Symbiotic Nitrogen Fixation in Legumes: Perspectives on the Diversity and Evolution of Nodulation by *Rhizobium* and *Burkholderia* Species

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### 89.1 INTRODUCTION

More than a decade ago, we wrote an update on the *Rhizobium*-legume symbiosis asking the question “what makes this symbiosis so special?” (Hirsch et al., 2001). Since that time, we have learned a great deal more about this important plant–microbe association. However, the story of symbiotic nitrogen fixation in legumes has become more complicated due to the discovery of several Betaproteobacteria, namely species of *Burkholderia* and *Cupriavidus*, which also establish nitrogen-fixing nodules on legumes. In this chapter, we expand our analysis of the *Rhizobium*-legume symbiosis to include the betarhizobia, specifically *Burkholderia*, and their relevance to this well-studied, beneficial interaction between bacterium and plant.

In particular, we examine the concept of host range, biogeography, as well as evolutionary history with respect to nodulation by *Burkholderia* and *Rhizobium* (see also Chapter 17). Phylogenetic analyses strongly indicate that both genera acquired the nodulation genes at approximately the same time as the legumes were evolving (Bontemps et al., 2010). We elaborate on this evolutionary theme as we delve into the even earlier history of these two nodulating and nitrogen-fixing bacterial families, with the goal of establishing an evolutionary description of this still incompletely understood symbiosis.
89.2 THE LEGUMES

The legumes (Fabaceae or Leguminosae) are the third largest family of flowering plants (>19,000 species); only Asteraceae and Orchidaceae are larger (Lewis et al., 2005). The family is monophyletic, but comprises three subfamilies (36 tribes), namely Caesalpinioideae, Mimosoideae, and Papilionoideae, which represent 22%, 10%, and 67%, respectively, of the family (Sprent, 2001). Of the three subfamilies, caesalpinoid legumes are less likely to be nodulated, but this is not because they evolved first (Sprent, 2007). Several caesalpinoid legumes considered to be “basal” are not found in the fossil flora in the early history of the family (Herendeen et al., 1992).

The structure of the flowers of the subfamily members differs significantly (Fig. 89.1), but legumes are incredibly diverse in many other features, including phytochemical profiles, nodulation status, and habitats. Legumes occupy deserts and grass-poor, succulent-rich, dry environments (S-biomes); tropical rain forests (R-biomes); grass-rich prairies and savannahs (G-biomes); temperate ecosystems (T-biomes); and agricultural systems derived from anthropogenic input. Numerous books and papers have described the biology, nodulation, and evolution of the legumes, so we describe only a few salient features in this chapter (Sprent, 2001; Doyle and Luckow, 2003; Lewis et al., 2005).

The legumes are believed to have evolved ca. 65–50 million years ago (Mya) in the late Cretaceous/early Tertiary after the K/T extinction (Lavin et al., 2005). This hypothesis is in part based on the discovery of legume fossils dating from the Paleocene (ca. 66–56 Mya; Herendeen et al., 1992) and also on molecular analysis developed from matK and rbcL phylogenies (Lavin et al., 2005). The evolutionary migration of legumes southward is postulated to have started north of the Tethys Sea, under warmer climate conditions, after the continents drifted apart (see Doyle and Luckow, 2003; Sprent, 2007). This boreotropical origin, deduced in part from the current habitats of legumes, is consistent with the migration of legumes from an S-biome to the R-, G-, and T-biomes (Lewis et al., 2005). Both molecular and fossil data suggest that legumes rapidly diversified into most of their major lineages within a relatively short period of time (ca. 55–50 Mya) (Herendeen et al., 1992; Doyle and Luckow, 2003; Lewis et al., 2005; Sprent, 2007, 2008). This is approximately the same time that the rest of the angiosperm families were evolving (Lewis et al., 2005). The boreotropical forest migration hypothesis has replaced the older Gondwanan theory in which legumes moved northward from their presumed sites of origin in Africa to South America and then, before the land masses drifted apart 165–145 Mya, to North America. Nonetheless, the boreotropical hypothesis leaves much unexplained, especially with regard to the evolution of legume nodulation (Sprent, 2008; see Chapter 3).

Although nodules have not been found in the fossil record, it is extremely likely that many of the legumes were already developing root nodules inhabited by nitrogen-fixing rhizobia by the time that the major lineages were established. The partnership between rhizobia and legumes is based on a pre-existing and ancient relationship, dating from the early Devonian (416–350 Mya) between arbuscular mycorrhizal fungi (AMF) and plants. The evolutionary origin of the genes important for root nodulation that was derived from the arbuscular-mycorrhizal (AM) symbiosis has been extensively studied and reviewed (Hirsch et al., 2001; Szczeglowsky and Amyot, 2003; Streng et al., 2011; and others; see also Chapters 42, 54, 55, 108, 110).

89.3 THE RHIZOBIA

“Rhizobia” is the collective term given to bacteria capable of inducing the development of and then populating nitrogen-fixing nodules (Sprent, 2001). A long-standing debate exists over whether legumes and rhizobia coevolved or whether the plants selected for symbiotic bacteria, but the presence of horizontal gene transfer and the lack of parallel speciation between legumes and rhizobia might argue against a coevolutionary trajectory (Martínez-Romero, 2009; Young and Johnston, 1989).

Up to 2001, only the Alphaproteobacteria in the family Rhizobiaceae had been classified as rhizobia. These bacteria

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Figure 89.1 Examples of flowers from the three legume subfamilies. (a) Caesalpinioideae: Chamaecrista fasciculata (N.A. Fujishige). (b) Mimosoideae: Calliandra hematocephala (A.M. Hirsch). (c) Papilionoideae: Swainsona formosa (W. Deng).
were first isolated from root nodules by Beijerinck (1890), and the name *Rhizobium* was suggested shortly afterwards by Frank (1889) (see additional references in Gyaneshwar et al., 2011; see Chapter 88). Until the 1980s, all the legume root-nodulating bacteria (RNB) were classified in the genus *Rhizobium*, which had been divided into fast and slow growers. After it became apparent that significantly greater differences than just growth rate existed, *Bradyrhizobium* (*Bradyrhizobiales*) was proposed by Jordan (1982) to encompass the slow-growing species whereas the name *Rhizobium* was retained for the fast growers. Using a molecular clock approach, the split between *Rhizobium* and *Bradyrhizobium* is estimated to have taken place 800–300 Mya depending on the gene analyzed (Turner and Young, 2000).

*Rhizobium* has also been subdivided into a number of distinct genera. Chen et al. (1988) isolated fast- and slow-growing rhizobia from nodules of soybean (*Glycine soja*) and from soil, and after analyzing cellular composition and by using methods such as DNA–DNA hybridization, serology, and phage typing, proposed a new genus, *Sinorhizobium*. Thus, the fast-growing isolate *Rhizobium fredii* was renamed *Sinorhizobium fredii*. In 2008, however, taxonomists questioned whether *Sinorhizobium* was distinct from the genus *Ensifer*, and the Judicial Commission of the International Committee on Systematics of Prokaryotes (2008) decided that it was not, and thus the name *Ensifer*, which had priority, was retained (see also Chapter 3). Later, other genera were described, including *Azorhizobium* (Dreyfus et al., 1988), which was placed in the family *Xanthobacteraceae*, and *Mesorhizobium* (Jarvis et al., 1997) in the *Phyllobacteriaceae*. The genus *Phyllobacterium* contains three rhizobial species that are closely related to *Mesorhizobium* (Valverde et al., 2005; Mantelín et al., 2006).

Concurrently, additional RNB from Rhizobiales were added to the list: *Devosia neptunia* (family *Hyphomicrobiaceae* (Rivas et al., 2003)); *Ochrobactrum* (Trujillo et al., 2005; Zurdo-Piñeiro et al., 2007) (family *Brucellaceae*); *Microvirga* (Ardley et al., 2012; see Chapter 23); and *Methyllobacterium* (*Methylbacteriaceae*) (Jourand et al., 2004). Each family that contains RNB also houses nonrhizobial species, some of which are pathogenic on animals or plants. For example, most of the *Xanthobacteraceae* are nonnodulating species and many *Brucellaceae* are human pathogens. As another example, *Bradyrhizobium* is closely related to the genus *Apifía*, which includes human pathogens (La Scola et al., 2002).

Other novel species of rhizobia recently isolated include *Shinella kummerowiae* from the herbal legume *Kummerowia stipulacea* in China (Lin et al., 2008). This genus is closely related to *Rhizobium* and the bacteria are often found as nodule occupants and in soil samples, but had not been shown previously to induce nodules. The diversity of organisms that function as RNB is summarized in Figure 89.2.

### 89.4 THE BETARHIZOBIA

In 2001, two genera of the family *Burkholderiaceae*, *Burkholderia* and *Cupriavidus*, were reported to nodulate legumes, but at that time were not shown to induce effective, that is, nitrogen-fixing nodules on their hosts (Moulin et al., 2001; see Chapter 17). Much of the original work on the *Burkholderia* symbiotic species focused on *B. tuberum* and *B. phytofirmans*, but it was quickly shown that neither species re-infected the hosts from which they were originally isolated (*Aspalathus carnosos* and *Machaerium lunatum*, respectively). Later studies showed that both genera established nitrogen-fixing nodules with a number of legume hosts. The majority of *Burkholderia* species have been isolated from nodules of Mimosoideae, especially the genus *Mimosa*. *Mimosa* spp. are native to the Neotropics although a few endemics live in the Paleotropics, mainly in Madagascar, and also extend into southeast tropical Africa and India (Lewis et al., 2005; see Chapter 17).

Some *Burkholderia* species are free-living and function as soil-dwelling microbes in bioremediation or act as plant growth-promoting bacteria (PGPB) living as epiphytes on plants (de Bruijn, 2013). Still others appear to be endophytes based on their isolation from both legume and nonlegume hosts following surface sterilization of plant tissues (Compton et al., 2008). However, many of these species have not been rigorously confirmed as either endophytes or PGPB based on experimental studies, with the exception of *B. phytofirmans* (Sessitsch et al., 2005).

Yabuuchi et al. (1992) split *Burkholderia* from *Pseudomonas* homology group II and named the genus for W.H. Burkholder, who isolated the etiological agent of onion rot (*Burkholder, 1942*). *Burkholderia* species, other than the RNB (shown in Fig. 89.3), are ubiquitous in the environment and are found in soil, rhizosphere, clinical samples, plants, fungus, insects, and animals (Compton et al., 2008). *Burkholderia* species have long been associated with plant and animal pathogenesis, and all of the initial species moved from *Pseudomonas* to *Burkholderia* demonstrated virulence toward numerous plants and animals (Compton et al., 2008). However, efforts have been initiated to differentiate the symbiotic and environmental *Burkholderia* species from the opportunistic, mammalian, and plant pathogens, and have led to the proposal that the former be assigned to a new group/genus because it is phylogenetically distinct from the *Burkholderia* group (Angus et al., 2014, Estrada-de Los Santos et al., 2013, Suárez-Moreno et al., 2012; see also Chapter 17).

### 89.5 THE ROOT-NODULATING *Burkholderia* SPECIES

To study the evolution of symbiotic genes, Bontemps et al. (2010) prepared a phylogenetic analysis using partial
Figure 89.2 Unrooted phylogenetic reconstruction of 16S ribosomal RNA of rhizobial and related nonrhizobial species by Neighbor Joining. Bootstrap percentage after 1000 replications is shown on nodes. Scale bar represents the number of substitutions per site. Colored nodes represent each of the main families with genera containing rhizobia (in bold). Purple, Rhizobiaceae; green, Brucellaceae; pink, Phyllobacteriaceae; olive, Xanthobacteraceae; turquoise, Hyphomicrobiaceae; blue, Methylobacteriaceae; orange, Bradyrhizobiaceae; red, Burkholderiaceae (see also Chapter 17).
sequences of the 16S, recA, nodC, and nifH regions of isolates from nodules of 47 species of *Mimosa* spp. growing in various regions of Brazil. All sequences were highly homologous to *Burkholderia* species from seven distinct clusters (clades). Four of the seven clusters observed by Bontemps et al. (2010) contained named species of *Burkholderia* RNB (cluster 2, *B. phymatum*; cluster 4, *B. mimosarum*; cluster 5, *B. nodosa*; and cluster 6, *B. tuberum*). However, three clusters represented novel species (clusters 1, 3, and 7). Based on recent evidence, cluster 3 is closely related to the recently described *B. diazotrophica* (Sheu et al., 2013); one isolate within this cluster, *Burkholderia* sp. mpa3.10 shares over 99% 16S and recA sequence homology to *B. diazotrophica* (Walker & Watkin, unpublished). Isolates from cluster 7 may be related to the recently sequenced *B. phenoliruptrix* (de Oliveira et al., 2012), and are also closely related to *B. fungorum*. Cluster 1 may contain the recently described *B. symbiotica*, which is the first *Burkholderia* sp. RNB characterized that is not closely related to other Brazilian species (Sheu et al., 2012).

The study by Bontemps et al. (2010) gave us important insight into the evolution of nodulation in *Burkholderia* by showing that it had a long and stable genetic history (Angus and Hirsch, 2010), which is most likely as old as that of the nodulating Rhizobiales. Phylogenograms reconstructed from partial nodC sequences place South American *Burkholderia* symbiotic genes in a monophyletic group that is highly divergent from all other nodC sequences and further suggest that any nod gene transfer event that occurred must have taken place at least 50–60 Mya (Bontemps et al., 2010). This transfer of symbiotic genes coincides with the period when the legumes themselves were diversifying into their major lineages (Fig. 89.4).

The legume genus *Mimosa* is almost exclusively nodulated by species of *Burkholderia* (see also Chapter 17). *Mimosa* most likely followed a boreotropical migration from north to south and this theory is supported by fossil evidence in North America and Europe, but palynological data have also been collected in Africa (Herendeen et al., 1992). However, disjunct populations of *Mimosa* in South Eastern Africa, Madagascar, and India may have originated from long-distance oceanic dispersal (Simon et al., 2011). Some of these introduced populations might be coincident with the southern hemisphere locations of the isolated RNB *Burkholderia* species (Fig. 89.3).

In contrast to the South American *Burkholderia* species, the South African *B. tuberum* strains nodulate papilionoid legumes. This nodulation preference is correlated with nodulation (*nod*) and nitrogen-fixation genes (*nif*) gene sequence organization, which differ between the South
African and South American RNB. One hypothesis posits that the establishment of *Mimosa* from long-distance oceanic dispersal may have led to the introduction of *B. tuberum* into South Africa, where it could have lost the ability to nodulate *Mimosa* in favor of the endemic Papilionoideae population following the lateral transfer of local nod genes (from alpha rhizobia?). *Mimosa* introduction has been observed in Australia with the accidental importation of South American *Burkholderia* species, including *B. diazotrophica* and *B. mimosarum* (Walker and Watkin, unpublished), on introduced *M. pigra* seeds (Parker et al., 2007).

The idea of introduced *Mimosa* species, however, does not adequately explain the distinct lineages of *Burkholderia* species found in South Africa that nodulate papilionoid legumes. The first lineage described is typified by the type strain *B. tuberum* STM678\textsuperscript{T}, originally isolated from *A. cariosa*, whereas a second lineage contains the recently described *B. rhynchosiae* (De Meyer et al., 2013a), which nodulates species of *Rhynchosia* (Garau et al., 2009). So far, no information is available about nod and nif gene organization in *B. rhynchosiae*, nor about how these symbiotic genes compare to those of *B. tuberum* STM678\textsuperscript{T} except for the fact that the nodA genes are 96% identical (Garau et al., 2009). Another lineage, *B. sprentiae*, is more closely related to *B. tuberum* STM678\textsuperscript{T}, but it nodulates *Lebeckia* spp. (De Meyer et al., 2013b). Numerous South African species have been recently isolated and many are in the process of being described (Beukes et al., 2013; De Meyer et al., 2014). Such studies will increase our understanding of this group of *Burkholderia* spp.

### 89.6 REGARDING THE EVOLUTION OF NODULATING *Burkholderiaceae* AND Rhizobiaceae

A limited fossil record exists for bacteria, except for cyanobacteria where fossils resembling these organisms have been reported from the Archean period of Earth history (Schopf, 2006; Javaux et al., 2010). Nonetheless, questions remain as to their actual identity and whether or not cyanobacterial photosynthesis evolved that early. In any case, most agree that marine bacteria probably evolved about 3.5 billion years ago (Gya). Undisputed microfossils from 1.2 Gya have been found (Horodyski and Knauth, 1994) (Fig. 89.4). Due to the debate about the exact nature of the fossils from 3.5 Gya, conclusions regarding bacterial evolution are based mainly on geochemical evidence, the detection of biomarkers, and isotope ratios (Fischer, 2008). The accumulation of such information has strongly suggested that bacteria achieved a terrestrial lifestyle (became terrabacteria) some 2.7–2.6 Gya (Watanabe et al., 2000) (Fig. 89.4). Moreover, it appears that many prokaryotic lineages were already evolved as early as 2.7–2.5 Gya, supporting the even older history for the earliest bacteria.
89.6 Regarding the Evolution of Nodulating *Burkholderiaceae* and *Rhizobiaceae*

Evolution of legumes; hypothesized horizontal transfer of *nod* genes

Breakup of Gondwana

Rise of terrestrial *Azospirillum* spp.

Rise of vascular plants; arbuscular mycorrhizal fungal fossils

Divergence of *Rhizobium* and *Bradyrhizobium*

Figure 89.4  Timeline showing some key points in the evolution of the legumes and their associated nitrogen-fixing bacteria. *(Source: Modified from Fischer (2008).)*

More recently, genome analysis has been used to study bacterial evolution. Wisniewsky-Dyé et al. (2011) proposed the divergence of terrestrial azospirilla from their aquatic *Rhodospirillaceae* relatives had occurred ca. 200–400 Mya based on their distinct genomes. Almost half of the terrestrial azospirilla genomes appear to have been derived by horizontal gene transfer from Rhizobiales and other Alphaproteobacteria (greatest percentage) and also from Burkholderiales, suggesting that these groups evolved long before *Azospirillum* diverged. Therefore, Rhizobiales and Burkholderiales were likely to have been among the bacterial lineages that colonized the land prior to the evolution of the vascular plants, the vast majority of which建立 a symbiotic association with AMF (Fig. 89.4). Both fossil and molecular evidence date the AMF association with plants to have been present ca. 430–350 Mya in the early Devonian (Simon et al., 1993; Taylor et al., 1995). Because many of the plant genes involved in establishing the AM symbiosis and the nitrogen-fixing nodule are conserved and also because the initial microbial signal molecules are chemically related (see references in Hirsch and Fujishige, 2012; see Chapters 55, 110), the idea that nitrogen-fixing rhizobia coopted the ancient mycorrhizal signaling pathway to ensure root cell entry and subsequent nodulation for the proliferation and protection of the bacteria is well accepted.

Thus, this brings up the question as to whether ancestral Rhizobiales and/or Burkholderiales could have been coparticipants in the origin of both plant–mycorrhizal associations and nitrogen-fixing symbioses. For the Betaproteobacteria, the answer is probably yes based on *Burkholderia* symbionts being present within AMF hyphae. *Candidatus Glomeribacteria gigasporarum* (CGG) is an obligate endobacterium that lives surrounded by a membrane within the cytoplasm of AMF cells, which themselves are obligate biotrophs and dependent on their plant hosts (Jargeat et al., 2004). However, no evidence for *nif* genes was uncovered (Jargeat et al., 2004), suggesting that the CGG ancestor was unlikely to be a diazotroph although it is sister to free-living *Burkholderia* species that fix nitrogen as well as to *B. rhizoxinica* HKI 0454T (Ghignone et al., 2004), an endophyte of the phytopathogenic fungus *Rhizopus*, the causal agent of rice-seeding blight (Partida-Martinez et al., 2007). In spite of its small genomes and lack of *nif* genes, some 250,000 CGG cells are estimated to live within one
fungal cell (Bianciotto et al., 1996) and at least 20,000 per spore (Lumini et al., 2007), strongly suggesting that these microbes provide benefits to their host. Indeed, the bacteria were found to improve the fitness of Gigasporaceae by promoting presymbiotic hyphal expansion and branching (Lumini et al., 2007).

Based on studies of various betaproteobacterial genomes, the difference in mutation accumulation in CGG differs from free-living Burkholderia species and from the Burkholderia endosymbionts of Rhizopus, strongly suggesting an ancient interaction with the fungus (Castillo and Pawlowska, 2010). Similar to insect symbionts, CGG and B. rhizoxinica have reduced genomes, but the fungal pathogen has a large number of genes associated with virulence (Lackner et al., 2011). Moreover, B. rhizoxinica can be grown in culture and re-infect its fungal host (Moebius et al., 2014). In contrast, CGG is unable to synthesize several essential amino acids and vitamins, and is totally dependent on its host for carbon, nitrogen, and phosphorous (Ghignone et al., 2012). The complex network seen in this tripartite symbiosis strongly suggests a different pattern of molecular evolution than that observed in the free-living Burkholderia species or B. rhizoxinica (Castillo and Pawlowska, 2010). Whether or not an ancient Burkholderia–AMF–plant symbiosis is the ancestor of the nodulation pathway (see Chapters 51, 59, 110) may be difficult to prove, but it is not clear whether any better candidate presents itself. To our knowledge, the evidence for a Rhizobiales endosymbiont living in association with AMF cells has not been reported.

89.7 FINAL COMMENTS AND PERSPECTIVES

Strong evidence for distinct Burkholderia populations in South America and Southern Africa exists, but so far not much is known about the Indian Burkholderia populations (Fig. 89.3). The Indian Burkholderia RNB may be closely related to RNB isolated from Dalbergia legume nodules in Madagascar although this strain was identified at the time as B. cepacia and lacks nod genes (Rasolomampianina et al., 2005). Although Burkholderia strains have been isolated from nodules of legumes growing in Australia, so far no nod genes have been detected in these (R. Walker, unpublished). For China and Europe, no reports of Burkholderia RNB from endemic legumes have been described. Clearly more isolations need to be made. Thus, it appears that Southern Africa and South America are the major centers for Burkholderia evolution (see also Chapter 17).

To explain these apparently disparate centers of origin of the RNB Burkholderiaceae, it is important to remember that ca. 200 Mya, South America, Africa, India, and Australia were all part of the single continent Gondwana. If Burkholderiaceae and Rhizobiaceae date from this time, and based on timing of the divergence of Bradyrhizobium and Rhizobium and the presence of Burkholderia in AMF (Fig. 89.4), it is a formal possibility that the two families were members of the Gondwanan microflora. Thus, it can be deduced that certain Burkholderia species such as B. tuberum originated when South Africa and South America were still connected, which might explain the disparate localization of B. tuberum with the mimosoid (South American) and papilionoid (South African) legumes after the continents split. The example of a South American B. tuberum is the strain Burkholderia CCGE1002, which was isolated from nodules of Mimosa occidentalis growing in Mexico and has characteristics typifying both the South African and South American strains (Mishra et al., 2012; Ormeño-Orrillo et al., 2012). Strain CCGE1002 is B. tuberum based on a concatenated sequence of 16S RNA and four housekeeping genes (Agapakis et al., unpublished), but not if based on nodA or nifH (Mishra et al., 2012). However, in contrast to South African B. tuberum STM678T and B. tuberum related strains such as B. rhynchosiae, B. dillworthii, and B. sprentiae (De Meyer et al., 2013a,b, 2014), CCGE1002 has a nod-nif gene arrangement upon a symbiotic plasmid that is similar to that of other Mimosa-nodulating Burkholderia strains except it has an integrase gene terminating the operon (Agapakis et al., unpublished). In addition, the nod and nif genes of the Mimosoideae-nodulating Burkholderia strains are not closely related to those of the Papilionoideae-nodulating South African B. tuberum strains (Mishra et al., 2012). Bontemps et al. (2010) placed the acquisition of the symbiotic genes by RNB at about the same time as when the legumes were diversifying. We propose that the presence of different legume subfamilies, Mimosoid versus Papilionoid, as well as the influence of different biogeographies on the two once connected continents facilitated the divergence of Burkholderia RNB into separate lineages, accounting for the Mimosa-nodulating strain CCGE1002 versus the Papilionoideae-nodulating B. tuberum STM678T and related species (see also Chapter 19).

Finally, which of the RNB evolved first, the alphaherzobia or betarhizobia? If Burkholderia species inhabited fungal cells at the time of the emergence of the vascular plants, this strongly suggests their ancient origin. The timeline implies that Burkholderia may have associated with plants some 500 Mya via the AM symbiosis, and in the process, recruited the capacity for an intimate interaction with the plant from AMF via an unknown mechanism. This hypothesis suggests that Burkholderia could have been the source of the nodulation genes via lateral transfer of genes if a nonreduced genome ancestor associated with the fungi. However, an alternative hypothesis, as previously mentioned (Hirsch et al., 2001), is that the original source of these genes was outside either Burkholderiaceae or Rhizobiaceae because of their lower G+C content compared to the rest of the genome. For both groups of RNB, the genome G+C
content is higher overall than that of the nodulation genes. Potential recruitment of nodB and nodC from other bacteria or fungi is a distinct possibility, but more elusive is the source of the gene-encoding NodA, the protein responsible for adding a fatty acyl chain onto the resulting free amino group of the nascent Nod factor.

Based on the isolations of Burkholderia RNB so far (Fig. 89.3), it appears that the RNB group is confined to the southern hemisphere except for CCGE1002, which was isolated from nodules in Mexico, as well as some undescribed isolates from China. However, based on fossil data, the distribution of many legume genera is very different from what it was predicted to be 65 Mya. The greatest diversity at the generic level then and now is in tropical America and Africa/Madagascar (Herendeen et al., 1992). Coincidentally, this distribution overlaps with that of the Burkholderia RNB. Of the North American fossil flora, many extant relatives are now restricted to South America possibly as a result of climatic constraints (Herendeen et al., 1992). A more extensive focus on Burkholderia RNB and their hosts is needed to obtain a more complete knowledge of legume and symbiont biogeography.

Exploring the origins of nodulation provides insight into the evolution and diversity of symbiosis more broadly. Interactions between plants and microbes have an ancient and important history. The role of the Burkholderia RNBs in that history, especially in the southern hemisphere, demonstrates the potential value of these strains in future agricultural applications as well as in our broad understanding of ecological and evolutionary relationships (see also Chapter 17).

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