# *Controversies in Science*

# Coevolution in *Rhizobium*-Legume Symbiosis?

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Legume nodules, specialized structures for nitrogen fixation, are probably the result of coevolution of plants and ancestral rhizobia. Among the evolutionary processes leading to legume radiation and divergence, coevolution with rhizobia might have occurred. Alternatively, bacteria could have been constantly selected by plants, with bacteria slightly influencing plant evolution (required to fulfill the criteria for a coevolutionary hypothesis). Evidence of bacterial effects on plant evolution is scarce but being searched for. Bacterial genetic plasticity may be indicative of the large capacity of *Rhizobium* to adapt to legumes. Events such as symbiotic replacement, easy recruitment of symbiotic bacteria by legume plants, and lateral transfer of symbiotic genes seem to erase the coevolutionary or selected relationships in rhizobial-legume symbiosis. In particular, the hypotheses proposed are (1) *Rhizobium* replaced *Bradyrhizobium* in a few hosts of the Phaseoleae tribe, *Phaseolus vulgaris* and *P. coccineus*; (2) *Rhizobium etli* as a species did not coevolve with bean; and (3) β-Proteobacteria replaced α-Proteobacteria in South American mimosas. Novel results on symbiosis suggest a more complex evolutionary process for nodulation that may include multiple organisms, such as mycorrhiza, nematodes, and other bacteria in addition to rhizobia.

## Coevolution

THE TERM COEVOLUTION was used to explain the interactions of butterflies and plants by Ehrlich and Raven in 1964, but a definition of coevolution was not published then. Coevolution includes several concepts (Thompson, 1994; Futuyma, 2005), and complex hypotheses on coevolution have been proposed (Thompson, 1999, 2005). Two of the many definitions are given below: "Coevolution, an evolutionary change in a trait of the individuals in one population in response to a trait of the individuals of a second population" (Janzen, 1980). "Reciprocal genetic change in interacting species owing to natural selection imposed by each on the other" (Futuyma, 1998). I will adhere to a common definition where two or more species jointly evolve in response to selection pressures they exert on one another.

Although nothing seems to have evolved in isolation, coevolution seems inadequate to explain many existing symbioses or mutalisms, and the term coevolution has been overused (Janzen, 1980; Mueller *et al.,* 2008). The applicability of the term in the Ehrlich and Raven pioneer paper on coevolution has been questioned as well as many other processes as critically pointed out in "When is it coevolution?" (Janzen, 1980). In addition to the alternative explanations for coevolution (Janzen, 1980), apparent coevolution may be an artifact of common biogeographical history due to covicariance (Smith *et al.*, 2008).

By establishing symbiotic relationships with eukaryotes, bacteria undergo evolutionary changes different from those of free living bacteria. If the relationship is long-standing and highly interdependent, then the evolutionary histories of both partners become mutually influenced. Coevolution of hosts and symbionts with long-term interdependence of the partners has been observed in the insect–endosymbiotic bacteria symbioses (Clark *et al.*, 2001; Baumann, 2005), in the gut symbioses of termites (Ohkuma *et al.*, 2001) and in mycorrhiza (Brundrett, 2002). Plant pathogenic bacteria provided a genetic basis for coevolution in the gene-for-gene hypothesis (Flor, 1956; Barrett, 1985), and the arms race hypothesis was recognized. Coevolution is a recurrent topic in biological interactions that deal from molecular coevolution as in coevolving positions in protein sequences (Caporaso *et al.*, 2008) and in compensatory substitutions for pathogenic mutations (Easton *et al.*, 2007), coevolution of host cationic antimicrobial peptide and microbial resistance (Peschel and Sahl, 2006), coevolution of rabbits and myxoma virus (Best *et al.*, 2000), to predator-prey coevolution in animals (Gross, 2008). Though many papers have been published on coevolution few deal with *Rhizobium*-legume symbiosis.

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### *Rhizobium* Symbiosis and Coevolution

The Leguminosae is one of the largest families of plants with over 19,000 species. Rhizobia are among the best studied bacteria, not only in terms of their diversity but also in terms of the molecular mechanisms of their interaction with hosts. *Rhizobium*-legume interaction (the terms *Rhizobium* or rhizobia will be used here to cover other genera of nodule-forming bacteria) has become a model for the study of symbiosis, and knowledge of its signaling pathways is accumulating. By fixing nitrogen, rhizobia allow plants to grow under poor nitrogen conditions and stimulate plant growth and yield. Nodulation and nitrogen fixation may have contributed to the large number of widely distributed legume species.

The formation of nitrogen-fixing nodules is a complex process involving bacteria-plant molecular exchanges and complex signaling processes. The refined mechanisms underlying the establishment of symbiosis suggest that a coevolutionary process led to the nitrogen-fixing nodule. Nodule formation is a plant property that seems to have coevolved in legumes and actinorhizal plants (that probably shared favorable traits for nodulation; Doyle, 1998) with their ancestral symbionts. *Rhizobium*-legume symbiosis is established every generation from soil bacteria, and key-lock models (Relic et al., 1994) or "handshaking" (Thompson, 2005) and "sweet talking" (Cullimore and Dénarié, 2003) have been proposed as means to select rhizobia and counterselect other bacteria.

Biogeographical analyses of reported data on *Rhizobium* indicate that symbiotic bacteria evolved with their hosts in the centres of legume origin and diversification (reviewed in Martínez-Romero and Caballero-Mellado, 1996). The coexistence of pea genotypes and specific *Rhizobium* strains in the same region (south Turkey) was a basis for establishing the notion of coevolution in the legume-*Rhizobium* associations in ecological times (Lie et al., 1987). Coevolution was considered as an explanation for the specificity in their interactions (Lie et al., 1987; Aguilar et al., 2004). A discussion on *Bradyrhizobium* and legume coevolution was published by Thompson (2005), based mainly on the effects of symbiosis on plant growth obtained only with particular combinations of bacterial strains and host legumes and by a not very clear argument on amino acid complementarity. The fact that *nif* genes (responsible for nitrogen fixation) seem to be older than *nod* (nodulation) genes (Hennecke et al., 1985) suggested that nitrogen-fixing capacity "is an ancient property which predisposed their progenitors for coevolution with legume hosts" (Provorov, 1998). A "tightly coordinated bacteria-plant coevolution for nodulation" is proposed based on "their parallel variation in the centers of plant origin" (Provorov, 1998; Provorov et al., 2008). A geographical mosaic model for coevolution was tested for *Amphicarpaea* symbioses, and the geographic differentiation seemed smaller in *Rhizobium*-legume symbiosis compared to pathogen interactions (Parker, 1999).

Coevolution may be reflected by the parallel and congruent phylogenies of the host and the bacterial endosymbiont (Futuyma, 2005), but this phylogenetic congruence may be due to a common biogeographic history not involving coevolution (Smith et al., 2008) or due to other causes. Bacterial and legume phylogenies have been found not to be parallel, and the explanation was that legume-*Rhizobium* interaction is not an obligate symbiosis (Young and Johnston, 1989). From reported data Thompson (2005) deduced that "molecular phylogenetic studies suggest that rhizobial lineages have not simply coevolved with different legume lineages." This observation is further supported with new examples of rhizobial lineages not coevolving with hosts—*Medicago sativa* (alfalfa) and *Sinorhizobium meliloti* (the most studied rhizobial species)—although specificity traits have been identified in both the plant and the bacteria and plant signals determine bacterial differentiation (Mergaert et al., 2006). Host specialization could have been by selection of biovars (different symbiotic specificities a single bacterial lineage may harbor) (Bena et al., 2005). The discovery of two new biovars of *S. meliloti*, one capable of efficiently nodulating *Medicago laciniata* (Villegas et al., 2006) and the other *Phaseolus* (Mnasri et al., 2007), suggests that it is the biovars and not the chromosomal lineages that coevolve with hosts, but further research is needed to support this proposal. The bacteria from *M. laciniata* nodules were indistinguishable by chromosomal markers from bacteria nodulating alfalfa but were distinct in the sequence of some symbiotic genes. *S. fredii*, *S. terangae*, *S. saheli*, *Mesorhizobium amorphae,* and *Mesorhizobium tianshanense* have different biovars with different specificities (Boivin et al., 1997; Lorquin et al., 1997; Donate-Correa et al., 2007; Mnasri et al., 2007; Rivas et al., 2007). We suppose that *Rhizobium etli* (the most common bean symbiont in its site of origin) as a species did not coevolve with bean; instead, biovar phaseoli seems to have evolved with beans (Plant Selection of Bacteria and Symbiont Replacement sections). On the other hand, *Sinorhizobium* chromosomal speciation leading to *S. medicae* (nodulating preferentially *Medicago truncatula*) and *S. meliloti* (*Medicago sativa* symbiont) and selection of *Medicago* species (*M. truncatula* or *M. sativa*) could have both occurred at the same time, within a large estimated range of time (Bena et al., 2005). This analysis should be extended to include the symbioses of *S. meliloti* (with different biovars) and its other hosts.

### Plant Selection of Bacteria

The environment, biotic and abiotic conditions may strongly influence the selection of bacterial strains or species that exist in the soil. From these bacteria, the plant selects particular strains to form nodules (Martínez-Romero et al., 1991; Mnasri et al., 2007; Rangin et al., 2008). Rhizobial diversity provides a pool of bacteria to be selected by the plants, and a single, few, or many bacterial cells may fit individual plant variability and also survive different environmental conditions fluctuating over time and space. *M. sativa* cultivars had a significant effect on the bacterial symbiont diversity (Paffetti et al., 1998). Different *Medicago* plant lines select their symbiotic partners differently (Rangin et al., 2008). *Rhizobium galegae* diversity matched the diversity of the hosts in the Caucasus, the center of origin of *Galega* plants (Andronov et al., 2003). Plant variability influenced the genetic structure of *Rhizobium leguminosarum* bv. viciae nodulating pea (Depret and Laguerre, 2008). Bacteria most suited for a host are more competitive in forming nodules and become dominant nodule occupants (Martínez-Romero et al., 1991; Rangin et al., 2008; Elliott et al., 2009). At nodule senescence the favored and competitive bacterial population may become enriched in soil

(Paau *et al.*, 1980; Aguilar *et al.*, 2004). *M. truncatula* might influence *Sinorhizobium* soil population according to its symbiotic preferences (Rangin *et al.*, 2008). The consequence of plant selection would be the enrichment of particular bacteria by the plant. If the bacteria contributed enough nitrogen to the plant, then there would be enough seed with good affinities for such bacteria. With this ongoing selection process at every plant generation, we could expect as an outcome increased specificity in *Rhizobium*-legume interactions, which explains why specific bacteria nodulating a legume are found locally. Soybean and its nodulating bacteria are found in Asia, where soybean originated (but not in other regions, where introduced soybeans required the introduction of bacterial symbionts); *R. etli* bv. phaseoli strains nodulating beans are found in the Americas, where beans originated (Segovia *et al.*, 1993); and some geographically isolated bean cultivars in Argentina are nodulated more efficiently by local bacteria than by outsiders (Aguilar *et al.*, 2004). In Argentina, the coevolution of plant and bacteria has been proposed (Aguilar *et al.*, 2004). To explain legume plant cooccurrence and their affinities, a hypothesis different from coevolution is proposed here in which plants select bacteria by having Nod D proteins with affinities for plant flavonoids (flavonoids are also key components in defense responses), by producing adequate Nod factor(s), by not eliciting defense responses (with adequate lipopolysaccharides or other extracellular components, type III secretion systems, Marie *et al.*, 2001), by growing with the nutrients provided by the plant (Hynes and O'Connell, 1990; Soedarjo *et al.*, 1994; Rosenblueth *et al.*, 1998), or by surviving phytoalexins, other plant antimicrobials and defense responses (González-Pasayo and Martínez-Romero, 2000). Bacteria resistant to antibiotics are easily obtained in the laboratory, and resistant pathogens are encountered shortly after any antibiotic is used in clinics. Similarly, plants may select bacteria resistant to phytoalexins and other antimicrobials. On the other hand, it seems unlikely that *Rhizobium* affects the evolution of some of the genes involved in antimicrobial production or plant defense that have important roles in pathogen interactions.

Nod gene phylogenies seem to follow legume phylogenies (Suominen *et al.*, 2001), and genes for Nod factor production in *Rhizobium* seem to be reshuffling to provide many variants of Nod factors to contend with plant variability. Purifying selection or a selective sweep was found to occur in the nodulation gene region in *S. meliloti* and *S. medicae* (Bailly *et al.*, 2006). Diversity of Nod factors produced may be a good indication of rhizobia adjusting to plants. While there is no strict correlation between type of Nod factor and plant specificity, there are preferences (Dénarié *et al.*, 1996; Roche *et al.*, 1996). It is remarkable that *Aeschynomene* plants do not need Nod factors for nodulation (Giraud *et al.*, 2007), suggesting that Nod factors were late acquisitions in nodule formation.

Further plant selection in the long term could lead to bacterial speciation. *Galega officinalis* and *G. orientalis* are preferentially nodulated by the respective biovars of *R. galegae*, which may be considered subspecies because of the large genetic differences between them (Terefework *et al.*, 2001). This raises the question, has subspeciation been host driven? Similarly, in *Mesorhizobium huakuii,* evolution of subspecies could be related to host specificity and geographical isolation (Nuswantara *et al.*, 1999).

## Plant Coevolution with *Rhizobium*?

The largely unexplored aspect of *Rhizobium*-legume coevolutionary hypothesis is if the bacteria actually influence the evolution of traits in the plant. Plant evolution may be in relation to reproductive, geographical, and environmental constraints, and there must be a stronger selective pressure by pathogens and factors other than rhizobia (Parker, 1999). However, "plants should experience constant selection to nodulate preferentially with bacteria that are especially beneficial" (Parker, 1999). We may imagine an ecological scenario where legume evolution driven by *Rhizobium* has occurred when plants colonized new environments, especially with low nitrogen and low rhizobial diversity and plant survivors were the variants of the population that better fitted the available rhizobia; rhizobial plasticity in turn provided new variants to be again selected by the new generation of plants that have symbiotic traits more suitable for the selected bacteria, and so on.

There have been very few studies on plant evolution driven by *Rhizobium*. Evidence for this has been searched for in plant phenotypes, generalists versus specialists (Parker, 1999), and in genes participating in the symbiotic process. A detailed and well-performed analysis of the evolution of NORK sequences using a large sample of *Medicago* species showed no evidence of selection of NORK gene that encodes a leucine-rich repeat kinase receptor controlling the infection by symbiotic rhizobia and endomycorrhizal fungi in legumes but not nematode infections. A positive selection in NORK tends not to be associated with changes in rhizobial specificity, indicating that this plant gene was probably not involved in evolving rhizobial preferences (De Mita *et al.*, 2007). In *Medicago* plants selection for specialization to single biovars was observed (Bena *et al.*, 2005), but the plant genes involved have not been identified. In *Galega* there was evidence for positive selection with the Nod factor receptor NFR5 (LysM type receptor) and a negative selection with symRK (equivalent to NORK) sequences (Lindström *et al.*, 2008), suggesting a possible coevolution of LysM type receptors and Nod factors. LysM domains may also be involved in pathogen recognition (Kaku *et al.*, 2006). The two biovars *orientalis* and *officinalis* of *R. galegae* may have coevolved with *G. orientalis* and *G. officinalis,* respectively. The codivergence in *Medicago-Sinorhizobium* symbiosis has been suggested (Bena *et al.*, 2005) without discarding a coevolutionary process, taking as a reference the supposedly coevolutionary process of bean and *R. etli* (Aguilar *et al.*, 2004) that could be alternatively explained as covicariance or as long-distance dispersal of rare genotypes.

While there is good evidence of plant selection of bacteria and bacterial symbiotic traits, there is little evidence of the effects of rhizobia on plant evolution or on the evolution of plant traits.

## Events Disrupting Selected or Coevolved Relationships

The different rhizobial species encountered in the nodules of a legume species may be due to the lack of specificity of legumes, especially in tropical trees (Moreira *et al.*, 1998; our own unpublished results), and due to the promiscuity of some bacteria such as *Sinorhizobium* sp. NGR234 (Perret *et al.*,

2000) and *Rhizobium tropici* (Hernandez-Lucas et al., 1995). Bacterial generalists seem to evolve with many host legumes. In addition, rhizobia may evolve in niches or sites independent of legumes. *Rhizobium* associations with nonlegumes and in other environments will have effects on *Rhizobium* evolution, but these aspects will not be covered here.

In spite of a seemingly constant plant selection of bacteria in *Rhizobium*-legume interactions, there is a wide degree of flexibility and novel symbiotic relationships seem to be frequently established. Symbiont replacement (or lateral transfer of symbionts within hosts), rapid selection of novel symbionts by legumes, and lateral transfer of symbiotic genes are probably responsible for blurring the selected or coevolutionary relationships of *Rhizobium* with their host legumes. New examples for the causes of this divorce in *Rhizobium*-legume symbiosis are given in the following sections.

### Symbiont Replacement

Symbiont replacement has been documented in insects (Lefevre et al., 2004; Conord et al., 2008; Lamelas et al., 2008) and in other symbioses. A case in study is presented, which was detected after years of work in this area. Many plants in the Phaseoleae tribe are nodulated by bradyrhizobia, and although these plants are generally considered promiscuous or of little specificity, they show preferences for some bacterial groups. Legume species in the following genera belonging to the Phaseoleae are nodulated by *Bradyrhizobium*: *Vigna*, *Pachyrhizus*, *Glycine max* (soybean), *Macroptilium* (siratro), *Amphicarpaea*, *Mucuna*, *Canavalia*, *Cajanus*, *Calopogonium*, *Centrosema*, *Clitoria*, *Galactia*, *Neonotonia*, and *Rhynchosia* (Xu et al., 1995; Minamisawa et al., 1997; Mandimba, 1998; Chen et al., 2000; Parker, 2001, 2002, 2004; Krasova-Wade et al., 2003; Parker et al., 2004; Menna et al., 2006; Appunu et al., 2008; Steenkamp et al., 2008; Vinuesa et al., 2008; Zhang et al., 2008). Several *Phaseolus* species, *P. parvulus*, *P. pedicellatus*, *P. pauciflorus*, *P. acutifolius*, and wild and cultivated *P. lunatus*, are also nodulated by *Bradyrhizobium* in their sites of origin (Parker, 2002; Ormeño-Orrillo et al., 2006). Remarkable exceptions are *P. vulgaris* and *P. coccineus* (both wild-type and cultivated varieties), which are nodulated by *Rhizobium* (*R. etli* and *R. gallicum*) and not by *Bradyrhizobium* (Martínez et al., 1985; Silva et al., 2003). *R. etli* and *R. gallicum* are related species, but *R. gallicum* seems older and more widely distributed than *R. etli* (Silva et al., 2005). There was probably a symbiont replacement in a few related *Phaseolus* species (*P. vulgaris* and *P. coccineus*), and *Rhizobium* would have substituted *Bradyrhizobium* in these hosts.

*R. etli* is the most common symbiont found in *P. vulgaris* nodules on both wild and cultivated varieties in native areas (Piñero et al., 1988; Segovia et al., 1993; Martínez-Romero, 2003), and it is more competitive for bean nodulation than other species (Martínez-Romero and Rosenblueth, 1990). Some *Phaseolus* accessions nodulate preferentially with selected *R. etli* strains, and regional *Rhizobium* isolates are more efficient with bean cultivars from the same region (Montealegre et al., 1995; de Oliveira et al., 1998; Martínez-Romero et al., 1998; Rosas et al., 1998; Bernal and Graham, 2001; Aguilar et al., 2004). We discovered a new biovar in *R. etli* and argued that *R. etli* bv. mimosae were older than bv. phaseoli, which have specificity for beans (Wang et al., 1999b). The analysis of the genome of *R. etli* bv. phaseoli led

to the supposition that the phaseoli symbiotic plasmid was a later acquisition in *R. etli* (González et al., 2006), which supports the hypothesis of bv. mimosae being older. Some American mimosas share geographic distribution with some *Phaseolus* species, and this provided opportunities to share symbiotic bacteria and substitute *Bradyrhizobium*. We suppose that *R. gallicum* and *R. etli* were mimosa symbionts that switched to nodulate *Phaseolus*. Plasmid plasticity probably led to the generation of new symbiotic plasmids such as the phaseoli, which are better suited for bean nodulation and competitiveness. The two types of plasmids, phaseoli and mimosae, exist alternatively in the same bacterial lineage in closely related strains (Wang et al., 1999b).

*R. etli* bv. mimosae *nod* genes resemble *R. gallicum* bv. gallicum *nod* genes (Elliott et al., 2009). The *R. gallicum nod* genes belong to a large group of *nod* genes that are found in American rhizobia nodulating mimosas (Fig. 5 in Rincón-Rosales et al., 2009). When *R. gallicum* strains were found in beans in France, they were considered natural mimosa symbionts since *Phaseolus* is not a native of France (Amarger et al., 1997). The analysis of *nod* genes supports this argument (Rincón-Rosales et al., 2009). Differences among *nod*C genes (Aguilar et al., 2004) and other differences in phaseoli plasmids have been reported (Martínez et al., 1985; Silva et al., unpublished), which may be linked to genetic traits involved in the adaptation to nodulating a specific bean cultivar (Aguilar et al., 2004). "The occurrence of coevolution of the bacterial species *R. etli* with *Phaseolus*" is proposed to have taken place (Aguilar et al., 2004), but we propose an alternative hypothesis: no coevolution but selection of bacteria by *P. vulgaris*, selecting *R. etli* variants with adequate symbiotic genes for bean nodulation with probable loss of mimosa symbiotic genes.

Rapid changes in a few genes may be responsible for adaptation of bacteria to hosts (Ebert, 1998). We performed an experiment with *P. vulgaris* cultivar Negro Xamapa in flasks with agar and Fahraues medium as described (Rogel et al., 2001) and *Burkholderia phymatum* STM815 as inoculum. This strain was described as a nodulating bacterium of mimosas (Elliott et al., 2007) originally recovered from *Machaerium lunatum* nodules (Moulin et al., 2001). Most nodules were small and white but a few red nodules could be recovered from bean plants, and this capacity was stably maintained in further inoculation experiments. The identity of the bacteria recovered from red bean nodules was confirmed as STM815.

*Rhizobium mongolense* (also described as *R. gallicum* bv orientale; Silva et al., 2005) nodulates *Medicago ruthenica* native to inner Mongolia (van Berkum et al., 1998), while other *Medicago* species are nodulated by *Sinorhizobium* (Eardly et al., 1990; Bena et al., 2005; Bailly et al., 2007; Silva et al., 2007). This could be another example of symbiotic replacement of *Sinorhizobium* by *Rhizobium*. *Burkholderia* nodulating mimosas in South America (Elliott et al., 2009) could also be examples of symbiont replacement where β-Proteobacteria displaced α-Proteobacteria, the most common legume symbionts.

### Recruitment of Novel Symbionts

"When species are introduced into new regions, they often leave behind their old enemies and mutualists" (Thompson, 2005). Some examples of rapid selection of new symbiotic bacteria by legumes are the following:

*Leucaena* plants are native to Mexico. They are fast-growing trees with a large capacity to fix nitrogen and have been introduced in different areas such as Hawaii, Asia, and South America. In these areas leucaenas are nodulated by bacteria different from those found in native areas (Wang *et al.*, 1999a; López-López *et al.*, 2009). Bradyrhizobia nodulating trees in the Philippines seem to be introduced and their recombinants may be among the novel bacteria nodulating these legumes (Andam and Parker, 2008). Some soybeans in Africa are able to be nodulated by native bradyrhizobia (Abaidoo *et al.*, 2000), and beans are nodulated by diverse bacteria in introduced areas (Martínez-Romero *et al.*, 1991; Herrera-Cervera *et al.*, 1999). Both in North America and South America, where alfalfa plants have been introduced, ineffective nodules have been found produced by *Rhizobium* strains such as Or 191 (Eardly *et al.*, 1992; Del Papa *et al.*, 1999). It seems that novel symbiotic associations with no coevolved bacterial symbionts have been frequently established in the history of legumes. This is in pace with one of the arguments raised by Janzen when questioning "When is it coevolution?"

## Lateral Gene Transfer

Symbiotic genes are plasmid borne or located in symbiotic islands and have an evolutionary history different from chromosomal genes (Haukka *et al.*, 1998; Toledo *et al.*, 2003; Vinuesa *et al.*, 2005b; Lloret *et al.*, 2007; Rincón-Rosales *et al.*, 2009). Being on plasmids symbiotic genes may be transferred among bacteria, and recombination and rearrangements in plasmids may lead to fast evolution of plasmids. In some cases, biovars may be equated to symbiotic plasmids or symbiotic islands (Wang *et al.*, 1999b; Vinuesa *et al.*, 2005a). The lack of correlation between chromosomal and symbiotic genes indicates that they have different evolutionary histories, which could mean that the "nodulation ability in rhizobia originated recently during the coevolution with the legume hosts" (Provorov, 1998).

The evolution of *Medicago* symbiotic bacteria is driven by horizontal gene transfer and recombination, mainly in the megaplasmid that carries symbiotic genes (Bailly *et al.*, 2006, 2007). The role of lateral transfer in the spread of symbiotic capabilities among bacteria has been recognized (Segovia *et al.*, 1991, 1993; Rogel *et al.*, 2001; Bailly *et al.*, 2007). One of the most remarkable examples of gene transfer occurring in nature is the transfer of nodulating capabilities of *Mesorhizobium loti* (in New Zealand) to native mesorhizobia, which became nodulating bacteria (Sullivan *et al.*, 1995; Sullivan and Ronson, 1998). Similar results were obtained in Australia, but the recipient *Mesorhizobium* were less efficient in fixing nitrogen than the original donor strain (Nandasena *et al.*, 2007). Some β-Proteobacterial species in the *Burkholderia* and *Cupriavidus* genera seemed to have long ago acquired *nod* genes from α-Proteobacteria (Moulin *et al.*, 2001; Amadou *et al.*, 2008). Lateral transfer of *nod* genes allows the creation of novel symbionts that are not coevolved partners to legumes but fortuitous recipients of symbiotic information (Sullivan *et al.*, 1995; Rogel *et al.*, 2006; Nandasena *et al.*, 2007). Lateral transfer of genes has been discussed with a different interpretation: "The plant-bacteria coevolution is suggested to be based on intensive intergenomic recombination and horizontal transfer of genes in rhizobia populations" (Provorov, 1998).

## New Perspectives and Conclusions

It is known that plants and their bee pollinators coevolved, but there are some remarkable changes in species interactions (Lunau, 2004). Some of the biological mechanisms disrupting partner relationships of plants and their bee pollinators have been described (Lunau, 2004) as have been analyzed for legume symbioses in the previous sections.

Interestingly, rhizobia use common plant signaling pathways that are used for mycorrhiza (Gianinazzi-Pearson and Dénarié, 1997) and nematodes (Weerasinghe *et al.*, 2005), with different overlapping components. Some authors consider that as mycorrhizal interactions were established prior to rhizobial nodules, in evolutionary times rhizobia used an already established signaling pathway. Among the components in rhizobial symbiosis not required for mycorrhizal symbioses are Nod factor receptors, probably the GRASS transcriptional factors (Kaló *et al.*, 2005; Smit *et al.*, 2005), and others that participate in nodule formation. Some GRASS factors are important for disease resistance in tomato (Mayrose *et al.*, 2006). The Nod factor receptors required in symbiosis with rhizobia are used by nematodes for infection (Weerasinghe *et al.*, 2005). Evolution of symbiotic traits in plants might have been driven by rhizobia, mycorrhiza, nematodes, and perhaps other bacterial endophytes and even pathogens all together. Multispecies coevolutionary processes are known to occur (Woolhouse *et al.*, 2002; Thompson, 2005), and in nature plants face all these organisms. A multiorganism coevolutionary hypothesis has not been previously considered for nodulation in *Rhizobium*-legume symbiosis and would be difficult to study.

Host selective pressures and lateral gene transfers are key mechanisms that shape the genetic structure of symbiotic microorganisms (Tibayrenc, 1996). "Coevolution has proven difficult to demonstrate rigorously" in host pathogen interactions (Woolhouse *et al.*, 2002). A non–*sensu stricto* coevolutionary process was described for plant and pathogen evolution considering that host plants evolved and the pathogens coevolved with them (Parlevliet, 1979). Such a process could be applicable in *Rhizobium*-legume symbiosis. Coincidentally, Barrett (1983) questioned the fundamentals that are taken for granted to support coevolutionary processes in mycorrhizal and lichen symbioses. Resistance (*R*) genes may have a role in rhizobial symbiosis (Samac and Graham, 2007). A gene-for-gene hypothesis similar to that described for plant pathogenic interactions is proposed for *Rhizobium*-legume coevolution (Provorov *et al.*, 2008) but needs further validation. More recently, a mathematical model was published, considering the effects of genuine rhizobial mutualists and symbiotic cheaters on seed production and concluding that frequency-dependent selection in cooperation with positive plant feedback is required to avoid rhizobial cheaters (Provorov and Vorobyov, 2008).

I conclude that in many cases there may not be a coevolution of legume and *Rhizobium* but a constant selection by the plant of microsymbionts that have large and fast capabilities for genetic change or for acquiring symbiotic genes (Flores *et al.*, 1988; Sullivan *et al.*, 1995; Mavingui *et al.*, 1997; Andam and Parker, 2008). Rapid changes in symbiotic plasmids or symbiotic islands could have been instrumental in enabling bacteria to adjust and adapt to the diversification burst of legumes that occurred during their radiation. Evolution of symbiotic

plasmids or islands or of symbiotic genes may occur independent of the chromosome and account for plant specificity (Aguilar *et al.*, 2004; Silva *et al.*, 2005; Vinuesa *et al.*, 2005b; Lloret *et al.*, 2007; Provorov *et al.*, 2008; Rincón-Rosales *et al.*, 2009).

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## Disclosure Statement

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