TITLE: Host-imposed Control Mechanisms in the Legume-Rhizobia Symbiosis

AUTHORS: Stephanie S. Porter[1], Simon Dupin[2], Ford Denison[3], Toby Kiers[2], Joel Sachs[4]

[1] School of Biological Sciences, Washington State University, Vancouver, Washington 98685, USA (Ph) 1(818) 522-9662
stephanie.porter@wsu.edu

[2] Amsterdam Institute for Life and Environment (A-LIFE), Section Ecology & Evolution, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, the Netherlands (Ph) +33 (0)6 67 35 49 90
sd.dupin@gmail.com
toby.kiers@vu.nl

[3] Department of Ecology, Evolution, and Behavior University of Minnesota, Saint Paul, MN 55108 (Ph)612-626-6462
denis036@umn.edu

[4] Department of Evolution, Ecology & Organismal Biology, University of California, Riverside, California 92521, USA
(Ph.) 1(951) 827-6357
joels@ucr.edu

ABSTRACT

Legumes are ecologically and economically important plants that contribute to nutrient cycling and agricultural sustainability, features tied to their intimate symbiosis with nitrogen-fixing rhizobia. However, rhizobia vary dramatically in quality, ranging from highly growth-promoting to nonbeneficial. Therefore, optimizing plant benefits from this symbiosis requires host mechanisms that select for beneficial rhizobia and limit losses to nonbeneficial strains. Here, we examine the considerable scientific progress made in decoding host control over rhizobia, empirically demonstrating both molecular and cellular mechanisms and their effects on symbiotic benefits. Pre-infection control requires plant production and detection of precise molecular signals to attract and select compatible rhizobia strains. Post-infection mechanisms leverage nodule- and cell-level compartmentalization of symbionts to enable host control over rhizobia development and proliferation *in planta*. These layers of host preferential allocation act as a series of sieves, each of which contributes to legume fitness by directing host resources to a narrowing subset of more-beneficial rhizobia.

INTRODUCTION

Legumes are among the most diverse and ecologically important plant families, colonizing habitats from deserts to rainforests[1]. Their global radiation is often linked to their ability to fix atmospheric N_2 with rhizobia bacteria - a trait that requires complex coordination between host and symbiont[2]. Symbiotic legumes contribute roughly half of all terrestrial nitrogen fixation[3], a quarter of agronomic production via crops such as soybean and alfalfa, and a third of human-consumed protein[4].

To fix N_2 with rhizobia, legumes have evolved novel organs called nodules, of which they can bear tens to hundreds across their roots systems (Box 1). The evolution of nodulation and symbiotic nitrogen fixation is of comparable complexity to the evolution of organs such as the eye, in that coordinated expression of multistep molecular pathways determine organogenesis and a functional phenotype[5–8]. Unlike the eye, the legume nodule is a symbiotic organ, whose evolution and function require successful integration between the host and bacteria[9]. Substantial progress has uncovered molecular mechanisms of nodule organogenesis and nitrogen fixation[8,10–12]. However, less is known about how legumes detect and appropriately respond to rhizobia symbionts that differ in quality.

Rhizobia encompass diverse alpha- and beta-proteobacteria, defined by their capacity to nodulate legumes and fix nitrogen[13]. Even closely related rhizobia strains show vast differences in the benefits they provide, with strains on any given host varying from highly beneficial to nonbeneficial. Moreover, nested within lineages of rhizobia that nodulate and benefit legumes are strains that lack symbiosis genes and might be adapted to saprophytic, commensal, or parasitic lifestyles[14–17]. Under longstanding theory, diverse unrelated symbionts are predicted to exhibit intense inter-strain competition, lowering host benefit and favoring evolution of host mechanisms to control infection and minimize symbiont exploitation[18]. Elucidating how hosts detect and respond to this variation in the quality of mutualistic microbes is equal in importance to host defense against pathogens, but only recently has become a focus of research advances. Below we describe mechanisms of infection and host control, understanding that most data come from few, well-studied host taxa (but see[19,20]), hence that not all mechanisms are reviewed.

LEGUME-RHIZOBIUM COOPERATION REQUIRES COORDINATION

Legume-rhizobia symbiosis is initiated with a coordinated exchange of molecular signals. Legume roots secrete flavonoids, cocktails of secondary metabolites that attract rhizobia and initiate nodulation[21,22]. Receptive rhizobia respond by secreting lipochitooligosaccharide nodulation (Nod) factors which are detected by plant LysM receptors[7]. Nod factors induce root hair curling which entraps rhizobia cells, from which an infection thread forms as an invagination of the plant cell membrane[7,23,24]. Rhizobia reproduce down this tubular structure, either as clones or multiple strains that found the nodule together[25] (Box 1), are internalized by developing nodule cells, and differentiate into intracellular bacteroids that fix N_2 into plant-available forms in exchange for fixed carbon[6,7,16,19,20].

Rhizobia only fix nitrogen inside legume nodules, which provide the microaerobic and energetic requirements of nitrogenase. A barrier limits oxygen flux into the nodule interior and maintains microaerobic concentrations, while leghemoglobin-facilitated diffusion supports rhizobia respiration[26]. Both partners bear marginal costs. The host supplies reductant, typically organic acids, that fuel N_2 fixation which requires 16 ATP to fix each N_2 molecule. N_2 fixation can compete with rhizobia resource storage and reproduction[27–30], and requires substantial allocation of a legume's fixed carbon[31], reducing host fitness if too many nodules are formed[32–34].

Measuring net benefits of symbiosis is challenging. Experimentalists typically inoculate legumes with single rhizobia strains and harvest biomass during vegetative growth. How well this predicts a strain’s contribution to host fitness under more natural conditions, where each plant hosts many strains, remains to be determined. Harvesting singly inoculated immature plants could obscure strains' differences in the speed, duration, and N-per-C efficiency of N_2 fixation, as well as impacts of rhizobia alteration of plant hormones[35]. Despite methodological uncertainties, it is clear that benefits from rhizobia vary among rhizobia genotypes and

conditions - including external nitrogen availability, light levels, and the host genotype[36–43,44–46]. But how do legumes detect and appropriately respond to rhizobia of varying quality as symbionts? We explore current evidence in the following sections (Fig 1).

PRE-INFECTION CONTROL AND PARTNER CHOICE

Pre-infection control mechanisms operate prior to significant resource investment (sensu[47–49]; Fig 2) and can enable hosts to preferentially associate with symbionts that confer greater benefit. Partner choice implies that i) host preference enhances plant fitness relative to random nodulation, and ii) filtering among strains occurs in advance of nodule organogenesis. Empirical research supports partner choice, in that some legumes can form more nodules with beneficial strains than with non-fixing ones[50], and that strain occupancy under mixed inoculations is correlated with the benefit of strains in single inoculation[51,52]. However, it is difficult to disentangle host effects from those of inter-strain competition among rhizobia, as nodule occupancy patterns are a joint phenotype that depends on the plant and rhizobia genotypes and their interactions[53].

Several approaches establish the capacity of legumes to distinguish among rhizobia strains that vary in quality. Split-root experiments eliminate direct interactions between strains to show that some hosts can initiate more nodules with a fixing strain than a non-fixing one[54]. Labeling rhizobia with fluorescence proteins or differentiating them genetically *in planta*, reveals that rhizobia strains that result in more plant biomass with single-strain inoculation also occupy more nodules in mixed inoculation, consistent with plant selection of more-beneficial strains[55,56]. Furthermore, stronger partner choice can lead to greater fitness benefit when a legume is presented with rhizobia that differ in quality[55]. Studies of wild legumes and co-evolved rhizobia

115 reveal additional examples consistent with partner choice (reviewed in[47]) -- yet mechanisms by
116 which plants detect and respond to rhizobia quality in advance of N_2 fixation are not well-
117 understood.

118 In experiments that expose legumes to N_2 fixing rhizobia and isogenic non-fixing
119 mutants, the strains show little difference in nodulation[28,57]. Thus, partner choice depends upon
120 reliable linkage between rhizobia alleles that encode signals to legumes in advance of N_2 fixation
121 and those that impact benefit to the legume[47]. Alleles that encode determinants of compatibility,
122 such as *nod* genes and Type-III secretion systems, often reside in the same mobile elements as
123 nitrogen fixation genes[16,58–60]. Host discrimination might maintain linkage among such alleles if
124 strains with compatible signals that confer high benefit have higher relative fitness than strains
125 that confer lower benefit[39,47,50,61,62]. However, evidence for such genetic linkage is lacking. In
126 fact, loci impacting symbiotic quality reside in genomic regions that are hotspots for gene gain,
127 loss, and recombination[16,17,63,64]. This means that elucidating the genetics of partner choice
128 remains challenging. One clear pattern is that hosts bear at least two mechanisms to
129 preferentially initiate nodules with superior strains, as described below.
130
131 **Fundamental Compatibility.** Like a match between a lock and key, a legume genotype's
132 fundamental compatibility with a rhizobia genotype is determined by responsiveness to
133 molecular signals produced by the rhizobia, including Nod factors, surface polysaccharides, and
134 Type-III effectors (Box 1). Fundamental compatibility contributes to partner choice if compatible
135 strains provide greater benefit than incompatible strains. For example, hosts possessing the *Rj4*
136 allele common in soybean are incompatible with many less-beneficial, chlorosis-inducing
137 *Bradyrhizobium* strains due to gene-for-gene resistance triggered by the effectors these strains

138 secrete[65–67]. Near-isogenic lines of soybeans bearing the alternate allele, *rj4*, are compatible with
139 these inferior strains, and display reduced fitness relative to *Rj4*-bearing soybeans when
140 inoculated with these strains[65,66]. Thus, the *Rj4* allele modifies soybean's fundamental
141 compatibility in a pattern consistent with partner choice where such ineffective strains are
142 common.

143 Variation in legume responsiveness to rhizobia Nod factors is another driver of fundamental
144 compatibility[24,68–70]. Legumes bear receptors (lysine motif receptor-like kinases; LysM-RLKs)
145 that determine which nod factors trigger legume cell calcium oscillations to initiate nodulation[70–
146 73] and can interact with rhizobia surface polysaccharides, which differ in their efficacy for
147 promoting early infection[23,74,75]. Furthermore, legume immune responses can terminate nodule
148 formation. Compatible rhizobia must possess mechanisms to evade legume immune responses[76].
149 For example, during nodule formation rhizobia can present surface exopolysaccharides to evade
150 bacteriocidal actions by the plant immune system, and can secrete effectors[23,77–80], such as
151 Nodulation outer proteins (Nops), to dampen legume defenses[24,74,81,82].

152 Nodule-specific cysteine-rich (NCR) peptides allow some legumes to discriminate amongst
153 rhizobia in a strain-specific manner[24]. By inundating rhizobia with a suite of defensin-like
154 antimicrobial NCR peptides[83,84], some legumes force rhizobia to differentiate into swollen, non-
155 reproductive, but nitrogen-fixing bacteroids. Swollen (perhaps nonreproductive) bacteroids are
156 found in many nodules with indeterminate growth but also in some determinate nodules,
157 including *Arachis* spp[85]. For incompatible rhizobia, NCR peptides' antimicrobial properties
158 terminate symbiosis[46], while for compatible rhizobia they are essential to preserve bacteroid
159 viability[86]. Rhizobia can resist host NCR peptides by cleaving them using host-range restriction
160 peptidases[84,87,88], membrane transport proteins[89] and extracellular polysaccharides[82]. However,

high specificity in strain recognition would not enable discrimination against mutants that retain the same molecular attributes but fix less nitrogen.

Legume taxa vary from specific to generalist in the rhizobia that they nodulate, with a tradeoff between generalism and the benefit gained from symbionts[40]. Consistent with a tradeoff, symbiotically promiscuous legumes, like the common bean *Phaseolus vulgaris*, allow extremely diverse bacteria to gain access to nodules, and can be dominated by strains that are not effective for fixing nitrogen[90]. Loss of specificity could be a byproduct of domestication, as the capacity to select beneficial rhizobia can vary markedly with breeding practices and could be tied to the relaxation of plant defenses[91,92]. New work in *Lotus spp.* has uncovered a quantitative trait locus that encodes promiscuity, opening the door to research to better understand its evolution and the mechanistic bases of generalism[93].

Few molecular mechanisms that restrict fundamental compatibility have been shown to confer an advantage to the host by excluding inferior symbionts. This could be tested using loss and gain of function experiments on hosts. While a host may be fundamentally compatible with a broad swath of symbionts, the ability to preferentially initiate nodulation with superior strains amongst diverse compatible options is a distinct trait, which we discuss next.

Realized Nodulation Compatibility. Legumes can preferentially initiate nodules with some fundamentally compatible rhizobia strains over others, which delineates a legume's realized nodulation. This contributes to partner choice if legumes selectively nodulate with superior compatible strains, as occurs in several wild systems. Surprisingly little is known about how legumes select strains to nodulate from among compatible partners[23]. From compatible strains in the rhizosphere, hosts can preferentially nodulate with rhizobia bearing specific early symbiont

recognition signaling pathways. For example, a host can preferentially nodulate with strains that express particular nod factor variants, even if other nod factor variants are compatible[94]. Pea plants exposed to mixed communities of compatible *Rhizobium leguminosarum*, tend to preferentially nodulate with strains bearing characteristic nodulation alleles, plasmid-encoded transporters, proteins involved in the biosynthesis of cofactors, and proteins related to metabolism[95]. In addition, rhizobia tRNA-derived small RNAs can silence target host genes to promote nodulation success in a specific, localized manner[96].

Overall, partner choice is susceptible to exploitation because rhizobia can rapidly evolve to be less beneficial yet express the same signals as more beneficial strains. Consistent with this susceptibility, legumes are typically unable to select superior partners when faced with novel or genetically manipulated rhizobia genotypes[47,57,97,98]. Even in natural communities, legumes nodulate with non-beneficial strains. In fact, some rhizobia evolve mixtures of Nod factors that allow them to colonize diverse legumes in which they fix little to no nitrogen[99,100] and others secrete no Nod factors, but hitchhike into nodules with strains that do[14,101]. Moreover, successful nodulation can be affected by competitive interactions among rhizobia in the rhizosphere, modulated by antibiotic[102] and antibiotic resistance traits[103]. To qualify as host-imposed partner choice, these interactions would need to be driven by a host's ability to generate rhizoplane conditions that favor more beneficial rhizobia[104,105], which remains unknown. Therefore, it is imperative that legumes detect and respond to rhizobia quality once nitrogen fixation has commenced, as discussed next.

SANCTIONS AND OTHER MECHANISMS OF POST-INFECTION CONTROL

206 Post-infection control mechanisms enable legumes to preferentially allocate resources to rhizobia
207 in nodules, based on net benefit provided to the host (Fig 3). Within post-infection control, hosts
208 may (i) exert sanctions or policing that lead to an accelerated and targeted senescence of nodules
209 or cells within nodules, and/or (ii) impose 'scaled rewards' to preferentially allocate resources to
210 higher-quality symbionts, which could cause nodules or cells containing more-beneficial
211 rhizobia being larger or better provisioned)[98,100,106,107]. While providing more or fewer resources
212 are two sides of the same coin[108] (i.e., an increase in resources to one nodule is likely linked to a
213 decrease in other nodules on the same plant), our framework highlights that hosts may also have
214 the *potential* to actively harm (e.g., attack) rhizobia[108,109].
215
216 **Compartmentalization.** Legume physical structures that spatially separate symbionts, namely
217 nodules and plant cells within them, facilitate host discrimination among rhizobia during
218 symbiosis[110] and reduce direct conflict among symbionts colonizing a single host[111]. Legume
219 control mechanisms should satisfy three criteria: (i) allowing hosts to distinguish among
220 compartments with more- vs less-beneficial rhizobia, (ii) enable the host to direct resources to
221 more beneficial rhizobia, ideally, even within coinfected nodules, to increase its fitness return on
222 resource investment, and (iii) minimize tissue damage to the host caused by sanctions on
223 rhizobia in those infected cells.
224 Nodules represent compartments for hosts to enact control mechanisms, containing
225 populations of rhizobia that the host can regulate independently. Nodule-level controls would be
226 most effective when nodules contain a single rhizobia strain[112]. If multiple strains share a nodule,
227 this could impair a host's ability to preferentially allocate resources to individual strains.
228 However, cases where ~20% of nodules are co-infected are common in the field[113–115] and the

laboratory[28,52,57,116–121], with some nodules containing up to six different strains[25]. One solution to this problem is if hosts can enact control at a finer spatial level. Recent work suggests that some legumes can discriminate, at least against completely ineffective rhizobia, even when as much as 50% of the host's nodules are coinfected with a mixture of effective and ineffective strains[25,50,116,122]. How is this achieved? In the next section we discuss possible control mechanisms.

Host control over resource supply. Preferential allocation of resources, such as carbon or oxygen, to individual nodules could be a mechanism by which hosts scale rewards to rhizobia performance. Such scaled rewards operate in other symbioses, such as those between plants and arbuscular mycorrhizal fungi[123,124]. L**Error! Bookmark not defined.**egumes can control nodule permeability to oxygen,[125,26,126] allowing hosts to scale rewards. Symbiotic rhizobia depend on precise levels of O_2 for aerobic respiration[127], requiring sufficient O_2 flux for ATP production but low enough levels to prevent damage to nitrogenase[128–130]. Nodule-level manipulations of rhizobia defection allows researchers to evaluate legume responses[131]. For example, when rhizobia were prevented from fixing N_2 using an Ar:O_2 atmosphere, soybean decreased the oxygen permeability of nodules, and oxygen-limited rhizobia decreased reproduction. However, these manipulations imperfectly mimic nodules with low N_2 fixation because nitrogenase still consumes resources in the N_2-free atmosphere, producing hydrogen gas rather than ammonia. Furthermore, it is not clear whether reduced O_2 influx was directly responsible for decreased rhizobia reproduction[132].

Nodules containing more effective nitrogen-fixing rhizobia receive greater carbon allocation by the legume. Regulation of carbon access to the nodule apoplastic space or to symbiotic cell

252 could control rhizobia and nodule growth. Legumes provide dicarboxylates, primarily succinate
253 and malate, as energy and electron source donors for N_2 fixation by rhizobia. Sucrose is brought
254 via phloem to the infected nodule cell and metabolized into malate by glycolysis pathways
255 (PEPC-MDH). Malate is used as a carbon skeleton to transport N out of the symbiotic cell and as
256 reductant power by rhizobia to fix N_2. A recent review of nodule metabolism reveals a central
257 role of malate[133]. However, there are few data on host control of apoplastic carbon metabolism
258 and its connection with endosymbiotic rhizobia fitness. Within nodules containing effective
259 nitrogen-fixing rhizobia, rhizobia proliferate rapidly, whereas nodules with less-beneficial
260 rhizobia tend to stay smaller, and the rhizobia within them divide slowly[97,98,114,116,132,134,135]. For
261 example, rhizobia that store more polyhydroxybutyrate (PHB) during symbiosis can survive
262 longer in the soil between hosts[136], but a more-beneficial PHB(-) *Rhizobium etli* knockout
263 provided bean plants with more nitrogen, presumably because PHB accumulation competes with
264 N fixation[29]. The PHB(-) strain produced roughly two-fold larger nodules than the less-
265 beneficial, wild-type strain, consistent with host sanctions against the latter[137].
266 While nodule-level sanctions could help optimize net legume benefits at the level of whole
267 nodules, it is unlikely that ineffective rhizobia in nodules with mixed infections could be targeted
268 by withholding O_2 or carbon[133,138]. While it is possible that the peribacteroid membrane could
269 limit carbon influx to particular bacteroids within a nodule[139,140], legume control of nodule
270 permeability to gases occurs at the nodule-level and carbon is metabolized via vascular bundles
271 that supply whole nodules. Therefore, control over oxygen flux is unlikely to serve as a
272 mechanism of post-infection control within a co-infected nodule (criterion (ii)).
273 **Host mediation of hormones, antimicrobials and amino acids.** Phytohormone
274 concentration is higher in nodules compared to other portions of roots[141,142] and may play a role

in the regulation of nodule metabolism. For example, together with CLE genes, cytokinines are important in the regulation of nodule numbers[143]. Plant hormones change in concentration throughout nodule developmental stages[144], and hormone transport and concentration in nodules is spatially controlled in the different cell types[145]. It is conceivable that spatial control of hormones by hosts could provide a mechanism by which a host could discriminate between beneficial and ineffective rhizobia in mixed nodules, though we lack research that addresses this possibility.

Some legumes secrete antimicrobial peptides that trigger terminal differentiation of bacteroids, which become incapable of reproduction[146–148] and can increase their N-per-C efficiency[149]. This does not appear to reduce the resource usage and reproduction of less effective strains as undifferentiated rhizobia clonemates persist in these nodules and are not subject to antimicrobial control[149] (violating criterion II). Pea and alfalfa, both of which host nonreproductive bacteroids[135] exert whole-nodule sanctions. Although sanctions against less-beneficial nonreproductive bacteroids in mixed nodules could conserve plant resources, they would not selectively harm the corresponding reproductive rhizobia[106].

Selective Nodule senescence. Nodule senescence is the natural process by which nodules break down[150]. Rhizobia should benefit from a delay in senescence as they can proliferate more within the nodule[151], whereas legume hosts would benefit from senescing nodules when costs of rhizobia outweigh benefits. Legumes display two types of nodule senescence. Developmental senescence entails nodule maturation and release of symbionts into the soil, often during fruit set, when legumes shift carbon resources towards seeds and away from nodules. In contrast, induced senescence is triggered if legumes experience stress, such as deficiencies of light[152], water[153], or failure of bacterial recognition by the plant[154]. Morphological changes associated with the two

298 types of nodule senescence differ. In the latter, changes progress faster and the contents of the
299 symbiosome are degraded without evidence for legume nutrient remobilization[155]. Several
300 morphological features of senescence are observed in ineffective nodules.
301 One of the first morphological changes during senescence is breakdown of the symbiosome
302 membrane[156], which releases bacteroids to the plant cytosol and exposes the rhizobia to the
303 hostile host cell environment[157]. In zones of senescence within indeterminate nodules, proteins
304 involved in the formation of vacuoles accumulate, and symbiosomes transform into vacuole-like
305 units[157,158]. Thus, vacuolar fusion machinery may be necessary for controlling symbiosome lysis.
306 A potentially critical step in nodule senescence is the neutralization of the peribacteroid space
307 that surrounds the symbiosome, an otherwise acidic environment which facilitates import of host
308 resources[159]. For instance, non-fixing rhizobia often fail to induce full peribacteroid space
309 acidification, potentially allowing the host to halt symbiosome development[159]. Senescence is
310 associated with induced expression of cysteine proteases, which mediate senescence[160]. In
311 *Astragalussinicus,* downregulated expression of nodule-specific cysteine protease *Asnodf32*,
312 delays nodule senescence and extends N_2 fixation[161]. Moreover, the ratio of reactive oxygen
313 species to scavenging enzymes increases during nodule senescence[156,160,162].
314 In experimental work, individual nodules that house ineffective rhizobia are selectively
315 senesced. Within *Acmispon* and *Lotus* nodules coinfected with beneficial and ineffective strains,
316 selective senescence was only associated with individual nodule cells housing ineffective
317 rhizobia[107]. Thus, selective senescence is a promising avenue for investigating host control as it
318 fulfills all three criteria described above.
319

Immunity. Plants respond to pathogens via inducing reactive oxygen species, altering gene expression, remodeling the plant cell wall, and producing antimicrobial compounds such as phytoalexins[163]. A common response is a rapid, localized programmed cell death that occurs where harmful microbes have invaded, known as a hypersensitive response. Many of these responses also occur during rhizobia infection[164,165], however, the role of immunity in legume sanctions is unresolved. Levels of responsive early gene expression are substantially lower in response to nod factors than to the elicitors of defense, suggesting a muted induction[166]. The early generation of reactive oxygen species is necessary for rhizobial infection and their increased production leads to increases in nitrogen fixation and a delay in senescence[167]. Development in established nodules is not affected by elicitation of immunity[164], though the repression of immunity could be reversed as nodules begin to senesce[168]. Lastly, processes similar to a hypersensitive response occur in legume-rhizobia interactions, but in a genotype-specific manner, independent of nitrogen fixing status[169]. Immune responses based on strain identity, rather than symbiotic performance, have the same challenge as partner choice, in that less-beneficial mutants could retain the identity signals of their more-beneficial ancestors. Moreover, it is unclear how a host immune response could differentially affect strains within the same nodule, violating criterion (ii).

Overall, post-infection control mechanisms are imperfect. Despite the opportunity for post-infection control to divert host resources away from less beneficial rhizobia, a legume that forms nodules with both a less-beneficial and a more-beneficial strain can have lower fitness than a legume that nodulates exclusively with the more-beneficial strain[51,170,56], though this cost is not ubiquitous[171]. In extreme cases, hosts have no control over parasitic rhizobia: the *Bradyrhizobium elkanii* strain USDA61 produces many nodules on soybean hosts, but fixes little

343 nitrogen[172], and rhizobitoxine-producing strains induce chlorosis[67]. Less-effective strains also
344 represent an opportunity cost for hosts, but the magnitude of these costs is poorly understood.
345 Little is known about how legumes sense rhizobia performance. Legumes could assess
346 rhizobia quality using absolute criteria by comparing nodule performance to a fixed threshold,
347 below which plants do not allocate resources to a strain. Alternatively, legumes could use
348 conditional criteria and compare rhizobia performance to that of other strains available to the
349 legume. Recent research reveals strong support for conditional sanctions: a rhizobium genotype
350 is sanctioned if it is co-inoculated with a strain that confers more benefit to the host, but not if it
351 is co-inoculated with a strain that confers less benefit to the host[55,98]. While the ability to
352 distinguish between cooperative and uncooperative rhizobia can impose no detectable cost on a
353 host legume[170], it could be that host-control is costly in some environments. Although the
354 metabolic costs of sensing nodule performance may be negligible, legume fitness could decrease
355 from shutting down *too* many nodules or allocating resources to suboptimal nodules.
356 Interestingly, legumes may differ in their ability to sanction, and this may be linked to breeding
357 and/or agricultural conditions under domestication[91,92,173], genetic bottlenecks during breeding,
358 or long term fertilization[174].
359

360 **CONCLUSION AND FUTURE PERSPECTIVE**

361 In conclusion, multiple mechanisms of preferential allocation act as a series of sieves, each
362 contributing to legume fitness by directing host resources to a narrowing subset of more
363 beneficial rhizobia[55]. This understanding is critical to future efforts to breed legumes with
364 improved host control. For instance, we must understand how different mechanisms of
365 preferential allocation to rhizobia interact. Are there trade-offs between host control traits that

could constrain their evolution, or could artificial selection improve multiple host control traits in parallel? If some host control traits sufficiently prevent legume exposure to inferior rhizobia, does this relax the selection that maintains other mechanisms of preferential allocation ? Host control is imperfect, meaning that hosts often reduce, but do not fully eliminate resources conferred to inferior symbionts[97,98]. Low costs and imperfect resource allocation could contribute to the evolution of multiple mechanisms of preferential allocation to rhizobia in legumes, which could provide promising novel targets for crop improvement. Understanding these mechanisms will also be important to engineering microbiomes or designing synthetic symbioses, both of which require the host's ability to control symbionts that differ in quality. Artificial selection and engineering efforts that fail to consider host control traits risk producing hosts that are overrun by less-beneficial symbionts.

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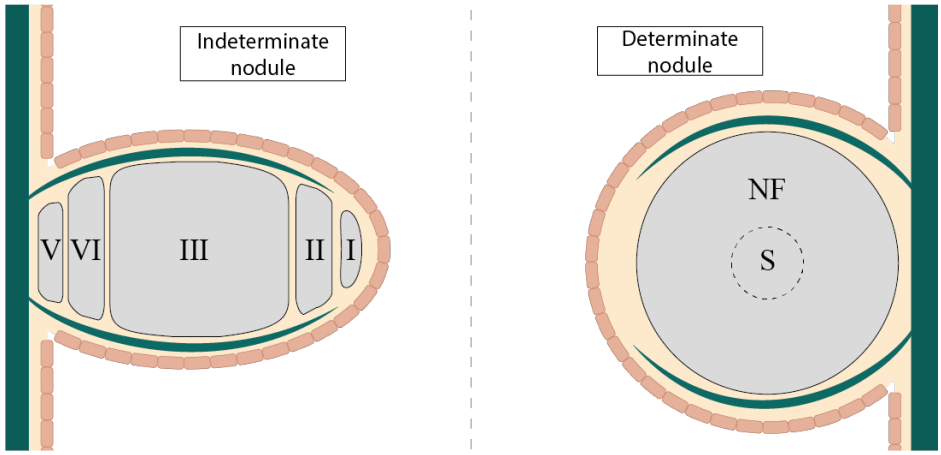
BOX 1. Typical biology of nodulation

Nodules are typically initiated when one or a few rhizobia cells enter root cortical cells via a crack or root hair and are encased by a plant-derived symbiosome membrane, within which they differentiate into bacteroids and can begin fixing nitrogen. Bacteroids typically reside as spatially structured groups within infected plant cells[117] and reach exceptionally high numbers, with 1-50 bacteroids within each symbiosome, and 10^3-10^4 symbiosomes in each infected host cell[107]. Only a subset of rhizobia in soil are compatible with any host, determined by host responsiveness to rhizobia molecular signals, including Nod factors, surface polysaccharides, and Type-III effectors[23,77–80].

Nodule development varies among legume taxa. **Determinate nodules**, such as in *Lotus japonicus*, lack a continuous meristem and are spherical, with a core of infected N_2 fixing cells (NF) surrounded by uninfected host cells. Determinate nodules host homogenous populations of bacteroids, cease growth after their development is complete, and allow nitrogen fixing rhizobia to escape back into the soil during nodule senescence, a process initiating from a senescent zone in the nodule center (S). **Indeterminate nodules**, such as in *Medicago truncatula*, grow throughout the functional association, with a spatial gradation of zones, including undifferentiated meristem cells (I), cells being invaded by bacteria and those undergoing symbiotic differentiation into a nitrogen fixing form (II, III), a nitrogen fixation zone (IV), and a senescent zone (V). In some hosts of each type[85], bacteroids terminally differentiate and cannot escape the nodule[177], but nonetheless a subset of undifferentiated rhizobia can be released upon nodule senescence[178]. In both nodule types, bacteroids within nodules can fix nitrogen, and greatly enhance plant fitness in return for host-derived carbon.



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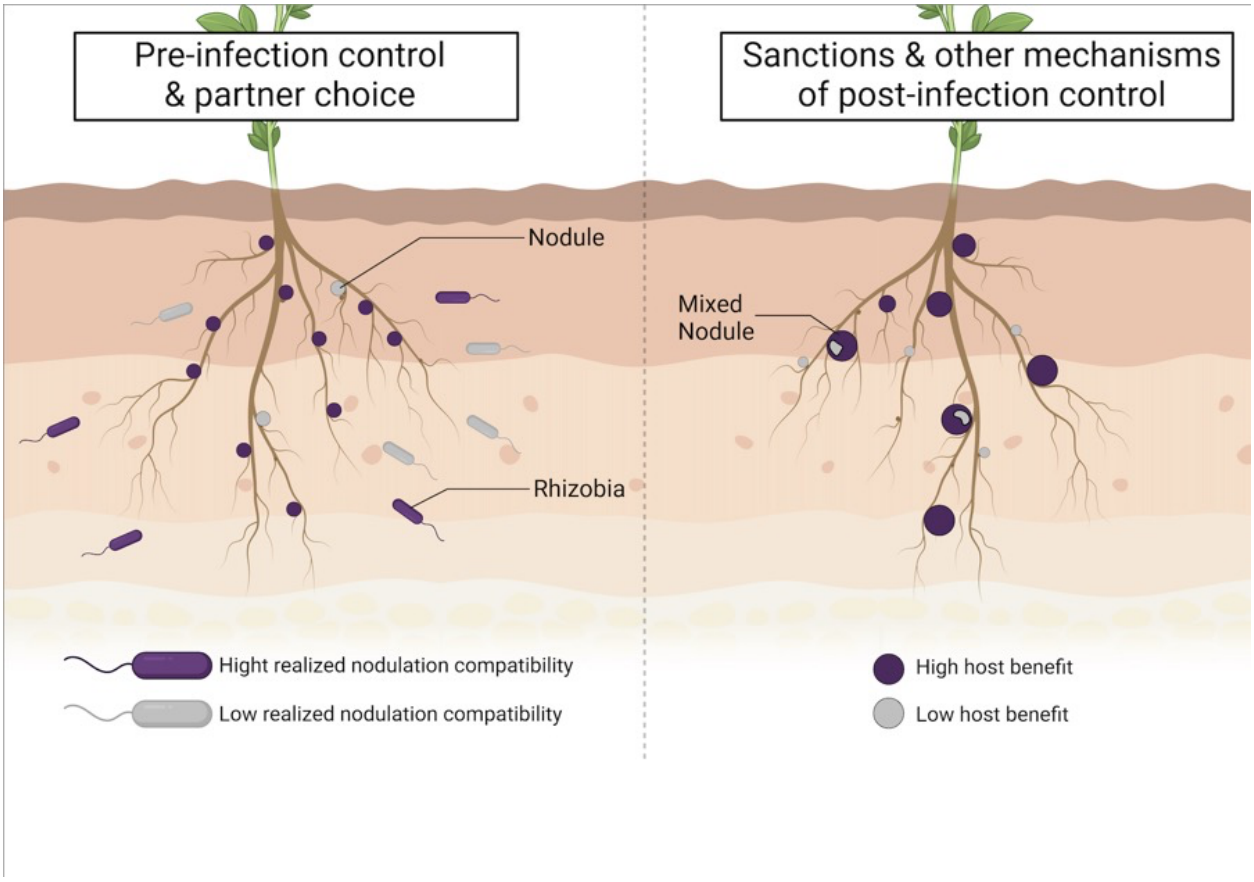

Figure 1. Legumes detect and respond to rhizobia of varying benefit via two broad classes of mechanisms. Pre-infection control and partner choice (left) occur if plant production and detection of molecular signals select for beneficial rhizobia (i.e., purple cells and nodules) and exclude less beneficial rhizobia (i.e., grey cells and nodules)[47–49]. Sanctions and other post-infection mechanisms (right) are enabled by compartmentalization of symbionts among nodules and infected plant cells[98,106,107]. Here, hosts control rhizobia development and proliferation *in planta*, such that nodules and plant cells infected with highly beneficial rhizobia to grow rapidly and those infected with less beneficial rhizobia grow slowly. Both classes of mechanisms can help optimize the benefits of symbiosis by favoring more beneficial rhizobia strains and selecting against those that provide little or no N_2 fixation.

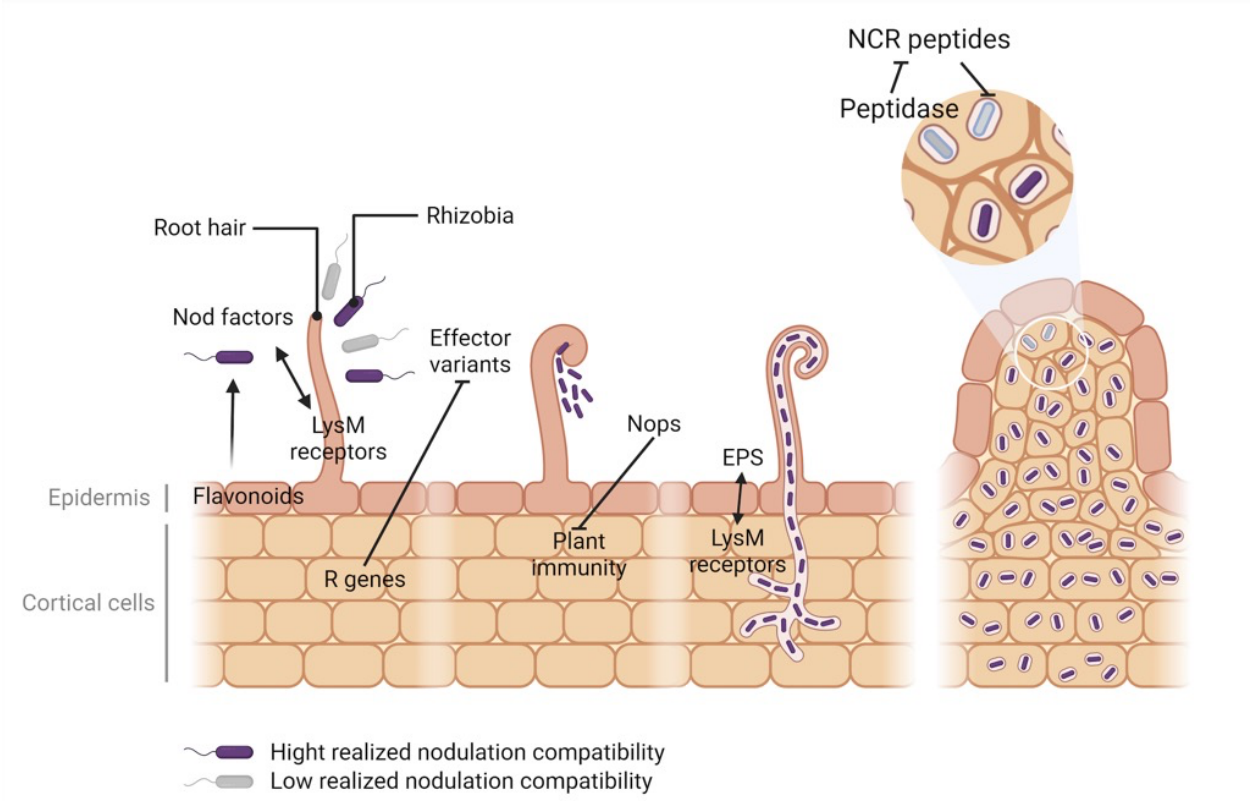

Figure 2. Mechanisms of pre-infection control and partner choice. Pre-infection control by legumes occurs via a multilayer response modulated by back-and-forth molecular communication between plants and rhizobia. The process is initiated by host secretion of species specific flavonoids[21,22], a response by compatible rhizobia via production of Nod factors, and detection of Nod factors by plant LysM receptors[7]. Compatible rhizobia, for instance with appropriate Nod factors or Type-III effector variants, must evade legume immune responses, via presenting surface exopolysaccharides (EPS) that minimize host bacteriocidal actions or secretion of Nodulation outer proteins (Nops) that dampen legume defenses[24,74,81,82]. Nodule-specific cysteine-rich (NCR) peptides allow some legumes to further discriminate among rhizobia using antimicrobial NCR peptides, though some rhizobia possess peptidases that can cleave and inactivate host NCR peptides[83,84].

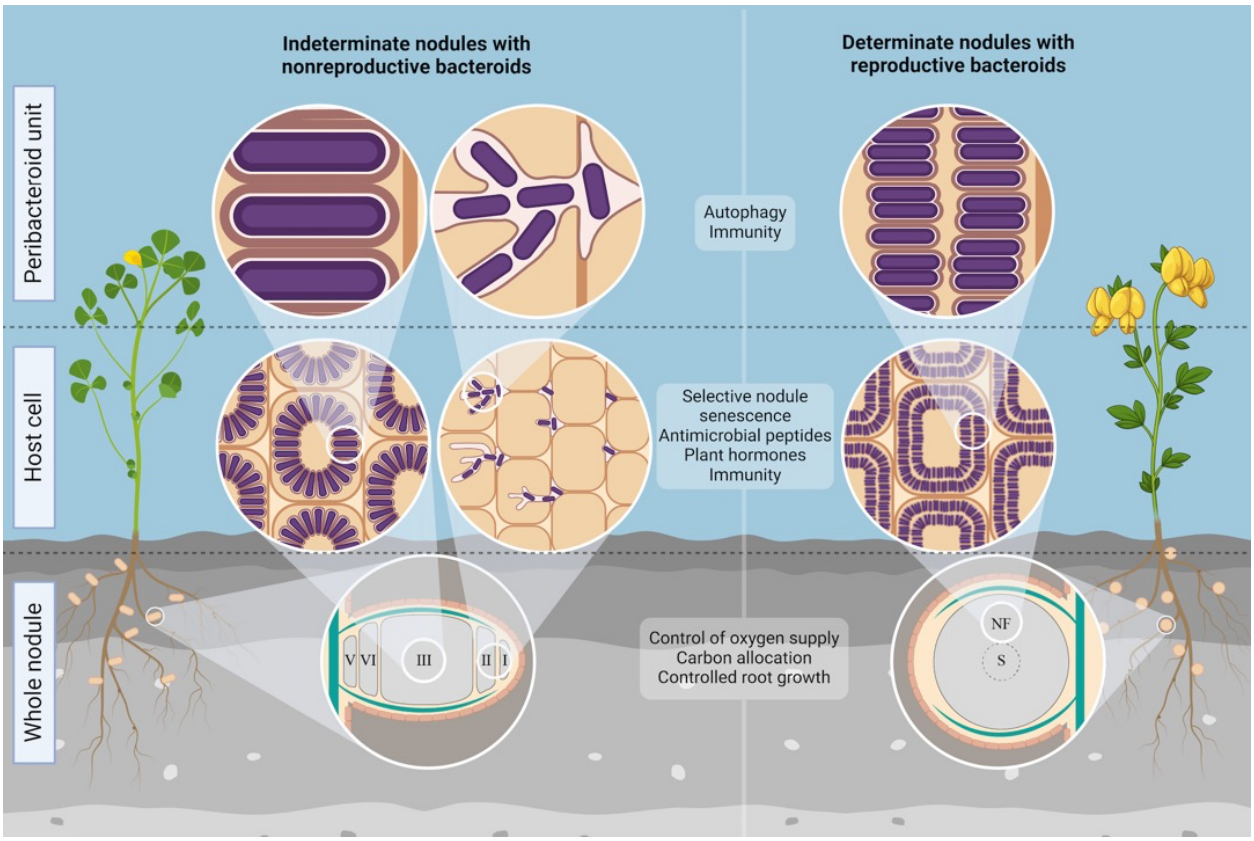

Figure 3. Post-infection control via sanctions and other mechanisms across the compartmentalized structure of the symbiosis. In **indeterminate nodules** (left, bottom), rhizobia (purple) often terminally differentiate into bacteroids in a spatial series (Zones II-V) and cannot escape the nodule[177], but undifferentiated rhizobia (Zone I) can be released upon nodule senescence[178]. Within peribacteroid units (shown encased in a dark brown peribacteroid membrane) are nitrogen fixing bacteroids that are often nonreproductive[128] (Zone III). These hosts appear to exert a form of whole-nodule sanctions. However, in a nodule infected by more than one strain, it is unknown how sanctions could target less-effective rhizobia because N_2 fixing bacteroids in Zone III are separated from reproductive clonemates in Zone I[106]. For many, but probably not all, **determinate nodules**[85] (right, bottom), N_2 fixing bacteroids in the nitrogen fixation Zone (NF) can escape back into the soil during nodule senescence by initiating a

449 senescent zone at the nodule core (S). In a nodule infected by more than one strain, sanctions can
450 directly target less-effective rhizobia because N_2 fixing bacteroids are reproductive[107]. Sanctions
451 likely occur via changes or breakdown of the symbiosome membrane[156], which releases
452 bacteroids to the hostile plant cytosol[157]. Host immunity and autophagy (i.e., degradation of
453 intracellular components, top) are both potential functions that could further mediate host
454 selection against ineffective rhizobia[165,168,169,179].
455

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