2$) atmosphere, thereby preventing N_2 fixation by a normally mutualistic rhizobial strain [35], while a control nodule was exposed to air ($N_2:O_2$).

We found that soybeans do monitor N_2 fixation of individual nodules and cut off O_2 supply to those that stop fixing N_2 . Within 48 h, the nodule interior O_2 concentration in the nonfixing nodules dropped to roughly half that in N_2 -fixing control nodules. After 10 days, nonfixing nodules were smaller and contained less than half as many rhizobia as control nodules [35]. Rhizobia prevented from fixing N_2 for a longer period, in a split-root experiment, also had lower subsequent survival rates in moist sand. In the absence of host sanctions, we would have expected nonfixing rhizobia to reproduce more and/or accumulate resources that would enhance their survival in soil. But host control of O_2 supply to the nodule interior (and perhaps other sanctions not yet identified) makes it unprofitable for rhizobia to cheat their legume host.

The first measurements of changes in the O_2 permeability of legume nodules were published in 1983 [36,37],

but the mechanism is still unknown. Nodule permeability to other gases, such as hydrogen [38] changes along with O₂ permeability, so some sort of physical diffusion barrier is involved. Electrophysiological measurements are consistent with turgor-driven changes in the size and shape of air channels between cells [39], but (reversible?) deposition of glycoprotein in intercellular spaces may be important in some species [40].

Host sanctions seem unlikely to change the symbiotic behavior of individual rhizobia, which is programmed by their DNA. A rhizobium lacking a functional nitrogenase – a “defect” that can lead to greater PHB accumulation [24] – would be unable to fix N₂, whatever the consequences of not doing so. Host sanctions would therefore affect the evolution of rhizobium populations, not the behavior of individual rhizobia.

Similarly, we consider it unlikely that anything like the “policing” seen in some social insects (e.g., workers eating the occasional eggs laid by other workers) happens in rhizobia. Even if a rhizobium could somehow determine how much N₂ other rhizobia nearby were fixing, and even if they could produce antibiotics that kill the cheaters without killing themselves, natural selection would be unlikely to favor policing behavior. If there is some metabolic cost to policing others, a mutant that avoids this cost would tend to displace a policing genotype. The tragedy of the commons would apply at the level of individual nodules as well as the whole plant. Of course, policing microbes might acquire resources from microbial cheaters they kill, but then they could also benefit by killing microbes that are not cheaters [41].

It is conceivable, however, that some rhizobia could have conditional (though still genetically programmed) strategies, such as modulating their N₂ fixation rate in response to sanctions imposed by their host, allocating just enough resources to N₂ fixation to avoid sanctions. Plant responses could be equally sophisticated. For example, legumes might modify sanctions against one nodule based on the amount of nitrogen derived from other nodules on a plant [31], at least as a side-effect of a response to overall N supply.

Given host sanctions, why does rhizobial cheating persist? In our experiments, rhizobia that stopped fixing N₂ produced only half as many descendants, at least inside nodules. Those descendants also had lower survival in soil, for reasons not yet known. If this host response is typical, we would expect mutualistic, N₂-fixing strains of rhizobia to completely displace nonfixing, parasitic strains within a few generations. Why has this not happened?

Mixed nodules may provide a haven for parasitic rhizobia. The nonfixing rhizobia that accumulated more PHB than N₂ fixers in the experiments of Hahn and Studer [24] were sharing a nodule with an N₂-fixing strain. Maybe the latter fixed enough N₂ to protect both strains from host sanctions. This would be analogous to a virus

that benefits from coinfecting with a second strain [42]. If sharing a nodule with an N₂-fixing strain is a key to the proliferation of nonfixing strains, whether or not they are closely related to common N₂-fixing strains, then we would expect the prevalence of nonfixing strains to be greater where mixed nodules are more common. Unfortunately, data on the frequency of mixed nodules under field conditions are scarce, especially in nonagricultural contexts [43].

4. Outlook

A complete understanding of rhizobium ecology and evolution must explain the coexistence of symbiotic rhizobia ranging from highly mutualistic N₂ fixers to nonfixing parasites, as well as nonsymbiotic strains that lack the ability to infect plants. The relative fitness of symbiotic and nonsymbiotic strains should depend on the odds of winning the nodulation lottery (a very small chance of producing many millions of descendants), which should vary with the abundance of symbiotic rhizobia relative to their legume host plants. Our limited data on the fitness effects of host sanctions suggest that they outweigh the benefits that rhizobia inside nodules might otherwise obtain from hoarding resources rather than fixing N₂. Some parasitic rhizobia may escape these sanctions by sharing nodules with more mutualistic strains.

There are still many unanswered questions, however. Do other legumes, especially wild species, impose sanctions severe enough to limit the spread of nonfixing rhizobia? How common are mixed nodules in the field? Are there consistent differences between determinate and indeterminate nodules (Fig. 1), either in sanctions or in the frequency of mixed nodules [11]? Do widespread nonfixing strains have genes for physiological adaptations that allow them to avoid host sanctions, perhaps by interfering with host signaling pathways that regulate the sanctions response? Finally, are the relative proportions of mutualistic, parasitic, and nonsymbiotic rhizobia in soils fairly stable over time? Or do they fluctuate, perhaps in a rock-paper-scissors sequence [44,45], as changes in population size change the relative payoff from alternative strategies? Most of these questions need to be addressed at multiple spatial and temporal scales. We encourage others to join us in exploring these and related topics.

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