



From North to South: A latitudinal look at legume nodulation processes[☆]

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ABSTRACT

Legumes and some nodulation processes evolved about 55–60 Ma. Since then they have radiated from their origin at either side of the Tethys seaway, to high latitudes in both the northern and southern hemispheres. In many cases this has involved different tribes and genera, and different nodule processes, but with the common feature that almost all legumes in the higher latitudes are potentially nodulated and, with the exception of some herbaceous species of *Chamaecrista*, nodulated caesalpinoid legumes are rare. This is not true of tropical regions where all three sub-families are found, with many of their species lacking the ability to nodulate. Whether or not this is related to the availability of combined nitrogen is a matter of current discussion. This review will consider the range of nodulation phenotypes (infection, morphology, structure) and show how many of these are confined to one or other hemisphere. How this might relate to the different genera and species of endophytic (nitrogen fixing) rhizobia in relation to soil conditions will also be discussed. Better knowledge of the wide variation in nodulation parameters is important for understanding the ecology of different regions and for management purposes. Nodule characters are of great potential use in defining taxonomic groupings in legumes.

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1. Introduction

One of the best known features of the Leguminosae is the ability of many of them to associate with soil bacteria, collectively known as rhizobia, which invade and colonise roots (rarely stems), forming specialised organs known as nodules. Within the nodule, the bacteria reduce (“fix”) nitrogen to ammonia that is passed over to the host plant for assimilation into organic compounds such as amino acids and nucleotides. Recent intensive studies on the so-called “model” legumes, *Medicago truncatula* Gaertn. and *Lotus japonicus* (Regel) K. Larsen have led to major advances in our understanding of how rhizobia interact with their hosts and induce the formation of nodules (Oldroyd et al., 2011). An unforeseen consequence of this excellent work is that it is often assumed that all legumes are infected via root hairs and form nodules of one of two types, having determinate (*Lotus*) or indeterminate (*Medicago*) growth. A further assumption that has been made is that any swelling on the root of a legume that looks like a nodule, is a nodule. The literature contains numerous false reports of nodulation of this type, mainly on caesalpinoid legumes such as *Eperua* Aubl. and *Mora*

Schomb.ex Benth. (Sprent, 2001) but also papilionoids such as *Pickeringia* Nutt. ex Torr. & A. Gray. The latter was reported to nodulate in the major work of Allen and Allen (1981) and because of its tribal placement (Thermopsidae) at the time, the report was not queried. However, when the genus was moved into the *Cladrastis* clade (Cordoso et al., 2012, the other members of which do not nodulate) we checked fresh material and found that the bumps on roots did not contain bacteria and had none of the structures that are associated with root nodules. So what are these features and how do they vary? The following sections give a brief summary – more details can be found in Sprent (2009) and over the next year or two will be entered on the new interactive database ILDON (Interactive Legume Database Of Nodulation: www.ildon.org.)

1.1. Morphological features of nodules

Corby (1988) gave detailed descriptions of the then known morphological types of nodule and grouped them according to legume taxonomy as it was understood at the time. His work is still a major source of information and his observations foreshadowed some later taxonomic changes, for example he noted that *Lotononis* (DC.) Eckl. & Zeyh. section *Listia* had nodules that circled their subtending root (as do *Lupinus* L. nodules), whereas nodules from other species in the genus were indeterminate. Using completely different criteria, *Listia* (E. Mey.) B.-E. Van Wyk. has now been reinstated as a separate genus (Boatwright et al., 2011). There are now no known exceptions to nodule morphology being consistent at the generic level. Fig. 1.1 illustrates the currently recognised range of nodule morphology.

[☆] Dedicated to Dr HDL (Tom) Corby, a pioneer of the study of legume nodule morphology in a taxonomic context, who died on the first day of the Johannesburg conference, a few weeks short of his 100th birthday.

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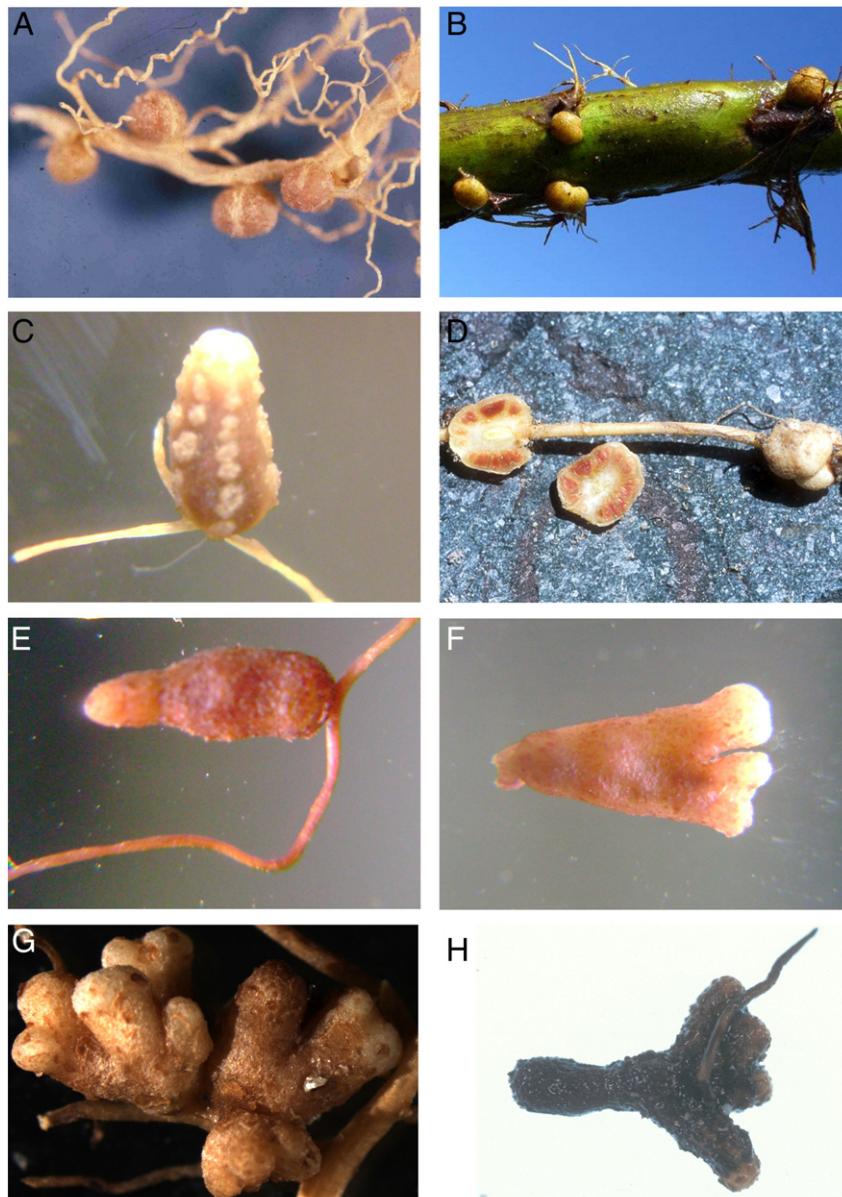


Fig. 1.1. Legume nodule morphology: A, determinate, desmodioid. These nodules are more-or-less spherical and have lenticels, usually as stripes, but occasionally as stars (see C); B, determinate, aeshynomenoid. These nodules are always associated with lateral or adventitious roots and do not have lenticels; C, Indigoferoid, rather like an indeterminate desmodioid nodule, its detailed structure has not yet been examined and has only so far been reported from Indigoferae; D, lupinoid; E, indeterminate unbranched nodules. Common in mimosoids and other groups; F, indeterminate with one or few branches, common in many papilionoids; G, indeterminate with many branches, found in all sub-families and (in some genera such as *Ormosia* and *Crotalaria* branching may be more than shown here. Note that many nodules of types F and G are unbranched when young; H, woody, most nodules that have fixation threads (see 1.3.D), both caesalpinoid and papilionoid, have a woody scleroid outer layer when mature. Reproduced from ILDON, with permission.

1.2. Modes of bacterial infection

There are basically three ways in which rhizobia gain entry into their hosts. The most common and best understood is root hair infection, where bacteria gain entry into (usually) curled root hairs and host cell wall material grows around the developing infection, forming what is known as an infection thread. This grows through the cortex of the root, branching repeatedly. At the same time cell divisions occur in the root cortex and some of the resulting daughter cells become invaded by branches of the infection thread. Bacteria are released from the tips of these infection threads, into host-membrane-bound units called symbiosomes. Within the symbiosomes, the bacteria differentiate into their N_2 -fixing form, in which they synthesise the nitrogenase enzyme, and are then known as bacteroids. There are a number of variants on this pattern, leading to the formation of either

determinate or indeterminate nodules. Within the former (which have evolved on two separate occasions, see Doyle, 2011) the products exported from N_2 fixation may be either ureides (phaseoloid legumes) or amides (many Loteae), see Sprent (2001).

The second mode of bacterial infection is found in dalbergioid legumes. A defining feature of this clade of legumes (Lavin et al., 2001) is the aeshynomenoid nodule, first described by Corby (1988). However, although Corby carefully drew these nodules with their associated lateral (occasionally adventitious) roots, he did not comment on this. Structural studies on the formation of aeshynomenoid nodules show that infection occurs at the sites of lateral root emergence, rather than by root hair curling, and infection threads are very rarely seen and then not in the infected region (e.g. James et al., 2001). The internal structure of the nodules, although they are determinate, is quite different from those formed following hair

infection (Section 1.3). Thirdly, some legumes, such as lupins and other members of the Genisteae, as well as the Crotalariaeae, are infected directly through the root epidermis. As with the dalbergioid legumes, infection threads are never formed in these nodules. Fig. 1.2 illustrates these infection modes.

1.3. Anatomical features of nodules

Although nodules may look similar from the outside, they can be quite different inside. For example the indeterminate nodules of Trifolieae and Fabaeae have a mixture of infected and uninfected

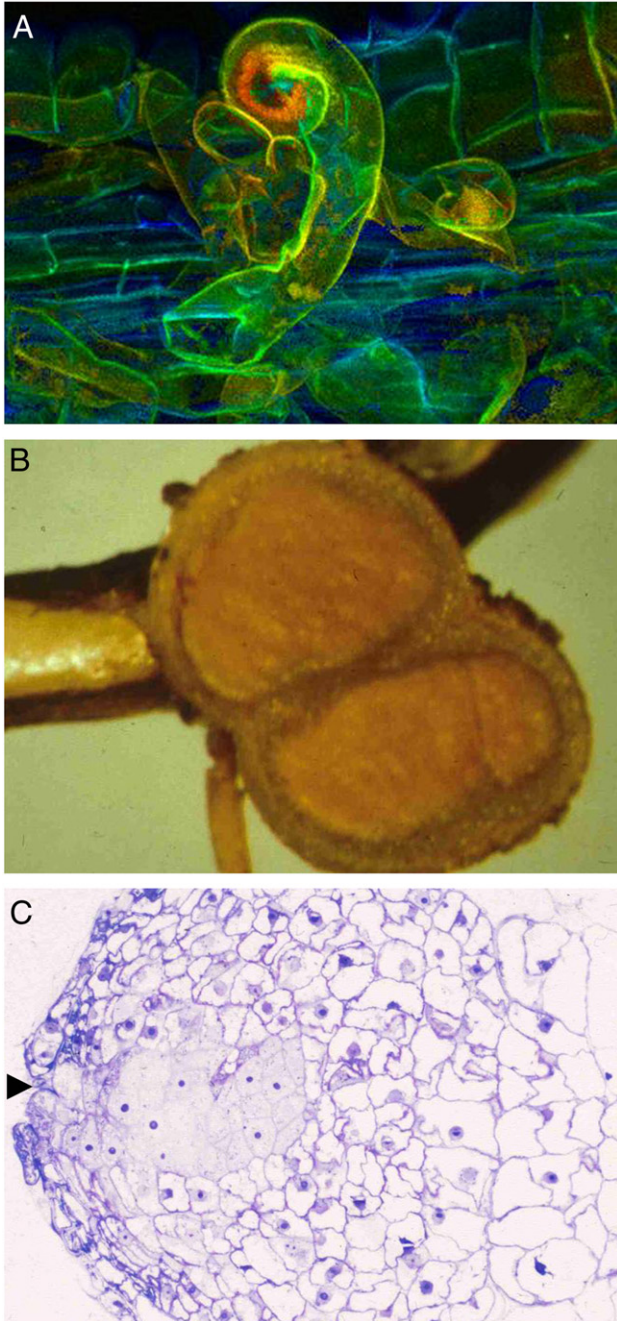


Fig. 1.2. Modes of infection. A, via root hairs with infection threads carrying bacteria to individual cells of the nodule primordium; B, crack infection, when rhizobia enter at the 'crack' where lateral or adventitious roots emerge. Infection threads not formed. Always found in aescynomenoid nodules; C, epidermal infection when bacteria enter between epidermal cells. Infection threads not formed. Occasionally infection thread-like structures are seen in early infections of types B and C, but these are not functional. Reproduced from ILDON with permission.

cells in the central region (as do all nodules described from mimosoid and caesalpinoid legumes), whereas in those of the Genisteae and Crotalariaeae that have been studied, such as *Lupinus* L., *Cytisus* Desf., *Listia* E. Mey., *Leobordea* Del., *Lotononis* (DC.) Eckl. & Zeyh. and *Crotalaria* L., the central tissue is uniformly infected. Determinate nodules of phaseoloid legumes and *Lotus* also have a mixture of infected and uninfected cells in their central tissue, (Sprent, 2009 and Fig. 1.3AB).

1.4. Structure of infected cells

The majority of legumes release their bacteria into symbiosomes (exceptions are noted below), but the latter vary considerably with no obvious phylogenetic grouping. Most commonly there are two or more bacteroids per symbiosome; these are of a similar size to their free-living ancestors and they retain some viability. It is quite common for them to have inclusions of the storage compound poly- β -hydroxybutyrate. (PHB) Trainer and Charles (2006) concluded that PHB was a feature of determinate, rather than indeterminate nodules, but Sprent (2009) pointed out that many indeterminate nodules may also have PHB, that not all determinate nodules have it and that this compound is often associated with nodules not being fully effective. The IRLC clade, which will be considered in detail later, has only one, greatly enlarged and pleiomorphic bacteroid per symbiosome and these bacteroids are not viable (i.e. they are unable to reproduce). The host plant controls which type of infected cell is formed. For example the same rhizobial strain produces rod shaped bacteroids in a phaseoloid type of nodule on *Vigna unguiculata* (L.) Walp. and enlarged spherical bacteroids in uniformly infected tissue on *Arachis hypogaea* L. (Sen and Weaver, 1984). In all caesalpinoid legumes, except herbaceous species of *Chamaecrista* Moench and in a few papilionoids, most notably tribe Brongniartieae, considered later, but also *Ormosia* Jacks, *Andira* Lam. and *Hymenolobium* Benth. bacteroids are retained within modified, often thin-walled infection threads, called fixation threads. These various structures are illustrated in Fig. 1.3CDEF.

1.5. Range of rhizobia that are found in nodules

Until relatively recently rhizobia were classified as either fast or slow growing, but as more sophisticated biochemical and molecular techniques have become available, many families and genera have now been defined. Fig. 1.4 shows the relationships between the currently recognised genera of legume nodulating bacteria. Almost every monthly issue of IJSEM, the International Journal of Systematic and Evolutionary Microbiology, has a new species described in it. In order to be accepted as a nodulating species, Koch's postulates have to be established, i.e. the strain of bacterium must be shown to be capable of nodulating the host species from which it was isolated and then be re-isolated from it. Nodules often contain endophytic bacteria that are not capable of nodulating their host (e.g. Li et al., 2008). All rhizobia properly established as able to nodulate and fix nitrogen with one or more species of legume belong either to the α or β branches of the Proteobacteria. The latter have only come to prominence in the last decade and are especially important in some tropical habitats and with certain genera, such as *Mimosa* L. The most common β rhizobial genus, *Burkholderia*, also has both plant and animal pathogens in it, which has caused a certain amount of panic from those worried about biosecurity, even though the symbiotic forms are clearly in a separate branch from the pathogenic ones and may be classified into a separate genus (Estrada-de Los Santos et al., 2013; see also review by Gyaneshwar et al., 2011). One strain of bacterium may nodulate several hosts and conversely one host may be nodulated by several different rhizobia, including in some cases both α and β forms. In other cases there is strict specificity between a rhizobial strain and a host genotype. Further general details can be found in Sprent (2009; although this is now rather out-of-date on the number

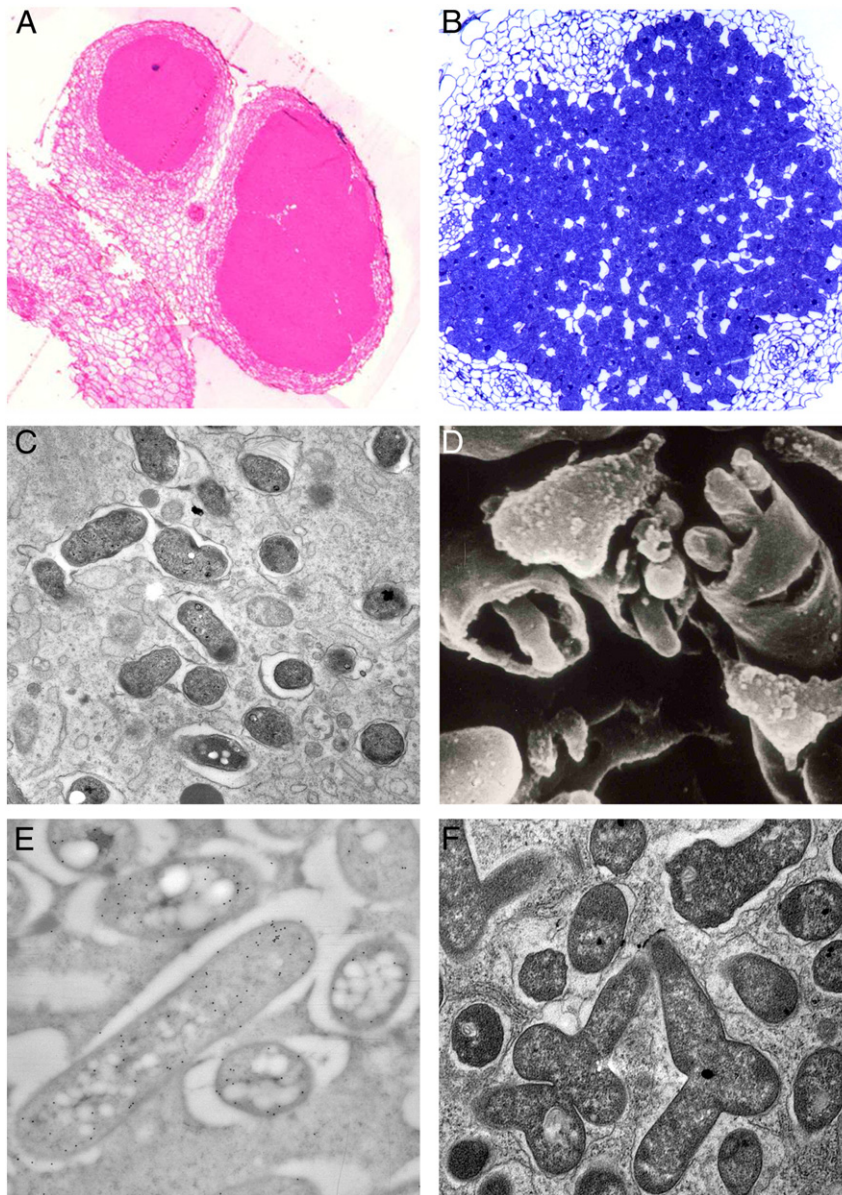


Fig. 1.3. Internal features of nodules. A, central infected tissue uniform; B, central tissue contains a mixture of infected and uninfected cells. C, bacteria located in symbiosomes; D, bacteria retained within fixation threads, which are modified infection threads from which bacteria are not released into symbiosomes. E, bacteroids not terminally differentiated. They can be seen in both longitudinal and transverse section, being basically rod-shaped; F, terminally differentiated bacteroids, greatly enlarged and pleomorphic. Reproduced from ILDON with permission.

of rhizobial genera and species). Biogeographical patterns of distribution of bacterial symbionts are beginning to emerge and a few examples of these will be given later.

1.6. The world's land masses and their movement through time

Fig. 1.5 shows what the world's landmasses are thought to have looked like at about the time legumes first evolved, at either side of the Tethys Seaway (Schrire et al., 2005). It can be seen that the northern hemisphere consisted of land masses that were close to each other and this has not changed appreciably with time, although climatic conditions have. In particular, there has been a series of glacial maxima, the last being about 18,000 years ago and this led to soils being more fertile than in other areas. Below the equator there were four main land masses plus what is now Antarctica. Of these, South America has moved slightly to the west, and a few million years ago became linked to North America. Africa moved

north, although there was not a great deal of room for manoeuvre. To the right of Africa is a piece of Asia, the Indian Plate, that moved north and collided with the main part of Asia, forming the Himalayas. The big question mark lies over Australia. It will be argued later that there may have been a land link between Australia, via Antarctica to South America. Since then Australia has moved slowly northwards towards Asia and there is now considerable evidence of exchange of legumes (and other flora) between Asia and Australia (Sniderman and Jordan, 2011). How these movements may be related to the evolution of nodulation in legumes as summarised in Doyle (2011); Fig. 1.6 will be discussed in subsequent sections.

2. The northern hemisphere

The Inverted Repeat Lacking Clade (IRLC) probably evolved about 39 Ma (Lavin et al., 2005). It is one of the largest clades in terms of number of species, around 4500, partly because it includes *Astragalus* L. with

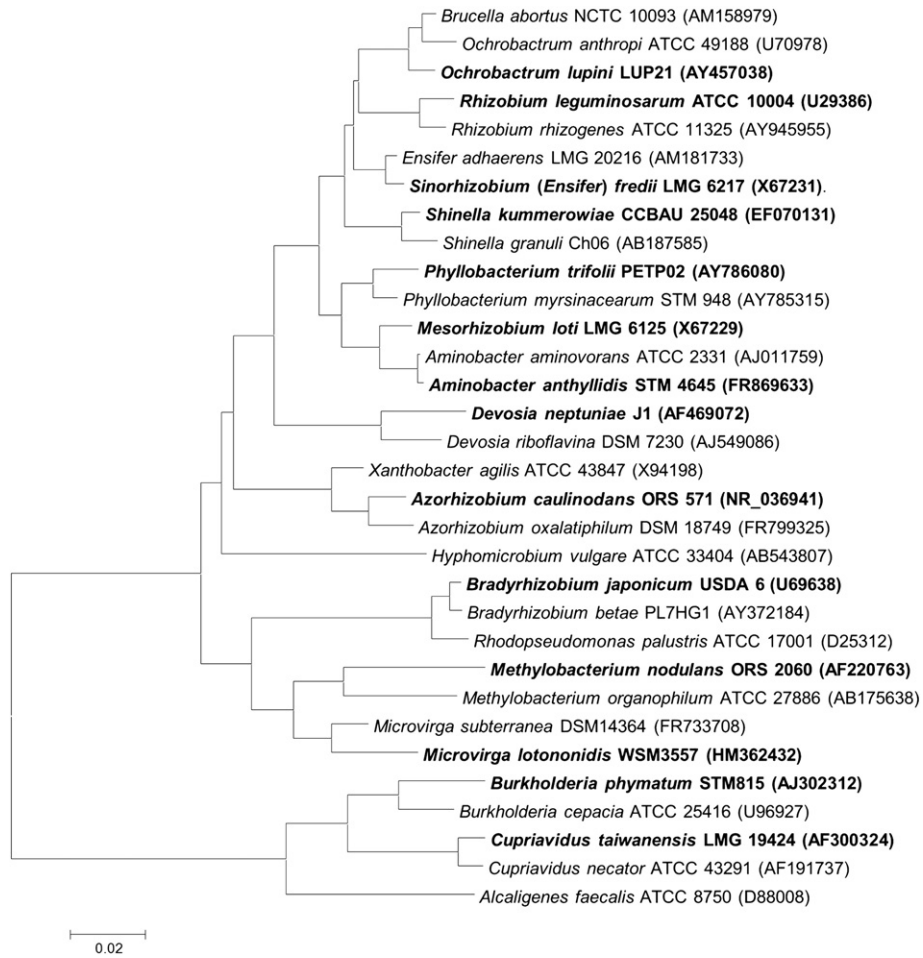


Fig. 1.4. Neighbour-joining phylogenetic tree, showing the relationships of selected genera of Alphaproteobacteria and Betaproteobacteria based on a 1342 bp alignment of the 16S rRNA genes. Rhizobial strains are in bold. Currently 14 genera of bacteria contain validly described species that are capable of eliciting nodules on legumes. GenBank accession numbers are in parentheses. Scale bar: 2% sequence divergence (two substitutions per 100 nucleotides).

about 2300 species. Its members, which are in tribes Galegeae, Hedysareae, Cicereae, Trifolieae, Fabae and the genus *Wisteria* Nutt. are largely found in the northern hemisphere although there are some Galegeae in the southern hemisphere and some species from the Trifolieae and Fabae went to South America 3–4 Ma

(Schaefer et al., 2012). In the few genera and species that have been studied in sufficient detail, the symbiosomes have a number of unique features (Kereszt et al., 2011). Firstly there is a high degree of endoreduplication in the bacteroids (and in the host cells) resulting in them being greatly enlarged. They are also usually

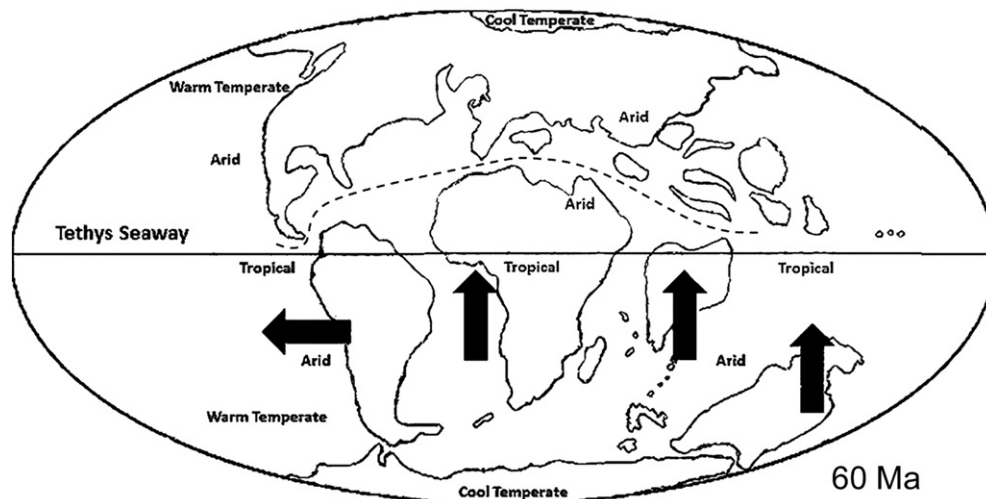


Fig. 1.5. Outline of where the world's land masses were at about the time when legumes evolved (60 Ma). Arrows indicate the direction of movement of the major masses below the equator. Modified from Schrire et al. (2005) Dotted line indicates the Tethys Seaway.

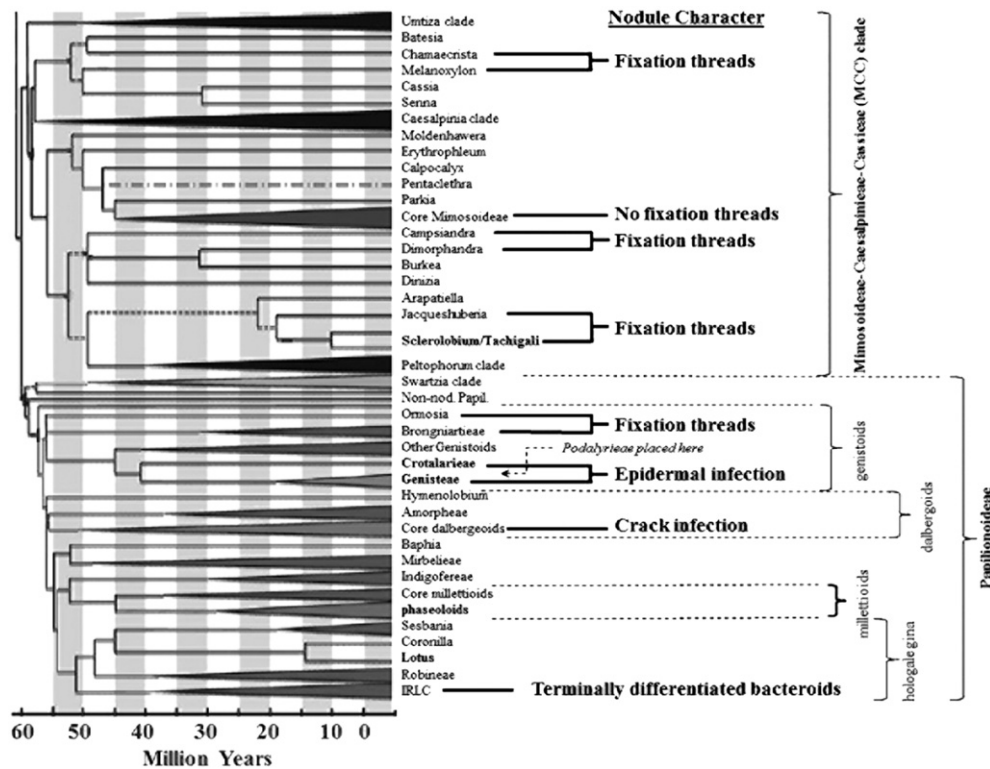


Fig. 1.6. Relationships between major legume groups and their time of evolution in relation to nodulation characters. Modified from Doyle (2011).

pleiomorphic, taking on the X and Y shapes, thought to be typical of bacteroids in general in much of the early work on nodule structure, which was largely based on temperate IRLC legumes such as clovers and peas. Bacteroids occur singly in each symbiosome, with the membrane very closely wrapped around them. The membrane is also different from those of other nodules, such as soybean, in that it is leakier and contains specific cysteine-rich peptides. These peptides resemble the defensin-like antimicrobial peptides, known to be involved in both plant and animal innate immunity. This helps the host plant achieve complete dominance over the bacteroids, which lose viability and are unable to carry out most normal metabolic functions, effectively becoming auxotrophic ammonia factories, with the necessary metabolites for the nitrogenase reactions being supplied by host cells (see review by Terpolilli et al., 2012). Since nodules in the IRLC clade are indeterminate, the less mature regions contain many undifferentiated bacteria that can be released into the soil to act as inoculants for other plants, so that loss of bacteroid viability is not a problem.

Does this extreme differentiation of bacteroids result in improved fitness? It has been shown, using various mutants (see review by Kereszt et al., 2011) that in *Pisum sativum* L., nodules fix more nitrogen per unit of carbon utilised and also produce more plant dry weight per unit of nodule material than the same strain of *Rhizobium leguminosarum* nodulating *Phaseolus vulgaris* L. whose bacteroids are not terminally differentiated. Since members of the IRLC clade are mainly found in former glaciated areas (see Section 1.6) they are likely to have access to soil N, possibly in greater quantities than elsewhere. For nodulation and nitrogen fixation to be a good option for the legumes, they may need to be particularly efficient in competing with assimilation of soil nitrogen, usually thought to be energetically less costly than nitrogen fixation. In that there are no known exceptions to nodulation in the IRLC clade and that nodulation is widespread in the field (see for example Ampomah et al., 2012), nodulation would appear to be an advantage. However, it is not known when the terminally differentiated bacteroids evolved in relation to the various glaciations that have

occurred in northern high latitudes. Evidence to date suggests that the rhizobia nodulating legumes in this area are mainly fast growing α -rhizobia.

3. The southern hemisphere

In Fig. 1.5 it can be seen that the major land masses in the southern hemisphere were widely separated when legumes first evolved and to a large extent they still are, in spite of the significant northerly movement of the Asian fragment and of Australia. Apart from Antarctica, large scale glaciation has not occurred and many of the soils are old and impoverished. Although parts of South Africa and Australia have very similar soils and complain about the ease with which they house each other's weeds, their native floras show major differences. South America's native sub-tropical and temperate flora is very different from either. This section will concentrate on two biodiversity hotspots, the Cape Floristic Region of South Africa (CFR) and the South West Australia Floristic Region (SWAFR). Both of these have very poor soils, both have legumes as a major component of their floras and almost all of these are potentially nodulated, inferring that nodulation is a useful attribute. Where they differ is that the CFR has a wide diversity of papilionoid, but no endemic mimosoid legumes, although these are widespread in other areas of the country, whereas the SWAFR has numerous mimosoid (mainly acacia) and papilionoid species.

3.1. The CFR

This houses several different legume tribes, but here we concentrate on two, the Crotalariae and Podalyrieae, because they have distinct and interesting nodulation characters.

Crotalariae currently consists of 15 genera (Table 1). Apart from the genus type, which is found in all continents (Le Roux, 2011), all are exclusively African and some, including *Aspalathus* L., are largely confined to the CFR. With the exception of *Listia* where they are lupinoid, nodules on all genera are indeterminate, usually branched

Table 1

Genera and numbers of species in tribe Crotalariaeae. Note that all are from southern Africa, except about 114 spp. of *Crotalaria* (Le Roux, 2011).

Genus	Total species
<i>Aspalathus</i>	278
<i>Bolusia</i>	5
<i>Calobota</i>	16
<i>Crotalaria</i>	690
<i>Euchlora</i>	1
<i>Lebeckia</i>	35
<i>Leobordea</i>	51
<i>Listia</i>	7
<i>Lotononis</i>	91
<i>Pearsonia</i>	13
<i>Rafnia</i>	19
<i>Robynsiophyton</i>	1
<i>Rothia</i>	2
<i>Wiborgia</i>	10
<i>Wiborgiella</i>	9

and where known (*Aspalathus*, *Lebeckia* Thunb., *Leobordea Lotononis*), have an epidermal infection with no infection threads and with uniformly infected central tissue (Fig. 1.3A). The latter poses some interesting questions, since the uninfected cells of legumes such as soybean are known to play a vital role in the assimilation and export of products of nitrogen fixation (by symplastic movement) as well as being the route by which carbon compounds such as sucrose are passed to the infected cells (by apoplastic movement: Brown et al., 1995 and references therein). Crotalarioid nodules appear to be very effective in fixing nitrogen (e.g. Ardley et al., 2013) so how do they manage without these uninfected cells? Within this tribe there is also great variation in nodulating bacteria. Even in *Crotalaria* there are species that are nodulated with low-specificity slow-growing bradyrhizobia and others that are nodulated by highly specific methylobacteria (α -rhizobial) (Sy et al., 2001). More recently, Ardley et al. (2012) have described *Microvirga lotononidis* and *Microvirga zambiensis*, two highly specific α -rhizobial species from nodules of *Listia angolensis*. *Lotononis* and *Leobordea* nodulate with species of *Bradyrhizobium*, *Mesorhizobium*, *Methylobacterium* and *Sinorhizobium* (Ardley et al., 2013). *Lebeckia* is nodulated by several species of the β -rhizobium *Burkholderia* (Howieson et al., 2013). So overall, there is more diversity in rhizobial endophytes within this one tribe than there appears to be in the whole of the ILRC clade in the northern hemisphere.

Podalyrieae is a small tribe of 8 genera, with the possibility of more being added (Table 2). Taxonomically it is placed in the core genistoid group (Van Wyk, 2005). All except *Calpurnia* are endemic to the CFR and we have been studying them for some years. *Cyclopia* Vent., species of which are used to make honeybush tea, was found to be nodulated by the β -rhizobial species *Burkholderia tuberum* (Elliott et al., 2007). We have found all other genera tested, except *Calpurnia* E. Mey. also to be

Table 2

Tribe Podalyrieae and possible relatives: nodulation of one or more species with the β -rhizobium *Burkholderia tuberum*.

Genus	Number of species	Nodulation with <i>B. tuberum</i>
<i>Amphithalea</i> Eckl.&Zeyh	42	+ve
<i>Calpurnia</i> E.Mey.	7	No nodules formed
<i>Cyclopia</i> Vent.	23	+ve
<i>Liparia</i> L.	20	+ve
<i>Podalyria</i> Willd.	19	+ve
<i>Stirtonanthus</i> B-E Van Wyk & A.L. Schutte	3	+ve
<i>Virgilia</i> Poir.	2	+ve
<i>Xiphotheca</i> Eckl.& Zeyh.	9	+ve
<i>Hypocalyptus</i> Thunb.	3	+ve
<i>Cadia</i> Forssk.	7	No nodules formed

nodulated by this species, as is *Hypocalyptus* Thunb. (a genus currently given its own tribe, but also endemic to South Africa) but not *Cadia* Forssk., that has been suggested to belong to Podalyrieae (Boatwright et al., 2008). Whether these podalyrioid legumes can also be nodulated by other rhizobia is not yet clear. However, their nodulation processes (root hair infection, indeterminate growth, and central tissue containing both infected and uninfected cells (Fig. 1.7) are not consistent with placement in the core genistoid group, where the Genisteae (Sprent, 2009) as well as the Crotalariaeae have non-hair infection and uniformly infected central tissue.

3.2. The SWAFR

This area has three major families, Myrtaceae, Leguminosae and Proteaceae. Apart from species of *Acacia* Mill., the legumes fall into three groups, tribe Brongniartieae, mirbelioids (tribes Mirbelieae + Bossiaeeae which are currently being rearranged (LPWG, 2013)) and Phaseoleae. All that have been examined are able to nodulate. For example, Lange (1961) sampled nodules from 59 species in 18 genera of mirbelioids, six species each from two genera of Brongniartieae and two genera of Phaseoleae. The numerous strains recovered were tested, not on their hosts of isolation, but on a number of species in what were then called cross-inoculation groups. All isolates were slow-growing and nodulated species such as *Vigna sinensis* (L.) Savi ex Hassk. Thus they were likely to be bradyrhizobia, a genus that was found to nodulate some of the same genera in Southeastern Australia (Lafay and Burdon, 1998). Lawrie (1983a) found both fast and slow-growing rhizobia in native legumes from the state of Victoria. These were fully authenticated, the first time this had been done on Australian native legumes. More recently Stepkowski et al. (2012) carried out a wide ranging study comparing bacteria nodulating native legumes from temperate Australia (in fact all were from Western Australia) and from tropical monsoonal Australia. Included in the temperate genera were 16 species of mirbelioids – none of which were present in the monsoonal area: most of the legumes there are from genera whose ancestors had probably migrated from Asia (Sniderman and Jordan, 2011). All isolates from the temperate areas were bradyrhizobia with distinct features and all were properly authenticated on their hosts. In an earlier paper Lawrie (1981) found field grown nodules to be very variable in terms of effectiveness. Apart from one paper on *Aotus ericoides* (Vent.) G. Don (mirbelioid; Lawrie, 1983b), there is no published information on infection processes, nodule morphology or structure in either mirbelioids or Australian Brongniartieae. In *Aotus*, it was inferred that there was root hair infection, but no conclusive evidence was given: growing nodules were indeterminate, branched and with central tissue containing both infected and uninfected cells. The unfortunate state of ignorance of nodule characters from SWAFR legumes reflects the fact that native papilionoid legumes are of no commercial interest and the main reason for studying their rhizobia has been to find out whether they nodulate exotic legumes being developed for agricultural use (e.g. Yates et al., 2004).

Tribe Brongniartieae as now understood consists of 16 genera (Table 3) with more likely to be added soon. Geographically they are found in Australia, South America, Central America and the Caribbean. *Cyclobium* Benth. and *Poecilanthus* Benth., were earlier placed in Millettieae where they appeared to be anomalous on nodule grounds, because their bacteroids were retained in fixation threads (De Faria et al., 1987). After these genera were moved to Brongniartieae (Ross and Crisp, 2005), we decided to look at the structure of nodules from Australian genera. So far we have examined *Hovea* R.Br. ex W.T. Aiton and *Templetonia* R. Br. ex W.T. Aiton and as both have fixation threads, similar to those of *Cyclobium* and *Poecilanthus* (a genus currently being revised) (Fig. 1.8), we are tentatively suggesting that this may be a tribal character. In view of

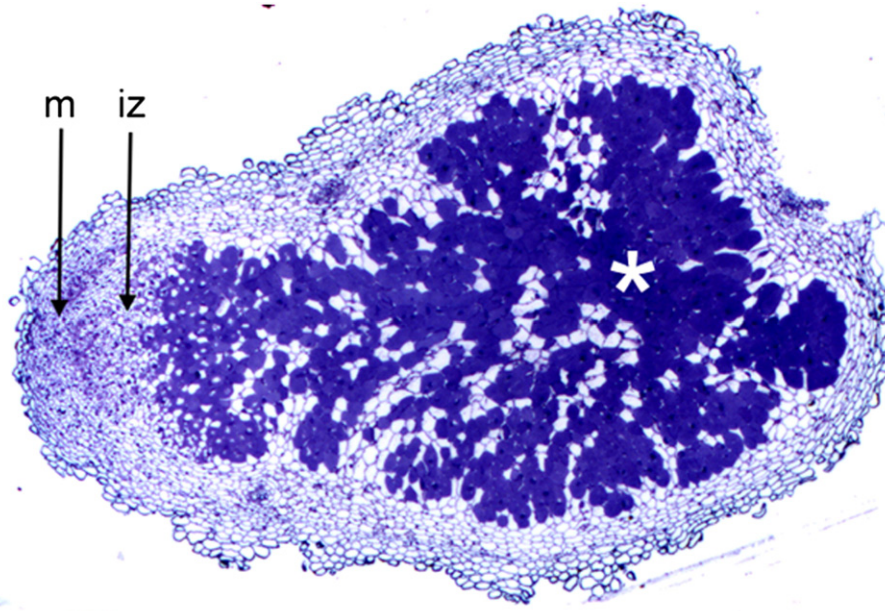


Fig. 1.7. Typical nodule of legumes from tribe Podalyrieae, as shown by *Podalyria calyprata* (Retz.) Willd. Note the apical meristem (m), invasion Zone (iz) and central tissue (asterisk) that contains both infected and uninfected cells.

their geographical distribution, we suggest that they may have arisen in what is now Antarctica and from there spread eastwards into Australia and northwards via South America (see Fig. 1.4). Further, we note the comment of Schrire et al. (2005) that “the crown clade of Mirbelieae/Hypocalypteae is remarkably old in legume terms, 55 Ma”. Taken together with the current distribution of the mirbelioid legumes, it suggests that this group, which is confined to Australia, evolved in the island continent before it was in contact with other land masses, but possibly after it had separated from mainland Antarctica. How it (or the Brongniartieae) got there from the Tethys Seaway is another question, but long-distance dispersal followed by speciation is possible.

There has been much attention given to the ways in which plants of the SWAFR cope with their impoverished soils, with most of the experimental data being on phosphorus and its acquisition in non-legumes (Lambers et al., 2011). However, many of the anatomical features of these plants are also found in nodulated legumes, for example sclerophyllous leaves (also common in legumes from the CFR) and protection against herbivores by structural means (thorns and prickles, high leaf mass per unit area). In addition, rather than using N-based chemical defences, they may use C-based ones, such as fluoroacetate (e.g. *Gastrolobium* R. Br. ex W.T. Aiton; Crisp et al., 2005). Many have cluster roots (Adams et al., 2002) as well

as being dual mycorrhizal (i.e. having both ecto and arbuscular mycorrhizas) (Sprent, 1994). Taken together, these features enable nodulated legumes to be important components of the SWAFR flora. The CFR legumes are arbuscular mycorrhizal and many have cluster roots (Sprent, 2009), but to our knowledge none is also ectomycorrhizal.

4. Chaos in the tropics

To many people the tropics are dominated by rain forests, but the largest fraction of the whole region is semi-arid to arid. Nodulated legumes may be very important in these areas, although the amount of nitrogen fixed is very variable (reviewed by Sprent and Gehlot, 2011). Many are also biodiversity hotspots, but because there is insufficient evidence about the role of nodulated legumes in overall ecosystem functioning, they will not be considered further here. On the other hand there has been a great deal of work carried out in rainforests. Here there is a pronounced longitudinal dimension, compounded by a great lack of information from some areas. Tropical South-East Asian forests are largely dominated by dipterocarps, with legumes being scarce. Other parts of Asia, such as India, have many native legumes, but not in rainforests. Sprent (2009) collated data from Borneo, West Africa and South American rainforests, where the information was sufficiently robust, and found that in all three, caesalpinoids were dominant: most cannot nodulate, but more are potentially nodulated in South America than the other two areas. In Africa, there are more non-nodulating mimosoids and papilionoids than in the other regions. Why is this? The situation is compounded by the fact that some genera, at least in the American rainforests have the ability to nodulate but may not do so in particular conditions (e.g. Barron et al., 2011; Hedin et al., 2009). Added to the fact that tropical rainforest areas are often rich in nitrogen, the need for nitrogen fixing organisms (nodulated legumes or other types) is not obvious. This paradox has been addressed by Hedin et al. (2009) who proposed a ‘leaky nitrostat model’, which will be considered by Lavin et al. (2013—in this issue). For the present purposes, we note that nearly all of the non-nodulated legumes from all three sub-families are found in the tropics, both wet, seasonally dry and arid (Sprent, 2009 and references therein).

Table 3

Genera in tribe Brongniartieae. The number will increase when current studies are published (see LPWG, 2013.) Number of species in a genus given in parentheses.

Genus	Geographical location	Presence of fixation threads
<i>Brongniartia</i> Kunth. (~63)	New world	?
<i>Cristonia</i> J.H.Ross (1)	Australia	?
<i>Cyclobium</i> Benth. (1)	New world	+ve
<i>Harpylce</i> Moc. and Sesse ex DC. (24)	New world	?
<i>Hovea</i> R.Br. ex W.T.Aiton (37)	Australia	+ve
<i>Lamprolobium</i> Benth. (2)	Australia	?
<i>Plagiocarpus</i> Benth. (1)	Australia	?
<i>Poecilanthus</i> Benth. (10–12)	New world	+ve
<i>Tabaroa</i> L.P.Queiroz, G.P. Lewis and M.F.Wocj. (1)	New world	?
<i>Templetonia</i> R.Br.ex W.T.Aiton (10)	Australia	+ve
<i>Thinicola</i> J.H.Ross (1)	Australia	?

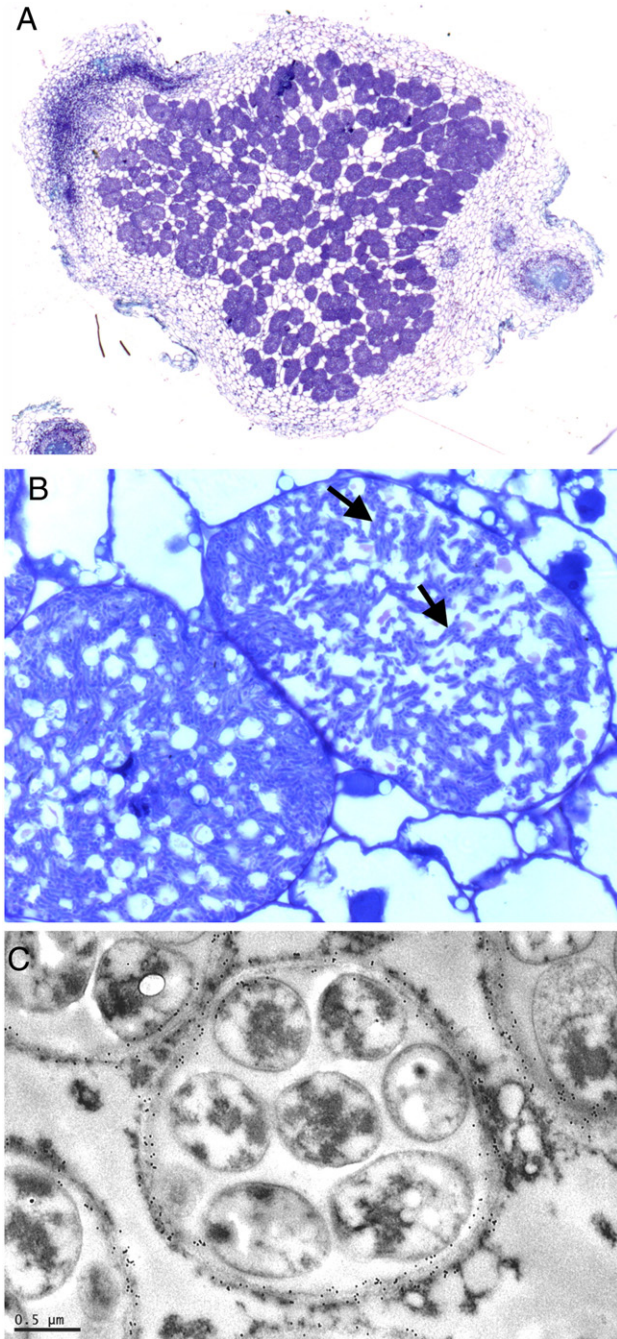


Fig. 1.8. *Templetonia* nodule. A, general structure which is similar to that shown in 3.1; B light micrograph showing fixation threads (arrows); C. electron micrograph of an infected cell showing bacteroids retained within fixation threads. The wall of the thread has been stained (black spots) with a monoclonal antibody (JIM5) specific for pectic cell-wall material.

5. Nodulation in an evolutionary context

Doyle (2011) made an in-depth study of when nodulation may have evolved in the different legume lineages. Here we take a geographical view of both the nodulation events and the various ways in which nodules may be formed, suggesting some routes by which differences came about. Although there is abundant evidence for recent evolution within tribes and genera, the basic processes of nodulation appear to have been laid down early. For example, 81 species of lupin evolved in the Andes within the last two million years (Hughes and Eastwood, 2006) and yet nodules from this area are structurally identical to those of the many other lupins studied

(see plate 2.13 in Sprent, 2009). Crisp et al. (2004) and Crisp and Cook (2007) showed that mirbelioid legumes have extended into increasingly arid areas of Australia over the last 2–4 million years and, as far as we know (see Section 3.2) they have retained their nodulation characters. This suggests that some nodule characters may be highly conserved, and as such they could be good markers for defining higher level taxonomic groups, as shown by Lavin et al. (2001) for dalbergioid legumes.

Sprent (2007) suggested that non-hair infection is an ancestral feature of nodulation and supported this contention with results from various studies with mutants of model legumes such as *L. japonicus*. However, in the first published study on infection and nodule development in a caesalpinoid legume, two species of *Dimorphandra* Schott, a genus currently being divided, (Fonseca et al., 2012) showed infection via root hairs. Following infection, further passage of rhizobia through the root cortex was rather disorganised, having much in common with the infection of roots of *Parasponia* (Cannabaceae), the only non-legume known to be nodulated by rhizobia. Later, fixation threads, typical of caesalpinoid and some basal papilionoid, but no known mimosoid legumes, developed. So what is the ancestral position and does it involve retention of bacteroids in fixation threads? All nodulated caesalpinoid legumes studied to date, all of which are within a single clade of this paraphyletic group (Table 4) with the possible exception of *Chidlowia*, for which there is no clear information on nodule structure (and which in some analyses is placed in Mimosoideae, Manzanilla and Bruneau, 2012), and herbaceous species of *Chamaecrista*, retain their bacteroids in fixation threads. Outside the Caesalpinioideae such structures have only been found in a few papilionoid legumes (de Faria et al., 1987). Recent advances in legume molecular phylogenetics have shed light on how these genera are grouped. *Andira* Lam. and *Hymenolobium* Benth. have been moved from the Dalbergieae to a separate position, nearer among the early-branching papilionoids. *Dahlstedtia* Malme currently remains in tribe Millettieae, but there have been changes in the genus, with several species of *Lonchocarpus* Kunth. being transferred to *Dahlstedtia* and others to *Muelleria* L.f. (da Silva et al., 2012). Included in this transfer to *Dahlstedtia* is *Lonchocarpus muehlbergianus* Hassl., whose nodules do not have fixation threads (Cordeiro et al., 1996). *Dahlstedtia* therefore remains an anomaly, whereas the situation in Brongniartieae (described above) is becoming clearer. In current thinking Brongniartieae is near the base of papilionoids (Wojciechowski et al., 2004) with *Ormosia* between it and the non-nodulating group (Fig. 1.5). The Legume Working Group (LPWG, 2013) regards *Ormosia* as being in need of further study. It has been known for many years to have profuse nodulation, with large, much divided indeterminate nodules, but with no information on their internal structure. We have now examined two species and find that *Ormosia* nodules have a type of fixation thread (Fig. 1.9), details of which are currently being studied. Thus its position as basal to Brongniartieae is entirely logical if fixation threads are a sign of an early type of nodule structure. Based on the similarities between caesalpinoid and basal papilionoid nodule structure, we suggest that it is premature to put nodulated caesalpinoids in with the mimosoids (see LPWG, 2013 and references

Table 4

Known caesalpinoid nodulating genera and number of species.

Cassiinae
<i>Chamaecrista</i> Moench >330 spp. – the eighth largest genus of legumes
Caesalpinieae
<i>Campsiandra</i> Benth. 19 spp.
<i>Chidlowia</i> Hoyle 1sp.
<i>Dimorphandra</i> Schott. 26 spp., but genus is being subdivided into 3 genera
<i>Erythrophleum</i> Afzel. ex R.Br. 10spp.
<i>Jacqueshuberia</i> Ducke 7spp. – new report on nodulation
<i>Melanoxylon</i> Schott 1sp.
<i>Moldenhawera</i> Schrad. 9spp.
<i>Tachigali</i> Aubl. 60–70 spp., (including those formerly in <i>Sclerolobium</i>)

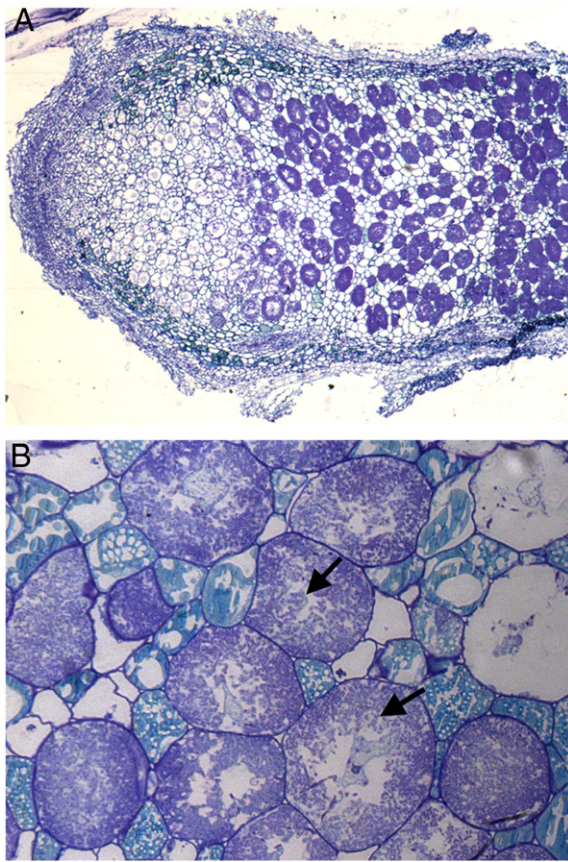


Fig. 1.9. Nodules of *Ormosia* spp. A, section through the tip of a branch of a nodule of *O. nitida* Vogel, showing general structure similar to that of indeterminate nodules with uninfected cells in the central tissue; B, detailed structure of infected cells from *O. macrocalyx* Ducke, with fixation threads (arrows) similar to those seen in Fig. 1.8 B.

therein), none of which has yet been shown to have any similarities in nodule structure.

This leaves the anomalous position of *Chamaecrista*, which may represent its own nodulation event and whose nodule structure covers the range of bacteroids from those retained within fixation threads (in some arborescent species) to those being fully released (in herbaceous species), with various intermediate forms (Naisbitt et al., 1992). This genus is the only nodulated caesalpinoid genus in which some species have colonised temperate areas and has been suggested as a good model for detailed study (Singer et al., 2009).

6. Summary and conclusion

Legume nodulation has evolved several times. Within the nodulating clade of Caesalpinioideae *Chamaecrista* is the largest successful nodulating genus. In Mimosoideae, all large genera can nodulate and nodule characters are rather uniform, but in Papilionoideae, where most genera can nodulate, they have diverged considerably, giving several different types of infection, nodule structure and development. These divergencies occurred early in the evolution of major tribes/clades such as Crotalariaeae and correlate with changes in land distribution, particularly with latitude. They have remained stable over time, even in genera such as *Lupinus* that have evolved rapidly in the last few million years. This makes nodulation, along with other morphological and molecular characters important as potential synapomorphies for supra-generic taxa, as has been done for the dalbergioid clade, which is supported by a distinct nodule type.

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References

- Adams, M.A., Bell, T.L., Pate, J.S., 2002. Phosphorus sources and availability modify growth and distribution of root clusters and nodules of native Australian plants. *Plant, Cell & Environment* 25, 837–850.
- Allen, O.N., Allen, E.K., 1981. *The Leguminosae: a source book of characteristics, uses and nodulation*. University of Wisconsin Press, Madison, Wisconsin and Macmillan Publishers Ltd. London.
- Ampomah, O.Y., James, E.K., Iannetta, P.P.M., Kenicer, G., Sprent, J.I., Huss-Dannel, K., 2012. Nodulation and ecological significance of indigenous legumes in Scotland and in Sweden. *Symbiosis* 57, 133–148.
- Ardley, J.K., Parker, M.A., de Meyer, S., Trengove, R.D., O'Hara, G.W., Reeve, W.F., Yates, R.J., Dilworth, M.J., Willems, A., Howieson, J.G., 2012. *Microvirga lupine* sp. nov., *Microvirga lotonoides* sp. nov., and *Microvirga zambeziensis* sp. nov., are Alphaproteobacterial root nodule bacteria that specifically nodulate and fix nitrogen with geographically and taxonomically separate legume hosts. *International Journal of Systematic and Evolutionary Microbiology* 62, 2579–2588.
- Ardley, J.K., Reeve, W.G., O'Hara, G.W., Yates, R.J., Dilworth, M.J., Howieson, J.G., 2013. Nodule morphology, symbiotic specificity and association with unusual rhizobia are distinguishing features of the genus *Listia* within the southern Africa crotalariaioid clade *Lotonoides* s.l. *Annals of Botany* 112, 1–15.
- Barron, A.R., Purves, D.W., Hedin, L.O., 2011. Facultative nitrogen fixation by canopy legumes in a lowland forest. *Oecologia* 165, 511–520.
- Boatwright, J.S., Savolainen, V., Van Wyk, B.-E., Schutte-Vlok, A.L., Forest, F., Van der Bank, M., 2008. Systematic position of the anomalous genus *Cadia* and the phylogeny of the tribe Podalyrieae (Fabaceae). *Systematic Botany* 33, 133–147.
- Boatwright, J.S., Wink, M., Van Wyk, B.-E., 2011. The generic concept of *Lotonoides* (Crotalariaeae, Fabaceae): reinstatement of the genera *Euchlora*, *Lebordea* and *Listia* and the new genus *Ezoloba*. *Taxon* 60, 161–177.
- Brown, S.M., Oparka, K.J., Sprent, J.I., Walsh, K.B., 1995. Symplastic transport in soybean root nodules. *Soil Biology and Biochemistry* 27, 387–399.
- Corby, H.D.L., 1988. Types of rhizobial nodule and their distribution among the Leguminosae. *Kirkia* 13, 53–123.
- Cordeiro, L., Sprent, J.I., McInroy, S.G., 1996. Some developmental and structural aspects of nodules of *Lonchocarpus muehlbergianus* Hassl. *Naturalia* (São Paulo) 21, 9–21.
- Cordoso, D., de Queiroz, L.P., Pennington, R.T., de Lima, H.C., Fonby, E., Wojciechowski, M.F., Lavin, M., 2012. Revisiting the phylogeny of papilionoid legumes: new insights from comprehensively sampled early-branching lineages. *American Journal of Botany* 99, 1991–2013.
- Crisp, M.D., Cook, L.G., 2007. A congruent molecular signature of vicariance across multiple plant lineages. *Molecular Phylogenetics and Evolution* 43, 1106–1117.
- Crisp, M., Cook, L., Steane, D., 2004. Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? *Philosophical Transactions of the Royal Society B* 359, 1551–1571.
- Crisp, M.D., Chappill, J.A., de Kok, R., Jobson, P., 2005. Mirbelieae. In: Lewis, G., Schrire, B., Mackinder, B., Lock, M. (Eds.), *Legumes of the World*, Royal Botanic Gardens Kew, Richmond, Surrey, pp. 339–353.
- Da Silva, M.J., de Queiroz, L.P., de Azevedo Tozzi, A.M., Lewis, G.P., de Sousa, A.P., 2012. Phylogeny and biogeography of *Lonchocarpus* sensu lato and its allies in tribe Millettieae (Leguminosae, Papilionoideae). *Taxon* 61, 93–108.
- De Faria, S.M., McInroy, S.G., Sprent, J.I., 1987. The occurrence of infected cells, with persistent infection threads in legume root nodules. *Canadian Journal of Botany* 45, 143–147.
- Doyle, J.J., 2011. Phylogenetic perspectives on the origin of nodulation. *Molecular Plant-Microbe Interactions* 24, 1289–1295.
- Elliott, G.N., Chen, W.-M., Bontemp, C., Chou, J.-J., Young, J.P.W., Sprent, J.I., James, E.K., 2007. Nodulation of *Cyclopia* spp. (Leguminosae, Papilionoideae) by *Burkholderia tuberum*. *Annals of Botany* 100, 1403–1411.
- Estrada-de los Santos, P., Vinuesa, P., Martínez-Aguilar, L., Hirsch, A.M., Caballero-Mellado, J., 2013. Phylogenetic analysis of *Burkholderia* species by multilocus sequence analysis. *Current Microbiology*. <http://dx.doi.org/10.1007/s00284-013-0330-9>.
- Fonseca, M.B., Peix, A., de Faria, S.M., Mateos, P.F., Rivera, L.P., Simões-Araújo, J.L., Costa Franca, M.G., dos Santos Isaias, R.M., Cruz, C., Velazquez, E., Scotti, M.R., Sprent, J.I., James, E.K., 2012. Nodulation in *Dimorphandra wilsonii* Rizz. (Caesalpinioideae), a

- threatened species native to the Brazilian Cerrado. *PLoS One* 7, e4920. <http://dx.doi.org/10.1371/journal.pone.0049520>.
- Gyaneshwar, P., Hirsch, A.M., Moulin, L., Chen, W.-M., Elliott, G.N., Bontemps, C., Estrada-de los Santos, P., Gross, E., dos Reis Jr., F.B., Sprent, J.I., Young, J.P.W., James, E.K., 2011. Legume-nodulating bacteria from the Betaproteobacteria: diversity, host range and future prospects. *Molecular Plant-Microbial Interactions* 24, 1276–1288.
- Hedin, L.O., Brookshire, J., Menge, D.N.L., Barron, A.R., 2009. The nitrogen paradox in tropical forest systems. *Annual Review of Ecology, Evolution, and Systematics* 40, 613–615.
- Howieson, J.G., de Meyer, S., Vivas-Marfisi, M., Ratnayake, S., Ardley, J.K., Yates, R.J., 2013. Novel *Burkholderia* bacteria isolated from *Lebeckia ambigua* – a perennial suffruticose legume of the fynbos. <http://dx.doi.org/10.1016/j.soilbio.2013.01.009>.
- Hughes, C., Eastwood, R., 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences USA* 103, 10334–10339.
- James, E.K., de Loureiro, M., Pott, A., Pott, V.J., Martins, C.M., Franco, A.A., Sprent, J.I., 2001. Flooding tolerant legumes from the Brazilian Pantanal. *New Phytologist* 150, 723–738.
- Kereszt, A., Mergaert, P., Kondorosi, E., 2011. Bacteroid development in legume nodules: evolution of mutual benefit or sacrificial victims? *Molecular Plant-Microbe Interactions* 24, 1300–1309.
- Lafay, B., Burdon, J.J., 1998. Molecular diversity of rhizobia occurring in native shrubby legumes in Southeastern Australia. *Applied and Environmental Microbiology* 64, 3989–3997.
- Lambers, H., Brundett, M.C., Raven, J.A., Hopper, S.T., 2011. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil* 348, 7–27.
- Lange, R.T., 1961. Nodule bacteria associated with the indigenous Leguminosae of South-Western Australia. *Journal of General Microbiology* 26, 351–359.
- Lavin, M., Pennington, R.T., Klitgaard, B.B., Sprent, J.I., de Lima, H.C., Gasson, P.E., 2001. The Dalbergioid legumes (Fabaceae): delimitation of a pantropical monophyletic clade. *American Journal of Botany* 88, 503–533.
- Lavin, M., Herendeen, P.S., Wojciechowski, M.F., 2005. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. *Systematic Biology* 54, 575–594.
- Lavin, M., et al., 2013. Stability structures tropical woody plant diversity more than seasonality: Insights into the ecology of high legume-succulent-plant biodiversity. *Systematic Biology* 89, 42–57 (in this issue).
- Lawrie, A.C., 1981. Nitrogen fixation by native Australian legumes. *Australian Journal of Botany* 29, 143–157.
- Lawrie, A.C., 1983a. Relationships among rhizobia from native Australian legumes. *Applied and Environmental Microbiology* 45, 1822–1828.
- Lawrie, A.C., 1983b. Infection and nodule development in *Aotus ericoides* (Vent.) G.Don, a woody native Australian legume. *Journal of Experimental Botany* 34, 1168–1180.
- Le Roux, M.M., 2011. A taxonomic study of the genus *Crotalaria* (Fabaceae, tribe Crotalariaeae) and a modified infrageneric classification system. (PhD thesis) University of Johannesburg, South Africa.
- Li, J.H., Wang, E.T., Chen, W.F., Chen, W.X., 2008. Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. *Soil Biology and Biochemistry* 40, 238–248.
- LPWG, 2013. Legume phylogeny and classification in the 21st century: progress, prospects and lessons. *Taxon* 62, 217–248.
- Manzanilla, V., Bruneau, A., 2012. Phylogeny reconstruction in the Caesalpinieae grade (Leguminosae) based on duplicate copies of the sucrose synthase gene and plastid markers. *Molecular Phylogenetics and Evolution* 65, 149–162.
- Naisbitt, T., James, E.K., Sprent, J.I., 1992. The evolutionary significance of the genus *Chamaecrista* as determined by nodule structure. *New Phytologist* 122, 487–492.
- Oldroyd, G.E., Murray, J.M., Poole, P.S., Downie, J.A., 2011. The rules of engagement in the legume–rhizobial symbiosis. *Annual Review of Genetics* 154, 1541–1548.
- Ross, J.H., Crisp, M.D., 2005. Brongniartieae. In: Lewis, G., Schrire, B., Mackinder, B., Lock, M. (Eds.), *Legumes of the World*, Royal Botanic Gardens Kew, Richmond, Surrey, pp. 253–259.
- Schaefer, H., Hechenleitner, P., Santos-Guerra, A., de Sequeira, M.M., Pennington, R.T., Kenicer, G., Carine, M.A., 2012. Systematics, biogeography, and character evolution of the legume tribe Fabaeae with special focus on the middle-Atlantic island lineages. *BMC Evolutionary Biology* 12, 250.
- Schrire, B.D., Lewis, G.P., Lavin, M., 2005. Biogeography of the Leguminosae. In: Lewis, G., Schrire, B., Mackinder, B., Lock, M. (Eds.), *Legumes of the World*, Royal Botanic Gardens Kew, Richmond, Surrey, pp. 22–54.
- Sen, D., Weaver, R.W., 1984. A basis of different rates of N₂-fixation by some strains of *Rhizobium* in peanut and cowpea nodules. *Plant Science Letters* 34, 239–246.
- Singer, S.R., Maki, S.L., Farmer, A.D., Hut, D., May, G.D., Cannon, S.B., Doyle, J.J., 2009. Venturing beyond beans and peas: what can we learn from *Chamaecrista*? *Plant Physiology* 151, 1041–1047.
- Sniderman, K.M.K., Jordan, G.J., 2011. Extent and timing of floristic exchange between Australian and Asian rain forests. *Journal of Biogeography* 38, 1445–1455.
- Sprent, J.I., 1994. Nitrogen acquisition systems in the Leguminosae. In: Sprent, J.I., McKey, D. (Eds.), *Advances in Legume Systematics 5: the nitrogen factor*, Royal Botanic Gardens Kew, Richmond, Surrey, pp. 1–16.
- Sprent, J.I., 2001. *Nodulation in Legumes*. Royal Botanic Gardens, Kew, Richmond, Surrey.
- Sprent, J.I., 2007. Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. *New Phytologist* 174, 11–25.
- Sprent, J.I., 2009. *Legume Nodulation: A Global Perspective*. Wiley-Blackwell, Oxford, UK.
- Sprent, J.I., Gehlot, H.S., 2011. Nodulated legumes in arid and semi-arid environments: are they important? *Plant Ecology and Diversity* 3, 211–219.
- Stepkowski, T., Watkin, E., McInnes, A., Gurda, D., Gracz, J., Steenkamp, E.T., 2012. Distinct *Bradyrhizobium* communities nodulate legumes native to temperate and tropical monsoon Australia. *Molecular Phylogenetics and Evolution* 63, 265–277.
- Sy, A., Giraud, E., Jourand, P., Garica, N., Willems, A., de Lajudie, P., Prin, Y., Neyra, M., Gillis, M., Boivin-Masson, C., Dreyfus, B., 2001. Methylophiles in symbiosis with legumes. *Journal of Bacteriology* 183, 214–220.
- Terpolilli, J.J., Hood, G.A., Poole, P.S., 2012. What determines the efficiency of N₂-fixing *Rhizobium*-legume symbioses? In: Poole, R.K. (Ed.), *Advances in Microbial Physiology*, 60, pp. 325–389.
- Trainer, M.A., Charles, T.C., 2006. The role of PHB metabolism in the symbiosis of rhizobia with legumes. *Applied Microbiology and Biotechnology* 71, 377–386.
- Van Wyk, B.-E., 2005. Podalyrieae. In: Lewis, G., Schrire, B., Mackinder, B., Lock, M. (Eds.), *Legumes of the World*, Royal Botanic Gardens Kew, Richmond, Surrey, pp. 267–271.
- Wojciechowski, M.F., Lavin, M., Sanderson, M.J., 2004. A phylogeny of legumes (Leguminosae) based on analysis of plastid *Matk* gene resolves many well-supported subclades within the family. *American Journal of Botany* 91, 1845–1861.
- Yates, R.J., Howieson, J.G., Nandasene, K.C., OHara, G.W., 2004. Bacteria from indigenous legumes in the north-west of Western Australia and their interaction with exotic legumes. *Soil Biology and Biochemistry* 36, 1319–1329.