



Tansley review

Biogeography of nodulated legumes and their nitrogen-fixing symbionts

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Summary

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In the last decade, analyses of both molecular and morphological characters, including nodulation, have led to major changes in our understanding of legume taxonomy. In parallel there has been an explosion in the number of genera and species of rhizobia known to nodulate legumes. No attempt has been made to link these two sets of data or to consider them in a biogeographical context. This review aims to do this by relating the data to the evolution of the two partners: it highlights both longitudinal and latitudinal trends and considers these in relation to the location of major land masses over geological time. Australia is identified as being a special case and latitudes north of the equator as being pivotal in the evolution of highly specialized systems in which the differentiated rhizobia effectively become ammonia factories. However, there are still many gaps to be filled before legume nodulation is sufficiently understood to be managed for the benefit of a world in which climate change is rife.

I. Introduction

Legumes (Leguminosae) are the third largest flowering plant family, important both economically and ecologically because of the major roles they play in natural and agricultural systems. Since the publication of the seminal book *Legumes of the World* (Lewis *et al.*, 2005), there have been major advances in our knowledge of legume taxonomy and also in the geographical range of legumes studied. These have included many new records of nodulation, detailed studies of nodule structure and a vast increase in the

number of genera and species of their symbiotic bacteria, known as rhizobia, identified. ‘Rhizobia’ is a general term used to cover all bacterial genera and species known to occupy root (and occasionally stem) nodule tissues, where they reduce nitrogen gas to ammonia. Nodulated legumes are found in all environments except open seas and are arguably more significant at high than low latitudes. There are also longitudinal differences in their occurrence, especially at low latitudes. Although the biogeography of legumes has been discussed in detail (Schrire *et al.*, 2005), no such study has been made of rhizobia or of host–rhizobial

interactions. This review aims to examine these topics, separately and together.

In the sections of this review, we have selected tribes of legumes from different areas to illustrate particular points. Because of their economic importance, grain legumes have been extensively covered elsewhere and will not be considered, unless there is a particular point about their symbiosis. One particular point is that legumes display a range of nodulation phenotypes (infection, morphology and structure) that are characteristic of different tribes and may be related to the evolution of nodulation in legumes. These are covered in depth in Sprent (2001, 2009) and Sprent *et al.* (2013).

Fig. 1 shows the main types of nodule morphology and Fig. 2 the main anatomical features of nodules. Further details can be found in Table 1, which is a glossary of commonly used terms.

II. Recent changes in the taxonomy of legume genera

Over the last 5 years there has been a major effort to put legume taxonomy on a better footing. To this end, the Legume Phylogeny Working Group (LPWG), which now has over 90 members, was set up and published its initial findings in *Taxon* in 2013 (LPWG, 2013). Since then, the LPWG has not only amassed more molecular data but also included morphological characters, such as the presence or absence of nodules, in its considerations. This has led to the production of another paper titled 'A new subfamily classification of the Leguminosae, based on a taxonomically comprehensive phylogeny' (LPWG, 2017). It is generally agreed that the earlier division of legumes into three subfamilies, Caesalpinioideae, Mimosoideae and Papilionoideae, is no longer appropriate, the Caesalpinioideae in particular being paraphyletic. Four of the recircumscribed Caesalpinioideae subfamilies (the Duparquetioideae, Cercidoideae, Detarioideae and Dialioideae) are non-nodulating members and will not be considered further. The so-called Mimosoideae-Caesalpinieae-Cassieae or MCC clade now includes all nodulating caesalpinioids as well as the Mimosoideae. The suggestion that mimosoids are part of the (remaining) caesalpinioids (Fig. 3), in the MCC clade, presents problems for those studying nodule characters, as will be discussed later in this section.

The classical picture of three subfamilies was based on the very different flower types (Fig. 4), with Caesalpinioideae often having actinomorphic flowers, Mimosoideae having complex heads or spikes of flowers, of which the numerous stamens are the most prominent feature, and Papilionoideae having zygomorphic, pea-like flowers. Occasional exceptions to the flower type of Papilionoideae were known, most notably for *Cadia* (Fig. 4a) (Citerne *et al.*, 2006). It is now known that various types of flower morphology are found in early branching papilionoids. For example, Cardoso *et al.* (2012) defined a group that comprises morphologically diverse genera, including the nearly papilionate and strongly bilaterally symmetric-flowered *Bowdichia* and *Diptotropis* s.l., as well as *Guianodendron* and *Leptolobium*, which have radial flowers with undifferentiated petals. These are now included in a new clade of legumes, the Leptolobieae (Cardoso *et al.*, 2013). Clearly, flower morphology is no longer a safe

taxonomic character, whereas nodule characters are arguably (this review) more reliable.

Only eight caesalpinioid genera are known to nodulate, *Chidlowia* having now been removed to Mimosoideae (LPWG, 2017); all have indeterminate, branched nodules. They include *Chamaecrista*, the eighth largest genus of legumes, with *c.* 330 species (Lewis *et al.*, 2005). It varies in habit from trees to shrubs and herbs and is one of the very few caesalpinioid legume genera to have extended into temperate areas. All properly tested species of *Chamaecrista* have been shown to nodulate. Caesalpinioid nodules, with the exception of some shrubby and herbaceous species of *Chamaecrista*, retain their nitrogen-fixing bacteroids within cell wall-bound structures, known as fixation threads (see also Section III 1). This feature is also found in a few papilionoid, but no mimosoid nodules (Sprent *et al.*, 2013). Earlier inclusion of *Chidlowia* in Caesalpinioideae (Sprent *et al.*, 2013) and its subsequent reassignment to Mimosoideae (Manzanilla & Bruneau, 2012; LPWG, 2017) have led us to re-examine its nodule structure and, although bacteroids were present, there were no clear signs of fixation threads. This key genus urgently needs further study. Only one detailed study of infection processes in caesalpinioid legumes has been published, for two species of *Dimorphandra* (Fonseca *et al.*, 2012), which shows that they are infected via root hair curling. A full list of nodulating caesalpinioid legumes is provided in Section IV.

The evidence, based mainly on chloroplast genes, but supported by work on nuclear genes, strongly supports the nesting of mimosoid legumes within the caesalpinioids in the MCC clade (Fig. 3). As intimated above, this is not consistent with the facts that nodule development and structure in these two groups are quite different. In particular, mimosoid legume nodules are all indeterminate, often branched, with an infection process involving root hairs and infection threads, with bacteroids being released into membrane-bound structures known as symbiosomes; fixation threads (Fig. 2) are never seen. Can these differences be reconciled? We believe that only taking into account whether or not a legume nodulates is insufficient for taxonomic purposes: nodule structure and infection processes are very important taxonomic characters, as was shown in defining the Dalbergioideae clade (Lavin *et al.*, 2001). Numerous examples of congruence between classic taxonomic and nodulation characters exist. Two examples are the separation of *Chamaecrista* from *Senna* and *Cassia* (see LPWG, 2013) and the revision of *Lotononis* with the reinstatement of *Listia*, a genus with lupinoid rather than indeterminate nodules (Boatwright *et al.*, 2011).

Although nodulation is common in mimosoid legumes, there are many exceptions. Some more basal genera do not nodulate, and others, including *Parkia*, have probably lost this ability. Current knowledge is summarized in Fig. 5. The two main nonnodulating groups (*Anadenanthera* and *Newtonia*) are from Africa, Asia and Madagascar. *Pentaclethra* is unusual in having both nodulating and nonnodulating members, the latter being in Africa. Apart from *Parkia*, all New World mimosoids appear to be nodulated. *Parkia* is a pantropical genus of *c.* 34 species, of which half have been reliably reported as unable to nodulate (Sprent, 2001). Most of these reports are from the New World. In view of its close relationship

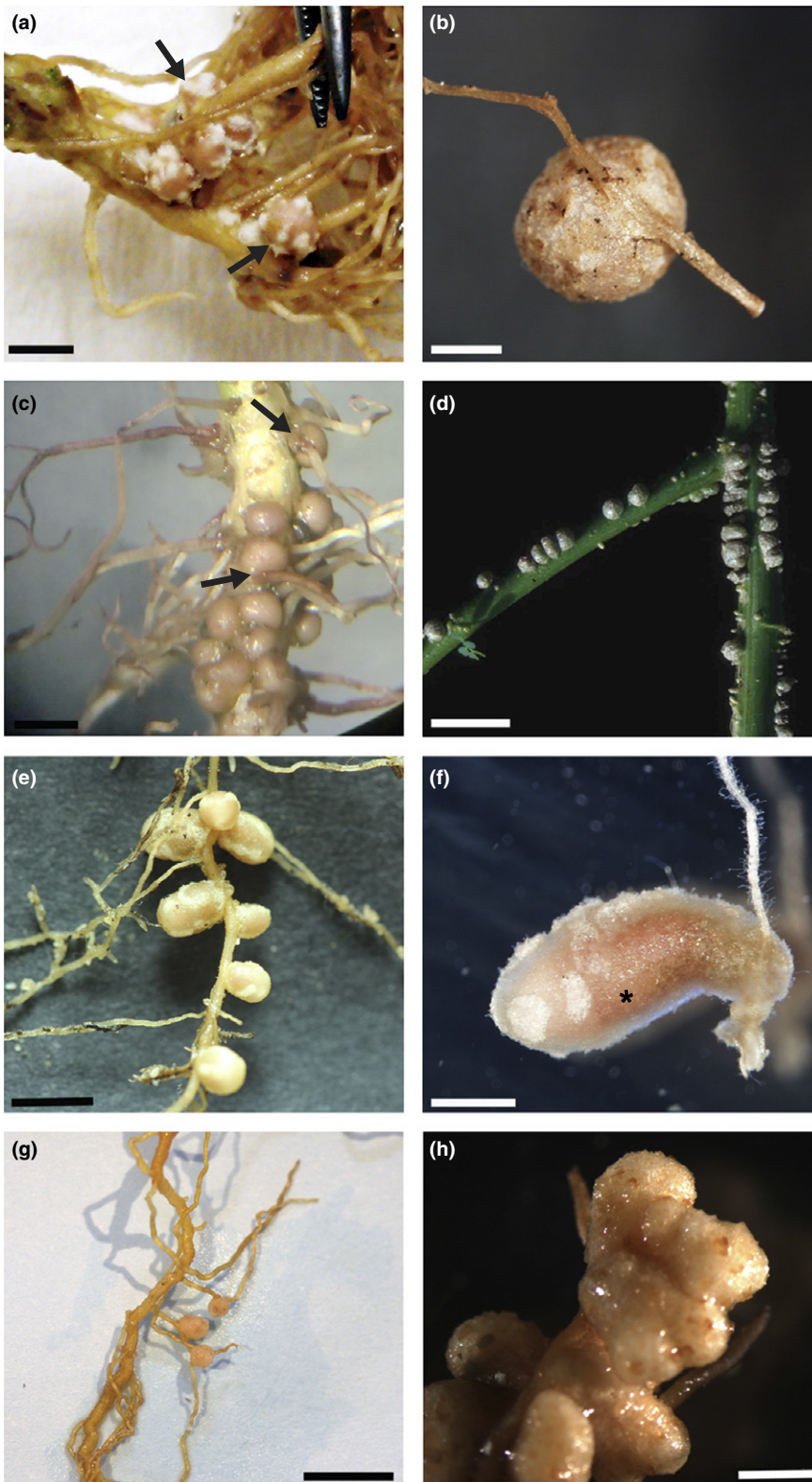


Fig. 1 Nodule morphologies across the Leguminosae (tribes indicated in parentheses). (a) Spherical determinate desmodioid-type nodules with lenticels (arrows) on the South African legume *Dipogon lignosus* (Phaseoleae) (photograph courtesy of Wendy Liu). (b) Determinate nodule on the Australian native *Hardenbergia comptoniana* (Phaseoleae). (c) Dalbergioid nodules on the upper root/lower stem of the neotropical species *Aeschynomene americana* (Dalbergiae); these nodules are always associated with lateral roots (arrows) (photograph courtesy of H. S. Gehlot). (d) Photosynthetic stem nodules on the West African semi-aquatic species *Sesbania rostrata* (Sesbaniae). (e) Indeterminate nodules on the roots of the Australian native *Chorizema cordatum* (Mirbelieae). (f) Indeterminate nodule of the Australian native *Chorizema rhombeum* (Mirbelieae). Note the pink colouration (*), which is caused by leghaemoglobin. (g) Indeterminate nodules on the roots of the Australian native *Templetonia retusa* (Brongniartae); the rhizobial symbionts in these are contained within cell wall-bound fixation threads (see Fig. 2i and j for examples). (h) Branched indeterminate nodule on the Brazilian native tree *Dimorphandra wilsonii* (Caesalpiniae); these also contain fixation threads. Bars: (a, c) 4 mm; (b, f) 1 mm; (d, g, h) 5 mm.

with major groups of nodulating mimosoids, it seems more likely that it has lost the ability to nodulate rather than never having it.

Within papilionoid legumes there have also been major changes in our understanding of taxonomic relationships, many resulting from the work of Cardoso *et al.* (2013), who

concentrated on the deep (first)-branching groups, up to and including the appearance of a 50-kb inversion (Fig. 6; Table 1). Several of the proposed new rearrangements are consistent with nodule characters; these will be considered first. Outside the clade with the 50-kb inversion, only some Swartzieae contain

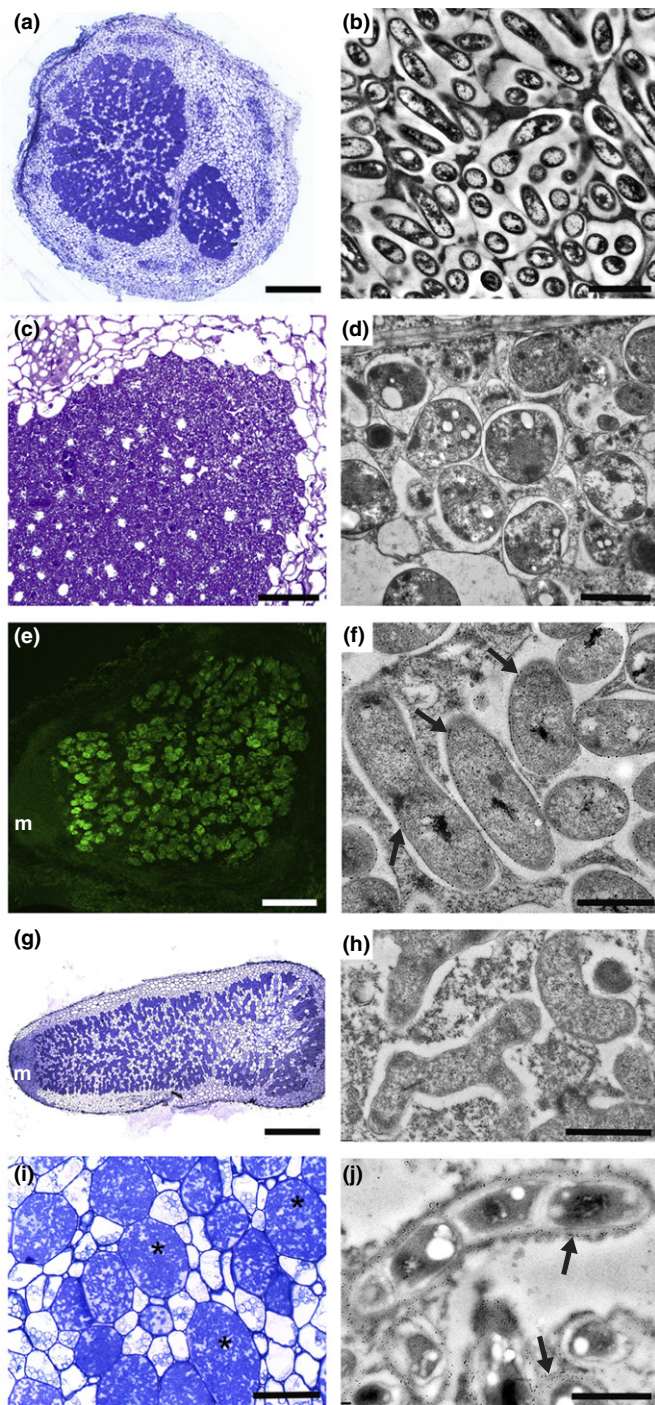


Fig. 2 Nodule structures across the Leguminosae (tribes indicated in parentheses). (a) Determinate desmodioid nodule of *Clitoria fairchildiana* (Phaseoleae). (b) Bacteroids within intermediate 'determinate-indeterminate' nodules on *Sesbania cannabina* (Sesbaniae) infected with *Rhizobium* (formerly *Agrobacterium*) strain IRBG74. Note that the bacteroids are undifferentiated and that there are several per symbiosome. (c) Crotalarioid nodule of *Listia angolensis* (Crotalariae) infected with *Microvirga lotononidis*. As with dalbergioid and lupinoid/genistoid nodule types there are no uninfected interstitial cells within the central zone. (d) Swollen differentiated spherical bacteroids within an indeterminate nodule on the New Zealand endemic species *Carmichaelia australis* (Galegae). (e) Indeterminate nodule on the mimosoid species *Piptadenia gonoacantha* (Mimoseae) infected with *Burkholderia phymatum* STM815GFP. The growing tip of the nodule, including the meristem, is marked with an 'm'. (f) Undifferentiated bacteroids within a nodule of '*Piptadenia*' *viridiflora* (Mimoseae) infected with *Burkholderia phymatum* STM815. The bacteroids are immunogold-labelled with an antibody specific to *Burkholderia* (arrows). Note the uninfected interstitial cells. (g) Indeterminate nodule of *Gastrolobium sericeum* (Mirbeliae). Unlike the 'classical' determinate nodule in (a), these nodule types have a persistently growing tip (m), with a meristem (also see e). (h) Differentiated pleomorphic bacteroids within a nodule of the Inverted Repeat Lacking Clade (IRLC) species *Vicia sylvatica* (Viciae). (i) Infected tissue within an indeterminate nodule on *Erythrophleum ivorense* (Caesalpiniae) infected with *Bradyrhizobium*; the bacteroids within the infected cells (*) are enclosed within cell wall-bound fixation threads (see j). (j) Bacteroids enclosed in fixation threads within a nodule on *Chamaecrista pumila* (Cassiae); the cell walls of the threads are immunogold-labelled with the monoclonal antibody JIM5 (arrows), which recognizes a pectin epitope. Bars: (a, g) 500 μm ; (b, d, f, h, j) 1 μm ; (c) 100 μm ; (e) 200 μm ; (i) 20 μm .

et al., 2013). Other nodule features that are typical of particular legume tribes have been discussed in Sprent *et al.* (2013). As pointed out there, tribal arrangements are not always consistent with nodule features. In particular, the association of Podalyriaceae with Crotalariae and Genisteae seems anomalous on the grounds of infection processes and nodule structure, the first having a root hair infection process and the latter two an epidermal infection process. The particular features of legumes in the Inverted Repeat Lacking Clade (IRLC) are discussed in Section VII.

How can we bring these patterns of nodulation into line with currently agreed legume phylogenetic groupings? Whereas chloroplast and nuclear genes have proved invaluable in taxonomic studies of plants, they take no account of symbiotic processes and understanding the genetic framework of these is essential. There is currently a lot of interest in this from specialists in symbiosis. For example, research from Delaux *et al.* (2014), based largely on arbuscular mycorrhizas, suggests that symbiosis can result in 'purifying selection on host gene networks'. This work is being extended to nodulation and the first of a series of comparative studies of complete genomes for paired nodulating and nonnodulating legume genera is being undertaken by a group led by Pierre-Marc Delaux at INRA, Toulouse, France. Data are expected in the near future.

There is general agreement that the ability to nodulate has been gained and lost several times. There is also an increasing body of evidence that there are common features in the evolution of various plant symbioses/associations, not only nodulation (both in legumes and in nonlegumes) and arbuscular mycorrhizal fungi (AMF), but other systems such as nematode galls (Genre & Russo, 2016). This suggests that a set of common symbiotic pathway proteins can be

nodulating species and these are now all found in one clade of the subdivided tribe. After the 50-kb inversion, the Exostyleae and Vatarioid groups do not nodulate. All other tribes do, apart from a few genera that have lost the ability, but their nodule characters vary widely. The Andira clade has two genera, both of which have nodules where bacteroids are retained in fixation threads, as noted for caesalpinoid species above. Many of the Brongniartieae also have nodules with fixation threads (Sprent *et al.*, 2013). The *Indigofera* nodule is unique in being rather like an indeterminate desmodioid nodule, with lenticels (Gehlot *et al.*, 2012; Sprent

Table 1 Glossary

50-kb inversion	A large (50-kb) inversion in the chloroplast genome, relative to the gene order found most commonly among land plants, which is synapomorphic for a clade that includes most of the Papilionoideae.
Arbuscular mycorrhiza	A symbiotic association in which fungi of the phylum Glomeromycota intracellularly colonize the host plant's roots, forming characteristic 'arbuscules' inside the host cell that facilitate nutrient exchange. Arbuscular mycorrhizas share a common symbiotic pathway with rhizobia.
Actinorhizal plants	Species of nonlegume plants belonging to the Rosales, Fagales and Cucurbitales within the Rosid I clade that are characterized by their ability to form a symbiosis with species of the Gram-positive nitrogen-fixing actinobacteria <i>Frankia</i> .
Bacteroid	The differentiated dinitrogen-fixing form of the rhizobial microsymbiont within a host nodule cell.
Common symbiotic pathway	The single plant signal transduction pathway that is proposed to act downstream of both mycorrhizal and rhizobial signal perception and upstream to allow infection and colonization of either symbiont.
Diazotrophy	The ability to fix and grow on N ₂ as a sole nitrogen source. Most rhizobia are not considered to be diazotrophs, as they are unable to fix N ₂ <i>ex planta</i> . Recorded exceptions are <i>Azorhizobium caulinodans</i> , strains of <i>Burkholderia</i> and strains of photosynthetic <i>Bradyrhizobium</i> .
Fixation thread	In many nodulating caesalpinoid and early-branching papilionoid legumes, rhizobia are not released into membrane-bound symbiosomes but are retained and fix nitrogen within specialized fixation threads inside the plant nodule cell.
Endoreduplication	Replication of the nuclear genome in the absence of cell division, leading to elevated nuclear gene content and polyploidy.
Infection	Rhizobial infection of the legume host most commonly occurs via root hair curling, followed by development of an infection thread. In some legumes, however, infection may proceed by epidermal or crack entry, with or without the formation of infection threads.
Infection thread	A tubule formed inside a root hair from an invagination of the plant cell wall and membrane, in which the infecting rhizobia grow and divide, and are contained. The infection thread ramifies as it grows through the root and enters the nodule primordium, after which the bacteria are released and enter the nodule cells.
Inverted Repeat Lacking Clade (IRLC)	A monophyletic clade of the Papilionaceae that is characterized by the loss of one of the two 25-kb inverted repeats in the chloroplast genome.
Lenticel	A loosely packed clump of cells with large intercellular spaces that regulates the gas permeability of nodules and typically shows as a white star or stripe on the nodule surface. Lenticels are a prominent feature of the determinate desmodioid type of nodule.
Nod factor	A lipochitooligosaccharide oligomer with various functional groups at the terminal or nonterminal residues that functions as a rhizobial signalling molecule. The particular Nod factor that is synthesized by the rhizobia, and recognized by the plant, varies between bacterial species and is a basis for host-symbiont specificity. Rhizobial Nod factors induce the plant signal transduction pathway that results in infection thread development and nodule morphogenesis.
Nodule	A plant organ that develops on the roots or (rarely) the stems of legumes, in which the N ₂ -fixing rhizobia are housed. Nodule morphology and structure vary according to the legume clade and can be a marker for legume phylogeny. The nodule morphology may be either determinate or indeterminate, depending on the persistence of the meristem, and the central tissue may or may not contain interstitial cells interspersed among infected cells. Desmodioid nodules (typically seen in <i>Desmodium</i> and other phaseoloid legumes) are determinate and have prominent lenticels. Lupinoid nodules (as seen in <i>Lupinus</i> and <i>Listia</i> spp.) are a type of indeterminate nodule where the nodule forms a collar around the subtending root.
Nodule-specific cysteine-rich (NCR) peptides	A large (c. 600) family of short (60–90 amino acids) polypeptides that carry a conserved signal peptide and a conserved cysteine motif. NCR peptides resemble plant antimicrobial peptides and govern terminal differentiation of rhizobia within nodules of IRLC legumes.
Non-protein amino acid-accumulating (NPAAA) clade	A clade in the Papilionoideae that is characterized by the accumulation of the nonproteinogenic amino acid canavanine in the seeds.
Symbiosome	A plant membrane-bound structure that encloses the rhizobial microsymbiont within the nodule cell. Symbiosomes can contain from one to several bacteroids.
Terminal bacteroid differentiation (TBD)	Terminally differentiated bacteroids have lost the capacity to resume cell division. They have characteristic features of cell enlargement, genome endoreduplication and increased membrane permeabilization.

recruited for various purposes, including nodulation, depending on the molecular and cellular context in which they act.

III. Latitudinal variations in the distribution of nodulated legumes

1. Latitudinal dimensions of nodulated legume biogeography: from pole to pole

Before current latitudinal distributions can be considered, it is necessary to consider where the different parts of the world were before legumes evolved. About 200 million years ago (Ma) there

were two large land masses, Laurasia and Gondwana (Schettino & Scotese, 2005). Between these was the Tethys Sea, which later separated the two land masses completely and which Schrire *et al.* (2005) hypothesized to be where legumes first evolved, c. 60 Ma. Thus, the Tethys Sea immediately imposed a major latitudinal rift between the Northern and Southern Hemispheres. Further, the parts of Laurasia to the north were fully interconnected, whereas Gondwana to the south was composed of several different land masses (see Fig. 1.3 in Sprent *et al.*, 2013). Australia was still firmly attached to Antarctica, from which it did not become detached until c. 33 Ma (Scotese, 2004; Crisp & Cook, 2013) (see Section III.2). About 3–4 Ma, North

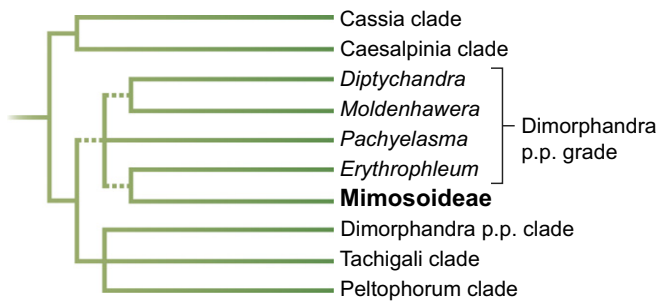


Fig. 3 The position of mimosoid legumes within the caesalpinioid group (sometimes called the Mimosoideae-Caesalpinieae-Cassieae or MCC clade), as shown by the Legume Phylogeny Working Group (LPWG, 2013), based on chloroplast markers.

and South America joined, which we speculate would have facilitated the southern migration of Laurasian species, as has been shown for some genera in tribes Trifolieae and Fabae (Schaefer *et al.*, 2012). This may also have happened for the (comparatively) few species of *Astragalus* (*c.* 100) now found in the Southern Hemisphere.

Clearly, between the poles and the equator there are major variations within the legume flora. In their consideration of the occurrence of nodulating trees, Menge & Crews (2016) used a cut-off point of 35° latitude to separate more tropical from more temperate regions. However, this is not consistent with some of the well-known groupings of legumes, especially as it excludes much of the Mediterranean area. Here, we examine legume biogeography, starting in the Arctic and proceeding southwards, considering Europe, North America and Asia down to the Tropic of Cancer. In the interests of space, only selected genera will be discussed.

Astragalus, with *c.* 2500 species, is the largest of all legume genera. *Oxytropis* (up to 400 species) is closely related and is found in areas of Northern Eurasia where *Astragalus* is most abundant. Both genera are in the IRLC group of legumes (see Section VII), with indeterminate branched nodules. *Hedysarum arcticum*, also in the IRLC, is found in the high Arctic, above 70°N, but is also common at lower latitudes. In a study of Swedish legumes above or near the Arctic Circle, Ampomah *et al.* (2012) found not only *Astragalus* and *Oxytropis* but also three species of *Trifolium*, and *Vicia cracca*. Close to the Arctic Circle (between 63 and 66°N) two species of

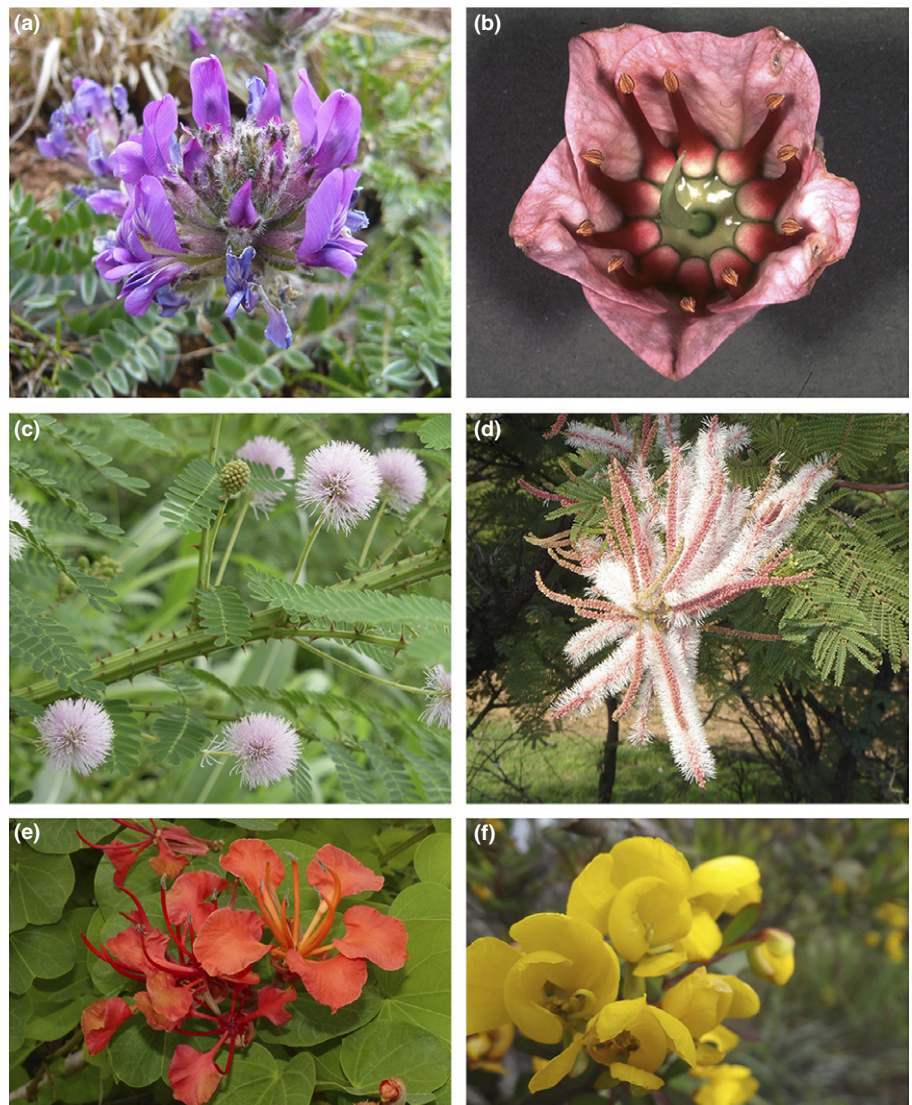


Fig. 4 Flower morphologies across the Leguminosae. (a) *Oxytropis halleri* (Astragaleae) (photograph courtesy of Natacha Frachon). (b) *Cadia purpurea* (Podalyriaceae) (photograph courtesy of Toby Pennington). (c) *Mimosa hamata* (Mimoseae) (photograph courtesy of H. S. Gehlot). (d) *Mimosa benthamii* (Mimoseae). (e) *Bauhinia galpinii* (Cercideae) (photograph courtesy of Gwil Lewis). (f) *Chamaecrista confertiflormis* (Cassieae) (photograph courtesy of Lander Alves).

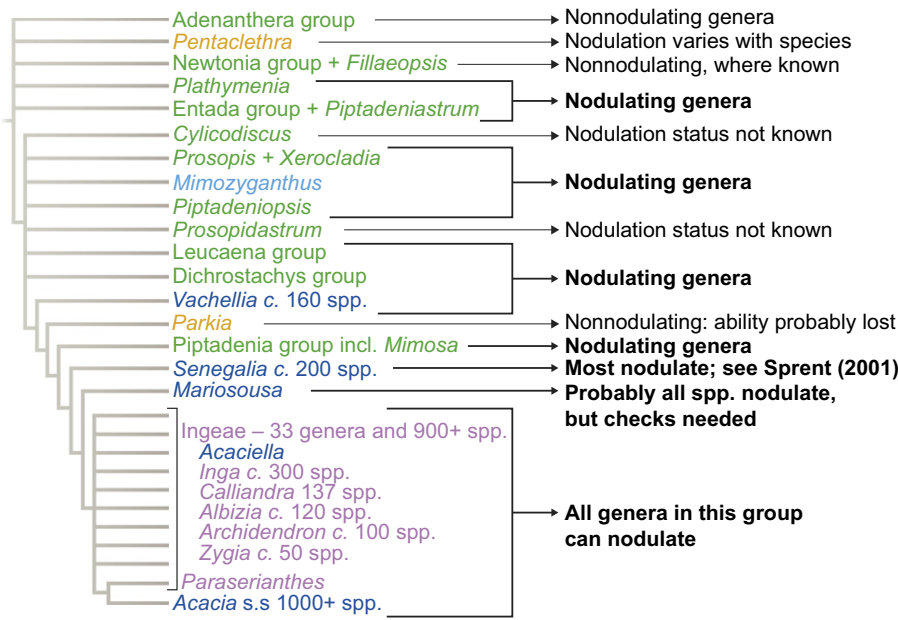


Fig. 5 Nodulation in mimosoid legumes: based on classification in the Legume Phylogeny Working Group (LPWG, 2013).

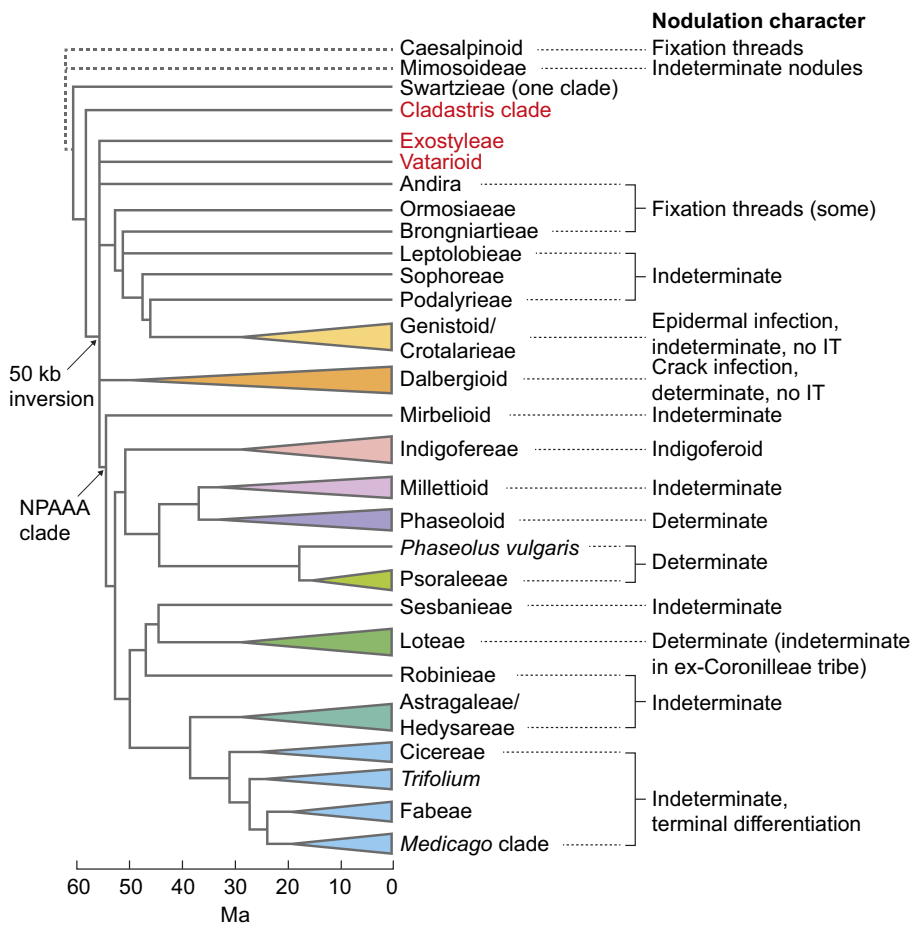


Fig. 6 Chronogram showing phylogenetic relationships between the major papilionoid and nodulating caesalpinoid and mimosoid legume groups and their time of evolution, in relation to nodulation phenotypes. Phylogenetic groupings are based on those given by the Legume Phylogeny Working Group (LPWG, 2013). The approximate dates of the nodes are taken from Lavin *et al.* (2005). The figure is modified from that shown in Doyle (2011) and Sprent *et al.* (2013). IT, infection thread; Ma, million years ago; NPAAA, nonprotein amino acid-accumulating.

Lathyrus were also found. Why these IRLC legumes, all of which are perennial herbs, have colonized Arctic regions is not clear, but Sprent *et al.* (2013) suggested that it might be related to their history of glaciation (see also Section VII). *Hedysarum* species as

herbs and shrubs are common in Northern China, some between 4000 and 5000 m altitude. The related genus *Caragana* is also found at higher altitudes and, in the form of shrubs or small trees, is a major component of the flora of the Tibetan plateau (see

Plate 2.12 in Sprent, 2009). The IRLC legume tribe is also prominent in the Mediterranean region, which is now considered.

The region around the Mediterranean Sea has been mined for forage legumes for centuries: these have been the foundation of the agricultural economies of both Australia (e.g. subterranean clover, *Trifolium subterraneum*, and many other *Trifolium* species) and New Zealand (largely white clover, *Trifolium repens*). So what is special about the flora of the Mediterranean region? Here we consider only two main groups of papilionoids. The first consists of genera in the IRLC and includes a number of species of *Astragalus*. In addition to the c. 250 species of *Trifolium*, species of *Trigonella*, *Melilotus*, and *Medicago* are important, internationally used forage/pasture plants. *Medicago* is a genus of 87 species that has recently been fully revised (Small, 2010). *Medicago sativa* (lucerne or alfalfa), which may have originated in China, was taken to North America by European colonists, as fodder for their horses. It is the mainstay of the pasture legume industry and is estimated world-wide to contribute over a trillion dollars to the economy (Small, 2010).

The other main papilionoid group, which extends out to Macaronesia, is the Genisteeae. Unlike the IRLC legumes, which have indeterminate nodules with a root hair infection pathway and central nodule tissue with a mixture of infected and uninfected cells, the Genisteeae have indeterminate nodules, infection is via the epidermis, infection threads are never formed and the central nodule tissue consists entirely of infected cells (Fig. 2). Members of this tribe are found in both the Northern and Southern Hemispheres. They include one tree genus, *Laburnum*, with the rest being shrubs or annual herbs. The genera *Cytisus*, *Genista*, and *Ulex* are most common in the area, often forming dense stands on very poor land, and are uniformly nodulated. Their economic potential has never been exploited, although their ecological role has been studied in parts of Macaronesia. In Australasia they are listed as noxious weeds and attempts to eradicate them are widespread. The genus *Lupinus* has many interesting features. First, it has a lupinoid type of nodule, in which nodules grow around the subtending root, a feature only found in one other genus, *Listia*, in the closely related tribe Crotalarieae (Fig. 2). Second, it is the only legume genus known not to form mycorrhizas. *Lupinus* also holds the record for rate of evolution of an angiosperm (Hughes & Eastwood, 2006), and can grow at altitudes of up to 5000 m, where humans fight for breath. The so-called 'cool season food legumes', members of tribe Fabeae, including *Vicia faba* and *Pisum sativum*, have been reviewed many times and will not be considered here.

Many of the legume genera considered above are also native to temperate North America. Although agricultural use of legumes is dominated by the introduced alfalfa and soybean (*Glycine max*), there are interesting indigenous potential forage and grain legumes, such as those in tribes Amorpheae and Phaseoleae, members of which are found from southern Canada to Mexico. Species of the genus *Dalea* are known as prairie clovers and are important in many ecosystems. *Apios americana* has tubers that are nutritionally similar to potatoes, as does *Pediomelum esculentum* (synonym *Psoralea esculentum*), the prairie turnip. *Chamaecrista fasciculata*, the partridge pea, is an annual caesalpinoid species used for forage.

Records of nodulation in the temperate legume flora of Asia are scarce, although much taxonomic work is currently being carried out in China and has been greatly assisted by the publication of volume 10 of *Flora of China* (Wu *et al.*, 2010). Among the genera for which there are no reports on nodulation are several in tribe Millettieae. Some of these have been transferred to the IRLC and urgently need to be studied (see Section VII).

In the tropics, the situation is complex and is different in wet and seasonally dry areas. In the former, legumes abound, but, unlike northern temperate areas where plants tend to be herbaceous or shrubby and are uniformly nodulated, many of the legumes are trees and many of them cannot nodulate. There is a distinct longitudinal dimension to this, so legumes from the wet tropics will be considered in Section IV. In the seasonally dry tropics, there are major differences between continents. In South America, many large mimosoid genera, such as *Mimosa* and *Inga*, have their centres of diversity. All mimosoid legumes studied to date have indeterminate nodules, with interstitial cells (Fig. 2). *Inga* has c. 300 species and has been used for centuries for a variety of purposes, including edible fruit, timber and shade (Pennington & Fernandes, 1998). All species examined have been shown to nodulate (Grossman *et al.*, 2005; da Silva *et al.*, 2014). *Mimosa* has two centres of diversity, one in Brazil and one in Mexico. These are nodulated by very different rhizobia (discussed in detail in Section VI). *Chamaecrista* also has Brazil as its centre of diversity, but many herbaceous species have spread around the world in tropical and warm temperate areas. All species sampled can nodulate (negative reports in the literature, cited in the United States Department of Agriculture (USDA)'s GRIN database, are from plants sampled in the dry season for taxonomic work; the material needs to be re-examined in the wet season). *Chamaecrista* nodule structures show a pseudo-evolutionary pattern, in that some (mainly arboreal) species retain their bacteroids in fixation threads, whereas most release them into symbiosomes (Naisbitt *et al.*, 1992) (Fig. 2).

In both South America and Africa, acacias (Mimosoideae) abound. The old nonmonophyletic genus *Acacia* has so far been divided into five genera (listed below, and see Fig. 5), and more may emerge. *Acacia* s.s., the second largest genus of legumes (c. 1000 spp.), is almost entirely confined to Australia; *Vachellia* (the earlier subgenus *Aculeiferum*) and *Senegalia* (the earlier subgenus *Acacia*) are both now separate from *Acacia*. Species of *Senegalia* extend into parts of the USA, where *Senegalia greggii* is unusual in being one of a group of species from both Africa and South America that has lost the ability to nodulate (Sprent, 2001). *Acaciella* is neotropical, while *Mariosousa* is restricted to tropical and subtropical regions of the southwestern USA, Mexico, and Central America.

The legume flora of Africa is extremely diverse. Some of the tribes are also common to the Northern Hemisphere, for example Genisteeae and Phaseoleae. Podalyrieae is, with one exception (*Calpurnia*), endemic to South Africa. Here, we concentrate on southern Africa, where some legumes have been widely studied and others with agricultural potential less so. The Cape Floristic Region (CFR) is a world biodiversity hotspot, with abundant papilionoid legumes that are known to nodulate and one detarioid legume (*Schotia afra*) that cannot nodulate. There are no mimosoid legumes in this region, unlike its Australian biodiversity hotspot counterpart,

the South West Australian Floristic Region (SWAFR), discussed in the next section. *Aspalathus linearis* (Crotalariaeae) and species of *Cyclopia* (Podalyriaeae) have long been used for production of Rooibos and Honeybush teas, respectively, which lack caffeine and are low in tannins. Both grow on extremely poor soils, which they mine with the help of both mycorrhizas and cluster roots, as well as being profusely nodulated. *Lebeckia ambigua* (Crotalariaeae) is being developed as a forage species for use in the Australian wheat belt (Howieson *et al.*, 2013). It can also grow on very poor soil, is drought tolerant, has cluster roots and is palatable to sheep. The monospecific perennial *Dipogon lignosus* is unusual for a phaseoloid legume in having both determinate (see Fig. 1a) and indeterminate nodules (Liu *et al.*, 2014). It has been introduced into Australia and New Zealand where it has become very invasive.

Elsewhere, important food crops such as cowpea (*Vigna unguiculata*) have been well studied; others such as the bambara groundnut (*V. subterranea*) are less so. Some of the problems and potential for using indigenous African legumes in agriculture have been discussed previously (Sprent *et al.*, 2009).

2. Australia vs the rest of the world

Australia is unique in many ways that affect the diversity of its legume genera, and how this diversity has varied over time. At first, Australia was connected to Antarctica, as part of Gondwana, when the climate was warm temperate. During this time it was possible for plants to move from Australia to South America and northwards. This may account for the presence of the legume tribe Brongniartieae in both Australia and South America (Sprent *et al.*, 2013). When Australia broke away from Antarctica (*c.* 33 Ma, although the exact time is uncertain; Crisp & Cook, 2013), a circum-polar current flowed between it and Australia, which then became both colder and drier. Evolution of species in the endemic mirbelioid legumes (all of which can nodulate) continued and still continues into very arid areas (Crisp *et al.*, 2004). Even within Australia there is a division between the east and west, which are separated by the arid desert of the Nullabor Plain; this allowed separate speciation in genera such as *Daviesia* (Mirbelieae). Species of *Acacia* dominate much of the large arid and semiarid areas of the Australian continent (Adams *et al.*, 2016). Recent revisions of the old genus *Acacia* (see Section III 1) have allowed more detailed study of the evolution of the new constituent genera. Miller *et al.* (2003) suggest that *Acacia* ss may have arrived from SE Asia *c.* 23 Ma, after the continent separated from Antarctica. Australia has several other genera of mimosoid legumes, all of which are known to nodulate.

The second event that could have been instrumental in forming Australia's unique flora was the formation of the so-called Wallace line that separated much of the southern land masses from the northern ones when Australia was still joined to Antarctica (Buerki *et al.*, 2014). It is possible that mirbelioids stayed below the Wallace line. After separating from Antarctica, Australia moved slowly northwards until it neared Asia, when it was possible for plants to be exchanged with that continent (Sniderman & Jordan, 2011). This exchange presumably would have included legumes and their associated rhizobia. Of Australia's many caesalpinoid species, all grow in the northern tropical or subtropical regions and species in

only two genera can nodulate: one species of *Erythrophleum* (*Erythrophleum chlorostachys*) and 14 species of *Chamaecrista*, some of which are endemic and others introduced. It is interesting to note that the Australian IRLC genera *Swainsona* (containing *c.* 70 taxa) and *Trigonella suavissima* are predominantly found in subtropical and/or arid zones (Brockwell *et al.*, 2010; Davis & Hurter, 2013).

Australia hosts a biodiversity hotspot, the SWAFR (Hopper & Gioia, 2004), which contains many mimosoid (*Acacia* ss) legumes, together with endemic mirbelioids and some endemic phaseoloids. There are no caesalpinoids in this area. Many of the endemics are unusual in thriving on low-phosphate soils (Suriyagoda *et al.*, 2010) and also in using carbon compounds rather than nitrogen compounds (such as alkaloids) for defence purposes. These include genera such as *Gastrolobium*, which produces fluoroacetate (Twigg *et al.*, 1996), the classic Krebs's cycle inhibitor that causes sheep nibbling it to die very quickly.

Two aspects of the occurrence of nodulated Australian phaseoloid legumes are as yet unexplained. First, the genus *Glycine* is best known for the species *G. max* (soybean), which is native to China, whereas the centre of diversity of the genus is in Australia. Second, the perennial genera *Hardenbergia* and *Kennedia* are Australian endemics, but how they got to Australia is unknown. *Kennedia* is also unusual in having indeterminate nodules (Sprent *et al.*, 2013).

IV. Longitudinal variations in the distribution of nodulated legumes

There are distinct differences in the legume floras at different longitudes. Few genera are native to all areas, *Indigofera* being one (Schrire *et al.*, 2009; Schrire, 2013). This section will concentrate on tropical regions – differences at higher latitudes have been mentioned in the previous sections.

There are major differences in the occurrence of nodulated legumes in the Old and New World floras. In the Old World, the only recorded nodulating caesalpinoid legumes are in the genera *Erythrophleum* and *Chamaecrista*. *Erythrophleum* has 10 species and is unusual for a nodulated caesalpinoid genus in that it is widespread, being found in several African countries, Madagascar, Australia and South East Asia. As elsewhere in the tropics, several herbaceous species of *Chamaecrista* are found in Africa. Mimosoid legumes are less common than in the New World and of these a higher proportion are unable to nodulate (see Section II and Fig. 5). However, in terms of numbers of nodulating species, former 'acacias' in the new genera *Vachellia* and *Senegalia* are particularly well represented in Africa. The monospecific *Chidlowia*, previously listed as being a typical nodulated caesalpinoid legume and now transferred to the Mimosoideae, is found in several West African countries. Papilionoids of all habits abound, ranging from huge buttressed trees, such as species of *Pterocarpus* (Dalbergieae), through to many lianas and shrubby and herbaceous genera. On the basis of a rather limited amount of evidence, we could find no detectable differences in the occurrence of non-nodulating papilionoids (Fig. 6) between the Old and New Worlds.

In the New World tropics, nodulated Caesalpinioideae are more common than in the Old World. Brazil is the centre of diversity of

Chamaecrista (Lewis *et al.*, 2005). Smaller genera include 10 species of *Campsiandra*, 26 species of *Dimorphandra*, seven species of *Jacqueshuberia*, one species of *Melanoxylon*, nine species of *Moldenhawera*, and 60–70 species of *Tachigali* (which now includes *Scerolobium*). Interestingly, these genera appear to have evolved at very different times, *Chamaecrista* and *Melanoxylon* being oldest at *c.* 50 Ma and *Tachigali* youngest at 10 Ma (Doyle, 2011). The latter is particularly interesting in that in lowland tropical forests species are co-dominant with papilionoids and mimosoids (Batterman *et al.*, 2013). This implies that, although their bacteroids are retained within fixation threads, they appear to be competitive with legumes having more ‘conventional’ nodule structures. There are also many non-nodulated caesalpinoid legumes in the New World tropics, for example *Bauhinia* and *Caesalpinia*. Large genera of mimosoids such as *Mimosa* and *Inga* have their centres of origin in the New World, where they continue to speciate (e.g. Pennington & Lavin, 2016). Other important woody mimosoids such as *Calliandra* and *Leucaena* are also common. Relatively speaking, papilionoids are less important, with notable exceptions such as *Andira* (Pennington, 2003) and many dalbergioid genera, including *Arachis*.

V. Evolution of legume nodulation

How legume nodulation evolved has been a subject of intense interest for decades, with major questions still unanswered, namely when, where and why? There has been much thought given to the groups of angiosperms in which nodulation occurs. The seminal paper of Soltis *et al.* (1995) laid the foundations, showing that nodulation is confined to a particular group of legumes and actinorhizal plants, the Rosid 1 clade. Considerable evidence has been provided that there is commonality between the symbiotic processes of legume and actinorhizal nodules, plus features in common with arbuscular mycorrhizal symbioses. Recent evidence suggests that there may be a set of common symbiotic proteins (CSPs) that could function in other interactions, such as the formation of nematode galls and fungal diseases. Homologues of CSPs are found in Charophytes and mosses and therefore may be extremely ancient (Genre & Russo, 2016). Further, the finding that the canonical nodulation genes *nodABC*, which encode the lipo-chito-oligosaccharide Nod factor backbone, are found in the actinorhizal microsymbiont *Frankia* (Persson *et al.*, 2015) opens up the possibility that rhizobia obtained them from this source.

When nodulation first evolved is a question that has given rise to much thought and argument (Doyle, 2016). There is a strong case for considering arbuscular mycorrhizas to be the mother of plant root endosymbioses: they have existed for 400 Myr and these symbioses are formed with 70–90% of land plant species (Parniske, 2008). Nitrogen-fixing root nodule symbioses are believed to have evolved within the Rosid 1 clade some 70 Ma (Doyle, 2011). Werner *et al.* (2014) proposed that a group of angiosperms evolved a predisposition towards the evolution of nodulation *c.* 100 Ma, but this paper has been strongly criticized because of the inaccurate information on the legume taxonomy used in it (Doyle, 2016; LPWG, 2017).

Perhaps more important is to consider whether there are several origins of nodulation among members of the Rosid 1 clade that have this predisposition. In his seminal paper, Doyle (2011) illustrated times at which various types of nodulated legumes seem to have first appeared. *Inter alia* it shows that some nodulating caesalpinoid genera (e.g. *Tachigali*) are relatively recent. The IRLC is also younger (*c.* 39 Ma) than older genera such as *Chamaecrista* (*c.* 50 Ma). So we cannot answer ‘when?’, but ‘where?’ is a question that we can partially answer. Schrire *et al.* (2005) suggested an area either side of the Tethys Sea, from where different legumes diversified into the Northern and Southern Hemispheres. This suggestion is still very relevant and consistent with the location of land masses *c.* 60 Ma.

There is no real consensus as to why nodulation evolved. In terms of numbers of species, nodulation has clearly been a successful strategy, as shown by the preponderance of nodulated legumes in the top 40 genera with the highest numbers of species (Lewis *et al.*, 2005). Together these account for well over half of all known legume species. Within them only *Senna* and *Bauhinia* cannot nodulate. The situation at the generic level is different, as there are many monotypic genera that cannot nodulate.

The ability of many legumes to nodulate may confer other benefits, enabling them to maximize available resources. The presence of nodules can modify root plasticity independent of any effects of nitrogen fixation (Goh *et al.*, 2016). Nodulated legumes appear to be particularly favoured by arid conditions (Pellegrini *et al.*, 2016). Adams *et al.* (2016) showed that nodulation can be correlated with greater water use efficiency in certain areas, an important property in the context of climate change, and Crisp *et al.* (2004) have also commented on the expansion of Australian legumes into more arid areas.

VI. Legume-nodulating bacteria – rhizobial diversity in a geographical context

The known diversity of rhizobia increases annually, and is the subject of several reviews, the most recent and comprehensive being that of Peix *et al.* (2015). It is not our intention to revisit this subject, nor the genetic basis of nodulation (Pueppke & Broughton, 1999; Perret *et al.*, 2000), the horizontal transfer of symbiosis-related genes (Remigi *et al.*, 2016) or the symbiovar concept (Rogel *et al.*, 2011), but instead to attempt to link, where possible, rhizobial genotypes with their geographical locations and/or legume tribes/genera.

At the time of writing, rhizobia consist of a diverse range of genera in the Alphaproteobacterial and Betaproteobacterial classes, and are termed ‘Alpha-rhizobia’ and ‘Beta-rhizobia’, respectively. Although there have been a few reports of rhizobial Gammaproteobacteria, these are unconfirmed (Gyaneshwar *et al.*, 2011; Moulin *et al.*, 2015). A phylogeny of the currently described and confirmed symbiotic rhizobial species is shown in Fig. 7. Although the number of so-far-described species does not currently reflect it (Peix *et al.*, 2015), in terms of the frequency of isolation by far the largest rhizobial genus is *Bradyrhizobium* (Bradyrhizobiaceae), members of which also nodulate the widest range of legume genera, as well as the nonlegume *Parasponia* (Parker, 2015). Among the

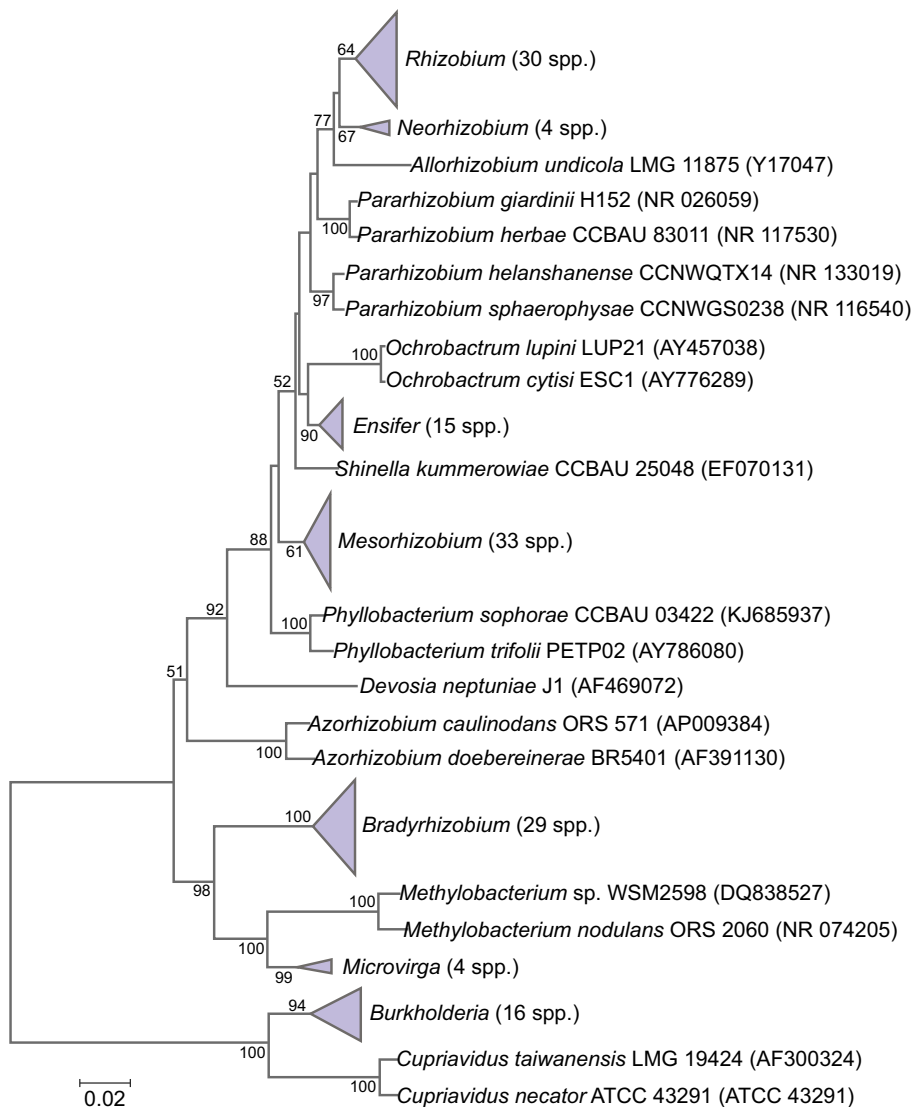


Fig. 7 Phylogenetic tree showing the relationships of currently described genera and species of Alpha- and Beta-rhizobia, based on aligned sequences of the 16S rRNA gene (1341-bp internal region). Phylogenetic analyses were performed using MEGA v.6 (Tamura *et al.*, 2013). The tree was built using the neighbour-joining method and the maximum composite likelihood model, using gamma distributed rates. A bootstrap analysis with 500 replicates was performed to assess the support of the clusters. The GenBank accession number is given for individual strains.

bradyrhizobia are a subgroup that nodulate some species of the wetland genus *Aeschynomene*, and which uniquely among the rhizobia use a Nod factor-independent infection process (Giraud *et al.*, 2007; Chaintreuil *et al.*, 2013). Bradyrhizobia are particularly dominant in Australia and in central South America, where they nodulate native legumes growing in the acidic and seasonally dry soils that predominate in these environments (Lafay & Burdon, 1998; Fonseca *et al.*, 2012; Stepkowski *et al.*, 2012). Bradyrhizobia are also the principal symbiont type isolated from nodulated 'basal' legumes in the old 'Caesalpinioideae', most of which are neotropical woody species (Fonseca *et al.*, 2012; Yao *et al.*, 2015). It has, therefore, been suggested that they are the ancestral symbionts of legumes, 'opening the door' for other rhizobial types as the legumes evolved (Parker, 2015). Indeed, in consideration of their dominance in some forest soils as nonsymbiotic saprophytes (Van Insberghe *et al.*, 2015), it can be hypothesized that bradyrhizobia were the first symbiosis-compatible Proteobacteria to be encountered by the newly evolving legumes as these plants started to spread across the globe.

Rhizobium and *Ensifer* (*Sinorhizobium*) in the family Rhizobiaceae are also very widespread, nodulating a large variety of legumes on all continents. *Rhizobium* is closely related to *Agrobacterium*, which is nested within it, but also to other pathogens and phytopathogens in the Rhizobiaceae (Peix *et al.*, 2015). This group of bacteria share the trait of harbouring their plant infection genes on mobile plasmids, symbiotic in the case of the rhizobia and tumour/hairy root-inducing in the case of phytopathogenic *Agrobacterium*. These plasmids can be exchanged, so that symbionts can become phytopathogens and vice versa (Cummings *et al.*, 2009; Remigi *et al.*, 2016). As with bradyrhizobia, *Rhizobium* and *Ensifer* are common in soils and are increasingly being isolated from nonlegumes (Peix *et al.*, 2015). In terms of environmental preferences, *Rhizobium* strains vary enormously, but *Ensifer* spp. are often found associated with legumes that are native to alkaline and semi-arid/saline soils (Yates *et al.*, 2004; Sankhla *et al.*, 2017 and references therein).

The third large genus in the Alpha-rhizobia is *Mesorhizobium* in the Phyllobacteriaceae. Like bradyrhizobia, many mesorhizobia do

not have sym-plasmids, but instead have mobile symbiotic 'islands' on their chromosome (Peix *et al.*, 2015). As with the above-mentioned genera, mesorhizobia are very widespread and nodulate a wide range of legumes. They are often common symbionts in legumes that prefer acidic soils (Lemaire *et al.*, 2015), but are also found as preferred microsymbionts of chickpea (*Cicer arietinum* L.) growing in alkaline soils in northwest China (Zhang *et al.*, 2012). The final 'classical' rhizobial genus is *Azorhizobium* in the Xanthobacteriaceae, but it is not so widespread, being quite specifically associated with a few species of the tropical genus *Sesbania*, most notably *Sesbania rostrata*, with which it forms photosynthetic stem nodules (James *et al.*, 1998; Fig. 1d). The symbiotic genes of azorhizobia are located on the chromosome. Unusually for rhizobia, *Azorhizobium caulinodans* has the ability to fix nitrogen *ex planta* (Dreyfus *et al.*, 1988).

Several 'nonclassical' symbiotic rhizobial strains have been confirmed in the Alphaproteobacteria over the last two decades, but the genera of all of these, except for *Methylobacterium* and *Microvirga* in the Methylobacteriaceae, are in the aforementioned families in the order Rhizobiales. These include *Aminobacter*, *Devosia*, *Ochrobactrum*, *Phyllobacterium* and *Shinella* (Peix *et al.*, 2015). In most cases they appear to be symbiotic with only one or two legume species that are already known to be associated with 'classical' rhizobia. *Methylobacterium* spp. and *Microvirga* spp. are the exceptions in that they appear to be highly specific symbionts of their legume hosts in the tribes Crotalariaeae and Genisteae (Renier *et al.*, 2011; Ardley *et al.*, 2012, 2013). *Microvirga vignae*, in contrast, is a microsymbiont of the promiscuous host *Vigna unguiculata* in a semi-arid region of Brazil (Radl *et al.*, 2014).

Beta-rhizobia consist of two genera: *Burkholderia* and *Cupriavidus*. Nodulating *Burkholderia* come exclusively from the nonpathogenic Plant Beneficial and Environmental ('PBE') cluster of this large and complex genus (Estrada-de los Santos *et al.*, 2016) and, although potential pathogens (e.g. *Burkholderia cepacia*-like organisms) have been isolated from nodules, none have yet been shown to be symbiotic. The nodulating burkholderias can be divided into two groups: South American strains with plasmid-borne sym-genes that nodulate *Mimosa* and other members of the Mimosoideae (Gyaneshwar *et al.*, 2011; Bournaud *et al.*, 2013), and South African strains with chromosome-borne sym-genes that nodulate diverse papilionoid species native to the Fynbos/Cape Core Subregion (Elliott *et al.*, 2007; Garau *et al.*, 2009; De Meyer *et al.*, 2016; Lemaire *et al.*, 2016). Both groups prefer acidic soils and higher altitudes, and probably originated as symbionts early in the evolution of legumes (Bontemps *et al.*, 2010; Bournaud *et al.*, 2013; Lemaire *et al.*, 2015, 2016). Their two centres of diversity are interesting, as they suggest that they had a common ancestor (Fig. 7), and the ancestors of the two groups probably diverged after the separation of South America from Africa. This is supported by the two locations having no currently described nodulating *Burkholderia* species in common, except for *Burkholderia tuberum*, which nodulates mimosoids in South America as symbiovar mimosae, and papilionoids in South Africa as symbiovar papilionoideae (Moulin *et al.*, 2015). In addition, apart from the notable exception of *B. phymatum* STM815^T (Moulin *et al.*, 2014; Lemaire *et al.*, 2016), the two groups of burkholderias cannot

nodulate each other's hosts (Gyaneshwar *et al.*, 2011; Lemaire *et al.*, 2016), which is not surprising, considering their highly divergent *nod* genes (and hence Nod factors) (De Meyer *et al.*, 2016; Remigi *et al.*, 2016).

Cupriavidus (formerly *Ralstonia*) *taiwanensis* was originally described as a symbiont of invasive *Mimosa* species in Taiwan, and has since been shown to have originated in the neotropics, probably acquiring its (plasmid-borne) symbiosis-related genes from its relative, *Burkholderia* (Amadou *et al.*, 2008; Gyaneshwar *et al.*, 2011; Moulin *et al.*, 2015; Remigi *et al.*, 2016). As its name suggests, *Cupriavidus* can be tolerant to heavy metals, such as copper, zinc and lead (Platero *et al.*, 2016 and references therein). Although generally less competitive than *Burkholderia* (Elliott *et al.*, 2009; Melkonian *et al.*, 2014), *Cupriavidus* can dominate as a *Mimosa* symbiont in environments with less acidic soils and/or which contain high (natural) concentrations of heavy metals. For example, new *Cupriavidus* species/strains are the sole symbionts isolated from native *Mimosa* species from mining areas in Uruguay (Platero *et al.*, 2016).

It should be noted that, although Beta-rhizobia have a very distinct set of hosts from Alpha-rhizobia, they are not mutually exclusive, with a number of promiscuous legumes (e.g. *Dipogon lignosus*, *Phaseolus vulgaris* and *Macroptilium atropurpureum*) able to nodulate with both types (Elliott *et al.*, 2007; Liu *et al.*, 2014; Dall'Agnol *et al.*, 2016). Moreover, it has been shown that even in legume genera noted for their very strong association with Beta-rhizobia, such as *Mimosa*, this association will break down in favour of Alpha-rhizobia if plant clades are separated between continents, and the clades subsequently evolve to adapt to nodulate with the 'local' rhizobia in soils that are radically different from their ancestral ones. This appears to have happened to *Mimosa* in India and Mexico, where the soils are considerably less acidic than in the main centre of diversity, central Brazil (Gehlot *et al.*, 2013; Bontemps *et al.*, 2016). Such a phenomenon may also occur within a biome over much shorter geographical distances, as has been observed in the South African Fynbos for the large endemic papilionoid genus *Aspalathus*, which is nodulated mainly by mesorhizobia/rhizobia, but contains a few species that prefer *Burkholderia* (Lemaire *et al.*, 2015, 2016).

Finally, although we have earlier expressed the opinion that the 'first' rhizobia were probably ancestral bradyrhizobia, *Burkholderia* may have at least an equal claim. In a study of Alpha- and Beta-rhizobial genomes, Aoki *et al.* (2013) concluded that the transfer of *nod* genes was from Beta-rhizobia to Alpha-rhizobia, on the basis that only the Beta-rhizobial genomes harboured both the *nodJ* genes and their nonsymbiotic paralogues (DRA-ATPase/permease genes). This is quite possible given the potentially ancient origin of symbiotic burkholderias, which has been estimated at > 50 Ma (Bontemps *et al.*, 2010), but does not explain why the vast majority of extant legume symbionts are Alphaproteobacteria. One possibility is that, shortly after a transfer of *nod* genes from Beta- to Alpha-rhizobia, there was a rapid expansion of legumes into environments that favoured Alpha-rhizobia more. It is known, for example, that *Burkholderia* predominate as symbionts in soils that are acidic and very poor in nutrients and often in montane environments, but that they can be outcompeted by other symbiont

types when concentrations of soil nutrients (particularly nitrogen) increase (Elliott *et al.*, 2009). This raises the possibility that Beta-rhizobia are effectively ‘relics’ of the original symbiont types that have been confined to very particular locations, and which have then co-evolved with legumes that have subsequently radiated out from these environments; the Brazilian Cerrado and the South African CFR are two excellent examples of this. There is no doubt that much further work on this fascinating question will be undertaken.

VII. Host plant control of rhizobia – the Inverted Repeat Lacking Clade (IRLC) legumes

The ‘temperate, herbaceous’ IRLC includes many economically important legumes such as common peas (*Pisum sativum*), lentils (*Lens culinaris*), chickpea, vetches (*Vicia* spp.), clover (*Trifolium* spp.) and lucerne (alfalfa) (*Medicago sativa*), as well as the model legume *Medicago truncatula* (Young *et al.*, 2011). This large group contains nearly one-third of all legume species, mainly because it includes *Astragalus*. Recent phylogenetic trees place *Glycyrrhiza*, *Callerya* and *Wisteria* as basal members of the IRLC, with the remaining members forming two large clades: one consisting of Hedysareae (including *Astragalus*) and the other containing Galega, Cicereae, *Trifolium*, Fabaeae and the ‘Trifolieae’ group which includes *Medicago*, *Melilotus* and *Trigonella* (Wojciechowski *et al.*, 2000; LPWG, 2013).

IRLC legumes evolved comparatively recently, with an estimated crown clade age of 39 Myr (Lavin *et al.*, 2005). Their centres of diversity are primarily in temperate regions of the Old World and they remain a predominantly Northern Hemisphere group (Wojciechowski *et al.*, 2000). While some *Trifolium* species are found in southern Africa and South America, they are not present in Australia; indeed, the endemic *Trigonella suavissima* is the only Australian representative of the Trifolieae (Brockwell *et al.*, 2010). However, tribe Astragaleae contains a number of indigenous Southern Hemisphere genera, including the endemic New Zealand *Carmichaelia*, *Montigena* and *Clanthus*, Australian *Swainsona* and South African *Lessertia* and *Sutherlandia*. Species of *Astragalus* are also found in South Africa (1) and South America (c. 110).

Symbiotic relationships in the IRLC are notable for (1) their symbiotic specificity and (2) the degree of control exerted by the host over the microsymbiont. Although *Bradyrhizobium* strains have been found in nodules of *Astragalus* (Laguerre *et al.*, 1997), the overwhelming majority of IRLC legumes are reported to nodulate with species of *Ensifer*, *Mesorhizobium* and *Rhizobium*. This specificity can be further refined: globally, nearly all *Medicago*, *Melilotus* and *Trigonella* spp. are nodulated by either *Ensifer medicae* or *Ensifer meliloti* (Béna *et al.*, 2005; Brockwell *et al.*, 2010), while *Trifolium* spp. and members of tribe Fabaeae are nodulated by *Rhizobium* spp. (Remigi *et al.*, 2016). This is in stark contrast to the situation in *Mimosa*, in which the microsymbiont varies according to edaphic conditions (see Section VI). Most studied IRLC legumes also have stringent requirements for their rhizobia to produce Nod factors with unsaturated fatty acyl chains (Debellé *et al.*, 2001).

The nodules of IRLC legumes are indeterminate, with interstitial cells (Fig. 2), and the symbiosomes of several well-studied species

display unique and characteristic features. Bacteroids of *Pisum*, *Medicago*, *Vicia* and *Oxytropis* are enclosed singly within each symbiosome, are greatly enlarged and often pleiomorphic in shape, and become terminally differentiated (i.e. they lose the capacity to grow and reproduce) (Mergaert *et al.*, 2006; Ampomah *et al.*, 2012). However, not all IRLC legumes have these features: *Biserrula pelecinus* symbiosomes, for example, have been shown to contain two to three nonswollen bacteroids (Nandasena *et al.*, 2004).

Terminal bacteroid differentiation (TBD) is under the control of the host plant and is governed by nodule-specific cysteine-rich (NCR) peptides that are similar to plant antimicrobial peptides (reviewed in Alunni & Gourion, 2016). These peptides were originally thought to be unique to IRLC hosts, until functionally equivalent NCR-like peptides, which are also associated with swollen bacteroids in the *Bradyrhizobium* microsymbionts, were found in *Aeschynomene* species (Czernic *et al.*, 2015). Apart from cell enlargement, terminal differentiation of bacteroids in IRLC legumes is associated with increasing permeabilization of the bacterial membrane and genome endoreduplication (up to 24C) (Mergaert *et al.*, 2006). Over 600 NCR genes have been detected in the genome of *M. truncatula* (Zhou *et al.*, 2013), suggesting fine-tuning of the process of bacteroid differentiation. TBD may be strain dependent, as *Mesorhizobium tianshanense* in symbiosis with the IRLC host *Glycyrrhiza uralensis* undergoes TBD (Montiel *et al.*, 2015), whereas *Ensifer fredii* strain HH103 does not (Crespo-Rivas *et al.*, 2016), implying that a particular rhizobial genotype is required for this type of host-mediated control.

Why do some (at least) IRLC legumes impose such severe controls on their rhizobial microsymbionts? It has been suggested that such control leads to more nitrogen fixed per unit of carbon utilized and also more plant dry weight produced per unit of nodule mass, thus providing the plant with an added fitness benefit (Oono & Denison, 2010; Kereszt *et al.*, 2011). In this regard, it is

Table 2 Outstanding problems/questions

We currently have no idea of the genetic basis of nodulation in legumes – chloroplast and nuclear genes do not provide answers consistent with known nodulation processes.
Why is there a preponderance of nonfixing legumes in the tropics, compared with temperate regions?
The different legume clades have different, and characteristic, nodule structures. Why and how have these evolved? Is one nodule type inherently more efficient in terms of N ₂ fixation than another?
Why have some legumes, notably those in the Inverted Repeat Lacking Clade (IRLC), imposed terminal differentiation on their rhizobia? Does terminal bacteroid differentiation (TBD) increase the efficiency of N ₂ fixation? What is the extent of TBD across the different legume phylogenetic clades and what are the environmental or genetic factors under which it operates? What selective pressures could lead to the evolution of TBD?
Why are some legume–rhizobia symbioses more specific than others and what governs the development of specificity? In legume phylogeny, why do we see a general trend of <i>Bradyrhizobium</i> being replaced by <i>Ensifer</i> , <i>Mesorhizobium</i> and <i>Rhizobium</i> strains as preferred microsymbionts?
What is the extent, and limit, of rhizobial diversity? What is the minimal chromosomal background required for evolution of a nonsymbiont to an N ₂ -fixing rhizobium? Can we modularize this process?

interesting to note that the rhizobial host range restriction peptidase (Hrrp), which can degrade a range of NCR peptides and improves bacterial proliferation and viability in *Medicago* nodules, can also inhibit nitrogen fixation (Price *et al.*, 2015). But this does not answer the question of why evolutionary pressures should have driven IRLC legume symbioses towards such an increase in the efficiency of nitrogen fixation. An answer may lie in the evolutionary biogeography of the IRLC legumes, which has taken place against the backdrop of numerous Northern Hemisphere glaciation cycles, beginning in the late Miocene and with the last glacial maximum occurring *c.* 17 500 BCE (Maslin *et al.*, 1998; Ray & Adams, 2001). Formerly glaciated areas are likely to be comparatively fertile, with possibly greater quantities of soil N. In such a situation, evolutionary fitness would accrue to those legumes that were competitive against plants that were able to derive N from soil, usually thought to be energetically less costly than nitrogen fixation (Vitousek & Field, 1999).

VIII. Conclusions and outstanding problems

The outstanding questions and problems that we now feel need to be addressed are listed in Table 2. The world is threatened by climate change, and global agricultural production will be required to double by 2050 to meet the projected demand for food and fibre. Currently, agricultural outputs rely heavily on the energy-intensive Haber–Bosch process to supply the reactive nitrogen that drives plant productivity. However, this comes at a high and environmentally unsustainable cost of fossil fuel use, greenhouse gas emissions and loss of biodiversity. A greater understanding of the range of legume–rhizobial symbioses, especially those found in harsh environments, is necessary to meet the challenge of providing the sustainable agricultural productivity that will be required in the 21st Century and beyond.

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