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Rhizobium sp. Strain NGR234 and *R. fredii* USDA257 Share Exceptionally Broad, Nested Host Ranges

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Genetically, *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 are closely related. Small differences in their nodulation genes result in NGR234 secreting larger amounts of more diverse lipo-oligosaccharidic Nod factors than USDA257. What effects these differences have on nodulation were analyzed by inoculating 452 species of legumes, representing all three subfamilies of the Leguminosae, as well as the nonlegume *Parasponia andersonii,* with both strains. The two bacteria nodulated *P. andersonii,* induced ineffective outgrowths on *Delonix regia,* and nodulated *Chamaecrista fasciculata,* a member of the only nodulating genus of the *Caesalpinieae* tested. Both strains nodulated a range of mimosoid legumes, especially the Australian species of *Acacia,* and the tribe *Ingeae*. Highest compatibilities were found with the papilionoid tribes *Phaseoleae* and *Desmodieae*. On *Vigna* spp. (*Phaseoleae*), both bacteria formed more effective symbioses than rhizobia of the "cowpea" (*V. unguiculata*) miscellany. USDA257 nodulated an exact subset (79 genera) of the NGR234 hosts (112 genera). If only one of the bacteria formed effective, nitrogen-fixing nodules it was usually NGR234. The only exceptions were with *Apios americana, Glycine max,* and *G. soja.* Few correlations can be drawn between Nod-factor substituents and the ability to nodulate specific legumes. Relationships between the ability to nodulate and the origin of the host were not apparent. As both *P. andersonii* and NGR234 originate from Indonesia/Malaysia/Papua New Guinea, and NGR234's preferred hosts (*Desmodiinae/ Phaseoleae*) are largely Asian, we suggest that broad host range originated in Southeast Asia and spread outward.

Fuchsius (1542) was probably the first to publish drawings of legume root nodules. Unfortunately, nodules were not mentioned in the accompanying text, and subsequent publications assigned them to everything from disease responses to storage organs (see Fred et al. 1932). Frank (1879) showed that sterilizing soil prevented nodule formation, while Hellriegel (1886) and Hellriegel and Wilfarth (1888) demonstrated that nodule formation results from an infection. Proof that bacteria were the causative agents came from Beyerinck (1888a, 1888b, 1888c, 1888d, 1888e), who prepared pure cultures of the nodule occupants and used them to infect legumes (Beyerinck 1890). Finally, Prazmowski (1889, 1890) inoculated *Pisum sativum* with pure cultures and showed that the bacteria penetrate legumes via infection threads in root hairs.

Specificity in legume-*Rhizobium* associations was also apparent at the end of the last century. Nobbe et al. (1891, 1895) found that bacteria isolated from *P. sativum* nodules were unable to nodulate plants belonging to the tribes *Genisteae* and *Hedysareae*. Ever since Hiltner and Störmer (1903) tried to classify rhizobia from various plant sources, numerous taxonomic proposals have been made (e.g., Fred et al. 1932). All strongly emphasized the host from which the rhizobia were isolated, which led to numerous problems (Wilson 1939; Lim and Burton 1982; Trinick 1982). Foremost among these is the unwieldy size of groups such as the "cowpea" miscellany, which originally had as its nucleus 21 legume genera (41 species). By definition, each of these "cowpea" rhizobia can nodulate *Vigna unguiculata* in addition to the host from which it was isolated. An additional 117 species were added to the group in the mid 1930s (Carroll 1934; Allen and Allen 1936). Two decades later, the majority of all nodulated legumes were included in this untidy and largely unworkable assemblage (Norris 1956).

Interestingly, work on the molecular basis of host specificity also began about 100 years ago. Hiltner (1900) showed that aqueous, bacteria-free filtrates from mature *P. sativum* nodules contained a substance that induced root-hair formation and deformation of the root hairs (Had) of *P. sativum*. Although many others sought these "Had-factors," the research only culminated in the report of Lerouge et al. (1990), who showed that the substances responsible for deformation are N-acylated oligomers of *N*-acetyl-D-glucosamine (see Relić et al. 1993). Since then, the "Had-factor" structures (now called Nod factors as they are the products of the nodulation genes; see Roche et al. 1991b) of a number of *Azorhizobium/ Bradyrhizobium/ Mesorhizobium/ Sinorhizobium/ Rhizobium* species have been elucidated (see Schultze et al. 1994; Fellay et al. 1995b; Dénarié et al. 1996; Long 1996; Spaink 1996; Hanin et al. 1999; Cohn et al. 1998).

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As Nod factors are responsible for root-hair curling (Hac), the induction of nodulation (Hai), and the entry of rhizobia into the roots, it should be possible to define host-range differences between rhizobia in terms of the genes involved in Nod-factor production.

Our work is concerned with the control of broad host range in legume-*Rhizobium* associations. As model rhizobia, we use *Rhizobium* sp. strain NGR234 (W. J. B.), and *R. fredii* USDA257 (S. G. P.). NGR234 was the only fast-growing strain among 30 isolates prepared from *Lablab purpureus* nodules in Papua New Guinea (soil pH 8.5) by M. J. Trinick (in 1965). Shortly afterward, Trinick recognized the potential of NGR234 and generously distributed it a decade in advance of publication (see Trinick 1980). Thus, Broughton and Dilworth (1971) inoculated *V. unguiculata* with either NGR234 or a *Bradyrhizobium* sp. to confirm that the plant encodes the globin portion of the leghemoglobin molecule. Reports followed on both the bacterium (Broughton et al. 1972) and development of *V. unguiculata* nodules induced by NGR234 (Broughton et al. 1978). Interest in NGR234 gradually increased following Trinick's comparison with other fast-growing rhizobia (Trinick 1980), and the nodulation characteristics of various derivatives as well as spontaneous resistance mutants including ANU237 (wild type), ANU240 (Sm^r), ANU265 (cured of the symbiotic plasmid), ANU280 (Sm^r Rif^r), and MPIK3030 (Sm^r) have been published (Kondorosi et al. 1982; Morrison et al. 1983; Pankhurst et al. 1983a, 1983b; Wong et al. 1983; Morrison et al. 1984; Broughton et al. 1984; Bachem et al. 1985; Broughton et al. 1985; Bachem et al. 1986; Bassam et al. 1986; Broughton et al. 1986; Dilworth et al. 1986; Horvath et al. 1987; Lewin et al. 1987a, 1987b; Nayudu and Rolfe 1987; Stanley et al. 1988; Williams et al. 1988; Wong et al. 1988). In the 10 years since the last of these reports, there has been an explosion of interest in NGR234 and related strains (see Perret, Jabbouri, and Broughton, *in press*, and Perret et al., *in press*). Although NGR234 has a short generation time and a G+C content characteristic of *Rhizobium* spp. (Broughton et al. 1972), its single subpolar flagellum (Padmanabhan et al. 1989) is more representative of those found on *Bradyrhizobium* spp.

In contrast, *R. fredii* USDA257 was isolated from a wild soybean (*Glycine soja*) plant growing near Wuking, China (Keyser and Griffin 1987; Keyser et al. 1982). Although generally considered to be a soybean symbiont (e.g., Dénarié et al. 1992), *R. fredii* nodulates a number of additional legume species (Broughton et al. 1984; Heron and Pueppke 1984; Keyser et al. 1982; Morrison et al. 1986; Stowers and Eaglesham 1984).

Data from DNA subtraction hybridizations confirmed that NGR234 and USDA257 are phylogenetically closely related, and share most of their genomic background (Perret et al. 1994). Homologies with insertion sequences (IS) and the absence from the USDA257 genome of the NGRRS-1 transposon-like repeat (Perret et al. 1997) suggest that many of the sequences "unique" to NGR234 are mobile elements that have accumulated since both bacteria diverged. Comparisons of the host ranges of NGR234 and USDA257 should thus allow correlations to be made between those genes (and the Nod-factor substituents) that are unique to NGR234 and its ability to nodulate specific plants. Furthermore, as the geographic and temporal origins of the two rhizobia are substantially different, nodulation capacity can be evaluated in terms of legume distribution and taxonomy. For these reasons, we tested the ability of both strains to nodulate a wide spectrum of plants.

RESULTS

Reproducibility of nodulation tests.

Rigorously controlled nodulation tests would have involved optimizing (i) growth conditions for each individual legume, (ii) inoculation conditions, especially by including a "benchmark" strain (i.e., one chosen for the particular plant because of its high nitrogen-fixing capacity), and (iii) harvest times for each legume. Given the number of legumes tested and the paucity of information available on many of them (including their rhizobial requirements), this was not practical. Rather, standardized conditions were developed that, though probably suboptimal for some plants, permitted reproducible tests in disparate laboratories.

Glycine max presents a good example of the kinds of problems presented by suboptimal growth conditions. Trinick (1980) reported that NGR234 effectively nodulates (i.e., is Fix^+) on *G. max* but did not specify the cultivar. Broughton et al. (1984) found that NGR234 formed ineffective nodules on *G. max* cv. Caloria (Nod^+), but that it failed to nodulate cv. Peking. Both sets of experiments were performed in large, "Leonard jar" assemblies (Leonard 1943) housed in glasshouses. Balatti and Pueppke (1990) concluded that *G. max* cv. McCall is not infected by NGR234 in plastic growth pouches. In a later study, Balatti et al. (1995) stated that "under uniform growth conditions in vermiculite, *Rhizobium* sp. NGR234 did not form normal Fix^+ nodules on any of a wide variety of soybean cultivars." This set of inoculation tests was performed on 89 different *G. max* cultivars grown in the same type of Magenta jar assemblies described here. It thus seems unavoidable that both abiotic and biotic factors (particularly cultivar and inoculum dosage) influence the spectrum of plants nodulated. In this sense, nodulation is a conditional phenotype.

Nodulation status of the Leguminosae nodule type.

About 57% of the legume genera have been examined for nodulation (approximately 3,500 species, 20% of the Leguminosae). Of these, only 23% of Caesalpinioid species have been found to nodulate, versus 90% in the Mimosoideae and 97% in the Papilionoideae (de Faria et al. 1989). It was thus important to compare the nodulation ability of the two rhizobia with the capacity of each individual legume to nodulate. To do this, a collation of the nodulation status of all the species tested (taken from Lim and Ng 1977; Allen and Allen 1981; de Faria et al. 1989; de Souza Moreira et al. 1992; Whitty et al. 1994; Athar 1993, 1996a, 1996b, 1997; and J. I. Sprent, *personal communication*) is presented in Table 1. Taxonomic entities not known to nodulate are shaded in the table.

Since nodule structure and function are largely determined by the plant (Dart 1977; Corby et al. 1983; Sprent and Sprent 1990), the nodulation capacities of the two bacteria were also compared with the types of nodules elicited. For simplicity, only three different types of nodule were recognized: Aeschynomenoid, determinate, and indeterminate (marked in pink, blue, and green, respectively, in Table 1). This information was taken from the publications of Corby et al. (1983), Crisp and Weston (1987), Sprent et al. (1989), Corby (1988),

James et al. (1993), Sutherland et al. (1994), Cordeiro and Sprent (1996), and Harrier et al. (1997), and from J. I. Sprent (*personal communication*).

Nodules formed on *Lupinus* spp. (*Genisteae*), which do not fit easily into any of these classes, were not classified. Aeschynomenoid nodules, which are a feature of the *Aeschynomeneae* (but also found in the *Dalbergieae*), are characterized by a direct, intercellular invasion process (Allen and Allen 1940; Dart 1977; Chandler 1978; Chandler et al. 1982). Rather than entering through the root hairs of, e.g., *Arachis* spp. and *Stylosanthes* spp., rhizobia penetrate the intercellular spaces. After the bacteria have gained entry into the root, they penetrate the cortical cells in a process that resembles pinocytosis (Meijer and Broughton 1982). A unique feature of Aeschynomenoid nodules is that the bacteroid-containing cortical cells continue to divide. Although a certain amount of dimorphism exists (Corby et al. 1983), determinate nodules (see cover) generally lack an apical meristem and are short-lived; the vascular strands fuse at the apex and they usually export ureides to the xylem (Sprent 1980). On the other hand, indeterminate nodules possess an apical meristem and export amino acids and amides (Corby et al. 1983). Although both types of nodules are found in the Papilionoideae, the indeterminate "caesalpinioid" type is the only one found in the Caesalpinioideae and Mimosoideae, suggesting that it is the plesiomorphic type (Doyle et al. 1997).

Legumes nodulated by NGR234 and USDA257.

In all, the ability of *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 to form root nodules was tested on a total of 452 legume species (Table 2), distributed among 194 genera in all three subfamilies of the Leguminosae (Polhill and Raven 1981; Corby et al. 1983; Polhill 1994). Twenty-four of the 153 Caesalpinioid genera, including all four tribes in this subfamily, were tested. The corresponding figures for the subfamily Mimosoideae are 20 out of 65 genera, distributed within four of the five recognized tribes. Far more nodulation tests were performed with papilionaceous genera, however. One hundred and fifty of 448 genera were examined, including every tribe except the *Carmichaelieae, Dipterygeae, Euchresteae*, and *Liparieae*. Among the Papilionoideae seven of the eight subtribes of the *Phaseoleae*, the largest tribe of the Leguminosae, and one that contains many species of commercial importance, were tested.

Subfamily Caesalpinioideae.

Chamaecrista, a mostly tropical and subtropical tribe, is the only large exception to the rule that Caesalpinioid legumes are not known to nodulate (Sprent 1994) (Table 1). It was therefore perhaps not surprising that USDA257 was Nod^+ and NGR234 Fix^+ on *C. fasciculata* (Table 1). Although *Delonix regia* is generally considered to be refractive to rhizobia (Allen and Allen 1981), Lim and Ng (1977) reported the presence of small, Fix^- nodules on this plant growing in Singapore. As NGR234 and USDA257 elicited similar nodules, we were able to confirm their observations.

Subfamily *Mimosoideae*.

NGR234 nodulated 28 of 53 species in this subfamily and in 16 cases the nodules were Fix^+. Nodulation by USDA257 was restricted to 18 of these species, but nitrogen was fixed in combination with only seven (Table 1). Thus, with the exception of the genera *Calliandra* and *Leucaena*, the nodulating abilities of the two strains for mimosoid legumes are quite similar. Both strains nodulated hosts such as *Albizia* spp., *Enterolobium contortisiliquum*, and *Desmanthus illinoensis*, all of which are thought to have symbiotic preferences for slow-growing bradyrhizobia (Allen and Allen 1981). The production of fully Fix^+ nodules by USDA257 on *Albizia lebbeck* and *A. procera* is noteworthy, because it shows that *R. fredii* can also effectively nodulate leguminous trees.

Interestingly, almost all the species of the *Acacia* subgenus *Heterophyllum* are Australian, and all tested here (*A. auriculiformis, A. cyanophylla, A. mangium, A. mearnsii, A. pendula, A. retionodes*, and *A. saligna*) nodulated with NGR234. Furthermore, with the exception of *A. pendula*, all also formed nodules with USDA257. Of the subgenus *Aculeiferum* (*A. aroma, A. ataxacantha, A. bonariensis*, and *A. macracantha*), only the two Argentinian species (*A. bonariensis* and *A. macracantha*) nodulated, and then only ineffectively, with NGR234. None of the African species nodulated with either strain. *A. ataxacantha* is believed not to nodulate, in common with a number of close relatives within the section Monocanthea of the subgenus *Aculeiferum* (Harrier et al. 1997).

Acacia spp. are known to form symbioses with both rhizobia and bradyrhizobia (Dreyfus and Dommergues 1981). Recently, the rhizobial requirements of a number of them have been shown to be even more diverse and include *Rhizobium saheli* and *R. teranga* bv. *sesbaniae*, species that produce arabinosylated and fucosylated Nod factors similar to those produced by *Azorhizobium caulinodans* (Lorquin et al. 1997a, 1997b). Although inoculation with NGR234 gave Fix^+ nodules with four *Acacia* spp. (*A. auriculiformis, A. pendula, A. retinodes*, and *A. saligna*), none of the 15 species tested fixed nitrogen with USDA257. Perhaps this indicates that NGR234 possesses host-specific nitrogen-fixation genes for *Acacia* that

Table 2. Analyses of the ability of *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 to nodulate members of the plant family Leguminosae[a]

	Tribes		Genera		Species		Total species nodulated	
Subfamily	**USDA**	**NGR**	**USDA**	**NGR**	**USDA**	**NGR**	**USDA**	**NGR**
Caesalpinoideae	2^N	$1^N, 1^F$	2^N	$1^N, 1^F$	2^N	$1^N, 1^F$		
Number tested		4		24		46		
Mimosoideae	$1^N, 2^F$	3^F	$4^N, 6^F$	$2^N, 10^F$	$11^N, 7^F$	$12^N, 16^F$		
Number tested		4		20		53		
Papilionoideae	$9^N, 9^F$	$3^N, 18^F$	$34^N, 33^F$	$37^N, 61^F$	$56^N, 59^F$	$83^N, 119^F$		
Number tested		26		150		353		452
Totals by taxonomic group	$12^N, 11^F$	$4^N, 22^F$	$40^N, 39^F$	$40^N, 72^F$	$69^N, 66^F$	$96^N, 136^F$	135 (30%)	232 (51%)

[a] A total of 452 species distrbuted among the three subfamilies of the Leguminosae were tested. Superscripts represent the number of taxa that formed Nod^+ or Fix^+ nodules. Both bacteria were also Nod^+ on *Parasponia andersonii* (Ulmaceae).

are either absent or inactive in USDA257, an observation that is consistent with the fact that *Acacia* prefers sulfated Nod factors (Lortet et al. 1996).

Subfamily Papilionoideae.

Both NGR234 and USDA257 are widely compatible with species of this subfamily (Table 1). USDA257 nodulated 115 Papilionoid species (out of 359 tested), and in 52% of the cases, nitrogen was fixed. NGR234 nodulated the same 115 species, as well as an additional 87 species distributed among 24 tribes of this subfamily (= 57% of all Papilionoideae tested). One hundred and nineteen of the 202 species (59%) nodulated by NGR234 fixed atmospheric nitrogen.

Although our data on some tribes are limited, analysis of Table 1 reveals distinctive patterns in the nodulation of Papilionoid legumes by the two strains. Thus, members of several tribes appear to be responsive only to NGR234. These include the *Abreae*, which is monotypic, the subtribe *Clitoriinae* of the *Phaseoleae*, the subtribe *Aeschynomeninae* of the *Aeschynomeneae*, the subtribe *Glycyrrhizinae* of the *Galegeae*, the

Fig. 1. Climatic map of the world showing the places of isolation of *Rhizobium* sp. strain NGR234 (Papua New Guinea) and *R. fredii* USDA257 (China). Also shown are the origins of some legume hosts and nonhosts of the two bacteria. Red spots indicate that both bacteria formed Fix^+ nodules on a legume originating from that region; green spots show that only NGR234 was able to nodulate the particular host; yellow spots mark origins of legumes unable to form nodules with either bacterium. Most of the data on origins of the legumes was taken from Allen and Allen (1981) and Mabberley (1987).

Table 3. Variation in the ability of three *Vigna* spp. to nodulate and fix nitrogen with three *Bradyrhizobium* and three *Rhizobium* strains[a]

	Bradyrhizobium japonicum			*Rhizobium* spp.		
Species/variety	**CB756**	**USDA76**	**USDA110**	**NGR234**	**NZP4010**	**USDA257**
V. mungo^{ROG}	Nod^+	$Fix^{+/-}$	Fix^+	Fix^+	Nod^-	Fix^+
V. radiata						
King^{WSA}	$Fix^{-/+}$	Nod^-	Fix^+	Fix^+	Nod^-	Fix^+
PUSa9173^{BSU}	Fix^+	Fix^+	Fix^+	Fix^+	Fix^+	$Fix^{+/-}$
RUM-1^{BSU}	Nod^-	Nod^-	Nod^-	Fix^+	Nod^-	$Fix^{+/-}$
Texproust^{BSU}	Nod^+	$Fix^{-/+}$	Fix^+	Fix^+	Nod^-	$Fix^{+/-}$
VC3890 A^{BSU}	Nod^+	$Fix^{-/+}$	Fix^+	Fix^+	Nod^-	$Fix^{-/+}$
VC4718 A^{BSU}	Fix^+	Nod+	Fix^+	Fix^+	Nod^+	Fix^+
WM-92^{BSU}	Fix^+	Nod^-	Fix^+	Fix^+	$Nod^{+/-}$	Fix^+
V. radiata subsp. *sublobata*^{BSU}	Nod^+	Fix^+	Fix^+	Nod^+	Nod^-	Nod^+
V. unguiculata						
CA B #5^{BSU}	Nod^+	Fix^+	NT	Fix^+	Nod^-	Fix^+
PI186465^{BSU}	Nod^+	Fix^+	Fix^+	$Fix^{+/-}$	Nod^-	Fix^+
R. Caloona^{WSA}	Nod^+	Nod^+	Nod^+	Fix^+	Nod^-	Fix^+
TVN963^{BSU}	Nod^+	Fix^+	Fix^+	Fix^+	Nod^-	Fix^+
UCR430^{BSU}	Nod^+	Fix^+	Fix^+	Fix^+	$Fix^{-/+}$	Fix^+
84S-2246-6^{BSU}	Fix^-	Fix^+	Fix^+	Fix^+	$Fix^{-/+}$	Fix^+
524 B^{BSU}	Nod^-	Fix^+	Fix^+	Fix^+	Nod^-	Fix^+

[a] Sources of seeds are as listed in the footnote to Table 1. NT = not tested. Phenotypes as in Table 2, except that in order of decreasing nitrogen-fixing efficiency they are Fix^+, $Fix^{+/-}$, and $Fix^{-/+}$. Nod^+ nodules did not fix nitrogen.

Podalyrieae, and the *Thermopsideae*. Both strains, but especially NGR234, are broadly compatible with members of the *Dalbergieae*, *Desmodieae*, *Millettieae*, and *Phaseoleae*. This is particularly evident in the *Phaseoleae* as NGR234 nodulates all species tested in the subtribes *Diocleinae*, *Glycininae*, *Kennediinae*, and, with two exceptions (*Physostigma reticulatum* and *Strophostyles helvola*), all other species of the *Phaseolinae*. In the *Phaseolinae*, 87% of the species formed nodules that were Fix$^+$. Perhaps adaptation to the *Phaseoleae* can be explained by the fact that NGR234 was isolated from

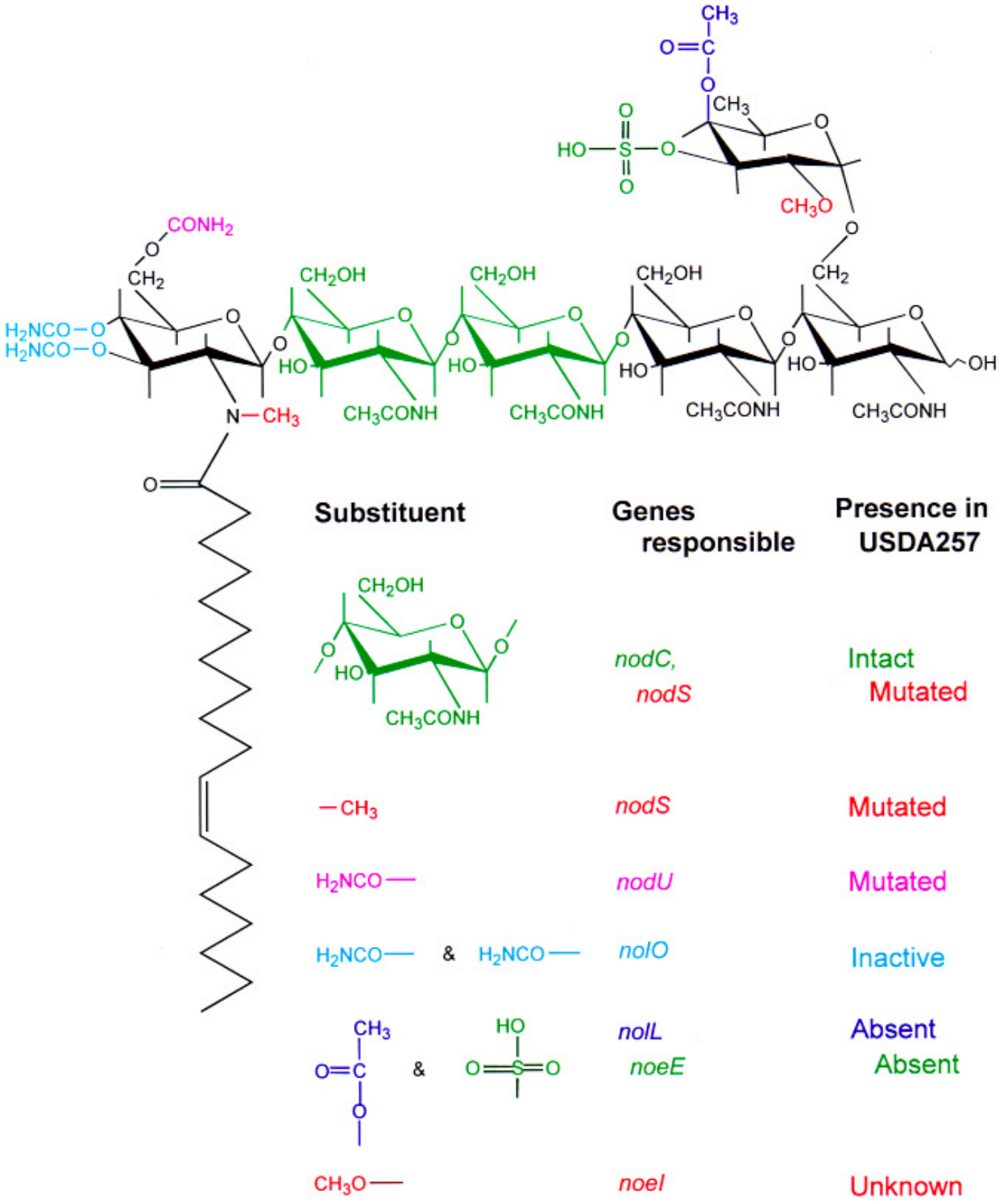


Fig. 2. Comparison of the structures of the Nod factors produced by *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 along with the known differences in nodulation and host-specificity genes between the two organisms. Data were taken primarily from Price et al. (1992), Bec-Ferté et al. (1994), and Jabbouri et al. (1998).

Lablab purpureus (subtribe *Phaseolinae*). Intriguingly, NGR234 nodulated all eight *Phaseolus* spp. as well as the 17 *Vigna* spp. tested (Table 1). In the latter case, all developed effective, nitrogen-fixing nodules. A member of the *Desmodiinae*, *Pycnospora lutescens*, is the only addition to the list of legumes known to nodulate.

Lack of nodulation in several taxonomic divisions is also significant. Included in this category are all members of two well-studied tribes: the *Trifolieae*, whose species are normally nodulated by *R. meliloti* and *R. leguminosarum* bv. *trifolii*, and the *Vicieae*, which are usually nodulated by *R. leguminosarum* bv. *viceae* (Trinick 1982). To see if exceptions to this "lack of nodulation rule" exist, the capacities of 40 different *Medicago* spp. were tested with *R. meliloti* as a positive control. Although *M. cancellata* and *M. papillosa* spontaneously produced nodules, neither plant responded to inoculation with NGR234 or USDA257.

Both strains failed to nodulate *Cicer arietinum*, the best-known member of the sole genus constituting the *Cicereae*. *C. arietinum* nodulates exclusively with a group of highly specific *Rhizobium* strains that have affinities to *R. leguminosarum* bv. *viceae* (Allen and Allen 1981). Non-nodulation also occurred within certain tribes. Thus, none of the *Astragalus* (tribe *Galegeae*) species tested (normally nodulated by a group of strains now classified as *R. haukuii*), no members of the genus *Onobrychis* (*Hedysareae*) (specific for a subgroup of bradyrhizobia), nor *Lotononis bainesii* (*Crotalarieae*) (recognized as a model of acute symbiotic specificity) (Norris 1956; Allen and Allen 1981; Chen et al. 1991) nodulated with either strain.

Nodulation of the nonlegume *Parasponia andersonii* (Ulmaceae).

Nodulated weeds were found in tea plantations in Papua New Guinea. Initially, they were identified as *Trema aspera* (Trinick 1973). Further studies showed that the specimens were incorrectly identified and in fact belong to *Parasponia rugosa* Bl. (see Akkermans et al. 1978; Akkermans and van Dijk 1981). Later it was shown that NGR234 nodulates (albeit ineffectively) a related species, *Parasponia andersonii* (Trinick and Galbraith 1980). Since, for evolutionary reasons, it is important to know if USDA257 can also nodulate *Parasponia* spp. (see Discussion), we attempted to perform similar nodulation experiments with plants of this group. Unfortunately, we were unable to germinate any of the batches of seeds obtained, but in collaboration with G. Webster and E. C. Cocking (Plant Genetic Manipulation Group, University of Nottingham, Nottingham, UK), we tested the nodulation capacity of the two bacteria on *P. andersonii* seedlings raised in tissue culture. Both nodulated this plant (Table 1).

Legume origins and nodulation capacity.

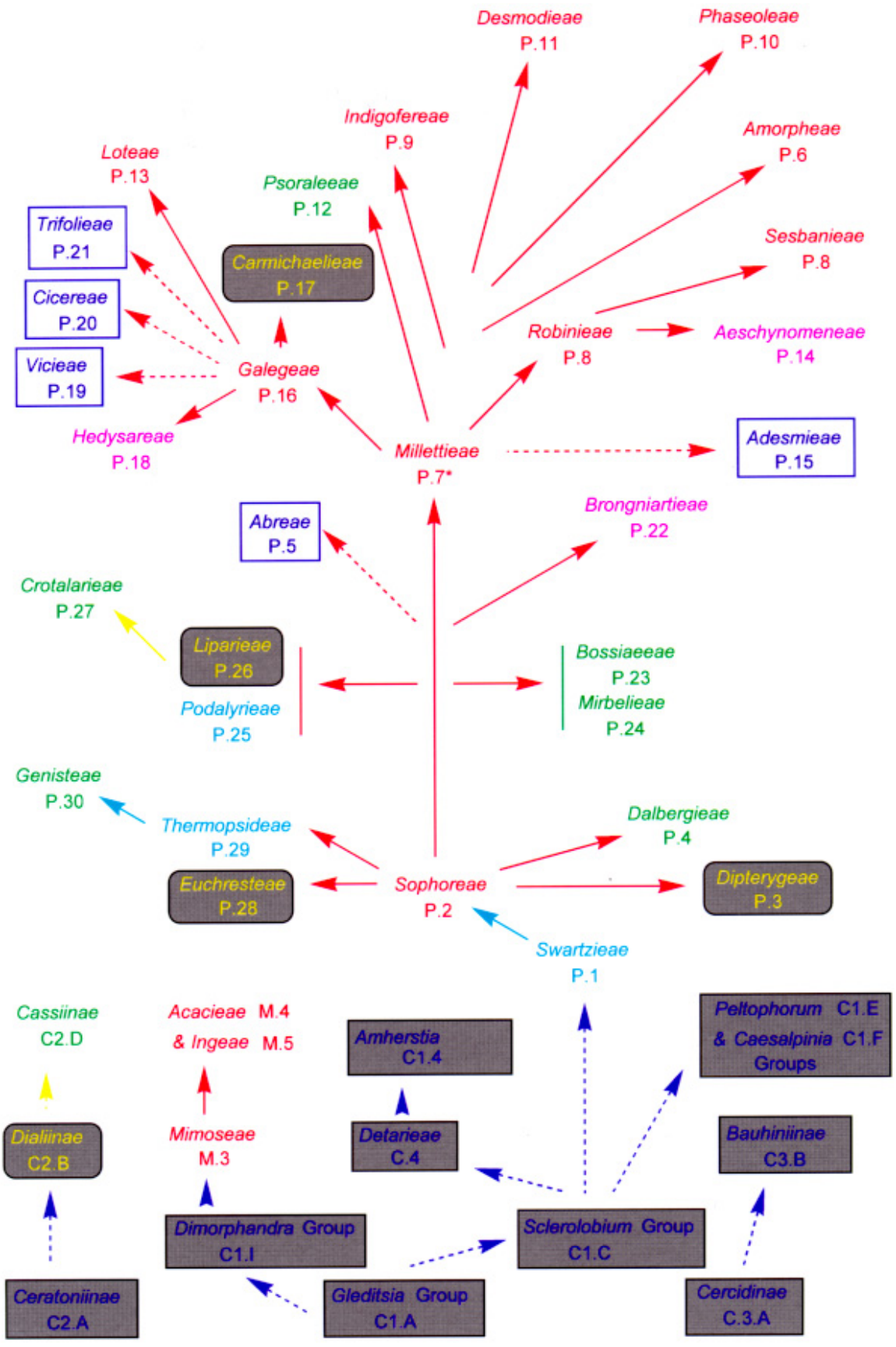
As NGR234 was isolated in Papua New Guinea, it is often regarded as a bacterium that is specific to tropical species. To examine whether correlations between the centers of origin and the nodulation capacities of the legumes exist, those species with known origins were grouped into three classes: hosts (i.e., Nod^+) to both NGR234 and USDA257; Nod^+ with NGR234 but Nod^- with USDA257; and, Nod^- with both bacteria. These data, together with the centers of origin of the legumes representing all three nodulation classes, were plotted on a map of the world that also displays regional variation in vegetation density (Fig. 1). One limitation of this analysis is that the origins of many legumes are difficult to trace, and are therefore not shown. Nevertheless, it is apparent that legumes that form nodules with both NGR234 and USDA257 can be found in most parts of the world. This includes such northern temperate species as *Amorpha fruticosa*, *Colutea arborescens*, *Lespedeza bicolor*, *Oxytropis halleri*, *Robinia pseudoacacia*, *Ulex europaeus*, and *Wisteria sinensis*. Equally, a number of southern temperate species form Nod^+ nodules with both bacteria. These include *Hardenbergia comptoniana*, *Kennedia rubicunda*, *Mirbelia dilatata*, *Oxylobium ellipticum*, and *Psoralea pustulata*. Finally, the tropical legumes *Cajanus* spp., *Codariocalyx motorius*, *Dolichos junghuhnianus*, *Flemingia* spp., *Macrotyloma axillare*, *Macroptilium* spp., *Psophocarpus tetragonolobus*, *Pycnospora lutescens*, *Tephrosia vogelii*, and *Sesbania grandiflora*, as well as many *Vigna*

Table 4. A simplified comparison of differential responses of selected legumes to inoculation with various broad-host-range rhizobia

	Nodulation in response to broad-host-range rhizobia[a]					
Inoculated	***Albizia* (*Rhizobium*)†**	***Desmodium* (*Rhizobium*)†**	***Glycine* USDA257‡**	***Lablab* NGR234‡**	***Macrotyloma* CB756***	***Mucuna* (*Bradyrhizobium*)†**
Clitoria sp.	+	+	–	+/–**	+	+
Colutea arborescens	–	+	+	+	NT	–
Desmanthus illinoensis	–	+	+	+	NT	–
Laburnum anagyroides	+	–	–	+	NT	+
Onobrychis vicifolia	+	+	–	–	NT	–
Phaseolus angularis	–	–	+	+	+	–
Pueraria phaseoloides	+	–	–	+	+	–
Vigna unguiculata	–	–	+	+	+	+

[a] Data are from Wilson (1939)†, Diatloff and Date (1978)*, and this work (Table 1)‡. NGR234 is Fix^+ with *Clitoria laurifolia*, but Nod^- with *C. ternatea***. + = plants were nodulated; – = nodules did not form; NT = not tested.

Fig. 3. Putative relationships among tribes of the Leguminosae and the ability of *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 to nodulate them. "Tree" was constructed from morphological analyses of Corby et al. (1983). Tribes in yellow letters on gray background have not been tested for their nodulation capacity. Tribes in dark blue (in black, shaded boxes) are not known to nodulate. Tribes in dark-blue framed in black are Nod^- with both bacteria; those in light-blue are Fix^+ with NGR234 but Nod^- with USDA257; those marked in pink are Nod^+ with both bacteria; those in green are Nod^+ with USDA257 and Fix^+ with NGR234; those in red are Fix^+ with both bacteria. Dotted blue arrows show absence of nodulation; dotted red arrows, loss of nodulation with NGR234/USDA257. Colors of the arrows match those of their immediate "progenitor."


Desmodieae
P.11
Phaseoleae
P.10
Indigofereae
P.9
Amorpheae
P.6
Loteae
P.13
Psoraleeae
P.12
Trifolieae
P.21
Carmichaelieae
P.17
Sesbanieae
P.8
Cicereae
P.20
Robinieae
P.8
Aeschynomeneae
P.14
Vicieae
P.19
Galegeae
P.16
Hedysareae
P.18
Millettieae
P.7*
Adesmieae
P.15
Abreae
P.5
Brongniartieae
P.22
Crotalarieae
P.27
Liparieae
P.26
Podalyrieae
P.25
Bossiaeeae
P.23
Mirbelieae
P.24
Genisteae
P.30
Thermopsideae
P.29
Dalbergieae
P.4
Euchresteae
P.28
Sophoreae
P.2
Dipterygeae
P.3
Swartzieae
P.1
Cassiinae
C2.D
Acacieae M.4
& Ingeae M.5
Amherstia
C1.4
Peltophorum C1.E
& Caesalpinia C1.F
Groups
Dialiinae
C2.B
Mimoseae
M.3
Detarieae
C.4
Bauhiniinae
C3.B
Dimorphandra Group
C1.I
Sclerolobium Group
C1.C
Ceratoniinae
C2.A
Gleditsia Group
C1.A
Cercidinae
C.3.A

spp., form nitrogen-fixing nodules with both bacteria. Even such Mimosoid legumes as *Dichrostachys cinerea* (Africa, India), *Albizia lebbeck* (tropical Asia), *Samanea saman* (tropical America), and *Paraserianthus falcataria* are Fix^+ with both species. Equally, Fix^+ hosts are found in high-rainfall regions (*Albizia* spp., *Codariocalyx motorius*, *Flemingia congesta*, *Indigofera tinctoria*, *Psophocarpus tetragonolobus*, some *Vigna* spp., etc.) and relatively dry parts of the world (*Colutea arborescens*, *Hardenbergia comptoniana*, etc.).

Legume growth habit and nodulation capacity.

Growth habit of the host plant also seems to bear little relationship to its propensity to be nodulated by either bacterium. NGR234, for example, fixes nitrogen in association with annuals from Asia, the Americas, Australia, southern Africa, and Europe (e.g., *Lablab purpureus*, *Phaseolus* spp., *Kennedia rubicundra*, *Psoralea* spp., and *Lotus* spp., respectively), as well as shrubs from temperate and tropical zones (e.g., *Desmanthus illinoensis*, *Dichrostachys cinerea*, *Enterolobium contortisiliquum*, and *Sophora* spp.). Both tropical (e.g., some *Acacia* spp., *Albizia lebbeck*, *Leucaena leucocephala*, *Mundulea sericea*, *Samanea saman*, and *Xeroderris stuhlmannii*), and temperate trees (*Erythrina crista-galli*, *Colutea arborescens*, *Hesperolaburnum platycarpum*, *Sophora davidii*, and *Robinia pseudoacacia*) are effectively nodulated by NGR234 (Table 1). Species as ecologically diverse as *Lotus corniculatus*, a forage legume known for its heat and drought tolerance, and *Neptunia oleracea*, a tropical aquatic legume, are also nodulated by NGR234.

Comparison of the USDA257 and NGR234 host ranges.

Perhaps the most striking correlation observed here is that all 135 legume species nodulated by USDA257 are also host to NGR234. Thus, with respect to nodulation, the host range of USDA257 is a subset nested entirely within that of NGR234. In the 135 combinations in which both strains nodulated the same plant, 30% of all the nodules were Nod^+Fix^- with both bacteria, 46% were Fix^+ with both bacteria, while NGR234 produced Fix^+ nodules on 64% of these plants. In other words, NGR234 also has a symbiotic advantage over USDA257 in terms of nitrogen fixation. *Apios americana*, *G. max*, and *G. soja* (wild soybean) constitute the

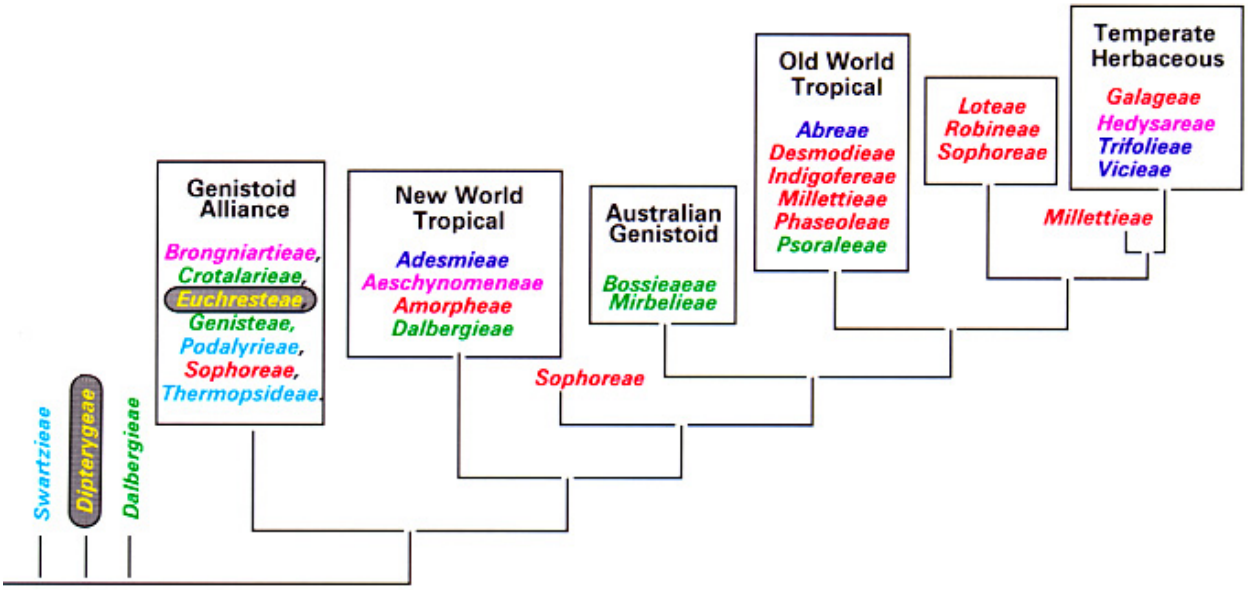


Fig. 4. Tribal relationships in the Papilionoideae, inferred from sequences of the chloroplast gene ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) strict consensus tree (Doyle et al. 1997). Color coding for the capacity of the various tribes to nodulate with NGR234/USDA257 is the same as shown in Figure 3.

Table 5. *Bradyrhizobiu/Rhizobium* strains used in this study

Strain	Host of isolation	Characteristics[a]	Reference
B. elkanii USDA76	*Glycine max*	Isolated from USDA74 by plant passage. Serogroup 76 type strain	Kuykendall et al. 1992
B. japonicum CB756	*Macrotyloma africanum*	Broad-host-range inoculant. Ap^r, Rif^r	Norris 1956; Diatloff and Date 1978
B. japonicum USDA110	*G. max*	Genetically best-characterized *B. japonicum* species. Sp^r	Kuykendall and Elkan 1976
Rhizobium sp. strain NGR234	*Lablab purpureus*	Broad host range. Rif^r	Trinick 1980
R. loti NZP4010	*Lotus divaricatus*	= NZP2037 cured of its plasmid, broad host range. Rif^r, Sm^r	Chua et al. 1985; Lewin et al. 1987b
R. fredii USDA257S1	*Glycine soja*	Km^r derivative of wild-type USDA257. Carries a silent Tn*5* insertion in the Sym plasmid	Heron et al. 1989
R. meliloti RCR2011	*Medicago sativa*	= SU47. Sm^r, Tc^r	Rosenberg et al. 1981

[a] Antibiotics used: Ap = ampicillin; Rif = rifampicin; Sm = streptomycin; and Tc = tetracycline.

Table 1. Host range of *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257. Classification of the Leguminosae follows that of Corby et al. (1983) and Polhill (1994). C, M, and P are abbreviations for the subfamily names CAESALPINIOIDEAE, MIMOSOIDEAE, and PAPILIONOIDEAE, respectively, while the numbers correspond to the tribes and genera of Polhill (1994). N denotes nodulation (but not nitrogen fixation), F that the nodules were effective, and a dash the absence of nodules. Shaded species have never been observed to nodulate, while the boxed species is the first report of nodulation. Legumes printed in green have indeterminate nodules, those in blue, determinate nodules, names in pink are species possessing Aeschynomenoid nodules, while the information available for those printed in black is either inconsistent or insufficient to determine the nodule type. Names in brackets are obsolete.

	Families, subfamilies, tribes, and species	Seed source[a]	Response to USDA 257	Response to NGR 234
	LEGUMINOSAE Juss.			
	SUBFAMILY CAESALPINIOIDEAE			
	Tribe ***Caesalpinieae***			
	A *Gleditsia* group			
C1.1	*Gymnocladus dioica* (L.) K. Koch	col^{HPP,SNU}	-	-
C1.2	*Gleditsia triacanthos* L.	col^{FSB,SNU}	-	-
	B *Acrocarpus* group			
C1.3	*Acrocarpus fraxinifolius* W. & A.	com^{ENA}	-	-
	E *Peltophorum* group			
C1.15	*Peltophorum africanum* Sond.		-	-
	Peltophorum dubium (Spreng.) Taub. [syn - *Peltophorum ferrugineum* (Decne.) Benth.]	48550^{KRG}	-	-
	Peltophorum pterocarpum (DC.) Heyne	com^{AOA}	-	-
C1.17	*Schizolobium parahyba* (Vell.) Blake	4525^{BTC}	-	-
C1.20	*Parkinsonia aculeata* L.	1569^{BTC}	-	-
C1.22	*Delonix regia* (Boj. ex Hook.) Raf.	com^{AHA,PPM}	N	N
	F *Caesalpinia* group			
C1.24	*Caesalpinia eriostachys* Benth.	4098^{BTC}	-	-
	Caesalpinia ferrea Mart.	com^{AHA,FBA}	-	-
	Caesalpinia pulcherrima (L.) Sw.	com^{AHA}	-	-
	Caesalpinia spinosa (Molina) Kuntze	Tara^{SCP}	-	-
C1.34	*Hoffmannseggia lactea* (Schinz) Schinz	90995^{KRG}	-	-
	I *Dimorphandra* group			
C1.45	*Burkea africana* Hook.	90685^{KRG}	-	-
	Tribe ***Cassieae***			
	A *Ceratoniinae*			
C2.1	*Ceratonia siliqua* L.	col^{PBE}	-	-
	D *Cassiinae*			
C2.16	*Cassia fistula* L.	2196^{BTC}	-	-
	Cassia javanica L. var. *indochinensis* Gagnepain	com^{FBA}	-	-

(*continued on the next page*)

Table 1. (*continued from the preceding page*)

	Families, subfamilies, tribes, and species	Seed source[a]	Response to USDA 257	Response to NGR 234
C2.17	*Senna hebecarpa* (Fernald) Irwin & Barneby [syn - *Cassia hebecarpa* Fernald]	com[FBA]	-	-
	Senna pallida (Vahl) Irwin & Barneby	col[PPM]	-	-
	Senna martiana (Benth.) Irwin & Barneby	48527[KRG]	-	-
	Senna notabilis (F. Muell.) B.R. Randell	com[AOA]	-	-
	Senna siamea (Lam.) Irwin & Barneby	2277/91[FZM]	-	-
	Senna spectabilis (DC.) Irwin & Barneby var. *spectabilis*	1426[BTC]	-	-
	Senna sulfurea (Collad.) Irwin & Barneby	col[UGC]	-	-
	Senna tora (L.) Roxb.	19152[KRG]	-	-
C2.18	*Chamaecrista desvauxii* (Collad.) Killip	18025[CCC]	-	-
	Chamaecrista fasciculata (Michx.) Greene	com[PWU]	N	F
	Chamaecrista nictitans (L.) Moench	19085[CCC]	-	-
	Chamaecrista rotundifolia (Pers.) Greene	7299[CCC]	-	-
	E *Labicheinae*			
C2.19	*Labichea cassioides* Gaud.	col[KPA]	-	-
C2.20	*Petalostylis labicheoides* R. Br.	com[AOA]	-	-
	Tribe ***Cercideae***			
	A *Cercidinae*			
C3.1	*Cercis occidentalis* Tor. ex A. Gray	col[UOG]	-	-
	Cercis siliquastrum L.	com[AHA]	-	-
C3.2	*Adenolobus pechuelli* (Kuntze) Torre & Hillc.	82389[KRG]	-	-
	B *Bauhiniinae*			
C3.4	*Bauhinia forficata* L. subsp. *pruinosa* (J. Vog.) Fortunato & Wunderlin [syn - *Bauhinia candicans* Benth.]	col[ARG]	-	-
	Bauhinia cunninghamii Benth.	com[AOA]	-	-
	Bauhinia galpinii N. E. Br.	col[DPA]	-	-
	Bauhinia rufescens Killip. & Macbride	col[BAD]	-	-
	Bauhinia syringifolia (F. Muell.) R. P. Wunderlin	col[ROG]	-	-
C3.4	*Piliostigma reticulatum* (DC.) Hochst.	col[BAD]	-	-
	Tribe ***Detarieae****			
	A *Cynometra* group			
C4.1	*Cynometra cauliflora* L.	col[PPM]	-	-
C4.8	*Schotia brachypetala* Sond.	com[AHA]	-	-
	Schotia latifolia Jacq.	com[ENA]	-	-
	D *Crudia* group			
C4.34	*Hardwickia binata* Roxb.	88/8177N[DNF]	-	-
	I *Amherstia* group			
C4.74	*Tamarindus indica* L.	com[FBA]	-	-

*Note: *Amherstia* has been placed in the *Detarieae* (Breteler 1995).

SUBFAMILY MIMOSOIDEAE

	Tribe ***Parkieae***			
M1.2	*Parkia javanica* (Lam.) Merr.	com[IFS]	-	-
	Parkia timoriana (DC.) Merr. [syn - *Parkia roxburghii* (DC.) Merr.]	com[IFS]	-	-
	Tribe ***Mimoseae***			
	A *Dinizia* group			
M3.1	*Dinizia excelsa* Ducke	1591[BTC]	-	-
	E *Adenanthera* group			
M3.9	*Adenanthera pavonina* L.	com[ENA,FBA]	-	-
	H *Prosopis* group			
M3.16	*Prosopis alba* Griseb.	col[ULA]	-	-
	Prosopis caldenia Burkart	col[ULA]	-	-
	Prosopis juliflora (Sw.) DC.	com[FBA]	-	-
	Prosopis nigra (Griseb.) Hieronymus	col[ULA]	-	-
	I *Piptadenia* group			
M3.20	*Stryphnodendron excelsum* Harms	1819[CCC]	-	-
M3.21	*Piptadenia moniliformis* Benth.	49052[KRG]	-	-
M3.26	*Anadenanthera colubrina* (Vell. Conc.) Brenan var. *colubrina*	48295 [KRG]	-	-
M3.27	*Mimosa aculeaticarpa* Ortega var. *aculeaticarpa*	col[PBE]	-	-
	Mimosa albida Kunth var. *willdenowii* (Poir.) DC.	col[BJF]	-	-
	Mimosa diplotrichia C.Wright ex Sauvalle var. *diplotrichia*	col[UBR]	-	-
	Mimosa pudica L.	com[NMU]	-	-
	K *Leucaena* group			
M3.30	*Leucaena diversifolia* Benth.	529[NHU]	-	N
	Leucaena lanceolata S. Wats.	17251[CCC]	-	-
	Leucaena leucocephala (Lam.) DeWit	Cunningham[YRA]	-	F
	Leucaena trichodes (Jacq.) Benth.	com[ENA]	-	N

(*continued on the next page*)

Table 1. (*continued from the preceding page*)

	Families, subfamilies, tribes, and species	Seed source[a]	Response to USDA 257	Response to NGR 234
	L *Dichrostachys* group			
M3.32	*Dichrostachys cinerea* (L.) W. & A.	19093[CCC]	F	F
M3.35	*Desmanthus illinoensis* (Michx.) MacMill. ex Robinson	col[PCU]	N	F
	Desmanthus virgatus (L.) Willd.	Marc[ASA]	-	F
M3.36	*Neptunia gracilis* Benth. var. *major* (Benth.) Windler	col[KPA]	-	-
	Neptunia natans (L.) Druce	col[BSS]	N	N
	Tribe ***Acacieae***[¶]			
M4.1	A subgen. *Acacia*			
	Acacia farnesiana (L.) Willd.	com[AHA]	-	N
	Acacia karroo Hayne	col[BJF]	-	-
	B subgen. *Aculeiferum*			
	Acacia aroma Gillis ex H.&A.	col[ULA]	-	-
	Acacia ataxacantha DC.	col[BAD]	-	-
	Acacia bonariensis Gillis ex H. & A.	col[ULA]	-	N
	Acacia macracantha H. & B. ex Willd.	col[ULA]	-	N
	Acacia polyacantha Willd.	col[ODS]	-	-
	Acacia senegal (L.) Willd.	273[BAD]	-	-
	C subgen. *Heterophyllum*			
	Acacia auriculiformis A. Cunn.	365[NHU]	N	F
	Acacia cyanophylla Lindl.	col[FNC]	N	N
	Acacia mangium Willd.	com[AHA]	N	N
	Acacia mearnsii De Wild.	col [NHU]	N	N
	Acacia pendula A. Cunn. ex. G. Don	com[FBA]	-	F
	Acacia retinodes Schltdl.	com[AOA]	N	F
	Acacia saligna (Labill.) Wendl.	com[AHA]	N	F

[¶]Division of the tribe *Acacieae* into subgenera follows the recommendations of Vassal (1981).

	Tribe *Ingeae*			
M5.1	*Inga mortoniana* J. León.	com[FSB]	F	F
M5.3	*Albizia julibrissin* Durazz.	col[CGC]	N	N
	Albizia lebbeck (L.) Benth.	14959[ACA]	F	F
	Albizia procera (Roxb.) Benth.	com[FBA]	F	F
	Albizia saponaria (Lour.) Miq.	col[UBR]	N	N
M5.5	*Samanea saman* (Jacq.) Merr.	21812[CCC]	F	F
M5.7	*Enterolobium contortisiliquum* (Vell.) Morong	col[ULA]	N	F
	Enterolobium timbouva Mart.	com[ENA]	-	F
M5.8	*Faidherbia albida* (Del.) A. Chev.	50418[KRG], 2067/89[FZM]	F	F
M5.9	*Calliandra houstoniana* (Miller) Standley var. *anomala* (Kunth) Barneby[§] [syn - *Calliandra grandiflora* Benth].	col[JTF]	-	N
	Calliandra houstoniana (Miller) Standley var. *calothyrsus* (Meissner & Barneby)[§] [syn - *Calliandra calothyrsus* Meissner]	com[AHA,IFS]	-	-
	Pithecellobium dulce (Roxb.) Benth.	com[IFS]	-	-
M5.14	*Paraserianthes falcataria* (L.) Nielsen	col[AHA,NHU]	F	F
	Paraserianthes lophantha (Willd.) Nielsen	com[AHA]	-	-

[§]see Barneby (1998)

	SUBFAMILY PAPILIONOIDEAE			
	Tribe ***Swartzieae***			
	D *Ateleia* group[¥]			
P1.13	*Ateleia ovata* Mohlenbr.	7362[CCC]	-	F

[¥]see Polhill (1994)

	Tribe ***Sophoreae***			
	B *Angylocalyx* group			
P2.12	*Castanospermum australe* A. Cunn.	com[AHA,FBA]	-	-
	F *Sophora* group			
P2.37	*Bolusanthus speciosus* (Bolus) Harms	com[ENA]	-	N
P2.40	*Maackia amurensis* Rupr. & Maxim.	col[SNU]	-	-
P2.41	*Cladrastis lutea* (Michx. f.) Koch	col[SNU]	-	-
P2.44	*Styphnolobium japonicum* Schott	col[JMF]	-	-
P2.45	*Sophora davidii* (Franch.) Pavol.	col[CGC]	F	F
	Sophora microphylla Aiton	col[CGC]	-	-
	Sophora tetraptera J. Mill.	com[ENA]	-	-
	Sophora tomentosa L.	com[AHA]	F	F
	Sophora velutina Lindl.	col[NHZ]	F	F
	Tribe ***Dalbergieae***			
	B *Dalbergia* group			
P4.5	*Dalbergia martinii* F. White	col[NHZ]	N	N
	Dalbergia retusa Hemsl.	2478[BTC]	-	F
P4.13	*Tipuana tipu* (Benth.) Kuntze	col[AOA,UGC]	N	N
P4.17	*Pterocarpus lucens* Guill. & Perr.	col[BAD]	N	F

(*continued on the next page*)

Table 1. (*continued from the preceding page*)

	Families, subfamilies, tribes, and species	Seed source[a]	Response to USDA 257	Response to NGR 234
	Tribe ***Abreae***			
P5.1	*Abrus precatorius* L.	col^{ILF,IFS}	-	-
	Tribe ***Amorpheae***			
P6.4	*Amorpha fruticosa* L.	col^{JTF,PBE}	F	F
P6.8	*Dalea candida* Willd.	col^{BMU}	-	F
	Dalea purpurea Ventenat	com^{PWU}	F	F
	Tribe ***Millettieae***[†]			
P7.14	*Derris robusta* (DC.) Benth.	com^{IFS}	-	-
P7.25	*Mundulea sericea* (Willd.) A. Chev.	84176^{KRG}	F	F
P7.23	*Millettia megasperma* Benth.	com^{AHA,SDS}	N	N
P7.34	*Pongamia pinnata* (L.) Pierre	com^{AOA}	-	-
P7.40	*Tephrosia cinerea* (L.) Pers.	9459^{CCC}	-	F
	Tephrosia rosea F. Muell. ex Benth.	com^{AOA}	-	F
	Tephrosia sessiliflora (Poir.) Hassl.	9155^{CCC}	-	F
	Tephrosia vogelii Hook. f.	387879^{SGU}	F	F
P7.43	*Wisteria frutescens* (L.) Poir.	com^{ENA}	-	N
	Wisteria sinensis (Sims) Sw.	col^{UOG}	N	N
P7.44	*Xeroderris stuhlmannii* (Taub.) Mend. & Sousa	col^{NHZ}	F	F

[†]Numbers in this tribe follow the alphabetical system of Corby et al. (1983) and have no taxonomic relevance.

	Tribe ***Robinieae***			
	A *Sesbania* group[‡]			
P8.1	*Sesbania sesban* (L.) Merr. [syn - *Sesbania aegyptiaca* Poir.]	com^{IFS}, 2296/91^{FZM}	-	-
	Sesbania bispinosa (Jacq.) W.F. Wight	col^{PPM}	N	N
	Sesbania cannabina (Retz.) Pers.	com^{AOA}	N	N
	Sesbania formosa (F.Muell.) N. Burb.	com^{AHA}	-	-
	Sesbania grandiflora (L.) Pers.	com^{AHA}	F	F
	Sesbania herbacea (Mill.) R. McVaugh	com^{SSU}	-	-
	Sesbania punicea (Cav.) Benth.	col^{ULA}	-	-
	Sesbania rostrata Brem. & Oberm.	col^{ODS}	-	N

[‡]Note - stem nodules (where they occur) are Aeschynomenoid.

	C *Gliricidia* group			
P8.6	*Gliricidia maculata* HBK	com^{IFS}	-	-
	D *Robinia* group			
P8.7	*Robinia hispida* L. var. *fertilis* (Ashe) R.T. Clausen [syn - *Robinia fertilis* Ashe]	com^{ENA}	N	F
	Robinia pseudoacacia L.	col^{FNC,FSB}	F	F
P8.8	*Coursetia caribaea* (Jacq.) M. Lavin	9392^{CCC}	-	-
	Tribe ***Indigofereae***			
P9.6	*Cyamopsis tetragonoloba* (L.) Taub.	Essex^{PCU}	-	-
P9.7	*Indigofera arrecta* Hochst. ex A. Rich.	18661^{ASA}	F	F
	Indifogera australis Willd.	col^{WBA}	N	N
	Indigofera glandulosa Willd.	col^{UGC}	-	-
	Indigofera jamaicensis Spreng.	9319^{CCC}	-	F
	Indigofera lespedezioides HBK	9048^{CCC}	-	-
	Indigofera tinctoria L.	com^{IFS}	F	F
	Tribe ***Phaseoleae***			
	A *Erythrininae*			
P10.1	*Erythrina abyssinica* DC.	col^{NHZ}	-	F
	Erythrina costaricensis M. Micheli	4153^{BTC}	F	F
	Erythrina crista-galli L.	col^{PBE,ULA}	-	F
	Erythrina fusca Lour.	21601^{CCC}	-	F
	Erythrina poeppigiana (Walpers) O.F. Cook	4520^{BTC}	-	-
	Erythrina variegata L.	com^{FBA,TSU}	-	N
	Erythrina vespertilio Benth.	com^{FBA}	-	F
P10.3	*Mucuna pruriens* (L.) DC.	com$^{GKM,\ IFS}$	-	-
P10.7	*Apios americana* Medik.	col^{HBU}	F	N
	C *Clitoriinae*			
P10.14	*Centrosema pubescens* Benth.	com$^{GKM,\ IFS}$	-	N
P10.16	*Clitoria laurifolia* Poir.	17366^{CCC}	-	F
	Clitoria ternatea L.	com^{TJU}	-	-
	D *Diocleinae*			
P10.18	*Dioclea guianensis* Benth.	7351^{CCC}	N	N
	Dioclea sericea HBK	8434^{CCC}	N	N
	Dioclea virgata (L.C. Rich.) Amshoff	18124^{CCC}	-	N
P10.21	*Canavalia ensiformis* (L.) DC.	col^{PCU}	N	N
	Canavalia rosea (Sw.) DC. [syn - *Canavalia maritima* Thouars]	com^{AHA,AOA}	N	F
P10.25	*Cratylia argentea* (Desv.) O. Kuntze	18957^{CCC}	-	N
P10.27	*Galactia jussiaeana* Kunth	8805^{CCC}	N	F
	Galactia latisiliqua Desv.	923^{CCC}	N	N
	Galactia striata (Jacq.) Urb.	464^{ASA}	-	N
	E *Glycininae*			
P10.32	*Pueraria lobata* (Willd.) Ohwi	col^{PTJ}	N	N
	Pueraria phaseoloides (Roxb.) Benth.	col^{IFS}	-	N
P10.35	*Glycine canescens* Herman	col^{PCU}	-	F
	Glycine max (L.) Merr.	McCall^{AAU,SSU,UUU}	N	N

(*continued on the next page*)

Table 1. (*continued from the preceding page*)

	Families, subfamilies, tribes, and species	Seed source[a]	Response to USDA 257	Response to NGR 234
	G. max	Peking[PCU]	F	N
	G. max	Preston[AAU]	-	-
	Glycine soja Sieb. & Zucc.	PI81762[NHU]	F	N
	Glycine tabacina (Labill.) Benth.	col[PCU]	-	F
	Glycine tomentella Hayata	col[PUU]	F	F
P10.36	*Teramnus labialis* (L. f.) Spreng.	60381[ASA]	-	F
	Teramnus uncinatus (L.) Sw.	87881[ASA]	N	F
P10.39	*Teyleria koordersii* (Backer) Backer	21157[CCC]	-	N
P10.40	*Neonotonia wightii* (Arn.) Lackey	Cooper's[WSA]	-	F
P10.44	*Amphicarpaea trisperma* Baker	col[HBC]	F	F
P10.45	*Calopogonium caeruleum* (Benth.) Sauv.	com[AHA,IFS]	-	F
	Calopogonium mucunoides Desv.	com [IFS]	N	N
P10.46	*Pachyrhizus erosus* (L.) Urb.	21039[CCC]	-	N
	Pachyrhizus tuberosus (Lam.) Spreng.	com[SPM]	-	F
	F *Kennediinae*			
P10.47	*Kennedia beckxiana* F. Muell.	com[ENA]	N	N
	Kennedia nigricans Lindley	com[ENA]	N	F
	Kennedia prostrata R. Br.	com[AHA,AOA]	N	F
	Kennedia rubicunda (Schneev.) Vent.	com[FBA]	N	F
P10.48	*Hardenbergia comptoniana* (Andr.) Benth.	com[ADU,WDA]	F	F
	Hardenbergia violacea (Schneer.) Stearn	com[ISM]	N	F
	G *Phaseolinae*			
P10.50	*Dysolobium apioides* (Gagnepain) Maréchal	4596[CCC]	N	F
P10.51	*Psophocarpus palustris* Desv.	com[IFS]	-	F
	Psophocarpus tetragonolobus (L.) DC.	com[SPM]	F	F
P10.56	*Otoptera burchellii* DC.	82448[KRG]	F	F
P10.61	*Lablab purpureus* (L.) Sweet	Rongai[WSA]	-	F
P10.64	*Dolichos junghuhnianus* Benth.	20030[CCC]	F	F
	Dolichos trilobus L.	21038[CCC]	-	F
P10.65	*Macrotyloma axillare* (E. Mey.) Verdc.	com[WSA]	F	F
	Macrotyloma uniflorum (Lam.) Verdc.	Leichardt[ASA]	-	F
P10.66	*Vigna aconitifolia* (Jacq.) Maréchal	214323[SGU]	-	F
	Vigna angularis (Willd.) Ohwi & Ohashi	col[PCU]	F	F
	Vigna caracalla (L.) Verdc.	146800[SGU]	-	F
	Vigna cylindrica Skeels	com[ASA]	F	F
	Vigna glabrescens Maréchal, Mascherpa & Stainer	207655[PBU]	N	F
	Vigna hosei (Craib) Backer	4983[CCC]	F	F
	Vigna lanceolata Benth.	CQ592[ASA]	F	F
	Vigna luteola (Jacq.) Benth.	330607,406347[SGU]	F	F
	Vigna minima (Roxb.) Ohwi & Ohashi	4985 [CCC]	F	F
	Vigna mungo (L.) Hepper	com[ROG]	F	F
	Vigna oblongifolia A. Rich.	60430[ASA]	F	F
	Vigna parkeri Baker	Shaw [ASA]	F	F
	Vigna radiata (L.) Wilczek	King[WSA], 305070, 197019, 227754[SGU]	F	F
	Vigna radiata subsp. *sublobata* (Roxb.) Verdc.	col [BSU]	N	N
	Vigna subterranea (L.) Verdc.	49A, 57B1[VBI]	F	F
	Vigna trilobata (L.) Verdc.	13671[ASA]	F	F
	Vigna umbellata (Thunb.) Ohwi & Ohashi	247691[SGU]	F	F
	Vigna unguiculata (L.) Walp.	Red Caloona[WSA]	F	F
	Vigna vexillata (L.) A. Rich.	406401, 406390[SGU]	F	F
P10.70	*Strophostyles helvola* (L.) Ell.	col[BMU]	-	-
P10.71	*Macroptilium atropurpureum* (DC.) Urb.	Siratro[WSA]	F	F
	Macroptilium bracteatum (Nels & Mart) Maréchal & Baudet	RLBB62[WSA]	F	F
	Macroptilium lathyroides (L.) Urb.	com[WSA]	F	F
	Macroptilium longepedunculatum (Benth.) Urb.	575[CCC]	-	N
P10.72	*Phaseolus acutifolius* A. Gray	214333[PCU]	-	N
	P. acutifolius	440813 [WPU]	-	N
	P. acutifolius	Tenuifolius [WPU]	-	N
	Phaseolus angustifolius Roxb. [syn - *Phaseolus anisotrichus* Schlect.]	312122[WPU]	-	N
	Phaseolus leptostachyus Benth.	325587[WPU] & 30677[CCC]	-	F
	Phaseolus coccineus L.	col[MKD]	N	N
	Phaseolus coccineus L. subsp. *polyanthus* (Greenman) Maréchal, Mascherpa, & Stainier	196813[WPU]	F	F
	Phaseolus polystachyus Britt., Stearns & Pogg	196813[WPU]	-	F
	Phaseolus vulgaris L.	Hilds Marona[UGC]	N	N
	Phaseolus vulgaris L. var. *aborigineus* (Burk.) Baudet	266910[WPU]	F	F
	H *Cajaninae*			
P10.73	*Cajanus cajan* (L.) Millsp.	ICP6443[ICI]	F	F
	Cajanus scarabaeoides (L.) Thou.	col[ASA],87[IPI]	F	F

(*continued on the next page*)

Table 1. (*continued from the preceding page*)

	Families, subfamilies, tribes, and species	Seed source[a]	Response to USDA 257	Response to NGR 234
P10.74	*Dunbaria circinalis* Backer	17329[CCC]	-	F
	Dunbaria nivea Miq.	17734[CCC]	-	F
	Dunbaria villosa Mak.	20649[CCC]	N	F
P10.77	*Flemingia congesta* Roxb.	com[WSA]	F	F
	Flemingia strobilifera (L.) Ait. f.	204[IPI]	F	F
P10.80	*Rhynchosia minima* (L.) DC.	18063[KRG]	-	F
	Rhynchosia phaseoloides (Sw.) DC.	col[SNU]	-	-
	Rhynchosia reticulata (Sw.) DC.	7152[CCC]	-	-
	Rhynchosia rothii Benth. ex Aitch.	30232[ASA]	-	F
	Rhynchosia sublobata (Schumach.) Meikle	77003[ASA]	F	F
P10.81	*Eriosema simplicifolium* (HBK) G. Don	7336[CCC]	-	-
	Eriosema violaceum (Aubl.) G. Don	18292[CCC]	-	N
P10.82	*Paracalyx scariosus* (Roxb.) Ali	207[IPI]	-	-
	Tribe ***Desmodieae***			
	B *Desmodiinae*			
P11.6	*Dendrolobium triangulare* Schindl.	23104[CCC]	F	F
P11.7	*Phyllodium elegans* (Lour.) Desv.	23230[CCC]	N	N
	Phyllodium pulchellum (L.) Desv.	13237[CCC]	-	-
P11.8	*Dicerma biarticulatum* (L.) DC.	18401[CCC]	N	N
P11.9	*Desmodium canadense* (L.) DC.	col[USU]	F	F
	Desmodium dichotomum DC.	47186 [ASA]	-	F
	Desmodium intortum Urb.	Greenleaf[YRA]	-	F
	Desmodium uncinatum (Jacq.) DC.	Silverleaf[YRA]	-	F
P11.10	*Codariocalyx gyroides* (Roxb.) Hassk.	13547[CCC]	F	F
	Codariocalyx motorius (Houtt.) Ohashi	23414[CCC]	F	F
P11.11	*Pseudarthria viscida* (L.) W. & A.	13701[CCC]	-	N
P11.12	*Pycnospora lutescens* (Poir.) Schindl.	17415[CCC]	F	F
P11.13	*Tadehagi triquetrum* (L.) Ohashi	13263[CCC]	-	-
P11.17	*Christia obcordata* (Poir.) Bakh.	20652[CCC]	-	-
P11.18	*Alysicarpus vaginalis* (L.) DC.	20580[CCC]	-	F
	C *Lespedezinae*			
P11.24	*Lespedeza bicolor* Turcz.	col[JTF]	F	F
P11.25	*Kummerowia stipulacea* (Maxim.) Makino	Summit[PCU]	F	F
	Kummerowia striata (Thunb.) Schindl.	Marion[PCU]	F	F
	Tribe ***Psoraleeae***			
P12.2	*Bituminaria bituminosa* (L.) C.H. Stirton	col[BBD]	-	-
P12.9	*Psoralea plumosa* F. Muell.	col[KPA]	-	F
	Psoralea pustulata F. Muell.	col[KPA]	N	F

	Families, subfamilies, tribes, and species	Seed source[a]	Response to USDA 257	Response to NGR 234
	Tribe ***Loteae***			
P13.2	*Anthyllis vulneraria* L.	55402[KRG]	-	N
P13.4	*Hymenocarpos circinnatus* (L.) Savi	27229[KRG]	-	-
P13.6	*Dorycnium herbaceum* Vill.	col[BGD]	-	-
P13.7	*Lotus corniculatus* L.	col[PCU]	N	F
	Lotus halophilus Boiss. & Sprun.	26004[KRG]	-	N
	Lotus japonicus (Regel) K. Larsen	Gifu[MLU,PKU]	F	F
	Lotus pedunculatus Cav.	Grassl. Maku[APN]	N	N
	Lotus tetragonolobus L.	col[HPP]	-	-
P13.11	*Coronilla varia* L.	col[KLB]	-	-
P13.12	*Securigera securidaca* (L.) Degen & Doerfler	col[NPF]	-	-
P13.13	*Hippocrepis ciliata* Willd.	26602[KRG]	-	-
P13.14	*Scorpiurus vermiculatus* L.	col[BGD]	-	-
P13.15	*Ornithopus compressus* L.	com[WSA]	-	-
	Tribe ***Aeschynomeneae***			
	B *Aeschynomeninae*			
P14.9	*Aeschynomene aspera* L.	col[IPI]	-	N
	Aeschynomene falcata (Poir.) DC.	Barjoo[ASA]	-	N
	Aeschynomene indica L.	col[IPI]	-	N
	D *Poiretiinae*			
P14.21	*Zornia brasiliensis* Vogel	14287[CCC]	-	-
	Zornia diphylla Pers.	70161[CCC]	-	N
	Zornia gemella (Willd.) Vogel	49269[KRG]	-	-
	Zornia glabra Desv.	8345[CCC]	-	-
	Zornia latifolia Sm.	100465[ASA]	-	N
	E *Stylosanthinae*			
P14.23	*Pachecoa prismatica* (Sessé & Moc.) Standl. & Schub.	18287[CCC]	N	N
P14.25	*Stylosanthes capitata* Vog.	55843[ASA]	-	-
	Stylosanthes guianensis (Aubl.) Sw.	Endeavor[WSA]	-	N
	Stylosanthes hamata (L.) Taub.	Verano[WSA]	-	N
	Stylosanthes humilis Kunth	Townsville[WSA]	-	N
	Stylosanthes scabra Vog.	Seca[ASA]	-	N
P14.26	*Arachis hypogaea* L.	NC4[CRU]	-	N
	Tribe ***Adesmieae***			
P15.1	*Adesmia bicolor* (Poir.) DC.	col[URU]	-	-
	Adesmia remyana Phil.	col[ARG]	-	-

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Table 1. (*continued from the preceding page*)

Families, subfamilies, tribes, and species		Seed source[a]	Response to USDA 257	Response to NGR 234
	Tribe ***Galegeae***			
	A *Coluteinae*			
P16.1	*Clianthus formosus* (G. Don) Ford & Vick	com[AHA]	-	-
P16.2	*Swainsona cyclocarpa* F. Muell.	Paradoxa[KPA]	-	-
	Swainsona formosa (G. Don) J. Thompson	com[ADU,NAA]	-	-
	Swainsona forrestii F. Muell. ex A. Lee	col[KPA]	-	F
	Swainsona maccullochiana F. Muell.	com[HCA]	-	-
	Swainsona procumbens (F. Muell.) F. Muell.	com[AHA]	-	-
P16.3	*Sutherlandia frutescens* (L.) R. Br.	col[BBD]	-	-
P16.5	*Colutea arborescens* L.	col[BGD,HPP]	F	F
	B *Astragalinae*			
P16.10	*Halimodendron halodendron* (Pallas) Voss	col[HBC]	N	N
P16.11	*Caragana arborescens* Lam.	com[FBA,JMF]	-	-
P16.15	*Astragalus alpinus* L.	col[NPF]	-	-
	Astragalus canadense L. [syn - *Astragalus cicer* L.]	com[PWU], col[CGC]	-	-
	Astragalus cruikschankii Griseb.	col[PPA]	-	-
P16.17	*Oxytropis campestris* (L.) DC.	col[BJF,HPP]	-	-
	Oxytropis halleri Bunge ex Koch	36108[KRG]	F	F
	Oxytropis uralensis DC. [syn - *Oxytropis arctica* R. Br.]	col[JMC]	-	-
P16.19	*Gueldenstaedtia stenophylla* Bunge	col [HBC]	-	N
	C *Galeginae*			
P16.20	*Galega officinalis* L.	36670[KRG]	-	-
	E *Glycyrrhizinae*			
P16.22	*Glycyrrhiza acanthocarpa* (Lindl.) J.M. Black	col[KPA]	-	-
	Glycyrrhiza glabra L.	col[KLB]	-	F
	Tribe ***Hedysareae***			
P18.2	*Hedysarum alpinum* L.	col[BBD]	N	N
	Hedysarum coronarium L.	com[TJU]	-	-
P18.6	*Onobrychis caput-galli* (L.) Lam.	col[BBD]	-	-
	Onobrychis viciifolia Scop.	col[CBC]	-	-
	Tribe ***Vicieae***			
P19.1	*Vicia benghalensis* L.	com[BWU]	-	-
	Vicia cracca L.	col[BJF]	-	-
	Vicia dasycarpa Ten.	com[FBA]	-	-
	Vicia faba L.	col[CGC]	-	-
	Vicia narbonensis L.	13167[ASC]	-	-
	Vicia sativa L. subsp. *nigra* (L.) Ehrh.	com[ILF]	-	-
	Vicia pannonica Crantz	Pannonica[ASC]	-	-
	Vicia villosa Roth subsp. *pseudocracca* (Bertol.) Ball	Glabrecens[ASC]	-	-
	Vicia vulgaris Moench	com[ASC]	-	-
P19.2	*Lathyrus japonicus* Willd.	col[JMC]	-	-
	Lathyrus magellanicus Lam.	col[PPA]	-	-
	Lathyrus odoratus L.	com[TJU]	-	-
P19.3	*Lens culinaris* Medik.	col[PCU]	-	-
P19.4	*Pisum sativum* L. subsp. *elatius* (Bieb.) Asch. & Gr.	com[ASC]	-	-
	Pisum sativum L.	Rondo[CRN]	-	-
	Tribe ***Cicereae***			
P20.1	*Cicer arietinum* L.	Annigeri[IPI]	-	-
	Tribe ***Trifolieae***✣			
P21.1	*Ononis natrix* L.	col[CGC]	-	-
P21.3	*Melilotus officinalis* Willd.	31169[KRG]	-	-
P21.4	*Trigonella foenum-graecum* L.	col[CGC]	-	-
	Trigonella gladiata M. Bieb.	55240[KRG]	-	-
P21.5	*Medicago arabica* (L.) Huds.	col[WPU]	-	-
	Medicago arborea L.	col[WPU]	-	-
	Medicago blancheana Boiss.	col[WPU]	-	-
	Medicago brachycarpa M. Bieb.	col[WPU]	-	-
	Medicago cancellata M. Bieb.	col[WPU]	N◆	N◆
	Medicago ciliaris (L.) Krocker	col[WPU]	-	-
	Medicago constricta Durien	col[WPU]	-	-
	Medicago coronata (L.) Bart.	col[WPU]	-	-
	Medicago disciformis DC.	col[WPU]	-	-
	Medicago doliata Carmign.	Muricata[WPU]	-	-
	Medicago granadensis Willd.	col[WPU]	-	-
	Medicago heyniana Greuter	col[WPU]	-	-
	Medicago italica (Miller) Fiori	col[WPU]	-	-
	Medicago laciniata (L.) Miller	col[WPU]	-	-
	Medicago lesinsii E. Small	col[WPU]	-	-
	Medicago littoralis Rohde ex Lois.	col[WPU]	-	-
	Medicago medicaginoides (Retz.) E. Smith	col[WPU]	-	-
	Medicago minima (L.) Bart.	col[WPU]	-	-
	Medicago monantha (C. A. Meyer) Trautv.	col[WPU]	-	-

(*continued on the next page*)

Table 1. (*continued from the preceding page*)

	Families, subfamilies, tribes, and species	Seed source[a]	Response to USDA 257	Response to NGR 234
	Medicago monspeliaca (L.) Trautv.	col[WPU]	-	-
	Medicago muricoleptis Tin.	col[WPU]	-	-
	Medicago noeana Boiss.	col[WPU]	-	-
	Medicago orbicularis (L.) Bart.	col[WPU]	-	-
	Medicago papillosa Boiss.	col[WPU]	N◆	N◆
	Medicago pironae Vis.	col[WPU]	-	-
	Medicago platycarpa (L.) Trautv.	col[WPU]	-	-
	Medicago polyceratia (L.) Trautv.	col[WPU]	-	-
	Medicago polymorpha L. var. *polymorpha*	col[WPU]	-	-
	Medicago rigidula (L.) All.	col[WPU]	-	-
	Medicago ruthenica (L.) Ledebour	col[WPU]	-	-
	Medicago sativa L.	Nitro[PCU]	-	-
	Medicago sativa L. subsp. *falcata* (L.) Arcang.	col[WPU]	-	-
	Medicago sativa L. subsp. *sativa*	col[WPU]	-	-
	Medicago sativa L. subsp. *xvaria* (Martyn) Arcang.	col[WPU]	-	-
	Medicago sativa L. subsp. *falcata* (L.) Arcang. var. *viscosa* (Reichenb.) Posp.	col[WPU]	-	-
	Medicago secundiflora Durien	col[WPU]	-	-
	Medicago shephardii Post	col[WPU]	-	-
	Medicago soleirolii Duby	col[WPU]	-	-
	Medicago tenoreana Ser.	col[WPU]	-	-
	Medicago truncatula Gaertn.	col[WPU], Jemalong[SAA]	-	-
	Medicago turbinata (L.) All.	col[WPU]	-	-
P21.6	*Trifolium repens* L.	Pitau[WSA]	-	-
	Trifolium subterraneum L.	Trikkala[BWU]	-	-
	Tribe ***Brongniarteae****			
P22.3	*Templetonia egena* (F. Muell.) Benth.	com[AHA]	-	-
	Templetonia retusa (Vent.) R. Br.	com[AHA]	-	N
P22.4	*Hovea acutifolia* G. Don	com[ENA]	N	N
	Hovea linearis (Sm.) R. Br.	com[AHA]	N	N
	Tribe ***Bossiaeae****			
P23.5	*Goodia lotifolia* Salisb.	com[AHA]	N	F
P23.6	*Bossiaea foliosa* A. Cunn.	com[AHA]	-	-
	Bossiaea heterophylla Vent.	com[AHA]	-	-
	Bossiaea obcordata Druce	com[AHA]	-	-
	Bossiaea rhombifolia Siebl.	com[AHA]	-	-
P24.1	Tribe ***Mirbelieae***			
	Gompholobium polyzygum F. Muell. [syn - *Burtonia polyzyga* (F. Muell.) Benth.]	com[AHA], col[KPA]	-	-
	Gompholobium latifolium Labill.	com[AHA]	N	N
	Gompholobium scabrum Smith	com[AOA]	-	-
P24.3	*Viminaria juncea* (Schrad.) Hoffmgg.	com[AHA]	-	-
P24.4	*Daviesia corymbosa* Sm.	com[AHA,FBA]	-	-
	Daviesia latifolia R. Br.	com[AHA]	-	-
P24.6	*Isotropis atropurpurea* F. Muell.	col[KPA]	-	-
P24.8	*Jacksonia scoparia* R. Br.	com[AHA]	-	-
	Jacksonia velutina Benth.	col[KPA]	-	-
P24.9	*Oxylobium ellipticum* R. Br.	col[KPA]	N	N
P24.10	*Chorizema cordatum* Lindley✹ [syn - *C. ilicifolium* Labill.]	com[AHA]	-	-
	Chorizema dicksonii Graham	com[AHA]	N	N
	Chorizema diversifolium A. DC.	com[AHA]	-	F
P24.11	*Mirbelia dilatata* R. Br.	com[AHA]	N	N
	Mirbelia pungens Don	com[AHA]	-	F
P24.13	*Gastrolobium bilobum* R. Br.	com[AOA]	N	N
P24.15	*Nemcia capitata* (Benth.) Domin [syn - *Oxylobium capitatum* Benth.]	com[AOA]	-	-
P24.16	*Brachysema latifolium* R. Br.	com[AOA]	-	-
P24.21	*Aotus ericoides* (Vent.) G. Don [syn - *Aotus villosa* (Andr.) Sm.]	com[AHA,AOA]	N	F
P24.23	*Pultenaea blakelyi* J. Thompson	com[AHA]	-	F
	Pultenaea microphylla DC.	com[AHA]	N	N
	Pultenaea daphnoides Wendl.	col[WBA]	N	N
	Pultenaea villosa Willd.	com[AHA,FBA]	N	N
P24.25	*Dillwynia glaberrima* Smith	com[AHA]	N	N
	Dillwynia juniperina Sieb.	com[AHA]	-	-

✣Nomenclature of the *Medicago* species is based on Small and Jompe (1989).
◆Spontaneous (i.e. pseudo-) nodules – see Truchet et al. (1989).

*Note: The "Templetonia group" has been transferred from the *Bossiaeae* to the *Brongniartieae*. In this system, the *Bossiaeae* only consists of the "Bossiaea group" (Crisp and Weston 1987).

✹Note: *Chorizema cordatum* was originally acquired as *Chorizema ilicifolium*, from which it segregates (Taylor and Crisp 1992).

(*continued on the next page*)

Table 1. (*continued from the preceding page*)

	Families, subfamilies, tribes, and species	Seed source[a]	Response to USDA 257	Response to NGR 234
	Tribe ***Podalyrieae***			
P25.3	*Virgilia capensis* (P. Bergius) T.M. Salter subsp. *oroboides* [syn - *Virgilia capensis* (L.) Lam]	com^{AHA}	-	F
	Virgilia divaricata Adamson	col^{ROG}	-	N
	Tribe ***Crotalarieae***			
P27.7	*Crotalaria juncea* L.	com^{IFS}	N	N
	Crotalaria sericea Retz.	col^{PCU}	N	F
P27.9	*Lotononis bainesii* Bak.	com^{WSA}	-	-
	Tribe ***Thermopsideae***			
P29.2	*Piptanthus concolor* Harrow	col^{HBC}	-	F
P29.3	*Anagyris foetida* L.	col^{BBD}	-	F
P29.4	*Thermopsis caroliniana* M. A. Curt.	col^{SNU}	-	-
P29.5	*Baptisia australis* (L.) R. Br.	col^{BNU,UOG}	-	-
	Tribe ***Genisteae***☼			
P30.8	*Lupinus albus* L.	Kijevsky mutant^{ASC}	-	-
	Lupinus angustifolius L.	col^{APN}	-	-
	Lupinus luteus L.	Jantar^{ASC}	-	-
	Lupinus nanus Dougl.	col^{APN}	-	-
	Lupinus pilosus L.	79170^{KRG}	-	N
P30.9	*Laburnum anagyroides* Medik. [syn – *Laburnum* x *watereri*]	col$^{CGC, JTF}$	-	N
	Laburnum vossii Hort.	com^{FBA}	-	N
P30.10	*Hesperolaburnum platycarpum* (Maire) Maire	84176^{KRG}	-	F
P30.15	*Cytisus hirsutus* L.	com^{FBA}	-	N
	Cytisus villosus Pourret	com^{FBA}	N	N
P30.15	*Chamaecytisus proliferus* (L.f.) Link subsp. *palmensis* (Christ) Kunkel	66477^{KRG}	-	N
P30.16	*Lembotropis nigricans* (L.) Griseb.	88510^{KRG}	N	N
P30.17	*Calicotome villosa* (Poir.) Link	31963^{KRG}	-	N
P30.19	*Spartium junceum* L.	col^{JTF}	N	N
P30.21	*Retama monosperma* (L.) Boiss.	84349^{KRG}	-	F
	Retama raetam (Forsskal) Webb	com^{FBA}	-	F
P30.22	*Genista pilosa* L.	col^{CGC}	-	-
	Genista tinctoria L.	col^{CGC}	-	-
P30.23	*Echinospartum horridum* (Vahl) Rothm.	col^{CGC}	-	-
P30.25	*Ulex europaeus* L.	col^{BBD,HPP}	-	N
	☼Note - *Lupinus* nodules are unusual, and for this reason have not been classified here.			
	ULMACEAE Mirbel.			
	Parasponia andersonii Planch.	tiss cul☿	N	N
	☿Note - Experiments were performed on plants raised in tissue culture.			

[a]**where**: col = collected seeds; com = commercial variety; names are those of varieties/cultivars; figures represent the supplier's accession number; and superscripts are abbreviations for the suppliers whose addresses are listed below.

[a]**Seed sources are as follows**: **AAU** = Agronomy Department, Iowa State University, Ames 50011-1010, U.S.A. (W. Fehr, J. Orf); **ACA** = Australian Tree Seed Centre, CSIRO Division of Forestry, PO Box 4008, Queen Victoria Terrace, Canberra 2600, AUS (C. Gardiner); **ADU** = Australian Products Inc., 7502 Greenvile - Suite 500, Dallas, TX 75231, U.S.A.; **AHA** = Australian Seed Co., PO Box 67, Hazelbrook, NSW 2779, AUS (A. J. Bourne); **AOA** = Australian Revegetation Corp., 42 Sarich Court, Osborne Park, 6017 AUS (W. Haines); **APN** = Applied Biochemistry Division, Department of Scientific and Industrial Research, Palmerston North, NZ; **ASA** = Australian Tropical Forages Genetic Resources Centre, CSIRO Division of Tropical Crops and Pastures, Cunningham Laboratory, 306 Carmody Rd., St. Lucia, 4067 AUS (B. Pengelly); **ASC** = Agritec Research, Breeding & Services Ltd., Zemedelska 16, 787 01 Sumperk-Temenice, CR (M. Hybl); **AWU** = W. Atlee Burpee Co., Warminster, PA 18974, U.S.A.; **BAD** = Botanisk Institut, Aarhus Universitet, Nordlandsvej 68, 8240, DK (K. Tybirk); **BBD** = Botanischer Garten und Botanisches Museum, Berlin-Dahlem, Königin-Luise-Straße 6-8, 14195 Berlin, D; **BGD** = Botanischer Garten, der Universität, Untere Karspüle 1, 37073 Göttingen, D; **BJF** = Botanical Gardens, University of Joensuu, PO Box 111, 80101 Joensuu 10, FI; **BMU** = Biochemistry Department, Kansas State University, Manhattan 65506, U.S.A. (L. Davis); **BNG** = Biology Department, The University, Newcastle Upon Tyne, NE1 7RU, GB; **BSS** = Hortus Botanicus Bergianus, University of Stockholm, PO Box 50017, 10405 Stockholm, S; **BSU** = Department of Plant Pathology, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul 55108, U.S.A. (N. D. Young); **BTC** = Banco Latinamericano de Semilas Forestales, CATIE, Turrialba, CR (E. Ledema); **BWU** = Bountiful Gardens, Willits, CA 95490, U.S.A.; **CBC** = Ceskoslovenska Akademie Ved, Ustav experimentalni botaniky, 37005 Ceske Budejovice, CZ; **CCC** = Centro Internacional de Agricultura Tropical (CIAT), Apdo. aéro 6713, Cali, CO (B.L. Maass, A. Ortiz, and R. Schultze-Kraft); **CGC** = Conservatoire et Jardin botaniques de la Ville de Genève, PO Box 60, 1292 Chambésy/Genève, CH (G. Lachard); **CNF** = Conservatoire et Jardins Botaniques de Nancy, 100 rue du Jardin Botanique, 54600 Villiers les Nancy, F; **CRN** = Cebe & Co.,

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Blaak 31, 3011 GA, Rotterdam, NL; **CRU** = Crop Science Department, North Carolina State University, Raleigh 27650, U.S.A. (J. C. Wynne); **CSI** = Conservatoire et Jardin Botanique de Mascarin, Domaine des Colimaçons, 97436 Saint-Leu, Ile de la Réunion, F; **DHD** = Danida Forest Seed Centre, Krogerupvej 3 A, 3050 Humlebk, DK (K. Poulsen); **DNF** = Département forestier CIRAD-Forêt, 45 bis, av. de la Belle Gabrielle, 94736 Nogent s/Marne, F; **DPA** = Darwin Botanic Gardens, PO Box 496, Palmerston 0831, AUS; **ENA** = Ellison Horticultural, PO Box 365, Nowra 2541, AUS; **FBA** = M.L. Farrar Pty. Ltd. (now AustraHort Pty. Ltd.), PO Box 1046 Bomaderry 2541, AUS; **FDA** = Fruit Spirit Botanical Garden, Dorroughby, 2480 AUS (P. Recher); **FNC** = Forestry Department, Ministry of Agriculture and Natural Resources, Nicosia, CY (S. P. Soteriou); **FSB** = Forest Research Insitute, 132 Blv. Kliment Ohridski, 1756 Sofia, BG (V. Bouzov and A. Delkov); **FSR** = FAO-UN, Avda. Santa Maria 6700, Casilla 10095, Santiage, RCH; **FZM** = Forest Research Institute of Malawi, PO Box 270, Zomba, MW (J. P. Gowela); **GKM** = Institut Penyelidikan Getah Malaysia, Biotechnology Division, 260 Jalan Ampang, Petisurat 10150, 50908, Kuala Lumpur, Malaysia; **HAN** = Hortus Botanicus Plantage, Plantage Middenlaan 2, 1018 DD Amsterdam, NL; **HBC** = Hortus Botanicus Pekinensis, Instituti Botanici Academiae Sinicae, Xiangshan, Beijing 100093, China (Z.-M. Zhang); **HBU** = Horticulture Department, Louisiana State University, Baton Rouge 70803, U.S.A. (W. J. Blackmon); **HCA** = Harper Seed Co., PO Box 315, Cannington 6107, AUS; **HPP** = Hortus Botanicus, Universitatis Posnaniensis, Dqbrowskiego 165, 60-594 Posnan, PL; **IAE** = International Livestock Centre for Africa, PO Box 5689, Addis Ababa, ETH (J. Hanson); **IFS** = Inland and Foreign Trading Co., Block 79A Indus Road, Singapore 0316, SGP; **ILF** = INRA-SAPF, 86600 Lusignan, F; **IPI** = ICRISAT, Patancheru, Andhra Pradesh 502 324, IND (A. M. Ghanekar); **JMC** = Jardin Botanique de Montréal, rue Sherbrooke, Montréal, Quebec H1X 2B2, CDN; **JMF** = Jardins Botaniques de Marseille, 48 Av. Clot-Bey, 13008 Marseille, F; **JRF** = Jardin Botanique, 7 rue de Trianon, 76100 Rouen, F; **JTF** = Jardin Botanique, Laboratoire de Biologie Végétale, 37042 Tours Cedex, F; **KPA** = Kings Park and Botanical Garden, West Perth 6005, AUS; **KLB** = Kruidtuin van de Stad Leuven, Hortus Botanicus Lovaniensis, 30 Kapucijnenvoer, 3000 Leuven, B; **KRG** = Royal Botanical Gardens Seed Bank, Wakehurst Place, Ardingly, Haywards Heath, West Sussex RH17 6TN, GB (J. Bone); **MKD** = Max-Planck-Institut für Züchtungsforschung, Carl von Linné Weg, 50829 Köln, D (E. Czerny); **MLU** = MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing 48825, U.S.A. (F. J. de Bruijn); **NAA** = Natseeds, 5 Reynolds Rd., Applecross, 6153, AUS; **NHU** = NifTAL Project, 1000 Holomua Av., Paia, Maui, HI 96779-9774, U.S.A.; **NHZ** = National Herbarium & Botanic Garden, PO Box 8100, Causeway, Harare, Zimbabwe (T. Muller); **NMA** = Native Plant Section, Woods & Forests, Bremer Rd., PO Box Murray Bridge, 5253 AUS (J. Fairlamb); **NMU** = Northrup King Co., Horticulture Div., PO Box 959 Minneapolis, MN 55440, U.S.A.; **NPF** = Muséum d'Histoire Naturelle-Cultures, 43 Rue Buffon, 75005, Paris, F; **NWU** = Nitrogen Fixing Tree Association (NFTA), PO Box 680, Waimanolo, HI 96795, U.S.A.; **OCC** = OSEVA, Zemedelska16, 78701 Sumperk-Temenice, CZ (M. Hybl); **ODS** = OSTROM, Laboratoire de Biologie des Sols, PB 1386 Dakar, SN; **PBE** = Parcs I Jardins, Escola de Jardineria, Av. Marquès de Comillas, s/n. Parc de Monthuïc, 08038 Barcelona, E; **PBU** = Plant Introduction Office, USDA-ARS-PSI-NGRL-PIO, Barc-West, Beltsville, MD 20705, U.S.A.; **PCU** = Department of Plant Pathology, University of Missouri, 108 Waters Hall, Columbia 65211, U.S.A. (S. G. Pueppke); **PKU** = Plant Molecular Genetics, 269 Ellington Plant Sci. Bldg., University of Tennessee, Knoxville 37901-1071, U.S.A. (P. M. Gresshoff); **PPA** = Universidad Nacional de la Patagonia, cc No. 786, 900 Comodoro Rivadavia, Patagonia, Argentina (M. Stronati); **PPM** = Pusat Penyelidikan Sains Kajihayat, Universiti Sains Malaysia, Pulau Pinang, MAL (Wong Chee-Hoong, Zairi Jaal); **PTJ** = Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, JP (U. Sankawa); **PUU** = Perennial Glycine Germplasm Collection, Department of Crop Science, University of Illinois, AE-110 Turner Hall, 1102 S. Goodwin Ave., Urbana 61801, U.S.A. (T. Hymowitz); **PWU** = Prairie Moon Nursery, Route 3, Box 163, Winona, MN 55987, U.S.A.; **ROG** = Rawlings Seeds, 5 Worlds End Lane, Green Street Green, Orpington, Kent BR6 6AA, GB (W. Rawlings); **SAA** = South Australian Seed Growers Cooperative, 135 Sth Terrace, Adelaide 5000, AUS; **SCP** = Sistemas Agroecologicos de Manejo Comunal en la Costa Norte del Peru, Los Alhelies 164, Santa Victoria, Chiclayo, PE; **SGU** = Southern Regional Plant Introduction Station (USDA), Athens, GA 30602, U.S.A.; **SNU** = Botanic Garden of Smith College, Northampton, MA 01063, U.S.A.; **SPM** = Soon Huat Seeds Co., 245 Beach St., 10300 Penang, MAL; **SPZ** = Seed Section, Department of Water Affairs and Forestry, Forestry Branch, PO Box 727, 0001 Pretoria, ZA.; **SSU** = Department of Soil, Water, and Climate, University of Minnesota, Borlaug Hall, 1991 Upper Buford Circle, St. Paul 55108, U.S.A. (P. H. Graham and M. J. Sadowsky); **TJU** = Thompson & Morgan Seed Co., Jackson, NJ 08527, U.S.A.; **TSU** = Tropical Seeds, PO Box 11122, Honolulu, HI 96828, U.S.A.; **UBR** = UPT Balai Pengembangan Kebun Raya-LIPI, Jl. Ir. H. Juanda 13, PO Box 309, Bogor, 16003, RI (Ir. Suhirman); **UGC** = Université de Genève, 1 ch. de l'Impératrice, 1292 Chambésy/Genève, CH (W. J. Broughton); **ULA** = Institute of Plant Physiology, cc 327, Universidad Nacional de La Plata, La Plata, 1900 Argentina (A. Arambarri); **UOG** = University of Oxford Botanic Garden, Rose Lane, Oxford OX1 4AX, GB; **USU** = Universidad de la Republica, Sayago-Montevido, Uruguay; **UUU** = USDA Soybean Germplasm Collection, USDA-Agricultural Research Service, University of Illinois, 232 EASB, 1101 West Peabody Drive, Urbana 61801, U.S.A. (R. Nelson); **VBI** = Department of Legume Inoculation, The Volcani Center, PO Box 6, Bet Dagan 50-250, Israel; **WBA** = Wildlife Reserve, LaTrobe University, Bundoora, Victoria 3083, AUS (B. C. Smith); **WDA** = West Australian Wildflower Seed Co., Darlington 6070, AUS (D. M. Wilson); **WPU** = Western Regional Plant Introduction Station, 59 Johnson Hall, Washington State University, Pullman 99164, U.S.A. (D. Stout); **WSA** = Wrightson Seeds., PO Box 357, Seven Hills 2147, AUS (S. Mitchell and H. Osborn); **YRA** = Arthur Yates Co., 25 Stanley St., Rockhampton 4700, AUS.

sole exceptions to these rules. On these plants, the nodules produced by USDA257 were Fix^+, while those produced by NGR234 were Nod^+Fix^-. Perhaps this is understandable with the *Glycine* spp., because their center of origin is in northeast China where *R. fredii* was first isolated (Hymowitz and Newell 1980; Keyser et al. 1982). It is less obvious with *A. americana*, however, as this species is indigenous to North America (Allen and Allen 1981).

NGR234, USDA257, and the "cowpea miscellany."

As mentioned above, both NGR234 and USDA257 are highly compatible with genera of the *Phaseoleae*, particularly the subtribe *Phaseolinae*, which includes the genus *Vigna*. Coincidentally, this is a defining characteristic of the "cowpea miscellany" (see above). To examine further these affinities, we compared the NGR234 and USDA257 host ranges with those of classic "cowpea" strains. Three different *Bradyrhizobium* strains were used, including CB756, which is widely used as an inoculant and is known to have a broad host range (Diatloff and Date 1978). Also included were USDA76 and USDA110, the latter of which is by far the best-studied *Bradyrhizobium* strain (see Göttfert 1993). We also included *R. loti* NZP4010 along with the fast-growing isolates because it has a moderately broad host range (Lewin et al. 1987b) even though its symbiotic genes are borne on the chromosome (Pankhurst et al. 1983b; Chua et al. 1985; Scott et al. 1996).

The nodulation capacities of all six bacteria were tested on three different *Vigna* spp. Only NGR234 and USDA257 were able to nodulate all plants (Table 3), and even then both were Fix^- on *V. radiata* subsp. *sublobata*. In comparison, NZP4010 only nodulated 30% of the test plants, while the nodulation capacities of the *Bradyrhizobium* strains were in the range of 80 to 90%. Since NGR234 and USDA257 nodulate more *Vigna* species and cultivars than conventional "cowpea" bradyrhizobia, they obviously possess the host-range requirements of this group and could, for this reason, be considered part of it.

Nodule morphology.

Although it is clear that the legume controls nodule structure, much useful symbiotic information can be obtained by comparing the effects of different rhizobia on nodulation of the same or different plants. As examples, Dilworth (1969) examined the effect of changing the plant species (*Lupinus luteus* and *Ornithopus sativus*) while maintaining a constant bacterial component (*Bradyrhizobium lupini*) on leghemoglobin synthesis. By inoculating *V. unguiculata* with either a *Bradyrhizobium* sp. or with NGR234, Broughton and Dilworth (1971) were able to confirm that the plant encodes the globin portion of the molecule. Extension of host-range studies, in which a nonreactive strain is complemented for nodulation by genetic loci derived from homologous rhizobia (Broughton et al. 1984, 1986), allowed identification of species-specific nodulation genes (Lewin et al. 1987a; 1990; Krishnan et al. 1992; Fellay et al. 1995a; Hanin et al. 1997; Jabbouri et al. 1998). Correlations have been made between the activity of these genes, their effect on Nod-factor structure, and their ability to nodulate specific plants (Jabbouri et al. 1995; Hanin et al. 1997; Quesada-Vincens et al. 1997; Jabbouri et al. 1998; Berck et al. 1999).

Some combinations of legume species that are Fix^+ with NGR234 and Nod^- with USDA257 have proven useful in elucidating Nod-factor structures and the plant requirement for nodulation. *Leucaena leucocephala* is an example. *L. leucocephala* not only possess indeterminate nodules, but it is one of only two Mimosoid plants that are Nod^- with USDA257 and Fix^+ with NGR234 (Table 1). For these reasons, it has been widely used in extension of host-range studies. It is now known that N-methylated Nod factors are necessary for nodulation of *L. leucocephala* and *L. diversifolia* (Fig. 2). Similarly, *Calopogonium caeruleum* and *Pachyrhizus tuberosus*, which have determinate nodules, are Nod^- with USDA257 and Fix^+ with NGR234 and have been helpful in the elucidation of Nod-factor structures (see below).

Model legumes.

Spurred on by the success attained when numerous research groups concentrated their efforts on *Arabidopsis thaliana* (L.) Heynh., molecular biologists have sought a comparable legume for genetic manipulation. Ideally, such a legume should (i) be diploid, (ii) have few, morphologically distinct chromosomes, (iii) possess a small genome, (iv) be autogamous, (v) possess simple and well-developed genetics, (vi) have a rapid generation time, (vii) be transformable, (viii) grow readily under laboratory conditions, and (ix) have the capacity to nodulate with a wide range of rhizobia. Obviously, model plants would be of more interest if they were also economically important.

Surprisingly, very few of the widely used or studied legumes meet these criteria. *Arachis hypogaea, G. max*, and *Medicago sativa* are tetraploids, while the genome of *P. sativum* is much larger than that of humans. Of the widely traded legumes, only *Phaseolus* and *Vigna* spp. possess relatively small genomes (500 to 600 Mbp/1C) and are true diploids (Arumuganathan and Earle 1991). Both are difficult to transform and regenerate, however. Partly for this reason, two other legumes have been adopted as models: *Lotus japonicus* (Handberg and Stougard 1992; Jiang and Gresshoff 1997; Stiller et al. 1997; Szczyglowski et al. 1998) and *Medicago truncatula* (Barker et al. 1990). *Rhizobium loti* is the symbiont of *L. japonicus*, while *M. truncatula* associates with *R. meliloti*. As the molecular genetics of *R. loti* are not very well developed, however, the finding that both NGR234 and USDA257 effectively nodulate *L. japonicus* removes this obstacle to the establishment of *L. japonicus* as a model system (Table 1). Many nodulation and other mutants of NGR234 exist, and the complete nucleotide sequence of the symbiotic plasmid (Freiberg et al. 1997) should make up for the lack of molecular information on *R. loti* strains. Of course, the ability of USDA257 and NGR234 to nodulate *G. max* and *V. unguiculata*, respectively, has also allowed intensive study of these hosts (e.g., Trese and Pueppke 1991; Krause et al. 1994; Arsenijević-Maksimović et al. 1997; Gehring et al. 1997).

Correlations between Nod factors and nodulation.

Although the concept of host specificity is nebulous, host-specificity of nodulation (hsn) loci, which are unique to specific rhizobia, direct the adjunction of unique groups to the core lipo-oligosaccharide Nod factors, and should thus permit nodulation of certain legumes. For example, stem nodules on *Sesbania rostrata* are only induced by *Azorhizobium caulinodans*, a bacterium that produces arabinosylated Nod factors (Mergaert et al. 1993). Host specificity, while not so stringent in other legume-*Rhizobium* combinations, can also restrict the

pool of possible microsymbionts to a few rhizobia. *R. leguminosarum* bv. *viceae*, which nodulates *P. sativum* and some *Vicia* spp., produces Nod factors in which the acyl-chain is highly unsaturated (Spaink et al. 1991). Perhaps the clearest examples concern *M. sativa* and *R. meliloti*. Wild-type *R. meliloti* Nod factors are sulfated {NodRm(IV,V)[Ac,S]}, while those of NodH$^-$ mutants, which are unable to nodulate *M. sativa*, are not {NodRm(IV,V)[Ac]}. Yet, NodH$^-$ mutants nodulate *Vicia sativa* subsp. *nigra*, which wild-type *R. meliloti* is unable to do (Roche et al. 1991a). Although these results are clouded by the varying phenotype of some NodH$^-$ mutants (Ogawa et al. 1991), they suggest that *M. sativa* requires sulfated Nod factors for nodulation while *V. sativa* does not tolerate them.

NGR234 and USDA257 represent the other extreme. As shown here, NGR234 is able to nodulate more than 50% of all legumes on which it was tested. Obviously, *nod* genes (and to a large extent, Nod factors) are involved in these varying patterns of nodulation. At first sight, some genes seem to function like *nodH*. *noeE*, which is involved in sulfation of the 2-*O*-methylfucose residue of NodNGR factors, is essential for nodulation of *Calopogonium caeruleum* (Hanin et al. 1997). Gaps within the *nod* box promoter region and the 5′ end of *nodS* of USDA257 render the strain incapable of nodulating *Leucaena diversifolia*, *L. leucocephala*, and *L. trichodes* (Krishnan et al. 1992). On the other hand, NGR234, which possesses a functional *nodSU* operon, readily nodulates these species (Table 1). Yet, at least three *L. leucocephala* isolates exist (94A3, 94A4, and WBM16) that apparently lack *nodSU* (Krishnan et al. 1992). This suggests either that N-methylated Nod factors (see Figure 2) are not always necessary for nodulation of *Leucaena* spp., or that another *N*-methyltransferase exists that has minimal homology to NodS. Nevertheless, adjunction of the *N*-methyl group to NodNGR factors is performed by the *N*-methyltransferase encoded by NodS (Jabbouri et al. 1995; Geelen et al. 1995).

nodZ and its role in fucosylation of Nod factors present an even greater paradox. All the usual *G. max* symbionts (*B. elkanii*, *B. japonicum*, *R. fredii*, but also NGR234) are fucosylated (see Hanin et al. 1998). Teleologically, one would have expected this fucose group (normally 2-*O*-methylfucose) to be a requirement for nodulation of *G. max*. Mutation of *nodZ* in the respective strains abolishes fucosylation of both NodBj and NodNGR factors but not nodulation of *G. max*, however (Stacey et al. 1994; Quesada-Vincens et al. 1997). Even more puzzling is the observation that nonfucosylated NodBj factors isolated from a NodZ$^-$ mutant are unable to deform root hairs of *G. soja*, a plant that *nodZ* mutants nevertheless nodulate (Stacey et al. 1994). In fact, the only Nod$^-$ phenotype observed so far concerns NGRΔ*nodZ* mutants that are Nod$^-$ on *Pachyrhizus tuberosus* (Quesada-Vincens et al. 1997), suggesting that fucosylated Nod factors are necessary for nodulation of this plant. As mentioned above, NodRf factors are also fucosylated (Fig. 2), and, on this basis, USDA257 would be expected to nodulate *P. tuberosus*, but this is not the case (Table 1). On *M. atropurpureum*, a NodZ$^-$ mutant of *B. japonicum* displays a delayed nodulation phenotype (Stacey et al. 1994), yet the same *nodZ* gene extends the host range of *R. leguminosarum* to include *M. atropurpureum* (Lopez-Lara et al. 1996). Mutation of *nodZ* has no effect on nodulation of *M. atropurpureum* by NGR234 (Quesada-Vincens et al. 1997).

Obviously, Nod-factor substituents alone are insufficient to explain host range. Host specificity can also be broken when Nod factors are present above certain "threshold" levels (Relić et al. 1994a). Probably, a complex interplay between the induction of *nod* genes by flavonoids, the excretion of variously substituted Nod factors, the relative and absolute Nod-factor levels, and feedback control of flavonoid accumulation (Schmidt et al. 1994) are all involved in nodulation of the different legumes.

DISCUSSION

Broad host range.

To the best of our knowledge, the host-range spectrum of individual *Rhizobium* strains has been the subject of only two other extensive investigations. The first, and certainly the most detailed, was that of Wilson (1939), who measured the abilities of 32 soil isolates to nodulate 160 different legumes. Broad-host-range rhizobia were found in both the Mimosaceae (present day Mimosoideae) and Fabaceae (Papilinoideae). All but two of the 32 isolates could nodulate more than 25 species, and three were extremely promiscuous in their symbiotic capacities. These included a *Rhizobium* isolate from *Albizia julibrissin* that nodulated 92 species, a *Rhizobium* isolate from *Desmodium canadense* that nodulated 93 species, and a presumed *Bradyrhizobium* isolate from *Mucuna pruriens* DC. subsp. *deeringiana* (Bort.) P. Hanelt (= *Stizolobium deeringianum* Bort.) that nodulated 105 legume species. Unfortunately, these strains were not examined further and, as far as we know, they have been lost. *Bradyrhizobium* sp. strain CB756, which was isolated from *Macrotyloma africanum* (R. Wilczek) Verdc. growing in Zimbabwe, is another broad-host-range organism. Although widely available, it has been tested on a relatively restricted set of legumes, for which only positively responding species were recorded (Diatloff and Date 1978).

A simplified comparison of the nodulation data available for these four rhizobia plus USDA257 and NGR234 is presented in Table 4. All six strains appear to be adapted for nodulation of members of the Mimosoid tribes *Acacieae*, *Ingae*, *and Mimoseae* as well as the Papilionoid tribes *Desmodieae*, *Genisteae*, *Robinieae*, but especially the *Phaseoleae*. Given the affinity of both NGR234 and USDA257 for the *Desmodieae*/*Phaseoleae*, it might have been expected that broad-host-range rhizobia would have preferred hosts within these tribes. Two out of the six strains failed to nodulate *V. unguiculata*, which is widely recognized as being promiscuous (Table 3; Lewin et al. 1987b; Hernandez-Lucas et al. 1995). None of the strains nodulated all plants, nor was any one plant nodulated by all strains. Nevertheless, NGR234 nodulated more of these "broad-host-range hosts" than any other rhizobia so far studied, and in this sense is the most promiscuous known rhizobia. Perhaps not surprisingly, in view of the hypothesis that defects in the host account for the lack of nodulation, none of the promiscuous rhizobia were able to overcome the recalcitrance of caesalpinioid legumes such as *Bauhinia*, *Caesalpinia*, and *Cassia* spp. to nodulate (see Allen and Allen 1981).

Molecular basis of broad host range.

Perhaps the most striking observation presented here is not that both bacteria have exceptionally broad host ranges, but

that every plant that is nodulated by *R. fredii* USDA257 is also host to *Rhizobium* sp. strain NGR234. The antithesis is not true: USDA257 only nodulates approximately 58% of the NGR234 hosts. And this is the conundrum: if USDA257 nodulates an exact subset of the NGR234 hosts and excretes an exact subset of NodNGR factors (see Figure 2), why cannot Nod-factor requirements be assigned to specific legumes? Obviously, correlations exist but they are far from perfect, and Nod-factor levels also play a role. Wild-type USDA257 secretes only one fortieth the amount of Nod factors produced by NGR234 (Relić et al. 1994a). Perhaps a combination of different levels of various Nod factors is responsible for the nodulation patterns.

Another intriguing observation is that if only one of the two bacteria forms an effective (i.e., nitrogen-fixing) association, it is almost always NGR234. The only exceptions to this rule concern *Apios americana, G. max,* and *G. soja*, where there is a reversal of the nitrogen fixation phenotypes: USDA257 is Fix^+ while NGR234 is Fix^-. Again, the reasons for these differences are not clear. Since *nod* genes are only expressed early in the symbiosis (Fellay et al. 1995b), it is unlikely that Nod factors themselves are responsible for the Fix^+/Fix^- phenotypes. Rhizobia are known to possess genes that permit full development of bacteroids and hence nodules (Wilson et al. 1987; Müller et al. 1995a, 1995b). Perhaps some of these differ between USDA257 and NGR234. Another possibility concerns the presence of genes homologous to the type three protein secretion system (TTSS) in both bacteria (Balatti et al. 1995; Freiberg et al. 1997; Gu et al. 1997; Viprey et al. 1998; Viprey et al., *in press*). The TTSS is an essential component of pathogenicity in both animal (e.g., *Salmonella*, *Shigella*, *Yersinia*) and plant (*Erwinia*, *Pseudomonas*, *Ralstonia*, and *Xanthomonas*) pathogens. Indications that genes encoding part of the TTSS are present in USDA257 developed from the work of Meinhardt et al. (1993). This and later studies showed that the *nolXWTUV* locus is responsible for the flavonoid-induced excretion (Krishnan and Pueppke 1993; Krishnan et al. 1995) of at least five proteins (Krishnan and Pueppke 1993). Mutation of *nolXWTUV* has cultivar- and host-specific effects on nodulation of certain legumes (Krishnan and Pueppke 1994; Kovács et al. 1995; Bellato et al. 1997). Sequence analysis of pNGR234*a* revealed that all genes necessary for elaboration of the TTSS are present in NGR234 (Freiberg et al. 1997). Insertional inactivation of several of them abolishes not only secretion of two proteins, but severely alters their ability to nodulate *Pachyrhizus tuberosus* (*Phaseoleae—Glycininae*) and *Tephrosia vogelii* (*Millettieae*) (Viprey et al. 199). It is thus possible that protein secretion is an important component of effectiveness in nitrogen fixation, and that differences in either the TTSS machinery itself or the exported proteins could help explain the variation in Fix^+/Fix^- phenotypes of USDA257 and NGR234.

Evolutionary implications.

It is even more difficult to explain the close genetic relationship between USDA257 and NGR234. *Rhizobium* populations are often heterogenous (e.g., see Broughton et al. 1987), yet no essential genes were found when genomic DNA of USDA257 was subtracted from that of NGR234 (Perret et al. 1994). DNA sequence homologies even extend to the nodulation genes. Only 19 bp of the 3.08-kb *nodABC* loci are different between the two strains (Relić et al. 1994b). Obviously, similar *nod* genes direct the synthesis of comparable Nod factors, and these are part of the molecular basis of the nested host ranges. Still, this does not explain the evolutionary pressures that lead to broad host range. Experiments with different *Vigna* spp. and cultivars showed that both strains are more "cowpea-like" than bacteria (normally slow-growing *Bradyrhizobium* spp.) of the "cowpea" miscellany. Hosts of the "cowpea miscellany" that include a range of tropical and subtropical *Phaseoleae* and *Desmodieae* are also the preferred hosts of both bacteria. In other words, two different bacteria (USDA257 and NGR234) isolated from different genera (*G. soja* and *Lablab purpureus*), at widely separated locations (China and Papua New Guinea), and at least a decade apart, acquired the ability to nodulate a group of Old World and Central American plants (Doyle et al. 1997).

Whether the ability of plants to nodulate arose once or a number of times during evolution is a matter of some controversy. Sprent and Sprent (1990) and Doyle (1994) suggest that it may have arisen several times within the Leguminosae. In contrast, Solitis et al. (1995) wrote of "the likelihood of only a single origin of the predisposition for root-nodule symbioses in angiosperms . . ."—a radically opposed idea. Of course, our limited data are insufficient to resolve this conflict, yet Figure 3 suggests that both NGR234 and USDA257 have acquired the ability to nodulate three distinct groups of plants, in the Caesalpiniodieae, Mimosoideae, and Papilionoideae. In the Papilionoideae this ability arose early, and was maintained through to the *Amorpheae, Robinieae, Indigofereae, Phaseoleae,* and *Desmodieae*. Once acquired, the capacity to symbiose with promiscuous rhizobia was apparently lost in only five tribes. Of these, it is difficult to draw conclusions on the *Abreae* and *Adesmieae,* since only one and two plants, respectively, were tested. It seems clear, however, that tribes P19 to P21 (*Vicieae, Cicereae,* and *Trifolieae*) have lost the ability to nodulate with NGR234 or USDA257. Since the microsymbionts of these plants generally secrete fewer, less-modified Nod factors, albeit with polyunsaturated acyl chains, this implies that narrow host range is a specialization that developed for certain plants in restricted niches.

It is possible to argue that Figure 3, which is based on morphological rather than molecular data, does not present the true relationships between the tribes. Accordingly, we sought molecular evidence of tribal associations, particularly those involving the crucial loss of nodulation that may have occurred between the *Galegeae* and the tribes *Cicereae, Trifolieae,* and *Vicieae*. Unfortunately, although much sequence data is now being published, it tends to deal with specific genera rather than whole subfamilies. Also, as Doyle et al. (1997) point out: "The chloroplast genome has provided . . . weak support for many clades in the [ribulose-1,5-bisphosphate carboxylase/oxygenase] *rbc*L tree suggests that data from additional genes will be required to construct rigourous hypotheses from some groups [of the Leguminosae]" (words in brackets have been added for clarity). In other words, it is not possible, at the present moment, to replace Figure 3 with one constructed from purely molecular data. Nevertheless, Doyle et al. (1997) present a molecular analysis of the tribal relationships among the Papilionoideae. These are shown in Figure 4, along with the capacity of the tribes to nodulate with NGR234 and USDA257. Although the *Cicereae* were not included in

Doyle et al.'s analysis, their data confirm our finding (Fig. 3) that the ability to nodulate with NGR234 or USDA257 has been lost in temperate, herbaceous legumes (their observation that the *Galageae* did not give rise to the *Trifolieae* and *Vicieae* is irrelevant to this argument).

It thus seems likely that symbiotic promiscuity is ancestral to restricted host range. Support for this hypothesis comes from the observation that NGR234/USDA257 also nodulate *Parasponia andersonii* (Ulmaceae). *Parasponia* spp. are small trees (up to 15 m high) that grow as pioneer plants in mountain areas of Indonesia, Malaysia, and Papua New Guinea (Akkermans and van Dijk 1981). In other words, *Parasponia-Rhizobium* symbioses evolved in NGR234's habitat. As there is a direct correlation between solar energy input and species diversity (Roy et al. 1998), tropical regions are probably driving evolution. In this scenario, bacteria like NGR234/USDA257 would have been the first to intimately associate with legumes.

Exactly what symbiotic genes, and therefore Nod factors, were necessary for these ancestral interactions is not apparent from the present data. Clearly, those additional Nod-factor substituents that NGR234 produces but USDA257 lacks are like "baroque" decorations—they enhance the general appeal of NGR234, but they are not necessary for basic promiscuity. Enzymes encoded by *nodS* (the *N*-methyltransferase), *nodU* (the 6-*O*-carbamoyl transferase, *nolO* (the 3- [or 4-]-*O*-carbamoyl transferase), *nolL* (an acetyltransferase), *noeE* (a sulfotransferase), and *noeI* (a 2-*O*-methyltransferase) simply extend an already broad host range. Insertional mutation of *nodZ*, which encodes the fucosyltransferase, suggests that it is also a "baroque" gene, since the mutants are only Nod^- on *Pachyrhizus tuberosus* (Quesada-Vincens et al. 1997).

If this logic reflects evolution, then the ancestral Nod factors were simply oligomers of chitin (probably trimers to pentamers), N-acylated with a C18 fatty acid, and were able to nodulate many plants. Modifications followed that in the case of NGR234 allowed it to nodulate even more plants. Northern migration of USDA257 was accompanied, however, by point mutations and genome re-arrangements that restricted its host range. Such changes, when they occurred in the *nodSU* operon, for example, deprived USDA257 of the ability to secrete N-methylated and 6-O-carbomoylated Nod factors, and thus the capacity to nodulate *Leucaena* spp. (Krishnan et al. 1992).

MATERIALS AND METHODS

The seeds listed in Table 1 were sterilized by immersion in concentrated H_2SO_4 for times ranging from 5 min (e.g., *Glycine*, *Phaseolus*, and *Vigna* spp.) to 4 h (*Delonix*, *Erythrina*, *Robinia*, etc.). They were then washed three times with sterile water, rinsed in 5% (vol/vol) H_2O_2 for 5 to 10 min, washed in sterile water, and placed on B & D agar (Broughton and Dilworth 1971) to germinate at 26°C. Special treatments were necessary to ensure germination of certain species. As examples, the embryo had to be freed by dissection from seeds possessing very thick testas (e.g., *Delonix*—performed after sterilization and imbibition), cold treatments were necessary with others (e.g., *Vicia* spp.), while heat treatment (immersion in boiling water) was used with *Swainsona*. Immersion in sterile water for a few days after sterilization, followed by transfer to agar plates, helped germination of some small-seeded legumes (e.g., *Hippocrepis ciliata*). When the radicals were approximately 2 cm long, the seedlings were planted into washed vermiculite held in Leonard jars of three different sizes. Magenta jars (250 ml capacity) were used for small seedlings (Lewin et al. 1990), modified wine bottle assemblies (volume = 750 ml) were used for medium to large seeds (*Arachis*, *Vicia faba*, etc.) (Broughton and John 1979), while Leonard jars made from 2.5-liter laboratory reagent bottles were used for extremely large seeds (e.g., *Castanospermum*, *Cynometra*, *Millettia megasperma*, etc.). All plants were raised at a day temperature of 30°C, a night temperature of 20°C, and a light phase of 16 h (including a 1-h stepped "sunrise" and a 1-h stepped "sunset"). The intensity of illumination was 350 µE s^{-1} m^{-2} PAR). Germinating seeds were inoculated with approximately 1×10^7 log-phase cells of one of the bacteria listed in Table 5 at planting (Pueppke 1983) or upon establishment (Lewin et al. 1990). Given the large number of legumes involved, and the paucity of information available on their rhizobial requirements, it was not possible to include positively nodulating controls for all plants in the experimental design. Rather, with the exception of the *Medicago* and *Vigna* spp., we simply asked whether either of the test bacteria could nodulate the plants listed in Table 1 (noninoculated plants served as negative controls). Since the rhizobial requirements of *Medicago* and *Vigna* spp. are well known, and as many species were tested, positive controls were inoculated with *R. meliloti* RCR2011 and *B. japonicum* USDA110, respectively. Each experiment was replicated, with one to 10 plants per treatment, depending on the availability of seeds and the size of the seedlings. Most nodulation experiments were performed at least twice. Plants were grown until the "controls" displayed clear symptoms of nitrogen deficiency (yellowing of the leaves, stunted growth), which varied between approximately 1 and 6 months, depending on the size of the seeds and the initial growth rate (*Desmodium* and *Castonospermum* represent the extremes). At harvest, the roots were examined for the presence/absence of nodules. Plants were listed as Nod^- when there was no apparent response to inoculation. Nod^+ was used to describe outgrowths/nodules that, irrespective of the internal structure, lacked leghemoglobin. Fix^+ nodules possessed organized internal structures and bacteroids. Mature Fix^+ nodules were red in color, fixed nitrogen, and caused the leaves to green.

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