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Horizontal Transfer of Symbiosis Genes within and Between Rhizobial Genera: Occurrence and Importance

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1. Introduction

Approximately 70% of the ca. 19,300 species in the Fabaceae (Leguminosae, the legume family) can fix atmospheric nitrogen (N\textsubscript{2}) via symbiotic bacteria (general term ‘rhizobia’) in root nodules [1,2]. Rhizobia reduce atmospheric N\textsubscript{2} to ammonia (NH\textsubscript{3}) through the enzyme nitrogenase, and this NH\textsubscript{3}, as ammonium (NH\textsubscript{4}\textsuperscript{+}), is transported to plant cells where it is assimilated into amino acids via the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway [3,4]. The ability to fix N\textsubscript{2} can give legumes an advantage under low soil nitrogen (N) conditions if other factors are favourable for growth [5,6]. Also, legume N\textsubscript{2} fixation can be a major input of N into a wide range of natural and agricultural ecosystems [7–10].
The most recent classification of the legumes identifies six legume sub-families, namely, the Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae, and Papilionoideae, but only species within the Caesalpinioideae and Papilionoideae nodulate [1]. In this classification, the sub-family Caesalpinioideae includes all members of the former sub-family Mimosoideae (now referred to as the Mimosoid clade), which contains most of the nodulating legumes within the Caesalpinioideae. For most legumes, the nodule formation process is initiated by the legume production of a mix of compounds, mainly flavonoids, which activate nodulation protein D (NodD) in rhizobia by stimulating the binding of NodD to nod gene promoters [11,12]. Different legumes produce different types and mixes of compounds, and this can be a point of legume rhizobium symbiosis specificity [13]. The NodD protein triggers the transcription of a range of genes within the rhizobium, including those required to produce nod factors, the signal molecules from the rhizobium which induce nodule morphogenesis in the legume [14]. These genes include nodABC which encode the enzymes required for the synthesis of the core nod factor structure of an N-acetyl glucosamine oligosaccharide backbone with a fatty acyl chain at the non-reducing end [12]. Nod factors differ in their length of the N-acetylglucosamine oligosaccharide backbone and length and saturation of the fatty acid chain. Other nod genes encode species-specific modifications to the Nod factor structure [12], and, related to this, specific nod genes have been shown to be major determinants of legume host specificity [15,16]. Rhizobia enter the roots of most legume species so far studied via root hair infection [2]. Host cell wall material grows around the developing ‘infection’ forming an infection thread which grows through the root cortex, branching repeatedly. Rhizobia are released from the tips of these infection threads into membrane-bound structures within the legume cells, called symbiosomes, where they differentiate into their N2-fixing form known as bacteroids in root nodules. Bacteroids differ in their level of differentiation and viability, and nodules can be indeterminate or determinate in growth, depending on the legume host [2,17,18]. Indeterminate nodules maintain meristematic activity, while determinate nodules have a transient meristem. All genera examined in the Caesalpinioideae and most tribes within the Papilionoideae had indeterminate nodules, but the Dalbergieae, Desmodieae, Phaseoleae, Psoraleeae, and some members of the Loteae had determinate nodules [2].

The nod genes and the nif genes, which encode the subunits of nitrogenase, are often carried on symbiotic islands or plasmids that can be transferred (horizontal—lateral—transfer) between different bacterial species within and across genera [19–22]. The symbiosis genes involved in horizontal transfer have phylogenies different from those of the core genome of their ‘host’ [17,22]. Here, the literature on legume–rhizobium symbioses in field soils was reviewed, and cases demonstrating incongruence between rhizobium core and symbiosis genes were collated. The occurrence and importance of horizontal transfer of rhizobial symbiosis genes within and between bacterial genera were assessed.

2. Framework and Assumptions of the Study

Rhizobia were aligned with their legume symbionts according to legume sub-family, tribe, genus, and nodule type. Where examined, bacteroids of genera in the Inverted Repeat-Lacking Clade (IRLC, Papilionoideae) were terminally differentiated and could not return to their bacterial form [2,23]. Also, several species in the IRLC were shown to have a high degree of rhizobial specificity related to specific symbiosis genes that have been shown to be transferred between rhizobial species [17], and this clade was considered separately.

The rhizobia genera used in the search were those validated in the International Journal of Systematic and Evolutionary Microbiology. These are the alpha proteobacterial genera in the families Rhizobiaceae (Rhizobium, Ensifer/Sinorhizobium, Allorhizobium, Pararhizobium, Neorhizobium), Bradyrhizobiaceae (Bradyrhizobium), Phyllobacteriaceae (Mesorhizobium, Phyllobacterium), Methylobacteriaceae (Methylobacterium, Microvirga), Brucellaceae (Ochrobactrum), Xanthobacteraceae (Azorhizobium), Hyphomicrobiaceae (Devosia), and the betaproteobacterial genera in the family Burkholderiaceae (Paraburkholderia/Burkholderia, and Cupriavidus) [17].
A comprehensive collation of published cases of phylogenetic incongruence between rhizobium core and symbiosis genes until 30 September 2017 was carried out. Articles were collected by searching the Institute for Scientific Information (ISI) Web of Science, using each legume genus known to nodulate partnered with each of the rhizobia genera, and each of the rhizobia genera partnered with 'horizontal gene transfer' and 'lateral gene transfer' as key words. Further searches were carried out on the literature quoted in the selected papers and on those papers listed as quoting the selected papers in ISI Web of Science. Only data for plants sampled under field conditions, for plants grown in soils taken from the field, or for plants supplied field soil extracts, were used. Bacteria isolated from legume nodules were accepted as rhizobia on the basis of the criteria described previously [17]. All cases of 'authenticated' rhizobia for which core and symbiosis gene sequences were presented were studied further, and the cases for which the authors considered there was incongruence between core and symbiosis gene sequences are discussed. The core and symbiosis genes that showed incongruence are given in Tables. Representative data are presented for Glycine max and Phaseolus vulgaris because of the large number of publications reporting incongruence between core and symbiosis gene sequences for these two species. In some studies, incongruence was tested statistically but, in most cases, this was determined from a visual assessment of phylogenetic trees of the two sets of genes. For example, Mesorhizobium strains isolated from New Zealand endemic Sophora spp. had diverse concatenated (gene sequences aligned head to tail) glinl-recA-rpoB gene sequences but similar concatenated nodA-nodC gene sequences (Figure 1), and, here, the housekeeping and symbiosis genes were considered incongruent [17]. Subsequent statistical analysis of these sequences indicated that the housekeeping and symbiosis genes were incongruent.

**Figure 1.** Comparative maximum likelihood phylogenetic analysis using housekeeping and symbiotic gene clusters from Mesorhizobium strains isolated from New Zealand endemic Sophora spp. Sequence alignment, alignment editing, and phylogenetic analysis were performed using MEGA7 [24]. The phylogenetic trees were built using the GTR model with G + I substitutions for the housekeeping genes (1083 bp) and Tamura 3-parameter model with G + I substitutions for the symbiosis genes (869 bp). The possibility of concatenation was investigated using the partition-homogeneity test with PAUP [25,26]. Each concatenation was investigated for 1000 replicates. All housekeeping genes were congruent with each other (p = 0.015), and both symbiosis genes were congruent with each other (p = 0.02). The housekeeping genes were shown not to be congruent with the symbiosis genes (p = 0.001). Bootstrap values after 500 replicates are expressed as percentages; values less than 50% are not shown. The scale bar indicates the fraction of substitutions per site. M: Mesorhizobium, S: Sophora, FS: Field Site.
3. Lateral Transfer of Symbiosis Genes

3.1. Rhizobia Associated with the Caesalpinioideae

There are reports of phylogenetic incongruence between core and symbiosis genes for Ensifer, Cupriavidus, Burkholderia, Rhizobium, and Devisia associated with legumes in the Mimosoid clade of the sub-family Caesalpinioideae (Table 1).

Table 1. Reported cases of phylogenetic incongruence between core and symbiosis genes for rhizobia associated with legumes in the sub-family Caesalpinioideae. All species have indeterminate nodules.

| Caesalpinioideae Mimosoid Clade | Rhizobia |
|---------------------------------|---------|
| Acaciella angustissima | Ensifer chiapanecum ITTG S70T and Ensifer mexicanum ITTG R7T had different gyrA, nodR, recA, rpoB, and rrs gene sequences but similar nifH and nodA sequences [27] |
| Leucaena leucocephala | Ensifer isolates formed three clades in both 16S rRNA and recA phylogenetic trees but only one clade in both nifH and nodC trees [28] |
| Mimosa caerulea, Mimosa nagentea, Mimosa ramulosa, Mimosa reptans, Mimosa schleidenii | Cupriavidus isolates separated into two groups on 16S rRNA, recA and gyrB sequences but grouped together on nifH and nodA sequences [29] |
| Mimosa diplotricha | Burkholderia caribensis TS182 characterized on 16S rRNA sequence group with Cupriavidus strains on nodA sequence [30] |
| M. diplotricha, Mimosa pudica | Burkholderia strains with diverse 16S rRNA gene sequences grouped together along with B. phymatum STM815T on nodA sequence [31] |
| M. pudica | Rhizobium altiplani BR 10423T had nifH and nodC sequences closely related (identical for nodC) to those of Rhizobium mesosamericanum CCGE 501T [32] |
| Neptunia natans | Devisia isolates characterized on 16S rRNA sequences had nifH and nodD sequences closely related to those of Rhizobium tropici CIAT899T [33] |
| Vachellia jacquemontii | Ensifer showed incongruence across all three of concatenated rrs-gthII-atpD-recA-duaK, nifH, and nodA gene sequences [34] |
| Vachellia macracantha | Ensifer sequences for nifH and nodC were incongruent with those for 16S rRNA [35] |
| Vachellia seyal, Vachellia tortilis | Ensifer isolates separated into seven groups on the basis of 16S rRNA, recA, gyrB, rpoB, atpD, gap and ynp gene sequences but were closely related with respect to their nodA and nodC gene sequences [36]. |

Five separate studies indicated that horizontal transfer of symbiosis genes had occurred between Ensifer spp. associated with species in the Caesalpinioideae [27,28,34–36]. For Ensifer associated with the Mexican native Leucaena leucocephala sampled in Panxi, China, gene sequences indicated that symbiotic genes of strains associated with introduced plants were transferred into indigenous strains in the soil [28]. Ensifer isolates from Vachellia macracantha sampled within the plant’s native range in Peru showed nifH and nodC sequences closely related to other American rhizobial strains, which adds support to the use of symbiotic genes as valuable indicators of geographical origin [35]. Gene sequences indicated diverse origins for the housekeeping genes nifH and nodA for seven Ensifer isolates representative of 73 isolates from Vachellia jacquemontii sampled within its native range in the Thar Desert of India [34]. The authors suggested that the stressful desert conditions, and stressful conditions in general, may favour frequent horizontal gene transfer. Alternatively, rather than promote its occurrence, stressful environments may represent a situation where the positive consequences of horizontal gene transfer in terms of natural selection are more significant, and thus horizontal gene transfer becomes more apparent within the rhizobial population.

Ten Cupriavidus rhizobia strains isolated from five Mimosa spp. in southern Uruguay showed symbioses and housekeeping gene sequence phylogenies that were not congruent [29]. The strains separated into two groups of five strains on their 16S rRNA sequences, and one strain selected from each of these groups differed substantially in its recA and gyrB sequences. However, both the nodA and nifH sequences for the ten strains grouped together in a cluster. Also, Rhizobium altiplani Br 10423T
isolated from *Mimosa pudica* in Distrito Federal in central Brazil had *nifH* and *nodC* sequences closely related (identical for *nodC*) to those of *Rhizobium mesoamericanum* CCGE 501\(^T\) [32].

*Mimosa* is a genus of ca. 550 species native to the Americas, South Asia, and Africa including Madagascar [1,2]. The evidence indicates that *Cupriavidus* is the main rhizobial symbiont of endemic *Mimosa* in southern Uruguay, but *Burkholderia* and *Rhizobium/Ensifer* are the main symbionts of *Mimosa* in central and southern Brazil and central Mexico, respectively [29,37–39]. In contrast with findings for *Mimosa Cupriavidus* symbionts in Uruguay, the symbiosis gene sequences for *Burkholderia* in Brazil and *Rhizobium/Ensifer* in Mexico were largely congruent with their respective 16S rRNA and housekeeping gene sequences [37,38]. This indicates that these symbiosis genes diverged over a long period within *Burkholderia* and *Rhizobium/Ensifer* without substantial horizontal transfer between species. Similarly, it was concluded that *nodC* and *nifH* sequences for *Burkholderia* isolated from the Piptadenia group (Piptadenia, Parapiptadenia, Pseudopiptadenia, Pityrocarpa, Anadenanthera, and Microlobius) (Mimoseae) have evolved mainly through vertical transfer, with rare occurrence of horizontal transfer [40]. Outside South America, *Burkholderia* strains isolated from *Mimosa diplotricha* in Yunan province in subtropical China showed diverse 16S rRNA sequences but grouped together, along with *Burkholderia phymatum* STM815\(^T\), on *nodA* sequences [31]. Also, *Burkholderia caribensis* TJ182 isolated from the invasive *M. diplotricha* in Taiwan and characterized on 16S rRNA sequence, grouped with *Cupriavidus* strains on *nodA* sequence, indicating that the *nodA* gene had been transferred from *Cupriavidus* to *Burkholderia* [30]. However, this transfer was not confirmed [41]. In both studies, it was concluded that it was likely that the *Burkholderia* ‘travelled’ with their invasive hosts from South America to South East Asia [30,31].

A more extreme case of incongruence between core and symbiosis genes was reported from India for *Devosia natans* isolated from the aquatic ‘water mimosa’ *Neptunia natans*. Here, the 16S rDNA sequences indicated that two strains isolated from *N. natans* were *Devosia*, but the *nifH* and *nodD* sequences were most closely related to those of *Rhizobium tropici* CIA T899\(^T\) [33]. This finding indicates that the horizontal transfer of symbiosis genes has occurred across genera from *Rhizobium* to *Devosia* at some stage.

### 3.2. Rhizobia Associated with the Papilionoideae

#### 3.2.1. The Inverted Repeat-Lacking Clade (IRLC)

*Ensifer, Mesorhizobium, and Rhizobium* are the main rhizobial symbionts of legumes in the IRLC, and there are several examples of phylogenetic incongruence between core and symbiosis genes for *Mesorhizobium* and *Rhizobium* associated with IRLC species (Table 2).

**Table 2.** Reported cases of phylogenetic incongruence between core and symbiosis genes for rhizobia associated with legumes in the inverted repeat-lacking clade (IRLC) of the legume sub-family Papilionoideae. All species have indeterminate nodules.

| Papilionoideae Tribes and Genera | Rhizobia |
|---------------------------------|---------------------------------|
| **Cicereae**                    | Mesorhizobium ciceri, *Mesorhizobium mediterraneum*, *Mesorhizobium muluense* and *Mesorhizobium* spp. with diverse 16S rDNA, *recA*, *atpD*, *glnII* and *gyrB* sequences had similar *nifH*, *nodA* and *nodC* sequences [42–47] |
| *Cicer arietinum*               | *Mesorhizobium* with diverse 16S rRNA, *recA* and *glnII* sequences had similar *nodC* gene sequences [48] |
| *Cicer canariense*             | Mesorhizobium with diverse 16S rDNA, *recA* and *glnII* sequences had similar *nodC* gene sequences [48] |
Table 2. Cont.

| Papilionoideae Tribes and Genera | Rhizobia |
|---------------------------------|----------|
| **Fabeae**                      |          |
| Lathyrus spp., Lens culinaris, Pisum sativum and Vicia spp. | Rhizobium fabeae, Rhizobium pisi, Rhizobium laguerreae, Rhizobium anhuiense, Rhizobium bangladeshense, Rhizobium binae, Rhizobium lentsis and Rhizobium spp. with diverse 16S rRNA and recA, atpD and glnII sequences had similar nifH, nodA and nodC sequences [49–54] |
| Lathyrus odoratus, Vicia hirsuta | Rhizobium multihospitium isolates had nifH and nodD sequences 100% similar to those of Rhizobium lusitanum P1–7T and Devosia neptuniae J1T [55] |
| **Galegeae**                    |          |
| Astragalus aksuensis, Astragalus sp., Oxytropis glabra, Oxytropis meinshauseni | R. multihospitium isolates had nifH and nodD sequences 100% similar to those of R. lusitanum P1–7T and D. neptuniae J1T [55] |
| Astragalus glycyphyllos          | Mesorhizobium isolates showing 16S rRNA sequences similar to M. ciceri, Mesorhizobium amorphae or Mesorhizobium septentrionale formed one clearly separated, closely related cluster for nodA, nodC, nifH and nod sequences [56] |
| Glycyrrhiza uralensis            | Mesorhizobium concatenated rrs-recA-rpoB, nifH, nodA and nodC sequences were not congruent [57] |
| Sphaerophysa salsula            | Mesorhizobium with diverse 16S rRNA sequences showed similar nifH sequences [58] | Mesorhizobium and Rhizobium identified on 16S rRNA sequences showed similar nifH sequences [58] |
| **Hedysareae**                  |          |
| Alhagi sparsifolia              | Mesorhizobium isolates separated into three groups on the basis of their rrs, dnaK and dnaJ sequences but their nodA and nodC sequences were closely related [59] |
| Alhagi sp., Caragana jubata, Halimodendron halodendron, | R. multihospitium isolates had nifH and nodD sequences 100% similar to those of R. lusitanum P1–7T and D. neptuniae J1T [55] |
| Caragana bicolor, Caragana erinacea, Caragana franchetiana, Caragana intermedia | Mesorhizobium isolates with diverse 16S–23S IGS 16S rRNA sequences and one Bradyrhizobium isolate (16S rRNA) from C. intermedia had similar nodC sequences [60] |
| Trifolium                       |          |
| Trifolium repens                | R. pisi sv. trifolii K3.22 characterised on the basis of 16S rRNA, atpD, dnaK, glnA, gyrB, recA and rpoB sequences had nodA, nodB, nodC and nodD sequences with high similarity to those of Rhizobium leguminosarum sv. trifolii [61]. Rhizobium spp. with diverse 16S rRNA and concatenated atpD-recA-glnII sequences had similar nifH and nodD sequences [62] |

Incongruence between housekeeping and symbiosis gene sequences indicates that within-genus horizontal transfer of symbiosis genes has occurred between Mesorhizobium strains associated with Cicer arietinum [42–47], Cicer canariense [48], Astragalus glycyphyllos [56], Glycyrrhiza uralensis [57], Sphaerophysa salsula [58], Alhagi sparsifolia [59], and five Caragana spp. [60], and between Rhizobium strains associated with Fabeae spp. [49–54], and Trifolium spp. [61,62] (Table 2). Considering the crop plants, evidence is strong that indigenous Mesorhizobium muleiense in Northwest China obtained its C. arietinum (chickpea) specific symbiotic genes from Mesorhizobium ciceri or Mesorhizobium mediterraneum associated with imported C. arietinum used as a crop [46]. Similarly, 83 isolates from Trifolium repens (white clover) grown in alkaline soils in subtropical China, identified as Rhizobium anhuiense, Rhizobium leguminosarum, and a novel Rhizobium genospecies on 16S rRNA and housekeeping
gene sequences, had nifH and nodC sequences similar to R. leguminosarum sv. trifolii introduced with the crop. This indicates that the symbiosis genes had been transferred from the R. leguminosarum sv. trifolii strain to the native soil bacteria [62].

In Xinjiang, China, isolates of Rhizobium multihospitium were obtained from a number of plant species from different tribes: Lathyrus odorata, Vicia hirsula (Fabaceae), Astragalus aksuensis, Astragalus sp., Oxytropis glabra, Oxytropis meinshausenii (Galegaeae), Alhagi sp., Caragana jubata, Halimodendron halodendron (Hedysareae) (Table 2); Robinia pseudoacacia (Robineae), Sophra alopecuroides (Sophoreae) (Table 3); Lotus frondosus and Lotus tenuis (Loteae) (Table 4). The nifH and nodD sequences of these isolates were 100% similar to those of Rhizobium lusitanum P1–7T and D. neptuniae J1T [55], and it was suggested that nifH and nodD genes of the three rhizobial species may have the same origin. Also, isolates from S. salsula identified as Rhizobium genotypes on 16S rRNA gene sequences showed similar nifH sequences to those of the Mesorhizobium isolates, while a Bradyrhizobium isolate (16S rRNA) from Caragana intermedia had a similar nodC sequence to the Mesorhizobium isolates [58,60].

Finally, within the IRLC, the pasture legume Biserrula pelecinus was introduced into western Australia from the Mediterranean region in 1994 and, as indigenous rhizobial populations in western Australia do not nodulate this legume, the seed was inoculated with M. ciceri sv biserrulac strain WSM1271 [63,64]. In 2000, Mesorhizobium strains, including WSM2073 and WSM2075 with 16S rRNA, dnaK and GST1 phylogenies different from strain WSM1271, were isolated from nodules of B. pelecinus grown in Western Australia and shown to nodulate the legume, although the bacteria were largely ineffective with regard to N₂ fixation [63,64]. Where tested, these strains had identical sequences for the symbiosis insertion regions with WSM1271, indicating that they had obtained their symbiosis genes via horizontal transfer of a symbiosis island from the inoculant within the space of six years. This quick transfer in the field was not a single event, as it was also demonstrated for commercially grown B. pelecinus inoculated with WSM1497 and resulted in three different Mesorhizobium lineages with identical symbiosis genes to the inoculant [64]. The ability of rhizobial strains that do not fix N₂ to produce nodules on B. pelecinus could result in decreased yield of the crop.

3.2.2. Papilionoideae with Indeterminate Nodules Excluding the IRLC

Phylogenetic incongruence occurs between core and symbiosis genes for Azorhizobium, Bradyrhizobium, Burkholderia, Ensifer, Mesorhizobium, Methylobacterium, Microvirga, Neorhizobium, Ochrobactrum, Rhizobium, and Phyllobacterium associated with Papilionoideae legumes with indeterminate nodules excluding the IRLC (Table 3).

| Papilionoideae Tribes (Genera) | Rhizobia |
|-------------------------------|---------|
| Crotalarieae                   |         |
| Aspalathus sp.                 | Rhizobium isolate characterized on 16S rRNA and concatenated recA-atpD-gyrB-glnA sequences had nifH and concatenated nodA-B-C sequences closely related to those of Mesorhizobium [65] |
| Aspalathus astroites, Aspalathus aurantiaca, Aspalathus bracteata, Aspalathus ciliaris, Aspalathus cordata, Aspalathus erieofolia, Aspalathus spicata | Mesorhizobium phylogenetic relationships between concatenated recA-atpD-gyrB-glnA and nodA-B-C sequences were incongruent [65] |
| Crotalaria podocarpa           | Methylobacterium nodulans ORS2060T nodA sequence groups with nodA sequences for Bradyrhizobium spp. [66] |
| Listia angolensis              | Microvirga lotonialis WSM3557T and Microvirga zambiensis WSM3693T nodA sequences were identical and clustered with Bradyrhizobium, Burkholderia and Methylobacterium nodA sequences [67] |
Table 3. Cont.

| Papilionoideae Tribes (Genera) | Rhizobia |
|--------------------------------|----------|
| **Genisteae** | | |
| Tephrosia purpurea | Burkholderia isolate characterized on concatenated 16S rRNA-recA-atpD sequences had a nifH sequence closely related to those of Ensifer spp. [68] |
| Tribes (Genera) Rhizobia | |
| **Cytisus** | | |
| Cytisus proliferus | Bradyrhizobium with diverse 16S–23S rRNA, atpD, glnII and recA sequences showed similar nifH and nodC sequences [69] |
| Cytisus scoparius | Bradyrhizobium 16S rRNA, 23S rRNA, dnaK, gyrB, rplC, rpoB, nifD, nifH, and nodC sequences indicated a highly heterogeneous ancestry [70] |
| Cytisus villosus | Bradyrhizobium with diverse 16S rRNA and concatenated glnII-recA sequences showed similar nifH and nodC sequences [71] |
| **Genista versicolor** | Bradyrhizobium with diverse 16S–23S ITS and atpD sequences showed similar nifH and nodC sequences for almost all strains [72] |
| **Lupinus** | | |
| Lupinus albus, Lupinus angustifolius, Lupinus luteus, Lupinus sp. | Bradyrhizobium with diverse 16S–23S ITS and 16S rRNA sequences clustered together on nodC sequences [73] |
| L. albus, L. angustifolius, L. luteus | | |
| **L. albus** | Bradyrhizobium with diverse concatenated atpD-glnII-recA sequences clustered together on nodA sequences [75] |
| **Lupinus honoratus** | Ochrobactrum lupini LUP21 \(^1\) nifH sequence showed 99.6% similarity to M. ciceri strains; its nodD sequence showed 86.4% similarity to *Rhizobium etli* CFN42 \(^1\) [76] |
| **Lupinus mariae-josephae** | Bradyrhizobium with diverse concatenated atpD-glnII-recA sequences separated into two distinct clusters on nodA and nodC sequences [77] |
| **Lupinus micranthus** | Bradyrhizobium with diverse concatenated 16S rRNA and concatenated atpD-glnII-recA sequences showed similar nodC gene sequences [78] |
| **Lupinus texensis** | Microvirga lupini Lut6 \(^1\) concatenated nifD-nifH sequence aligned close to *R. etli* CFN42 \(^1\); its nodA sequence was placed in a clade that contained strains of *Rhizobium, Mesorhizobium* and *Ensifer* [67] |
| **Hypocalpytus** | | |
| Hypocalpytus sophoroides, Hypocalpytus oxalidifolius, Hypocalpytus colutoides | Burkholderia phylogenies inferred from nifH and nodA sequences were incongruent; *Burkholderia* phylogenies inferred from nifH and nodA sequences were incongruent with those from 16S rRNA and recA sequences [79] |
| **Loteae** | | |
| Coronilla varia | Mesorhizobium phylogenies for 16S rRNA, nifH and nodC sequences were incongruent [80] |
| **Ornithopus** | | |
| Ornithopus compressus, Ornithopus sativus | Bradyrhizobium with diverse 16S–23S rRNA ITS and dnaK, atpD, glnII and recA sequences clustered together on nodA, nodZ and nodL sequences [73,81] |
| **Millettieae** | | |
| Tephrosia falciformis, Tephrosia leptostachya, Tephrosia purpurea, Tephrosia villosa, Tephrosia wallichii | Ensifer 16S rRNA and concatenated recA-atpD-glnII-dnaK sequences grouped with *Ensifer saheli* LMG 7837 \(^1\) and *Ensifer kotiensis* LMG 19225 \(^1\) but nifH, nodA and nodC sequences clustered with *Ensifer fredii* USDA 205 \(^1\) [62] |
| **Podalyrieae** | | |
| Cyclopia buxifolia, Cyclopia germinoides, Cyclopia glabra, Cyclopia intermedia, Cyclopia longifolia, Cyclopia maculata, Cyclopia meyeriana, Cyclopia pubescens, Cyclopia sessiflora, Cyclopia subtomenta | Burkholderia phylogenies inferred from nifH and nodA sequences were incongruent; phylogenies inferred from nifH and nodA sequences were incongruent with those from 16S rRNA and recA sequences [79] |
Rhizobia phylogenetic relationships between concatenated nodA-atpD-gyrB-glnII sequences were largely incongruent [86] and nodA-B-C sequences were largely incongruent with respect to their phylogenetic relationships between concatenated nodA sequences and nodA sequences were incongruent with those from 16S rRNA and nodC sequences [79].

**Table 3. Cont.**

| Papilionoideae Tribes (Genera) | Rhizobia |
|-------------------------------|----------|
| **Podalyria calyptrata**       | Burkhodaria phylogenetic relationships between concatenated recA-atpD-gyrB-glnA and nodA-B-C sequences were largely incongruent [86]. Burkhodaria phylogenies inferred from nifH and nodA sequences were incongruent; phylogenies inferred from nifH and nodA sequences were incongruent with those from 16S rRNA and recA sequences [83]. |
| **Virgilia oroboides**         | Burkhodaria phylogenies inferred from nifH and nodA sequences were incongruent; phylogenies inferred from nifH and nodA sequences were incongruent with those from 16S rRNA and recA sequences [79]. |
| **Robineae**                   |          |
| *R. pseudocacia*               | *R. multihospitium* isolates had nifH and nodD sequences 100% similar to those of *R. lusitanum* P1–7 and *D. neptuniae* J1 [55]. |
| **Sesbanieae**                 | Rhizobium strain IRBG74 characterised on concatenated 16S rRNA–rpoB-fusA sequence harboured a sym-plasmid containing nifH and nodA genes similar to those of Ensifer strains that nodulate this legume [21]. Rhizobium/Agrobacterium and Ensifer characterized on concatenated recA-atpD-glnII sequences had similar Ensifer nifH and nodA sequences [84]. |
| *Sesbania cannabina*           |            |
| *Sesbania sesban*              | Ensifer isolates separated into three groups on the basis of concatenated 16S rRNA-recA-gyrB-rpoB-atpD-gap-pnp sequences but were closely related with respect to their nifH and nodC sequences [36]. |
| **Sophoreae**                  |            |
| *Sophora alopecuroides*        | *R. multihospitium* isolates had nifH and nodD sequences 100% similar to those of *R. lusitanum* P1–7 and *D. neptuniae* J1 [55]. |
| *Sophora chathamica*, *Sophora fulcida*, *Sophora godleyi*, *Sophora longicarinata*, *Sophora microphylla*, *Sophora prostrata*, *Sophora tetrapetra* | Mesorhizobium with diverse concatenated recA-glnII-rpoB sequences had similar nifH, nodA and nodC sequences [85, 86]. |
| *Sophora flavesens*            | Rhizobium mongolense isolate characterized on concatenated atpD-glnII-recA sequences had nodC sequence similar to isolates characterized as *M. septentrionale* [87]. *E. fredii* isolate characterized on concatenated atpD-glnII-recA sequences had nodC sequence identical to *Mesorhizobium temperatum* [87]. Phyllobacterium sophorae isolate characterized on concatenated atpD-glnII-recA sequences had nodC sequence closely related to *M. septentrionale* [87]. Mesorhizobium and Rhizobium phylogenetic relationships between concatenated atpD-glnII-recA and nodC sequences were incongruent [87]. |
| **Thermopsideae**              | Ensifer arboris and Neorhizobium galegae characterized on 16S rRNA and concatenated recA-atpD-rpoB-thrC sequences aligned with *Ensifer meliloti* ATCC9930 on nifH and nodC sequences [88]. Phyllobacterium giardinii characterized on 16S rRNA and concatenated recA-atpD-rpoB-thrC sequences aligned with *R. leguminosarum* sv. *viciae* USDA 2370 on nifH and nodC sequences [88]. Rhizobium/Agrobacterium radiobacter characterized on 16S rRNA and concatenated recA-atpD-rpoB-thrC sequences aligned with *E. fredii* USDA205 on nifH and nodC sequences [88]. |
| *Ammopiptanthus nanus*, *Ammopiptanthus mongolicus* | Marhemizobium isolates with diverse 16S–23S rDNA ITS, 16s rRNA and glnII sequences had identical nodC sequences closely related to Mesorhizobium tianshanense USDA 3592 [89]. |

Four studies carried out on species within the Crotalarieae found evidence of horizontal transfer of symbiosis genes between different genera of rhizobia. *Rhizobium* isolated from Aspalathus sp.
grown in the Cape Fynbos biome in South Africa and characterized on 16S rRNA and housekeeping gene sequences had nifH and nodA,B,C sequences closely related to those of *Mesorhizobium* [65]. *Methylobacterium nodulans ORS2060* isolated from *Crotalaria podocarpa* in Senegal, grouped with *Bradyrhizobium* spp. on *nodA* sequences [66]. *Microvirga lotonidis* WSM3557 and *Microvirga zambiensis* WSM3693 isolated from *Listia angolensis* in Zambia had identical *nodA* sequences which clustered with strains of *Bradyrhizobium*, *Burkholderia*, and *Methylobacterium* [67]. *Burkholderia*, isolated from *Rafnia triflora* in the Core Cape subregion of South Africa and characterized on 16S rRNA and housekeeping gene sequences, had a nifH sequence closely related to those of *Ensifer* spp. [68].

Lemaire and co-workers specifically assessed the degree of horizontal transfer of nodulation genes within rhizobia genera of a range of legumes endemic to the Cape Fynbos biome in South Africa [65]. It was concluded that *Mesorhizobium* strains isolated from *Aspalathus* spp., *Argyrolobium* spp. (*Genistae, Table 3*), *Otholobium* spp., and *Psoralea* spp. (*Psoraleeae, Table 4*), and *Burkholderia* isolated from *Podalyria calytrata* (*Table 3*) show high degrees of horizontal transfer of nodulation genes among closely related species. In associated studies, a *Mesorhizobium* isolate from *Psoralea* sp., characterized on 16S rRNA and housekeeping gene sequences, aligned closely to *Ensifer* on *nifH* sequence, and a *Mesorhizobium* isolate from *Psoralea oligophylla* aligned closely to *Burkholderia* on *nodA* sequence (*Table 4*) [68]. Also, for *Burkholderia* isolated from *P. calytrata*, different branching patterns were found among numerous isolates for *recA* and *nodA* phylogenies [83]. In a separate study of *Burkholderia* isolates from *Hypocalyptus* spp. (*Hypocalyptaeae*) and *Cyclopia* spp., *P. calytrata* and *Virgilia oroboides* (*Podalyrieae*) sampled in the Cape Floristic Region of South Africa, phylogenies inferred from *nifH* and *nodA* sequences were incongruent and, generally, phylogenies inferred from *nifH* and *nodA* sequences were incongruent with those from 16S rRNA and *recA* sequences [79]. These findings confirm that horizontal transfer of symbiosis genes is common in South African *Burkholderia*, which is in contrast with findings for South American *Burkholderia* rhizobial symbionts [37,40].

There is also strong evidence that horizontal transfer of symbiosis genes has occurred between different *Bradyrhizobium* spp. associated with *Cytisus* spp. [69–71], *Genista versicolor* [72] and *Lupinus* spp. [73–75,77,78] in the tribe *Genistae* (*Table 3*). In particular, most *Bradyrhizobium* isolates from native *Lupinus* spp. (and native *Genistae* species in general [90]) in Europe form a distinct lineage, ‘Clade II’, on the basis of their *nodA* gene sequences [75,81]. Also, *Bradyrhizobium* isolates from native *Cytisus villosus* in Morocco had diverse 16S rRNA and housekeeping gene sequences, but all showed similar *nifH* and *nodC* sequences which were closely related to those of *Bradyrhizobium japonicum* sv. *genisteearum* [71]. These findings indicate that horizontal transfer of symbiosis genes has played a role in the development of specific relationships between *Genistae* spp. and *Bradyrhizobium* spp. over wide areas. *Bradyrhizobium* isolated from invasive *Cytisus scoparius* in the United States had housekeeping genes similar to indigenous *Bradyrhizobium*, but their *nodC*, *nifD*, and *nifH* sequences were highly similar or identical to those of a *Bradyrhizobium* strain from Spain [70]. It appears, therefore, that indigenous North American *Bradyrhizobium* had acquired symbiosis genes from *Bradyrhizobium* symbionts of European *C. scoparius* via horizontal gene transfer.

*Microvirga* and *Ochrobactrum*, which are rare as rhizobial symbionts, can nodulate specific *Lupinus* spp. [17,67,76]. The *nifD* and *nifH* sequence for *Microvirga lupini* *Lut6* isolated from *Lupinus texensis* aligned closely to *Rhizobium etli* CFN 42T, while its *nodA* sequence was placed in a clade that contained strains of *Rhizobium, Mesorhizobium, and Ensifer* [67]. The *nifH* sequence for *Ochrobactrum lupini* LUP21 isolated from *Lupinus honoratus* in Argentina showed 99.6% similarity to two *Mesorhizobium* strains, while its *nodD* sequence showed 86.4% similarity to *R. etli* CFN 42T [76]. Thus, evidence is strong that *M. lupini* *Lut6* and *O. lupini* LUP21T obtained their symbiosis genes from other, more common rhizobial genera.

Incongruence between housekeeping and symbiosis genes indicate that horizontal gene transfer of symbiosis genes has occurred between *Mesorhizobium* strains from *Coronilla varia* (Loteae) grown in Shaanxi province, China [80], *Bradyrhizobium* isolates from *Ornithopus* spp. (Loteae) sampled in Europe and western Australia [75,81], and *Ensifer* strains from *Tephrosia* spp. (Millettiaeae) in the
Indian Thar Desert [82]. New Zealand endemic *Sophora* spp. are nodulated by diverse *Mesorhizobium* spp. with similar symbiosis genes (Figure 1) [85,86,91,92]. Generally, *Mesorhizobium* isolates from the same field site grouped together on housekeeping gene sequences [85,86]. This apparent link between housekeeping gene sequences and field site in association with almost identical symbiosis genes is consistent with the proposal that horizontal transfer of symbiosis genes to *Mesorhizobium* strains adapted to local soil conditions has occurred, but this requires further testing. The relationship between New Zealand endemic *Sophora* species and *Mesorhizobium* with particular symbiosis genes is highly specific and contrasts with findings for *Sophora alopeucoideae* and *Sophora flavescens* sampled in China, which are nodulated by *Ensifer*, *Mesorhizobium*, *Phyllobacterium*, and *Rhizobium* with a wide range of *nodC* and *nifH* gene sequences [87,93]. The data indicate that symbiosis genes of rhizobia associated with *S. alopeucoideae* and *S. flavescens* are primarily maintained by vertical transfer, but there is also evidence for occasional horizontal gene transfer of symbiosis genes within and between rhizobia genera associated with these legume species [87,93].

Horizontal transfer of symbiosis genes has occurred across genera in the case of *Agrobacterium* sp. strain IRBG74 originally isolated from the aquatic legume *Sesbania cannabina* and subsequently shown to effectively nodulate *Sesbania sesban* and seven other *Sesbania* species [21]. Housekeeping gene sequences identified strain IRBG74 as a close relative of the plant pathogen *Agrobacterium radiobacter (=*Agrobacterium tumefaciens*). However, it did not contain *vir* genes but harboured a sym-plasmid containing *nifH* and *nodA* genes with sequences similar to those of *Ensifer* spp. which nodulate *S. cannabina*. In a separate study, *Rhizobium*/*Agrobacterium* and *Ensifer* isolates from *S. cannabina* with diverse housekeeping gene sequences had similar *nifH* and *nodA* sequences, and one *Rhizobium*/*Agrobacterium* strain showed highly similar *nifH* and *nodA* sequences to strain IRBG74 [84].

Within the Thermopsideae, *Ammopiptanthus nanus* and *Ammopiptanthus mongolicus* sampled across nine sites in three regions of China were nodulated by *Ensifer*, *Neorhizobium*, *Pararhizobium*, and *Rhizobium* [88]. For strains characterized to species level on 16S rRNA and housekeeping gene sequences, *Ensifer arboris* and *Neorhizobium galegaeae* strains aligned with *Ensifer meliloti* ATCC9930T on *nifH* and *nodC* gene sequences, *Phyllobacterium giardinii* strains aligned with *R. leguminosarum* sv. *viciae* USDA 2370T on *nifH* and *nodC* gene sequences, and *R. /A. radiobacter* strains aligned with *E. fredii* USDA 205T on *nifH* and *nodC* gene sequences [88]. For *Anagyris latifolia* grown in soil samples collected from within natural populations of the legume growing in the Canary Islands, *Mesorhizobium* isolates with diverse 16S–23S rDNA ITS, 16S rRNA and *gltII* gene sequences had identical *nodC* sequences closely related to *Mesorhizobium tianshanense* USDA 3592T [89].

3.2.3. Papilionoideae with Determinate Nodules

Phylogenetic incongruence between core and symbiosis genes has been described for *Bradyrhizobium*, *Ensifer*, *Mesorhizobium*, *Microvirga*, *Pararhizobium*, *Phyllobacterium*, and *Rhizobium* associated with Papilionoideae legumes with determinate nodules (Table 4).

**Table 4.** Legume–rhizobia symbioses of species in the sub-family Papilionoideae with determinate nodules.

| Papilionoideae Tribes and Genera | Rhizobia |
|---------------------------------|---------|
| **Dalbergieae**                 | **Bradyrhizobium guangdongense** CCBAU 51640T, **Bradyrhizobium guangxiense** CCBAU 53363T, **Bradyrhizobium sp.** P1237 and **Bradyrhizobium sp.** CH81 had identical *nodA* sequences [94]; **Bradyrhizobium** with diverse 16S–23S rRNA ITS and concatenated *atpD-recA* sequences showed similar *nodA* sequences [95] |
| **Arachis hypogaea**            | **Rhizobium** characterized on 16S rRNA and concatenated *recA-atpD-gltII* sequences aligned with *Ensifer* sp. on *nodC* sequences [96] |
| **Desmodieae**                  | **Rhizobium and Pararhizobium** characterized on 16S rRNA and concatenated *recA-atpD-gltII* sequences aligned with *Ensifer* sp. on *nodC* sequences [96] |
Table 4. Cont.

| Papilionoideae Tribes and Genera | Rhizobia |
|----------------------------------|----------|
| **Phaseoleae**                   | **Bradyrhizobium** strains with clearly separated 165 rRNA sequences showed identical or similar nifH, nodC and nodY-nodA sequences [97]; **Ensifer** strain characterized on 165 rRNA sequence showed similar nifH, nodC and nodY-nodA sequences to B. japonicum [97]; *Bradyrhizobium* with diverse 16S rRNA and concatenated recA-glnI-atpD sequences showed identical nifH and nodC sequences [98]; **Ensifer** sojae CCBAU 05684T and E. fedii USDA 205T showed identical nodC sequences [99]; **Bradyrhizobium daqingense** CCBAU 15774T, **Bradyrhizobium laonginense** USDA 3622T and B. japonicum USDA 6T showed identical nifH and nodC sequences [100]; **Ensifer** isolate classified on 165 rRNA sequence showed 99% similarity to **Bradyrhizobium guanxingense** in nodD1 and nifD sequences [101]. |
| **Glycine max**                  | **Ensifer** and **Rhizobium** with diverse 165 rRNA and concatenated recA-atpD-glnI sequences formed a single **Ensifer** lineage on nifH and nodA sequences [102]. |
| **Glycine soja**                 | **R. etli**, **Rhizobium gallicum**, R. leguminosarum sv. phaseoli and **Pararhizobium gardnii** characterized on 165 rRNA sequences had similar nodC sequences and a strain characterized as **Rhizobium** aligned with E. meliloti on nodC sequence [103]; R. lusitanum P1–7T had nifH and nodC sequences similar to D. neptuniae LMG 21357T and R. tropici CIAT 899T [104]; R. etli and R. leguminosarum characterized on 165 rRNA sequences showed similar nifH and nodC sequences to R. etli CFN 42T [105]; **Rhizobium** with diverse 165 rRNA and concatenated atpD-glnI-recA sequences clustered together on nifH and nodC sequences [106]; **Pararhizobium gardnii** characterized on 165 rRNA and concatenated recA-glnII-atpD sequences aligned with Ensifer on nodC sequence [107]. |
| **Phaseolus vulgaris**           | **Rhizobium** characterized on 165 rRNA and concatenated atpD-recA sequences had a nodC sequence similar to **Ensifer** strains [108]. |
| **Vigna angularis**             | **Bradyrhizobium** characterized on sequences of the 16S rRNA, nodD1 and nifD genes and the ITS region aligned with **Ensifer** on nodA sequences [109]. |
| **Vigna radiata**               | **Bradyrhizobium** with diverse concatenated rrs-recA-glnI sequences showed similar nodA sequences [110]; *Mesorhizobium vignae* BR3299T aligned with *Mesorhizobium* and **Rhizobium** on nifH sequence and *Microvirga lotononis*, M. zamhensis, **Bradyrhizobium**, *Burkholderia* and *Methylobacterium* on nodA sequences [111]; E. *fedii* characterized on sequences of 165 rRNA, concatenated recA-glnI-gyrB-truA-thrA-SMc00019 and IGS were substantially diverged from E. *fedii* on nifH, nodC and *rhrST*-1 sequences [112]. |
| **Vigna unguiculata**           | **Mesorhizobium** phylogenetic relationships between concatenated recA-atpD-gyrB-glnA and nodA-B-C sequences were incongruent [65]. |
| **Psoraleae**                   | **Mesorhizobium** phylogenetic relationships between concatenated recA-atpD-gyrB-glnA and nodA-B-C sequences were incongruent [65]. |
| **Otholobium bracteolatum**, Otholobium hirtum, Otholobium virgatum, Otholobium zeyheri** | **Mesorhizobium** isolate characterized on concatenated 165 rRNA-recA-atpD sequence aligned closely to *Burkholderia* on nodA sequence [68]. |
| *Psoralea asarina*, *Psoralea congesta*, *Psoralea laxa*, *Psoralea rigidula** | **Mesorhizobium** isolate characterized on concatenated 165 rRNA-recA-atpD sequence aligned closely to **Ensifer** on nifH sequence [68]. |
| *Psoralea* sp.                  | **Mesorhizobium** with diverse 16S rRNA, atpD and recA sequences clustered together on nodC gene sequences [113,114]. |
| **Loteae**                      | **Transfer of symbiotic island between** **Mesorhizobium loti** inoculum and indigenous **Mesorhizobium** strains [19,20]; **Rhizobium multispinatum** isolates had nifH and nodD sequences 100% similar to those of *R. lusitanum* P1–7T and D. neptuniae J11 [59]. |
| *Lotus berthelotii*, *Lotus callis-viridis*, *Lotus corniculatus*, *Lotus campestris*, *Lotus purpureus*, *Lotus scoposus*, *L. tenus** | **Mesorhizobium** with diverse 16S rRNA, atpD and recA sequences clustered together on nodC gene sequences [113,114]. |
| *L. corniculatus**              | **Transfer of symbiotic island between** **Mesorhizobium loti** inoculum and indigenous **Mesorhizobium** strains [19,20]; **Rhizobium multispinatum** isolates had nifH and nodD sequences 100% similar to those of *R. lusitanum* P1–7T and D. neptuniae J11 [59]. |
| *R. frondosus*, *L. tenus**     | **Mesorhizobium** with diverse 16S rRNA sequences clustered together on nifH and nodC sequences [115]. |
Considering crop legumes with determinate nodules, incongruence between housekeeping and symbiosis genes indicates that within-genus horizontal transfer of symbiosis genes has occurred between \textit{Bradyrhizobium} spp. associated with \textit{Arachis hypogaea} (peanut) [94,95], \textit{Bradyrhizobium}, and \textit{Ensifer} spp. associated with \textit{G. max} (soybean) [97–100] and \textit{Vigna unguiculata} (cowpea) [110,112], \textit{Rhizobium} spp. associated with \textit{P. vulgaris} (common bean) [103,105,106] and \textit{Mesorhizobium} associated with \textit{Lotus corniculatus} (bird’s-foot trefoil) [19,20]. The data indicate that, in particular cases, symbiosis genes of rhizobia associated with all these species have transferred to indigenous soil bacteria. The transfer of symbiosis genes from \textit{Mesorhizobium loti} used as inoculum on \textit{L. corniculatus} in New Zealand to indigenous \textit{Mesorhizobium} strains has been studied in detail [19,20]. Here, it was shown that the chromosomal symbiotic element of \textit{M. loti} ICMP 3153 is transferrable between \textit{Mesorhizobium} strains and can be fully functional in the recipient strain. For \textit{G. max} in Brazil, an indigenous \textit{Bradyrhizobium elkanii} strain and an indigenous \textit{Ensifer fredii} strain had similar \textit{nifH}, \textit{nodC}, and \textit{nodY-nodA} gene sequences to those of \textit{B. japonicum} used as inoculant. This is strong evidence that horizontal transfer of symbiosis genes had occurred between \textit{Bradyrhizobium} inoculum and indigenous strains of \textit{Bradyrhizobium} and \textit{Ensifer} [97]. An \textit{Ensifer} isolate from \textit{G. max} in north-eastern Afghanistan had identical \textit{nodD1} and \textit{nifD} sequences to those of \textit{Bradyrhizobium} \textit{yuanningense} [101].

For \textit{P. vulgaris}, strains of \textit{R. leguminosarum} sv. \textit{phaseoli}, \textit{R. gallicum}, and \textit{Pararhizobium giardinii}, isolated from plants grown in France, and \textit{R. etli}, isolated in Mexico and Belize, had highly similar \textit{nodC} sequences, and a strain characterized as \textit{Rhizobium} on its 16S rRNA sequence aligned with \textit{E. melliloti} on its \textit{nodC} sequence [103]. \textit{R. lusitanum} P1–7T isolated from \textit{P. vulgaris} in Portugal had \textit{nifH} and \textit{nodD} sequences similar to \textit{R. tropici} CIA T 899T and \textit{D. neptuniae} LMG 21357T [104]. In a related study, a \textit{Pararhizobium giardinii} strain characterized on 16S rRNA and housekeeping gene sequences aligned with \textit{Ensifer} spp. on \textit{nodC} sequence [107].

Data are presented for three \textit{Vigna} crop species (Table 4). For \textit{Vigna} \textit{angularis} (adzuki bean), grown in the sub-tropical region of China, \textit{Bradyrhizobium}, and \textit{E. fredii} were major, and \textit{Rhizobium}, \textit{Mesorhizobium}, and \textit{Ochrobactrum} were minor rhizobial symbionts [108]. Here, 16S rRNA, housekeeping and \textit{nodC} gene phylogenies were congruent, except that one \textit{Rhizobium} strain aligned with \textit{E. fredii} strains on \textit{nodD} sequences. In a related study on \textit{Bradyrhizobium} isolates from \textit{V. radiata} (mungbean) and \textit{V. unguiculata} grown in subtropical China, 16S rRNA, housekeeping and \textit{nodC} gene phylogenies were mainly congruent [116]. Similarly, for \textit{V. radiata} grown in three agro-ecological regions in Nepal, \textit{Bradyrhizobium} 16S–23S RNA IGS, 16S rRNA, \textit{nodA}, \textit{nodD1}, and \textit{nifD} sequences were mainly congruent, but five \textit{Bradyrhizobium} strains aligned with \textit{E. melliloti} on \textit{nodA} sequences [109]. In contrast, overall phylogenies for core and nodulation genes for \textit{Bradyrhizobium}, isolated from \textit{V. unguiculata} at a range of sites in Botswana and Roodeplaat, South Africa, were incongruent [110]. It was concluded that horizontal gene transfer has significantly influenced the evolution of \textit{V. unguiculata} root-nodule bacteria in these African countries [110]. Also, for \textit{M. vignae} BR3299T isolated from \textit{V. unguiculata} in north-east Brazil, the \textit{nifH} gene sequence aligned with \textit{Mesorhizobium} and \textit{Rhizobium} strains, which supports the proposal that \textit{M. vignae} BR3299T obtained its \textit{nifH} gene via horizontal transfer from \textit{Rhizobium} [111].

Examples of horizontal transfer of symbiosis genes within and between rhizobial genera were also found for non-crop species with determinate nodules. For \textit{Desmodium} spp. (\textit{Desmodieae}) growing in Panxi, Sichuan, China, \textit{nodC} sequences for one \textit{Pararhizobium}, one \textit{Rhizobium}/\textit{Agrobacterium}, and two \textit{Rhizobium} isolates characterized on 16S rRNA and housekeeping gene sequences were identical to those for \textit{Ensifer} strains isolated from \textit{L. leucocephala} in China (Panxi) and Brazil [96]. It was concluded that horizontal transfer of \textit{nodC} genes had occurred between the different genera, but that further work was required to determine the direction of gene transfer [96]. Evidence was also found for lateral transfer of symbiosis genes between rhizobial genera associated with \textit{G. soja} (‘wild soybean’). Here, \textit{Ensifer} and \textit{Rhizobium} isolates characterized on 16S rRNA and housekeeping gene sequences formed a single \textit{Ensifer} lineage on \textit{nifH} and \textit{nodA} sequences [102].

Across two studies, diverse \textit{Mesorhizobium} isolated from seven \textit{Lotus} species in the Canary Islands and characterized on 16S rRNA, \textit{atpD}, and \textit{recA} gene sequences clustered together close to \textit{M. loti} on
nodC sequences [113,114]. Similarly, diverse *Mesorhizobium* (16S rRNA) isolated from *L. tenuis* grown in three soils of the Salado River Basin Buenos Aires Province, Argentina, clustered together in a large clade on *nifH* and *nodC* sequences, again close to *M. loti* [115]. An *Aminobacter* (Phyllobacteriaceae) strain was also reported to nodulate *L. tenuis* and have a *nodC* sequence similar to the *Mesorhizobium* strains. This is the first report of an *Aminobacter* rhizobial strain, however, and requires verification. Similarly, *Geobacillus* (Phylum Firmicutes), *Paenibacillus* (Firmicutes), and *Rhodococcus* (Actinobacteria) were reported as rhizobial symbionts of *L. corniculatus* [117]. It was stated that these bacterial species had similar *nodA* gene sequences to *Mesorhizobium* isolates from the same plants and that they had obtained their *nodA* gene via horizontal transfer from the *Mesorhizobium*. This report also needs to be verified using authentification experiments and whole-genome sequencing.

4. Recombination of Symbiotic Islands

Incongruence between rhizobium core and symbiosis genes is strong evidence that horizontal transfer of symbiosis genes has occurred between strains, but it tells little about when the transfer took place. However, there are several cases linked to rhizobia associated with invasive species or use of rhizobial inoculum on crop species where the transfer of symbiosis genes between rhizobial species has been monitored. Here, we briefly consider the recombination of symbiotic islands and focus on two studies in which symbiosis genes were shown to transfer between a *Mesorhizobium* strain used as a crop inoculant and indigenous soil *Mesorhizobium*. The structure and physiology of *R. leguminosarum* symbiotic plasmids is considered in a separate paper in this Special Issue of *Genes* [118].

Firstly, in an important early study, the chromosomal symbiotic element of *M. loti* strain ICMP 3153 used as inoculum on *L. corniculatus* in New Zealand was shown to have transferred to indigenous *Mesorhizobium* strains and was fully functional in the recipient strains [19,20]. Subsequent work on *M. loti* strain R7A, a derivative of strain ICMP 3153, has shown that the symbiosis island is a single 502 kb Integrative Conjugal Element (ICE*MLSymR7A*) which integrates into a phenylalanine transfer RNA (phe-tRNA) gene [119–121] (Figure 2). The recombination reaction is driven by the recombination directionality factor S (RdfS) on the attachment sites attL and attR, resulting in the excised ICE*MLSymR7A*. The attB and attP sites facilitate the reintegration of the ICE into the chromosome, driven by integration factor S (IntS). In a separate study, the full genome sequence was reported for *M. ciceri* strain CC1192, which is the sole inoculant used on *C. arietinum* in Australia [122]. The *nod*, *nif*, and *fix* genes appear to be located on a 419 kb symbiosis island integrated within the chromosome of strain CC1192. This symbiosis island shows a similar structure to that of *M. loti* R7A strain.

![Figure 2. Recombination and circularization of Mesorhizobium loti R7A integrative and conjugative elements (ICE*MLSymR7A*) [20,119–121]]. The recombination is initiated by IntS (integration factor S) at the attachment sites attB and attP to integrate the ICE*MLSymR7A* in the chromosome and produce the attachment sites attL and attR. The recombination directionality factor S (RdfS) together with IntS stimulates excision of the ICE and forms the attachment sites attP and attB. phe-tRNA = phenylalanine transfer RNA. The ICE*MLSymR7A* is coloured blue, and the remaining chromosome is coloured grey. The schematic diagram is not drawn to scale.
The second example is the chromosomal symbiotic element of *M. ciceri* sv biserrula strain WSM1271, which was used as inoculum on *B. pelecinus* in western Australia. Again, the symbiotic island was shown to have been transferred from the inoculum to indigenous *Mesorhizobium* strains, but, here, the recipient strains were ineffective or poorly effective on N\textsubscript{2} fixation of the host plant [63,64]. In this case, the symbiosis ICE exists as three separate chromosomal regions when integrated in their host (alpha, beta, and gamma in Figure 3) [121,123]. These regions occupy three different recombinase attachment sites that do not excise independently but recombine in the host chromosome to form a single contiguous region prior to excision and conjugative transfer. Nine additional tripartite ICEs were identified in diverse mesorhizobia, and transfer was demonstrated for three of them [123]. Single-part ICE and tripartite ICEs appear to be widespread in the *Mesorhizobium* genus [122,123].

![Figure 3](image-url)

Figure 3. Comparative genome analysis using the circular BLASTN alignment in BRIG (BLAST Ring Image Generator, [124] of WSM2073 and WSM2075 against WSM1271. The three ICE\textsubscript{MC}Sym\textsubscript{1271} regions are indicated and summarised from Haskett et al. [123]. It highlights the transfer of the symbiosis island from the *Biserrula pelecinus* inoculant strain WSM1271 to non-symbiotic recipient strains that turn into poorly effective symbionts. ICE\textsubscript{MC}Sym\textsubscript{1271} consists of three separate regions (alpha, beta, and gamma) when integrated in the chromosome but excises as one circular plasmid and re-integrates in the recipient chromosome [123].
5. Occurrence and Importance of Horizontal Transfer of Rhizobial Symbiosis Genes

Andrews and Andrews [17] reviewed the literature on legume–rhizobia symbioses in field soils and related genotypically characterized rhizobia (genus level) to the taxonomy of the legumes (species level) from which they were isolated. Symbioses were described for approximately 450 legume species over 255 separate studies, and phylogenetic incongruence between rhizobium core and symbiosis genes was reported in 73 (~30%) of these studies (Tables 1–4). In the current review, we have listed examples of phylogenetic incongruence between rhizobium core and symbiosis genes for strains of 14 of the currently accepted 15 genera of rhizobia. The exception is Allorhizobium, for which no sequences for symbiosis genes are available for comparison with those in the databases. Thus, horizontal transfer of symbiosis genes between rhizobial strains is of common occurrence and is not restricted to specific rhizobial genera.

Phylogenetic comparisons of gene sequences indicated that horizontal transfer of symbiosis genes was more common within than between genera. In several cases such as Bradyrhizobium associated with native Genisteae species in Europe [90] and Mesorhizobium associated with Sophora spp. throughout New Zealand [85,86], within-genus horizontal transfer of nod genes has occurred across many bacterial species and is associated with legume divergence over a wide range of habitats and over long time periods. In 27 studies (~35% of those listed here), gene sequences indicated that horizontal transfer of symbiosis genes had occurred between different rhizobia genera. In most cases, this involved gene transfer between the common alphaproteobacterial genera Bradyrhizobium, Ensifer, Mesorhizobium, and Rhizobium. However, evidence is strong that these genera provided the symbiosis genes for the less common rhizobia genera Devosia [33], Methylobacterium [66], Microvirga [67,111], and Ochrobactrum [76], which thrive under particular environmental conditions. It seems certain that within- and between-genera horizontal transfer of symbiosis genes to indigenous soil bacteria has aided the diversification and establishment of legumes in different habitats.

The data indicate that horizontal transfers of symbiosis genes between alpha- and beta-proteobacteria and between the beta-proteobacteria Burkholderia and Cupriavidus are of rare occurrence. Only two cases (in one study) were found for horizontal transfer of symbiosis genes between alpha- and beta-proteobacteria. Specifically, in the Core Cape subregion of South Africa, Burkholderia isolated from R. triflora had a nifH sequence closely related to those of Ensifer spp., and a Mesorhizobium isolate from P. oligophylla aligned closely to Burkholderia on nodA sequence [68]. There are no confirmed reports of horizontal transfer of symbiosis genes between Burkholderia and Cupriavidus, which may at least in part be related to the genera not overlapping extensively in their biogeographic ranges [17,29,38]. For Burkholderia, findings indicate that within-genus horizontal transfer of symbiosis genes is rare for South American species but common in South African species [37,40,65,79]. The reasons for this regional difference are not clear, but the patterns across different rhizobial genera indicate that ‘lateral transfer of symbiosis traits is an important evolutionary force among rhizobia of the Cape Fynbos biome’ [65]. The potential rapidity of these evolutionary-scale changes in bacterial genomes was illustrated by the example from western Australia, where populations of indigenous Mesorhizobium, originally unable to nodulate the pasture legume B. pelecinus, were isolated from nodules and displayed symbiosis genes obtained from the commercial inoculant within six years [63,64].

In relation to the legume host, horizontal transfer of symbiosis genes to rhizobia and non-rhizobial bacteria that are adapted to local soil conditions is likely to increase the likelihood of establishment of a successful legume–rhizobia symbiosis in these soils as long as the recipient bacteria can induce functional N2-fixing nodules on the legume. The ecological success of the transfer is enhanced by the strong selection the plant exerts towards efficient infection and nodulation and the action of error-prone DNA polymerases that accelerate adaptation to symbiosis after gene transfer [22].

By considering all of the examples described here, we can conclude that horizontal transfer of symbiosis genes between rhizobial strains is of common occurrence, is widespread geographically, is not restricted to specific rhizobial genera, and occurs within and between rhizobial genera. The transfer of symbiosis genes to bacteria adapted to local soil conditions can allow these bacteria
to become rhizobial symbionts of previously incompatible legumes growing in these soils. This, in turn, will have consequences for the growth, life history, and biogeography of the legume species involved, which provides a critical ecological link connecting the horizontal transfer of symbiosis genes between rhizobial bacteria in the soil to the above-ground floral biodiversity and vegetation community structure.

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