

## 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)  $\beta$ -Proteobacteria replaced  $\alpha$ -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 mutualisms, 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 covariance (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.

### 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 (Futuyama, 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. terangaie*, *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

(Paa *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* (Bailey *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 covariation 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* (siratiro), *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 mimosasymbionts 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 mimosasymbionts 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 *nodC* 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 mimosasymbiotic 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  $\beta$ -Proteobacteria displaced  $\alpha$ -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  $\beta$ -Proteobacterial species in the *Burkholderia* and *Cupriavidus* genera seemed to have long ago acquired *nod* genes from  $\alpha$ -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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### References

- Abaidoo, R.C., Keyser, H.H., Singleton, P.W., and Borthakur, D. (2000). *Bradyrhizobium* spp. (TGx) isolates nodulating the new soybean cultivars in Africa are diverse and distinct from bradyrhizobia that nodulate North American soybeans. *Int J Syst Evol Microbiol* **50**, 225–234.
- Aguilar, O.M., Riva, O., and Peltzer, E. (2004). Analysis of *Rhizobium etli* and of its symbiosis with wild *Phaseolus vulgaris* supports coevolution in centers of host diversification. *Proc Natl Acad Sci USA* **101**, 13548–13553.
- Amadou, C., Pascal, G., Mangenot, S., Glew, M., Bontemps, C., Capela, D., Carrere, S., Cruveiller, S., Dossat, C., Lajus, A., Marchetti, M., Poinot, V., Rouy, Z., Servin, B., Saad, M., Schenowitz, C., Barbe, V., Batut, J., Medigue, C., and Masson-Boivin, C. (2008). Genome sequence of the beta-rhizobium *Cupriavidus taiwanensis* and comparative genomics of rhizobia. *Genome Res* **18**, 1472–1483.
- Amarger, N., Macheret, V., and Laguerre, G. (1997). *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii* sp. nov., from *Phaseolus vulgaris* nodules. *Int J Syst Bacteriol* **47**, 996–1006.
- Andam, C.P., and Parker, M.A. (2008). Origins of *Bradyrhizobium* nodule symbionts from two legume trees in the Philippines. *J Biogeogr* **35**, 1030–1039.
- Andronov, E.E., Terefework, Z., Roumiantseva, M.L., Dzyubenko, N.I., Onichtchouk, O.P., Kurchak, O.N., Dresler-Nurmi, A., Young, J.P.W., Simarov, B.V., and Lindström, K. (2003). Symbiotic and genetic diversity of *Rhizobium galegae* isolates collected from the *Galega orientalis* gene center in the Caucasus. *Appl Environ Microbiol* **69**, 1067–1074.
- Appunu, C., N'Zoue, A., and Laguerre, G. (2008). Genetic diversity of native bradyrhizobia isolated from soybeans (*Glycine max* L.) in different agricultural-ecological-climatic regions of India. *Appl Environ Microbiol* **74**, 5991–5996.
- Bailly, X., Olivieri, I., Brunel, B., Cleyet-Marel, J.-C., and Bena, G. (2007). Horizontal gene transfer and homologous recombination drive the evolution of the nitrogen-fixing symbionts of *Medicago* species. *J Bacteriol* **189**, 5223–5236.
- Bailly, X., Olivieri, I., De Mita, S., Cleyet-Marel, J.-C., and Béna, G. (2006). Recombination and selection shape the molecular diversity pattern of nitrogen-fixing *Sinorhizobium* sp. associated to *Medicago*. *Mol Ecol* **15**, 2719–2734.
- Barrett, J.A. (1983). Plant-fungus symbioses. In *Coevolution*. D.J. Futuyama and M. Slatkin, eds. (Sinauer Associates, Sunderland, MA), pp. 137–160.
- Barrett, J.A. (1985). The gene-for-gene hypothesis: parable or paradigm. In *Ecology and Genetics of Host-Parasite Interactions*. D. Rollinson and R.M. Anderson, eds. (Academic Press, New York), pp. 215–225.
- Baumann, P. (2005). Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu Rev Microbiol* **59**, 155–189.
- Bena, G., Lyet, A., Huguet, T., and Olivieri, I. (2005). *Medicago-Sinorhizobium* symbiotic specificity evolution and the geographic expansion of *Medicago*. *J Evol Biol* **18**, 1547–1558.
- Bernal, G., and Graham, P.H. (2001). Diversity in the rhizobia associated with *Phaseolus vulgaris* L. in Ecuador, and comparisons with Mexican bean rhizobia. *Can J Microbiol* **47**, 526–534.
- Best, S.M., Collins, S.V., and Kerr, P.J. (2000). Coevolution of host and virus: cellular localization of virus in myxoma virus infection of resistant and susceptible European rabbits. *Virology* **277**, 76–91.
- Boivin, C., Ndoye, I., Lortet, G., Ndiaye, A., De Lajudie, P., and Dreyfus, B. (1997). The *Sesbania* root symbionts *Sinorhizobium saheli* and *S. teranga* bv. *sesbaniae* can form stem nodules on *Sesbania rostrata*, although they are less adapted to stem nodulation than *Azorhizobium caulinodans*. *Appl Environ Microbiol* **63**, 1040–1047.
- Brundrett, M.C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytol* **154**, 275–304.
- Caporaso, J.G., Smit, S., Easton, B.C., Hunter, L., Huttley, G.A., and Knight, R. (2008). Detecting coevolution without phylogenetic trees? Tree-ignorant metrics of coevolution perform as well as tree-aware metrics. *BMC Evol Biol* **8**, 327.
- Chen, L.Sh., Figueredo, A., Pedrosa, F.O., and Hungria, M. (2000). Genetic characterization of soybean rhizobia in Paraguay. *Appl Environ Microbiol* **66**, 5099–5103.
- Clark, J.W., Hossain, S., Burnside, C.A., and Kambhampati, S. (2001). Coevolution between a cockroach and its bacterial endosymbiont: a biogeographical perspective. *Proc R Soc Lond* **268**, 393–398.
- Conord, C., Despres, L., Vallier, A., Balmand, S., Miquel, C., Zundel, S., Lemperiere, G., and Heddi, A. (2008). Long-term evolutionary stability of bacterial endosymbiosis in Curculionoidea: additional evidence of symbiont replacement in the Dryophthoridae family. *Mol Biol Evol* **25**, 859–868.
- Cullimore, J., and Dénarié, J. (2003). How legumes select their sweet talking symbionts. *Science* **302**, 575–578.
- Del Papa, M.F., Balague, L.J., Sowinski, S.C., Wegener, C., Segundo, E., Abarca, F.M., Toro, N., Niehaus, K., Puhler, A., Aguilar, O.M., Martínez-Drets, G., and Lagares, A. (1999). Isolation and characterization of alfalfa-nodulating rhizobia present in acidic soils of central Argentina and Uruguay. *Appl Environ Microbiol* **65**, 1420–1427.
- De Mita, S., Santoni, S., Ronfort, J., and Bataillon, T. (2007). Adaptive evolution of the symbiotic gene *NORK* is not correlated with shifts of rhizobial specificity in the genus *Medicago*. *BMC Evol Biol* **7**, 210.
- Dénarié, J., Debelle, F., and Promé, J.C. (1996). *Rhizobium* lipochitooligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis. *Annu Rev Biochem* **65**, 503–535.
- de Oliveira, W.S., Meinhardt, L.W., Sessitsch, A., and Tsai, S.M. (1998). Analysis of *Phaseolus-Rhizobium* interactions in a subsistence farming system. *Plant Soil* **204**, 107–115.
- Depret, G., and Laguerre, G. (2008). Plant phenology and genetic variability in root and nodule development strongly influence genetic structuring of *Rhizobium leguminosarum* biovar *viciae* populations nodulating pea. *New Phytol* **179**, 224–235.

- Donate-Correa, J., León-Barrios, M., Hernández, M., Pérez-Galdona, R., and del Arco-Aguilar, M. (2007). Different *Mesorhizobium* species sharing the same symbiotic genes nodulate the shrub legume *Anagyris latifolia*. *Syst Appl Microbiol* **30**, 615–623.
- Doyle, J.J. (1998). Phylogenetic perspectives on nodulation: evolving views of plants and symbiotic bacteria. *Trends Plant Sci* **3**, 473–478.
- Eardly, B.D., Materon, L.A., Smith, N.H., Johnson, D.A., Rumbaugh, M.D., and Selander, R.K. (1990). Genetic structure of natural populations of the nitrogen-fixing bacterium *Rhizobium meliloti*. *Appl Environ Microbiol* **56**, 187–194.
- Eardly, B.D., Young, J.P.W., and Selander, R.K. (1992). Phylogenetic position of *Rhizobium* sp. strain Or 191, a symbiont of both *Medicago sativa* and *Phaseolus vulgaris*, based on partial sequences of the 16S rRNA and *nifH* genes. *Appl Environ Microbiol* **58**, 1809–1815.
- Easton, B., Maxwell, P., Isaev, A., and Huttley, G. (2007). A probabilistic method to identify compensatory substitutions for pathogenic mutations. 5th Asia Pacific Bioinformatics Conference (APBC 2007), Hong Kong City (Hong Kong-SAR of China), 15–17 Jan 2007. Resource Location: <http://www.cs.hku.hk/apbc2007>.
- Ebert, D. (1998). Experimental evolution of parasites. *Science* **282**, 1432–1435.
- Ehrlich, P.R., and Raven, P.H. (1964). Butterflies and plants: a study in coevolution. *Evolution* **18**, 586–608.
- Elliott, G.N., Chen, W.-M., Chou, J.-H., Wang, H.-C., Sheu, S.-Y., Perin, L., Reis, V.M., Moulin, L., Simon, M.F., Bontemps, C., Sutherland, J.M., Bessi, R., de Faria, S.M., Trinick, M.J., Prescott, A.R., Sprent, J.I., and James, E.K. (2007). *Burkholderia phymatum* is a highly effective nitrogen-fixing symbiont of *Mimosa* spp. and fixes nitrogen ex planta. *New Phytol* **173**, 168–180.
- Elliott, G.N., Chou, J.-H., Chen, W.-M., Bloemberg, G.V., Bontemps, C., Martínez-Romero, E., Velázquez, E., Young, J.P.W., Sprent, J.I., and James, E.K. (2009). *Burkholderia* spp. are the most competitive symbionts of *Mimosa*, particularly under N-limited conditions. *Environ Microbiol* **11**, 762–778.
- Flor, H.H. (1956). The complementary genic systems in flax and flax rust. *Adv Genet* **8**, 29–54.
- Flores, M., González, V., Pardo, M.A., Leija, A., Martínez, E., Romero, D., Piñero, D., Dávila, G., and Palacios, R. (1988). Genomic instability in *Rhizobium phaseoli*. *J Bacteriol* **170**, 1191–1196.
- Futuyma, D.J. (1998). *Evolutionary Biology* (Sinauer Associates, Sunderland, MA).
- Futuyma, D.J. (2005). *Evolution*, 2nd edition (Sinauer Associates, Sunderland, MA).
- Gianinazzi-Pearson, V., and Dénarié, J. (1997). Red carpet genetic programmes for root endosymbioses. *Trends Plant Sci* **2**, 371–372.
- Giraud, E., Moulin, L., Vallenet, D., Barbe, V., Cytryn, E., Avarre, J.-C., Jaubert, M., Simon, D., Cartieaux, F., Prin, Y., Bena, G., Hannibal, L., Fardoux, J., Kojadinovic, M., Vuillet, L., Lajus, A., Cruveiller, S., Rouy, Z., Mangenot, S., Seguren, B., Dossat, C., Franck, W.L., Chang, W.-S., Saunders, E., Bruce, D., Richardson, P., Normand, P., Dreyfus, B., Pignol, D., Stacey, G., Emerich, D., Vermeglio, A., Medigue, C., and Sadowsky, M. (2007). Legumes symbioses: absence of nod genes in photosynthetic bradyrhizobia. *Science* **316**, 1307–1312.
- González, V., Santamaría, R.I., Bustos, P., Hernández-González, I., Medrano-Soto, A., Moreno-Hagelsieb, G., Janga, S.C., Ramírez, M.A., Jiménez-Jacinto, V., Collado-Vides, J., and Dávila, G. (2006). The partitioned *Rhizobium etli* genome: genetic and metabolic redundancy in seven interacting replicons. *Proc Natl Acad Sci USA* **103**, 3834–3839.
- González-Pasayo, R., and Martínez-Romero, E. (2000). Multi-resistance genes of *Rhizobium etli* CFN42. *Mol Plant-Microbe Interact* **13**, 572–577.
- Gross, L. (2008). Predators make (temporary) escape from co-evolutionary arms race. *PLoS Biol* **6**, e75.
- Haukka, K., Lindstrom, K., and Young, J.P.W. (1998). Three phylogenetic groups of *nodA* and *nifH* genes in *Sinorhizobium* and *Mesorhizobium* isolates from leguminous trees growing in Africa and Latin America. *Appl Environ Microbiol* **64**, 419–426.
- Hennecke, H., Kaluza, K., Thöny, B., Fuhrmann, M., Ludwig, W., and Stackebrandt, E. (1985). Concurrent evolution of nitrogenase genes and 16S rRNA in *Rhizobium* species and other nitrogen fixing bacteria. *Arch Microbiol* **142**, 342–348.
- Hernandez-Lucas, I., Segovia, L., Martínez-Romero, E., and Pueppke, S.G. (1995). Phylogenetic relationships and host range of *Rhizobium* spp. that nodulate *Phaseolus vulgaris* L. *Appl Environ Microbiol* **61**, 2775–2779.
- Herrera-Cervera, J.A., Caballero-Mellado, J., Laguerre, G., Tichy, H.V., Requena, N., Amarger, N., Martínez-Romero, E., Olivares, J., and Sanjuan, J. (1999). At least five rhizobial species nodulate *Phaseolus vulgaris* in a Spanish soil. *FEMS Microbiol Ecol* **30**, 87–97.
- Hynes, M.F., and O'Connell, M.P. (1990). Host plant effect on competition among strains of *Rhizobium leguminosarum*. *Can J Microbiol* **36**, 864–869.
- Janzen, D.H. (1980). When is it coevolution? *Evolution* **34**, 611–612.
- Kaku, H., Nishizawa, Y., Ishii-Minami, N., Akimoto-Tomiya, C., Dohmae, N., Takio, K., Minami, E., and Shibuya, N. (2006). Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proc Natl Acad Sci USA* **103**, 11086–11091.
- Kaló, P., Gleason, C., Edwards, A., Marsh, J., Mitra, R.M., Hirsch, S., Jakab, J., Sims, S., Long, S.R., Rogers, J., Kiss, G.B., Downie, J.A., and Oldroyd, G.E.D. (2005). Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. *Science* **308**, 1786–1789.
- Krasova-Wade, T., Ndoye, I., Braconnier, S., Sarr, B., de Lajudie, P., and Neyra, M. (2003). Diversity of indigeneous bradyrhizobia associated with three cowpea cultivars (*Vigna unguiculata* (L.) Walp.) grown under limited and favorable water conditions in Senegal (West Africa). *Afr J Biotech* **2**, 13–22.
- Lamelas, A., Pérez-Brocal, V., Gómez-Valero, L., Gosalbes, M.J., Moya, A., and Latorre, A. (2008). Evolution of the secondary symbiont "*Candidatus serratia symbiotica*" in aphid species of the subfamily lachninae. *Appl Environ Microbiol* **74**, 4236–4240.
- Lefevre, C., Charles, H., Vallier, A., Delobel, B., Farrell, B., and Heddi, A. (2004). Endosymbiont phylogenesis in the dryophthoridae weevils: evidence for bacterial replacement. *Mol Biol Evol* **21**, 965–973.
- Lie, T.A., Ktan, D.G., Engin, M., Pijnenborg, J., and Anlarsal, E. (1987). Coevolution of the legume-*Rhizobium* association. *Plant* **100**, 171–181.
- Lindström, K., Chizhevskaya, E., Fewer, D., and Andronov, E. (2008). Co-evolution in the *Rhizobium*-legume symbioses. PS4-19. Book of abstracts. 8th European Nitrogen Fixation Conference, August 30–September 3, 2008, Gent, Belgium.
- Lloret, L., Ormeño-Orrillo, E., Rincón, R., Martínez-Romero, J., Rogel-Hernández, M.A., and Martínez-Romero, E. (2007). *Ensifer mexicanus* sp. nov. a new species nodulating *Acacia angustissima* (Mill.) Kuntze in Mexico. *Syst Appl Microbiol* **30**, 280–290.

- López-López, A., Rosenblueth, M., Martínez, J., and Martínez-Romero, E. (2009). Rhizobial symbioses in tropical legumes and non-legumes. In *Soil Biology and Agriculture in the Tropics*. P. Dion, ed. (Springer, Berlin), (in press).
- Lorquin, J., Lortet, G., Ferro, M., Mear, N., Dreyfus, B., Prome, J.C., and Boivin, C. (1997). Nod factors from *Sinorhizobium saheli* and *S. teranga* bv. *sesbaniae* are both arabinosylated and fucosylated, a structural feature specific to *Sesbania rostrata* symbionts. *Mol Plant-Microbe Interact* **10**, 879–890.
- Lunau, K. (2004). Adaptive radiation and coevolution—pollination biology case studies. *Org Divers Evol* **4**, 207–224.
- Mandimba, G.R. (1998). Response of soybean crops to *Mucuna pruriens* green manure in symbiosis with cowpea bradyrhizobia in Congo. *Biol Agric Hort* **16**, 15–23.
- Marie, C., Broughton, W.J., and Deakin, W.J. (2001). *Rhizobium* type III secretion systems: legume charmers or alarmers? *Curr Opin Plant Biol* **4**, 336–342.
- Martínez, E., Pardo, M.A., Palacios, R., and Cevallos, M.A. (1985). Reiteration of nitrogen fixation gene sequences and specificity of *Rhizobium* in nodulation and nitrogen fixation in *Phaseolus vulgaris*. *J Gen Microbiol* **131**, 1779–1786.
- Martínez-Romero, E. (2003). Diversity of *Rhizobium-Phaseolus vulgaris* symbiosis: overview and perspectives. *Plant Soil* **252**, 11–23.
- Martínez-Romero, E., and Caballero-Mellado, J. (1996). *Rhizobium* phylogenies and bacterial genetic diversity. *Crit Rev Plant Sci* **15**, 113–140.
- Martínez-Romero, E., Hernandez-Lucas, I., Pena-Cabriaes, J.J., and Castellanos, J.Z. (1998). Symbiotic performance of some modified *Rhizobium etli* strains in assays with *Phaseolus vulgaris* beans that have a high capacity to fix N<sub>2</sub>. *Plant Soil* **204**, 89–94.
- Martínez-Romero, E., and Rosenblueth, M. (1990). Increased bean (*Phaseolus vulgaris* L.) nodulation competitiveness of genetically modified *Rhizobium* strains. *Appl Environ Microbiol* **56**, 2384–2388.
- Martínez-Romero, E., Segovia, L., Mercante, F.M., Franco, A.A., Graham, P., and Pardo, M.A. (1991). *Rhizobium tropici*, a novel species nodulating *Phaseolus vulgaris* L. beans and *Leucaena* sp. trees. *Int J Syst Bacteriol* **41**, 417–426.
- Mavingui, P., Flores, M., Romero, D., Martínez-Romero, E., and Palacios, R. (1997). Generation of *Rhizobium* strains with improved symbiotic properties by random DNA amplification (RDA). *Nat Biotechnol* **15**, 564–569.
- Mayrose, M., Ekengren, S.K., Melech-Bonfil, S., Martin, G.B., and Sessa, G. (2006). A novel link between tomato GRAS genes, plant disease resistance and mechanical stress response. *Mol Plant Pathol* **7**, 593–604.
- Menna, P., Hungria, M., Barcellos, F.G., Bangel, E.V., Hess, P.N., and Martínez-Romero, E. (2006). Molecular phylogeny based on the 16S rRNA gene of elite rhizobial strains used in Brazilian commercial inoculants. *Syst Appl Microbiol* **29**, 315–332.
- Mergaert, P., Uchiyumi, T., Alunni, B., Evanno, G., Cheron, A., Catrice, O., Mausset, A.-E., Barloy-Hubler, F., Galibert, F., Kondorosi, A., and Kondorosi, E. (2006). Eukaryotic control on bacterial cell cycle and differentiation in the *Rhizobium*-legume symbiosis. *Proc Natl Acad Sci USA* **103**, 5230–5235.
- Minamisawa, K., Onodera, S., Tanimura, Y., Kobayashi, N., Yuhashi, K.-I., and Kubota, M. (1997). Preferential nodulation of *Glycine max*, *Glycine soja* and *Macroptilium atropurpureum* by two *Bradyrhizobium* species *japonicum* and *elkanii*. *FEMS Microbiol Ecol* **24**, 49–56.
- Mnasri, B., Mrabet, M., Laguerre, G., Aouani, M.E., and Mhamdi, R. (2007). Salt-tolerant rhizobia isolated from a Tunisian oasis that are highly effective for symbiotic N<sub>2</sub>-fixation with *Phaseolus vulgaris* constitute a novel biovar (bv. *mediterraneense*) of *Sinorhizobium meliloti*. *Arch Microbiol* **187**, 79–85.
- Montealegre, C., Graham, P.H., and Kipe-Nolt, J.A. (1995). Preference in the nodulation of *Phaseolus vulgaris* cultivar RAB39. *Can J Microbiol* **41**, 992–998.
- Moreira, F.M.S., Haukka, K., and Young, J.P.W. (1998). Biodiversity of rhizobia isolated from a wide range of forest legumes in Brazil. *Mol Ecol* **7**, 889–895.
- Moulin, L., Munive, A., Dreyfus, B., and Boivin-Masson, C. (2001). Nodulation of legumes by members of the beta-subclass of Proteobacteria. *Nature* **411**, 948–950.
- Mueller, U.G., Dash, D., Rabeling, C., Rodrigues, A., Brown, J. (2008). Coevolution between attine ants and actinomycete bacteria: A reevaluation. *Evolution* **62**, 2894–2912.
- Nandasena, K.G., O'Hara, G.W., Tiwari, R.P., Sezmiş, E., and Howieson, J.G. (2007). *In situ* lateral transfer of symbiosis islands results in rapid evolution of diverse competitive strains of mesorhizobia suboptimal in symbiotic nitrogen fixation on the pasture legume *Biserrula pelecinus* L. *Environ Microbiol* **9**, 2496–2511.
- Nuswantara, S., Fujie, M., Yamada, T., Malek, W., Inaba, M., Kaneko, Y., and Murooka, Y. (1999). Phylogenetic position of *Mesorhizobium huakuii* subsp. *rengi*, a symbiont of *Astragalus sinicus* cv. Japan. *J Biosci Bioeng* **87**, 49–55.
- Ohkuma, M., Noda, S., Hongoh, Y., and Kudo, T. (2001). Coevolution of symbiotic systems of termites and their gut microorganisms. *RIKEN Rev* **41**, 73–74.
- Ormeño-Orrillo, E., Vinuesa, P., Zuñiga-Dávila, D., and Martínez-Romero, E. (2006). Molecular diversity of native bradyrhizobia isolated from Lima bean (*Phaseolus lunatus* L.) in Peru. *Syst Appl Microbiol* **29**, 253–262.
- Paau, A.S., Bloch, C.B., and Brill, W.J. (1980). Developmental fate of *Rhizobium meliloti* bacteroids in alfalfa nodules. *J Bacteriol* **143**, 1480–1490.
- Paffetti, D., Daguin, F., Fancelli, S., Gnocchi, S., Lippi, F., Scotti, C., and Bazzicalupo, M. (1998). Influence of plant genotype on the selection of nodulating *Sinorhizobium meliloti* strains by *Medicago sativa*. *Antonie Van Leeuwenhoek* **73**, 3–8.
- Parker, M.A. (1999). Mutualism in metapopulations of legumes and rhizobia. *Am Nat* **153**, S48–S60.
- Parker, M.A. (2001). Case of localized recombination in 23S rRNA genes from divergent *Bradyrhizobium* lineages associated with neotropical legumes. *Appl Environ Microbiol* **67**, 2076–2082.
- Parker, M.A. (2002). Bradyrhizobia from wild *Phaseolus*, *Desmodium*, and *Macroptilium* species in northern Mexico. *Appl Environ Microbiol* **68**, 2044–2048.
- Parker, M.A. (2004). rRNA and *dnaK* relationships of *Bradyrhizobium* sp. nodule bacteria from four Papilionoid legume trees in Costa Rica. *Syst Appl Microbiol* **27**, 334–342.
- Parker, M.A., Doyle, J.L., and Doyle, J.J. (2004). Comparative phylogeography of *Amphicarpea* legumes and their root-nodule symbionts in Japan and North America. *J Biogeogr* **31**, 425–434.
- Parlevliet, J.E. (1979). Components of resistance that reduce the rate of epidemic development. *Annu Rev Phytopathol* **17**, 203–222.
- Perret, X., Staehelin, Ch., and Broughton, W.J. (2000). Molecular basis of symbiotic promiscuity. *Microbiol Mol Biol Rev* **64**, 180–201.
- Peschel, A., and Sahl, H.-G. (2006). The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nat Rev Microbiol* **4**, 529–536.

- Piñero, D., Martínez, E., and Selander, R.K. (1988). Genetic diversity and relationships among isolates of *Rhizobium leguminosarum* biovar phaseoli. *Appl Environ Microbiol* **54**, 2825–2832.
- Provorov, N.A. (1998). Coevolution of rhizobia with legumes: facts and hypotheses. *Symbiosis* **24**, 337–368.
- Provorov, N.A., and Vorobyov, N.I. (2008). Equilibrium between the “genuine mutualists” and “symbiotic cheaters” in the bacterial population co-evolving with plants in a facultative symbiosis. *Theor Popul Biol* **74**, 345–355.
- Provorov, N.A., Vorobyov, N.I., and Andronov, E.E. (2008). Macro- and microevolution of bacteria in symbiotic systems. *Russ J Genet* **44**, 6–20.
- Rangin, C., Brunel, B., Cleyet-Marel, J.-C., Perrineau, M.-M., and Béna, G. (2008). Effects of *Medicago truncatula* genetic diversity, rhizobial competition, and strain effectiveness on the diversity of a natural *Sinorhizobium* species community. *Appl Environ Microbiol* **74**, 5653–5661.
- Relic, B., Perret, X., Estrada-Garcia, M.T., Kopcinska, J., Golonowski, W., Krishnan, H.B., Pueppke, S.G., and Broughton, W.J. (1994). Nod factors of *Rhizobium* are a key to the legume door. *Mol Microbiol* **13**, 171–178.
- Rincón-Rosales, R., Lloret, L., Ponce, E., and Martínez-Romero, E. (2009). Rhizobia with different symbiotic efficiencies nodulate *Acaciella angustissima* in Mexico including *Sinorhizobium mexicanum*. *FEMS Microb Ecol* **67**, 103–117.
- Rivas, R., Laranjo, M., Mateos, P.F., Oliveira, S., Martínez-Molina, E., and Velázquez, E. (2007). Strains of *Mesorhizobium amorphae* and *Mesorhizobium tianshanense*, carrying symbiotic genes of common chickpea endosymbiotic species, constitute a novel biovar (*ciceri*) capable of nodulating *Cicer arietinum*. *Lett Appl Microbiol* **44**, 412–418.
- Roche, P., Maillet, F., Plaz Janet, C., Debelle, F., Ferro, M., Truchet, G., Prome, J.-C., and Dénarié, J. (1996). The common *nodABC* genes of *Rhizobium meliloti* are host-range determinants. *Proc Natl Acad Sci USA* **93**, 15305–15310.
- Rogel, M.A., Hernández-Lucas, I., Kuykendall, L.D., Balkwill, D.L., and Martínez-Romero, E. (2001). Nitrogen-fixing nodules with *Ensifer adhaerens* harboring *Rhizobium tropici* symbiotic plasmids. *Appl Environ Microbiol* **67**, 3264–3268.
- Rogel, M.A., Torres, C., Lloret, L., Rosenblueth, M., Hernández-Lucas, I., Martínez, L., Martínez, J., and Martínez-Romero, E. (2006). Lateral transfer of *Rhizobium* symbiotic plasmids leading to genomic innovation. In *Biology of Plant-Microbe Interactions*, Vol. 5. F. Sánchez, C. Quinto, I.M. López-Lara, O. Geiger, eds. (International Society for Molecular Plant-Microbe Interactions, St. Paul, MN), pp. 310–318.
- Rosas, J.C., Castro, J.A., Robleto, E.A., and Handelsman, J. (1998). A method for screening *Phaseolus vulgaris* L. germplasm for preferential nodulation with a selected *Rhizobium etli* strain. *Plant Soil* **203**, 71–78.
- Rosenblueth, M., Hynes, M.F., and Martínez-Romero, E. (1998). *Rhizobium tropici* *teu* genes involved in specific uptake of *Phaseolus vulgaris* bean-exudate compounds. *Mol Gen Genet* **258**, 587–598.
- Samac, D.A., and Graham, M.A. (2007). Recent advances in legume-microbe interactions: recognition, defense response, and symbiosis from a genomic perspective. *Plant Physiol* **144**, 582–587.
- Segovia, L., Piñero, D., Palacios, R., and Martínez-Romero, E. (1991). Genetic structure of a soil population of nonsymbiotic *Rhizobium leguminosarum*. *Appl Environ Microbiol* **57**, 426–433.
- Segovia, L., Young, J.P.W., and Martínez-Romero, E. (1993). Reclassification of American *Rhizobium leguminosarum* biovar phaseoli type I strains as *Rhizobium etli* sp. nov. *Int J Syst Bacteriol* **43**, 374–377.
- Silva, C., Kan, F.L., and Martínez-Romero, E. (2007). Population genetic structure of *Sinorhizobium meliloti* and *S. medicae* isolated from nodules of *Medicago* spp. in Mexico. *FEMS Microbiol Ecol* **60**, 477–489.
- Silva, C., Vinuesa, P., Eguiarte, L.E., Martínez-Romero, E., and Souza, V. (2003). *Rhizobium etli* and *Rhizobium gallicum* nodulate common bean (*Phaseolus vulgaris*) in a traditionally managed milpa plot in Mexico: population genetics and biogeographic implications. *Appl Environ Microbiol* **69**, 884–893.
- Silva, C., Vinuesa, P., Eguiarte, L.E., Souza, V., and Martínez-Romero, E. (2005). Evolutionary genetics and biogeographic structure of *Rhizobium gallicum* sensu lato, a widely distributed bacterial symbiont of diverse legumes. *Mol Ecol* **14**, 4033–4050.
- Smit, P., Raedts, J., Portyanko, V., Debelle, F., Gough, C., Bisseling, T., and Geurts, R. (2005). NSP1 of the GRAS protein family is essential for rhizobial Nod factor-induced transcription. *Science* **308**, 1789–1791.
- Smith, C.L., Godsoe, W.K.W., Tank, S., Yoder, J.B., and Pellmyr, O. (2008). Distinguishing coevolution from covariance in an obligate pollination mutualism: asynchronous divergence in Joshua tree and its pollinators. *Evolution* **62**, 2676–2687.
- Soedarjo, M., Hemscheidt, T.K., and Borthakur, D. (1994). Mimosine, a toxin present in leguminous trees (*Leucaena* spp.), induces a mimosine-degrading enzyme activity in some *Rhizobium* strains. *Appl Environ Microbiol* **60**, 4268–4272.
- Steenkamp, E.T., Stepkowski, T., Przymusiak, A., Botha, W.J., and Law, I.J. (2008). Cowpea and peanut in southern Africa are nodulated by diverse *Bradyrhizobium* strains harboring nodulation genes that belong to the large pantropical clade common in Africa. *Mol Phylogenet Evol* **48**, 1131–1144.
- Sullivan, J.T., Patrick, H.N., Lowther, W.L., Scott, D.B., and Ronson, C.W. (1995). Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. *Proc Natl Acad Sci USA* **92**, 8985–8989.
- Sullivan, J.T., and Ronson, C.W. (1998). Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a *phe-tRNA* gene. *Proc Natl Acad Sci USA* **95**, 5145–5149.
- Suominen, L., Roos, C., Lortet, G., Paulin, L., and Lindstroem, K. (2001). Identification and structure of the *Rhizobium galegae* common nodulation genes: evidence for horizontal gene transfer. *Mol Biol Evol* **18**, 907–916.
- Terefework, Z., Kajjalainen, S., and Lindstroem, K. (2001). AFLP fingerprinting as a tool to study the genetic diversity of *Rhizobium galegae* isolated from *Galega orientalis* and *Galega officinalis*. *J Biotechnol* **91**, 169–180.
- Thompson, J.N. (1994). *The Coevolutionary Process* (University of Chicago Press, Chicago), p. 387.
- Thompson, J.N. (1999). Specific hypotheses on the geographic mosaic of coevolution. *Am Nat* **153**, S1–S14.
- Thompson, J.N. (2005). *The Geographic Mosaic of Coevolution* (University of Chicago Press, Chicago).
- Tibayrenc, M. (1996). Towards a unified evolutionary genetics of microorganisms. *Annu Rev Microbiol* **50**, 401–429.
- Toledo, I., Lloret, L., and Martínez-Romero, E. (2003). *Sinorhizobium americanum* sp. nov., a new *Sinorhizobium* species nodulating native *Acacia* spp. in Mexico. *Syst Appl Microbiol* **26**, 54–64.
- van Berkum, P., Beyene, D., Bao, G., Campbell, T.A., and Eardly, B.D. (1998). *Rhizobium mongolense* sp. nov. is one of three rhizobial genotypes identified which nodulate and form

- nitrogen-fixing symbioses with *Medicago ruthenica* [(L.) Ledebour]. *Int J Syst Bacteriol* **48**, 13–22.
- Villegas, M.C., Rome, S., Maure, L., Domergue, O., Gardan, L., Bailly, X., Cleyet-Marel, J.-C., and Brunel, B. (2006). Nitrogen-fixing sinorhizobia with *Medicago laciniata* constitute a novel biovar (bv. *medicaginis*) of *S. meliloti*. *Syst Appl Microbiol* **29**, 526–538.
- Vinuesa, P., León-Barrios, M., Silva, C., Willems, A., Jarabo-Lorenzo, A., Pérez-Galdona, R., Werner, D., and Martínez-Romero, E. (2005a). *Bradyrhizobium canariense* sp. nov., an acid-tolerant endosymbiont that nodulates endemic genistoid legumes (Papilionoideae: Genisteae) from the Canary Islands, along with *Bradyrhizobium japonicum* bv. *genistearum*, *Bradyrhizobium* genospecies alpha and *Bradyrhizobium* genospecies beta. *Int J Syst Evol Microbiol* **55**, 569–575.
- Vinuesa, P., Rojas-Jiménez, K., Contreras-Moreira, B., Mahna, S.K., Prasad, B.N., Moe, H., Selvaraju, S.B., Thierfelder, H., and Werner, D. (2008). Multilocus sequence analysis for assessment of the biogeography and evolutionary genetics of four *Bradyrhizobium* species that nodulate soybeans on the Asiatic continent. *Appl Environ Microbiol* **74**, 6987–6996.
- Vinuesa, P., Silva, C., Werner, D., and Martínez-Romero, E. (2005b). Population genetics and phylogenetic inference in bacterial molecular systematics: the roles of migration and recombination in *Bradyrhizobium* species cohesion and delineation. *Mol Phylogenet Evol* **34**, 29–54.
- Wang, E.T., Martínez-Romero, J., and Martínez-Romero, E. (1999a). Genetic diversity of rhizobia from *Leucaena leucocephala* nodules in Mexican soils. *Mol Ecol* **8**, 711–724.
- Wang, E.T., Rogel, M.A., García-De los Santos, A., Martínez-Romero, J., Cevallos, M.A., and Martínez-Romero, E. (1999b). *Rhizobium etli* bv. *mimosae*, a novel biovar isolated from *Mimosa affinis*. *Int J Syst Bacteriol* **49**, 1479–1491.
- Weerasinghe, R.R., McK. Bird, D., and Allen, N.S. (2005). Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus*. *Proc Natl Acad Sci USA* **102**, 3147–3152.
- Woolhouse, M.E.J., Webster, J.P., Domingo, E., Charlesworth, B., and Levin, B.R. (2002). Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nat Genet* **32**, 569–577.
- Xu, L.M., Ge, C., Cui, Z., Li, J., and Fan, H. (1995). *Bradyrhizobium liaoningense* sp. nov., isolated from the root nodules of soybeans. *Int J Syst Bacteriol* **45**, 706–711.
- Young, J.P.W., and Johnston, A.W.B. (1989). The evolution of specificity in the legume-*Rhizobium* symbiosis. *Trends Ecol Evol* **4**, 341–349.
- Zhang, Y.F., Wang, E.T., Tian, C.F., Wang, F.Q., Han, L.L., Chen, W.F., and Chen, W.X. (2008). *Bradyrhizobium elkanii*, *Bradyrhizobium yuanmingense* and *Bradyrhizobium japonicum* are the main rhizobia associated with *Vigna unguiculata* and *Vigna radiata* in the subtropical region of China. *FEMS Microbiol Lett* **285**, 146–154.

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