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## Small-Subunit rRNA Genotyping of Rhizobia Nodulating Australian *Acacia* spp.

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**The structure of rhizobial communities nodulating *Acacia* in southeastern Australia from south Queensland to Tasmania was investigated by a molecular approach. A total of 118 isolates from nodule samples from 13 different *Acacia* species collected at 44 sites were characterized by small-subunit (SSU) ribosomal DNA (rDNA) PCR-restriction fragment length polymorphism analysis. Nine rhizobial genomospecies were identified, and these taxa corresponded to previously described genomospecies (B. Lafay and J. J. Burdon, *Appl. Environ. Microbiol.* 64:3989–3997, 1998). Eight of these genomospecies belonged to the *Bradyrhizobium* lineage and accounted for 96.6% of the isolates. The remaining genomospecies corresponded to *Rhizobium tropici*. For analysis of geographic patterns, results were grouped into five latitudinal regions regardless of host origin. In each region, as observed previously for rhizobial isolates taken from non-*Acacia* legumes (Lafay and Burdon, *Appl. Environ. Microbiol.* 64:3989–3997, 1998), rhizobial communities were dominated by one or two genomospecies, the identities of which varied from place to place. Despite this similarity in patterns, the most abundant genomospecies for *Acacia* isolates differed from the genomospecies found in the non-*Acacia*-derived rhizobial collection, suggesting that there is a difference in nodulation patterns of the Mimosoideae and the Papilionoideae. Only two genomospecies were both widespread and relatively abundant across the range of sites sampled. Genomospecies A was found in all regions except the most northern sites located in Queensland, whereas genomospecies B was not detected in Tasmania. This suggests that genomospecies A might be restricted to the more temperate regions of Australia, whereas in contrast, genomospecies B occurs in different climatic and edaphic conditions across the whole continent. The latter hypothesis is supported by the presence of genomospecies B in southwestern Australia, based on partial SSU rDNA sequence data (N. D. S. Marsudi, A. R. Glenn, and M. J. Dilworth, *Soil Biol. Biochem.* 31:1229–1238, 1998).**

The bacteria inducing nitrogen-fixing nodules on leguminous plants (family Fabaceae) all belong to the alpha subdivision of the proteobacteria but represent at least six genera, *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Allorhizobium*; these taxa are relatively distantly related to one another, and each is more closely related to nonnodulating taxa (12, 23, 49). Additionally, a number of *Rhizobium* isolates group in the *Agrobacterium* lineage (13, 43, 49).

In recent years, studies of natural populations of rhizobia isolated from a variety of legume hosts around the world have revealed considerable genetic diversity and led to the description of two new genera, *Azorhizobium* (17) and *Allorhizobium* (12), as well as several new species of *Rhizobium* (1, 9, 45), *Mesorhizobium* (14, 23), and *Sinorhizobium* (15, 37). Furthermore, based on genotyping, a number of new lineages have been identified (5, 24, 31, 42).

In Australia, both fast-growing and slow-growing rhizobia occur naturally, and *Bradyrhizobium* species (slow growers) are predominant throughout the continent (2, 3, 27, 28, 39, 44). Recent molecular approaches have shown that various genomospecies (i.e., species characterized only at the genomic level) belonging to the genera *Rhizobium*, *Mesorhizobium*, and *Bradyrhizobium* are represented among rhizobia symbiotically

associated with a variety of native legume hosts in Western Australia (31) and that *Bradyrhizobium* genomospecies occur in Queensland soils (29).

In a previous study aimed at analyzing the effect of the identity of the associated host legume, as well as geographic origin, on the structure of Australian native rhizobial communities, we examined 745 strains from 32 legume species in southeastern Australia (24). Using a molecular systematics approach combining small-subunit (SSU) ribosomal DNA (rDNA) PCR-restriction fragment length polymorphism (RFLP) analysis and sequencing, we identified 21 genomospecies, all but one of which are still undescribed. No clear specificity between rhizobial genomospecies and legume taxa was observed, although some preference for particular genomospecies was suggested for three legume species. One of these species was the only non-Papilionoideae taxon (*Acacia obliquinervia*; subfamily Mimosoideae) from which nodule samples had been obtained.

In the present study we tried to further analyze the possible specificity of host species belonging to the Mimosoideae for rare rhizobial genomospecies. With about 850 species naturally occurring in Australia (11), the genus *Acacia* overwhelmingly represents the family Mimosoideae in this part of the world. Acacias are widespread on the Australian continent, where they are a dominant component of many ecosystems, whether dominance is measured in terms of structural position, numbers, or overall biomass. They occur as dominant understory species in many tall and open forests in mesic areas (2) and also are the dominant vegetation in arid zone woodlands (2,

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TABLE 1. Origins of *Acacia* collection rhizobial isolates

Host <i>Acacia</i> species	Site	State <sup>a</sup>	Latitude (°S)	Longitude (°E)	Region <sup>b</sup>	No. of isolates
<i>A. cangaiensis</i>	Cangai	NSW	29°30'	152°29'	I	3
<i>A. cincinnata</i>	Gympie	Qld	26°11'	152°40'	I	1
<i>A. dealbata</i>	Ben Lomond	NSW	30°01'	151°40'	I	2
	Kandos	NSW	32°52'	149°58'	II	5
	Captains Flat	NSW	35°35'	149°27'	III	1
	Bemboka	NSW	36°38'	149°35'	III	2
	Erinundra	Vic	37°20'	148°55'	IV	1
	Licola	Vic	37°38'	146°37'	IV	1
	Inglis River	Tas	41°08'	145°37'	V	2
	Mt. Elephant	Tas	41°37'	148°15'	V	8
	Snug	Tas	43°04'	147°15'	V	5
<i>A. deanei</i>	East Goondiwindi	NSW	28°32'	150°20'	I	2
<i>A. decurrens</i>	Picton	NSW	34°11'	150°37'	III	2
<i>A. fulva</i>	Howes Valley	NSW	32°51'	150°51'	II	1
<i>A. glaucocarpa</i>	Gayndah	Qld	25°37'	151°37'	I	3
<i>A. implexa</i>	Moonan Flat	NSW	31°55'	151°14'	II	2
	Pyalong	Vic	37°07'	144°52'	IV	2
	Glenmaggie Lake	Vic	37°55'	146°47'	IV	4
<i>A. irrorata</i>	Wauchope	NSW	31°28'	152°44'	II	3
	Barrington National Park	NSW	31°59'	151°55'	II	1
	Gloucester	NSW	32°01'	151°58'	II	2
<i>A. mearnsii</i>	Berrima	NSW	34°29'	150°20'	III	1
	Lake George	NSW	34°59'	149°24'	III	3
	Cooma	NSW	36°14'	149°08'	III	2
	Wattle Circle, Omeo Highway	Vic	37°05'	147°35'	IV	1
	Grampians	Vic	37°08'	142°26'	IV	3
	Northeast Kyneton	Vic	37°15'	144°30'	IV	4
	Cann River	Vic	37°31'	149°10'	IV	6
	Tantanoola	SA	37°42'	140°27'	IV	9
	Apsley River bridge	Tas	41°52'	148°11'	V	1
	Boyer	Tas	42°47'	147°06'	V	1
<i>A. melanoxylon</i>	Bli Bli	Qld	26°37'	153°02'	I	4
	Singleton	NSW	32°34'	151°10'	II	1
	Mt. Lindsay	NSW	34°29'	150°35'	III	2
	Mt. Coree	ACT	35°19'	148°48'	III	2
	Goanna Creek	Vic	37°18'	148°43'	IV	5
	The Highlands	Vic	37°38'	144°24'	IV	2
	Gellibrand River	Vic	38°32'	143°33'	IV	2
	Mt. Gambier	SA	37°52'	140°47'	IV	4
	Lileah	Tas	40°58'	145°10'	V	1
	Nabowla	Tas	41°10'	147°22'	V	3
	Huon River	Tas	43°06'	146°43'	V	5
<i>A. parramattensis</i>	Tarago	NSW	35°04'	149°39'	III	1
<i>A. parvipinnula</i>	Howes Valley	NSW	32°51'	150°51'	II	2

<sup>a</sup> ACT, Australian Capital Territory; NSW, New South Wales; Qld, Queensland; SA, South Australia; Tas, Tasmania; Vic, Victoria.

<sup>b</sup> Region I, Queensland and northern New South Wales; region II, New South Wales between latitudes 31°S and 34°S; region III, southeastern New South Wales and Australian Capital Territory; region IV, Victoria and South Australia; region V, Tasmania.

32). A few species occur in rain forests (4). Australian acacias have considerable potential for agroforestry, for fuelwood production, and for improvement of impoverished soils (36, 39). Indeed, the interactions that they have with root nodule bacteria can be responsible for substantial levels of nitrogen fixation (21).

In this study, we used isolates that were collected during a joint project of the Australian Centre for International Agricultural Research, CSIRO Plant Industry, and CSIRO Forestry & Forest Products. This project was aimed at assessing the potential of temperate Australian *Acacia* species for use in a range of plantation and farm forestry situations in Australia, China, and Vietnam, where rapid growth is essential (8). In this study we used the same identification procedure that was used in our previous study of rhizobial communities in Australia and we compared the *Acacia* isolates with rhizobial

strains associated with native, non-*Acacia* legumes (24). We also took advantage of the availability of this isolate collection to explore further the nature and structure of rhizobial communities for a larger geographic and climatic range in Australia.

#### MATERIALS AND METHODS

**Rhizobial strains.** We characterized 118 isolates collected from 13 *Acacia* species at 44 sites in six Australian states (Australian Capital Territory, New South Wales, Queensland, South Australia, Tasmania, and Victoria). This group was a subset of a more extensive collection of rhizobial isolates generated during a joint project of the Australian Centre for International Agricultural Research, CSIRO Plant Industry, and CSIRO Forestry & Forest Products (Table 1). The *Acacia* species examined covered the range of species growing in different ecological habitats in southeastern Australia. The nodulation ability of each isolate was verified by inoculation onto sterilely grown seedlings of siratro (*Macroptilium*

*atropurpureum*), a universally promiscuous host. After 12 weeks of growth in a glasshouse, nodules were found on the root systems of all inoculated plants.

**DNA preparation.** Bacterial DNA was prepared by the method described by Sritharan and Barker (40). Bacteria were grown on yeast-mannitol agar medium (46), and colonies were collected, suspended in 100  $\mu$ l of 10 mM Tris (pH 8.0)–1 mM EDTA–1% Triton X-100, and boiled for 5 min. After a single chloroform extraction, 5  $\mu$ l of each supernatant was used in the amplification reaction.

**SSU rRNA gene amplification.** Primers corresponding to positions 8 to 28 and 1498 to 1509 (26) in the *Escherichia coli* SSU rRNA sequence (7) were used for amplification of the SSU rRNA genes by PCR. PCR were carried out in 100- $\mu$ l mixtures containing 5  $\mu$ l of template DNA solution, 50 pmol of each of two primers, each deoxyribonucleoside triphosphate (Boehringer Mannheim) at a concentration of 200  $\mu$ M, and 2.5 U of Amplitaq DNA polymerase (Perkin-Elmer) in Amplitaq DNA polymerase reaction buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM MgCl<sub>2</sub>). Amplifications were performed with a Hybaid Omnigene thermocycler by using the following temperature profile: an initial cycle consisting of denaturation at 95°C for 5 min, annealing at 52°C for 120 s, and extension at 72°C for 90 s; 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 52°C for 60 s, and extension at 72°C for 60 s; and a final extension step consisting of 72°C for 5 min.

**SSU rDNA PCR-RFLPs.** Ten-microliter aliquots of PCR products were digested with restriction endonucleases as described by Laguerre et al. (25). A combination of four enzymes (*Hha*I, *Hinf*I, *Msp*I, *Rsa*I), which distinguished rhizobial species (24, 25), was used. Restricted fragments were separated by electrophoresis on 3% NuSieve 3:1 agarose gels at 80 V for 5 h and were visualized by ethidium bromide staining.

## RESULTS

**Rhizobial diversity.** Eight *Bradyrhizobium* and one *Rhizobium* genomospecies were detected among the 118 isolates collected from the 13 species of *Acacia* (Fig. 1; Table 1). All nine genomospecies had previously been characterized in a study of rhizobial communities in southeastern Australia, and all of them except genomospecies Q corresponded to undescribed species (24). Four genomospecies related to *Bradyrhizobium japonicum* (genomospecies A, B, F, and H) accounted for 33.1, 21.2, 21.2, and 12.7% of all of the isolates, respectively. Together, these genomospecies accounted for 88.2% of the isolates, although only two (genomospecies A and B) were widespread in many *Acacia* species (11 and 7 hosts, respectively). Genomospecies D, I, and O, which belong to the same cluster of closely related *Bradyrhizobium* genomospecies, occurred far less frequently (three times, five times, and once, respectively). Genomospecies P, affiliated with *Bradyrhizobium elkanii*, was found only once, and genomospecies Q, corresponding to *Rhizobium tropici*, was found only four times, although it was widespread and was recovered from three host species at four locations.

**Host specificity.** Most of the isolates assessed (75.4%) were obtained from nodules occurring on *Acacia dealbata*, *Acacia mearnsii*, or *Acacia melanoxylon*; between six and eight genomospecies were identified on each of these species (Table 2). The combinations of nodulating genomospecies varied from site to site for these three species, as well as for *Acacia irrorata* and *Acacia implexa*, for which we also genotyped isolates obtained from several sites. The number of genomospecies obtained from *A. dealbata*, *A. implexa*, *A. irrorata*, *A. mearnsii*, or *A. melanoxylon* was positively correlated with the number of sites or geographical regions from which rhizobia were collected for each of these species ( $r^2 = 0.98$ ,  $P < 0.001$ ). The numbers of isolates obtained from other *Acacia* species were not sufficient to allow separate consideration on a host species basis (Table 2).

**Geographic distribution.** Rhizobial occurrence was also considered independent of host origin. Sites were grouped into five geographic regions on the basis of latitude (Table 1; Fig. 1). In any one region, the distribution of rhizobial genomospecies was biased toward one or two major types (Fig. 1). The distribution of rhizobial genomospecies in the five regions was assessed by considering each of the most abundant genomospecies (genomospecies A, B, F and H) individually and grouping the less frequently occurring ones for each region (Table 3). A  $\chi^2$  test showed that the different genomospecies had significantly different distributions ( $P < 0.001$ ).

The distributions of genomospecies H and F were patchy; these genomospecies were present at noticeable frequencies in some areas but totally absent from regions I and III, respectively. Moreover, even within a region the distribution was often uneven. For example, six of the seven genomospecies H isolates characterized in region IV were obtained from Tantanoola in South Australia. In contrast, genomospecies A and B were dominant in regions I through IV. Interestingly, the frequencies of rhizobial types in the two latitudinally extreme regions were notably different (Fig. 1). Genomospecies A occurred at a low frequency and genomospecies B was absent in Tasmania (region V). In contrast, genomospecies F was dominant among the Tasmanian strains but was absent from the most northerly area (region I).

**Comparison with rhizobia nodulating non-*Acacia* legumes.** In our previous study, nodules were collected from only one *Acacia* species, *A. obliquinervia* (the only representative of the Mimosoideae among the 32 legume species sampled). Although the rhizobial genomospecies isolated from *A. obliquinervia* were the same as those nodulating the other legumes sampled in that study, a slightly different frequency distribution was observed for this host (24) (Table 4). On the other hand, at Island Bend in New South Wales, where *A. obliquinervia* was present, genomospecies A was the most common species on all species except *A. obliquinervia*. On this host species, genomospecies P accounted for 58.3% of the isolates recovered and genomospecies A accounted for only 16.7% of the isolates recovered (24). In contrast, in the present study, which was confined to samples from *Acacia* species, genomospecies P accounted for only 11.2% of all isolates (Table 4). As a consequence, we compared the rhizobial frequency distributions of the two collections (*Acacia* derived and non-*Acacia* derived) (Table 4). A  $\chi^2$  test revealed that the two distributions are significantly different ( $P < 0.001$ ).

## DISCUSSION

A number of rhizobial species, both fast-growing and slow-growing species, have been isolated from a broad range of *Acacia* species in countries other than Australia (18, 22, 34, 35, 37, 50). Indeed, members of four of the formally described genera (*Rhizobium*, *Mesorhizobium*, *Sinorhizobium*, and *Bradyrhizobium*) occur among the rhizobia nodulating *Acacia*. Taken together, the previous reports suggest that at least in light of current information, there is evidence that the range of genomospecies is greater in other parts of the world than in Australia. Indeed, within Australia, *Rhizobium*, *Mesorhizobium*, and *Bradyrhizobium* appear to be the only three genera represented among *Acacia* rhizobial isolates, and one genus, *Brady-*

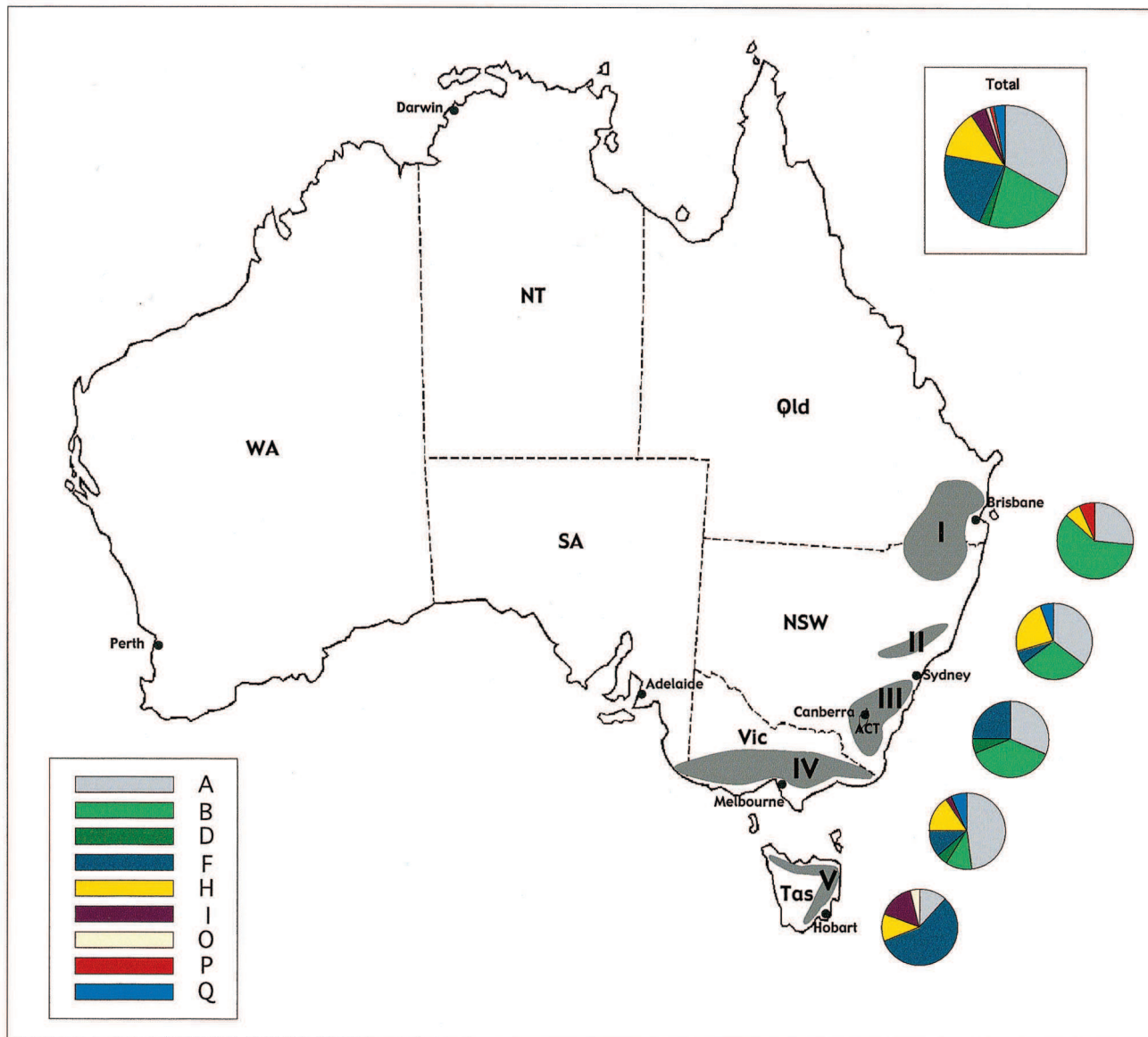


FIG. 1. Rhizobial community structures in the five regions sampled. Rhizobial genomespecies A through Q, characterized for the *Acacia* isolates, are color coded. Region I, Queensland and northeastern New South Wales; region II, New South Wales between latitudes 31°S and 34°S; region III, southeastern New South Wales and Australian Capital Territory; region IV, Victoria and South Australia; region V, Tasmania. Abbreviations: ACT, Australian Capital Territory; NSW, New South Wales; Qld, Queensland; SA, South Australia; Tas, Tasmania; Vic, Victoria; WA, Western Australia; NT, Northern Territory.

*rhizobium*, is largely dominant throughout the Australian continent. Both fast-growing and slow-growing rhizobia have been isolated from a wide range of *Acacia* species in diverse environments in southeastern Australia (2, 3, 28). The rhizobia nodulating *Acacia longifolia* var. *sophorae* (28) in Victoria were all fast-growing strains, whereas slow-growing isolates were also recovered from the same *Acacia* species in New South Wales (3). In contrast, only slow-growing rhizobia were isolated from a range of *Acacia* species in southwestern Australia (27). Fewer studies have investigated *Acacia* rhizobia in northern Australia, and only slow-growing isolates have been isolated so far (6, 39). However, beyond the slow-growing or fast-growing characteristic, the true nature of the root nodule

bacteria occurring on *Acacia* in Australia is poorly understood. Recently, a study using partial SSU rRNA sequence analysis conducted in southwestern Australia (31) confirmed that both types of rhizobia nodulate *Acacia saligna* and revealed that rhizobial strains in that part of Australia are related to *B. japonicum*, *Rhizobium leguminosarum* subsp. *phaseoli*, or *R. tropici*.

We used SSU rRNA PCR-RFLP analysis to characterize 118 rhizobial isolates collected from 13 *Acacia* species at 44 sites in eastern Australia from southern Queensland to Tasmania. SSU rDNA alone is not appropriate for formal definition of prokaryote species (30, 47). Two prokaryotes are unlikely to have more than 60 to 70% DNA similarity and hence be related at the species level, when their SSU rDNA se-

TABLE 2. Frequency distribution of various rhizobial genomospecies among isolates from *Acacia* species samples obtained in southeastern Australia

Host	Frequency of rhizobial genomospecies (%)									No. of isolates	No. of sites	Region(s) <sup>a</sup>	
	A	B	D	F	H	I	O	P	Q <sup>b</sup>				
<i>A. cangaiensis</i>	100.0										3	1	I
<i>A. cincinnata</i>								100.0			1	1	I
<i>A. glaucocarpa</i>		100.0									3	1	I
<i>A. dealbata</i>	7.4	11.2	3.7	25.9	25.9	18.5	3.7		3.7		27	9	I,II,III,IV,V
<i>A. deanei</i>	50.0	50.0									2	1	I
<i>A. decurrens</i>	100.0										2	1	III
<i>A. fulva</i>	100.0										1	1	II
<i>A. implexa</i>	37.5	50.0		12.5							8	3	II,IV
<i>A. irrorata</i>	33.3	50.0		16.7							6	3	II
<i>A. mearnsii</i>	51.6	16.1	3.2	6.5	19.4				3.2		31	10	III,IV,V
<i>A. melanoxylon</i>	19.4	19.4	3.2	48.3	3.2				6.5		31	11	I,II,III,IV,V
<i>A. parramattensis</i>	100.0										1	1	III
<i>A. parvipinnula</i>	100.0										2	1	II

<sup>a</sup> Region I, Queensland and northern New South Wales; region II, New South Wales between latitudes 31°S and 34°S; region III, southeastern New South Wales and Australian Capital Territory; region IV, Victoria and South Australia; region V, Tasmania.

<sup>b</sup> Genomospecies Q is *R. tropici*.

quences have less than 97% homology (41). However, levels of DNA similarity can greatly vary, from 10 to 100%, at SSU rDNA homology levels greater than 97% (41). Thus, a very high level of SSU rDNA similarity, as high as 99.8%, can be observed for different species (20). In contrast, heterogeneity between SSU rDNA sequences has been documented in the seven rRNA operons of *E. coli* (10). Nevertheless, despite not being a sufficient taxonomic criterion (47), SSU rDNA remains one of the most reliable indices of organismal phylogeny (48) and allows rapid identification of a large number of strains (24). Our results confirmed that *Acacia* species are nodulated by both fast-growing and slow-growing rhizobia and showed that all genomospecies identified thus far have been found previously among rhizobial strains nodulating shrubby legumes in southeastern Australia (24). Indeed, the strains recovered from *Acacia* corresponded to 9 of the 21 genomospecies identified in our previous study. Among the *Bradyrhizobium* strains, the six genomospecies detected (genomospecies A, B, D, F, I, and O) are part of a cluster of closely related lineages affiliated with *B. japonicum* (24). Genomospecies P is related to *B. elkanii*, whereas genomospecies H constitutes an independent lineage within this group (24). Additionally, some isolates corresponded to genomospecies Q (i.e., *R. tropici*). As already observed for a range of shrubby legume species, *Bradyrhizobium* species were dominant overall (96.6% of the strains isolated from *Acacia* hosts).

The nine genomospecies isolated from the 13 species of *Acacia* examined here were also by far the genomospecies most frequently recovered from nodules collected from the roots of the 32 shrubby legumes analyzed previously by us (24), where they represented 98.1% of all isolates (Table 4). The absence of the 12 other genomospecies among the *Acacia* isolates assessed in the present study is most likely a reflection of the smaller sample size (118 isolates, compared to 745 isolates in the previous study 24) since the missing rhizobial types were only very rarely recovered from shrubby legume nodules. Despite the wider geographical range, as well as more diverse climatic and edaphic conditions, we did not identify any additional rhizobial genomospecies, either already described

genomospecies or new genomospecies. This contrasts with results obtained by Marsudi et al. (31) for southwestern Australia. Only two of the partial SSU rRNA sequences which these authors obtained for *Acacia* rhizobia were similar to our sequences. One of the partial SSU rRNA sequences obtained by Marsudi et al. (31) corresponded to genomospecies Q (*R. tropici*). The only other genomospecies common to both studies was genomospecies B (sequence AF000622 for strain BDT51 in reference 31).

To analyze geographic patterns, we grouped our results into five latitudinal regions regardless of host origin. In a study of rhizobial isolates taken from non-*Acacia* legumes, we previously observed that rhizobial communities are frequently dominated by one or two genomospecies whose identities varied from place to place (24). This pattern was also apparent in the *Acacia*-derived rhizobial data presented here. Despite the similarity in the patterns, the identity of the most abundant genomospecies differed depending on the origin of the rhizobial collection (*Acacia* derived versus non-*Acacia* derived). This confirms our earlier hypothesis that *A. obliquinervia* is nodulated selectively by one rhizobial genomospecies regardless of its frequency at the site where nodule samples are obtained (24) and is consistent with the suggestion that the Mimo-

TABLE 3. Frequency distribution of various rhizobial genomospecies in five regions

Region <sup>a</sup>	Frequency of rhizobial genomospecies (%)					No. of isolates
	A	B	F	H	Others <sup>b</sup>	
I	26.6	60.0	0.0	6.7	6.7	15
II	35.3	29.4	5.9	23.5	5.9	17
III	31.3	37.5	25.0	0.0	6.2	16
IV	47.7	11.4	11.4	15.9	13.6	44
V	11.5	0.0	57.7	11.5	19.3	26

<sup>a</sup> Region I, Queensland and northern New South Wales; region II, New South Wales between latitudes 31°S and 34°S; region III, southeastern New South Wales and Australian Capital Territory; region IV, Victoria and South Australia; region V, Tasmania.

<sup>b</sup> *Bradyrhizobium* genomospecies D, I, O, and P and *R. tropici* (genomospecies Q).

TABLE 4. Frequency distribution and numbers of strains of various rhizobial genospecies in the *Acacia* and the BDV collections<sup>a</sup>

Collection	Frequency of rhizobial genospecies (%)									
	A	B	D	F	H	I	O	P	Q <sup>b</sup>	Others <sup>c</sup>
<i>Acacia</i>	33.1 (39) <sup>d</sup>	21.2 (25)	2.5 (3)	21.2 (25)	12.7 (15)	4.2 (5)	0.8 (1)	0.8 (1)	3.4 (4)	0.0 (0)
BDV (non- <i>Acacia</i> )	58.3 (427)	10.5 (77)	2.5 (18)	3.8 (28)	3.3 (24)	2.6 (19)	0.8 (6)	11.1 (82)	5.2 (38)	1.9 (14)
BDV ( <i>A. obliquinervia</i> )	16.7 (2)	0.0 (0)	0.0 (0)	58.3 (7)	0.0 (0)	8.3 (1)	0.0 (0)	0.0 (0)	0.0 (0)	16.7 (2)

<sup>a</sup> See reference 24 for an explanation of the BDV collection.

<sup>b</sup> Genospecies Q is *R. tropici*.

<sup>c</sup> *Bradyrhizobium* genospecies C, E, G, J, K, L, M, and N, *Rhizobium* genospecies R, and *Mesorhizobium* genospecies S, T, and U (24).

<sup>d</sup> The numbers in parentheses are numbers of strains.

soideae and the Papilionoideae may behave somewhat differently because of independent evolution of nodulation (16).

Only two genospecies were both widespread and relatively abundant at the range of the sites samples (Fig. 1; Tables 3 and 4). Genospecies A was found in all five regions but not at the most northerly sites (region I), where only genospecies B and P were found. The absence of genospecies A at sites located north of Brisbane, although somewhat significant since one of the three *Acacia* species sampled there had been found to associate with this genospecies at other sites, should, however, be regarded with caution considering the small sample size available (Table 1). Thus, we cannot rule out the possibility that genospecies A, the most abundant genospecies in eastern Australia (24; this study), occurs in all parts of the regions sampled in this study. However, its range may not be pan-continental as no corresponding SSU rDNA sequence was recovered either from *Acacia*-nodulating rhizobia obtained in southwestern Australia (31) or, in accordance with the results presented here, from Queensland soil (29).

The other most widespread rhizobial species, genospecies B, was found in all regions other than Tasmania. Given the much larger sample size for Tasmania, the absence of genospecies B there may reflect either true absence or a very low level of occurrence due to poor adaptation to distinctly different climatic and edaphic conditions. Despite this, if we take into account the results of Marsudi et al. (31), genospecies B could still be the most widespread genospecies on continental Australia. However, a comparison of full-length sequences would be desirable to provide further confirmation that genospecies B does actually occur in southwestern Australia.

Despite the apparent lower phylogenetic diversity, particularly in comparison to rhizobial communities in Africa, Australia isolates constitute an important source of rhizobial diversity since all but one genospecies that we characterized have not been found elsewhere. Furthermore, insofar as rhizobial communities are concerned, a large part of the continent remains unexplored. This is particularly true of tropical areas, and studies in tropical Africa, South America, and Southeast Asia have previously shown higher diversity (12, 14, 19, 33, 34, 38). In order to evaluate fully the diversity of rhizobia in Australia, it will be necessary to investigate the tropical north portion of the continent, where other species or even genera may occur.

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