Chapter 1

Nodulation in a Taxonomic Context

In 2005, Lewis et al. published their comprehensive and beautiful book 'Legumes of the World', in which all genera then known are described and at least one species of each illustrated. In addition, the book contains a very interesting account of legume biogeography, which will be the subject of Chapter 2. The general terminology of Lewis et al. (2005) will be followed here, i.e. the family Leguminosae (or Fabaceae as some prefer) is divided into three sub-families, each of which is divided into tribes. Table 1.1 summarises these tribes and the numbers of genera and species within them. Since, with a very few known exceptions, detailed where appropriate, nodulation is a generic characteristic, for the purposes of the following discussion all species within a genus are presumed to nodulate, even though the number recorded as nodulated may be far less than the total (Tables 1.4 to 1.17). At various times when genera have been divided, it has unwittingly also been on presence or absence of nodulation (Table 1.2). Many of the more recently described genera in sub-families Mimosoideae and Papilionoideae have been segregated from others that can nodulate, but often there is no information on the nodulation status of the new combinations. Whilst, because of their taxonomic position, many of these are likely to be able to nodulate, in view of the examples given in Table 1.2, it certainly cannot be taken for granted.

In all three sub-families, there is active research on tribal and generic details. The authors of the various chapters in Lewis et al. (2005) present the current situation as they see it, pointing out anomalies without taking a position, because one of their aims is to stimulate research. In this sense, their book can be described as covering 'work in progress'. In some cases nodulation characteristics may add some clarity, and this will be attempted in the present chapter. A further complication is that the current ideas of taxonomy, phylogeny and evolution are not entirely congruent with known major alterations in the chloroplast genome. Doyle (1995) outlined the major changes of importance for legumes, of which two are relevant here. Chronologically the first is the inversion of a 50kb section of chloroplast DNA. This is absent from Caesalpinioideae and Mimosoideae, but present in most Papilionoideae. The second is the loss of a large duplicated and inverted section of the genome, present in nearly all land plants, and known as the inverted repeat (IR). Liston (1995) carried out an extensive survey of this feature of legumes, which is largely confined to the more advanced papilionoid

Table 1.1 Summary of tribes, genera and species in the three subfamilies of Leguminosae, mainly as given in Lewis et al (2005), except that Mimozygantheae is included here in Mimoseae and some recent changes in Crotalarieae are included. Numbers of species are approximate

Caesalpinioideae			
Tribe	Genera	Species	Comments
Caesalpinieae	56	429	7 gen. known to nodulate 171 spp.
Cassieae	21	73	One nodulating genus 330 spp.
Cercideae	12	335	May be a separate branch
Detarieae	82	747	None known to nodulate
Mimosoideae			
Tribe			
Acacieae	1	1450+	Subdivision in hand
Ingeae	36	950	~300 in *Inga*
Mimoseae	41	870	~500 in *Mimosa*
Papilionoideae			
Tribe			
Abreae	1	17	
Amorpheae	8	246	165 in *Dalea*
Bossiaeeae	6	72	
Brongniartieae	10	151	
Cicereae	1	43	
Crotalarieae	12	1120	Subject to revision
Dalbergieae	49	1324	250 in *Dalbergia*
Desmodieae	30	527	275 in *Desmodium*
Dipterygeae	3	22	Non-nodulating
Euchrestieae	1	4	
Fabeae	5	328	Formerly Vicieae
Galegeae	24	2929	2300+ in *Astragalus*
Genisteae	25	561	225 in *Lupinus*
Hedysareae	12	426	140–180 in *Hedysarum*
Hypocalypteae	1	3	
Indigofereae	7	768	700 in *Indigofera*
Loteae	22	282	Includes Coronillieae
Millettieae	45	908	350 in *Tephrosia*
Mirbelieae	25	687	
Phaseoleae	89	1576	230 in *Rhynchosia*
Podalyrieae	8	125	
Psoraleae	9	185	
Robinieae	11	71	
Sesbanieae	1	60	
Sophoreae	45	295	Polyphyletic
Swartzieae	17	258	Polyphyletic
Thermopsideae	6	45	
Trifolieae	6	425	

Table 1.2 Generic changes that have unwittingly included nodulation. Nodulating genera in bold type. They include one genus from each sub-family

Old genus	New genera	Reference
Cassia	*Cassia, Senna,* ***Chamaecrista***	Irwin & Barneby, 1982
Newtonia	*Newtonia,* ***Pseudopiptadenia***	Lewis & Lima, 1991
Sophora	***Sophora,*** *Styphnolobium*	Sousa & Rudd, 1993

tribes, but which also occurs in some others, causing some difficulties for classification, discussed later. As each of these two changes is thought to have occurred only once, it is hoped that when more genera have been analysed for them, their presence/absence will help clarify some anomalies. Although very important for legume phylogeny, there are no known nodulation characteristics involved in these chloroplast genome changes (or, indeed, in the chloroplast genome at all).

In the following sections, brief reference will be made to nodule morphology and structure (Figs. 1.1 and 1.2), and to the bacteria inducing nodules, detailed in Chapter 4. Basically, bacteria nodulating legumes are known collectively as rhizobia, and they fall within several families of two branches (α and β) of phylum Proteobacteria. Earlier they were often categorised in terms of fast or slow growth and these terms will also be used here.

1.1 Caesalpinioideae

This sub-family has long been known to contain the smallest proportion of nodulated species (Allen & Allen, 1981). However, it is worth re-examining the distribution of nodulation in the light of currently described tribes. Cercideae and Detarieae are basally branched from the rest of Caesalpinioideae (Fig. 1.3): neither has known nodulated members and Detarieae is uniformly ectomycorrhizal. Most legumes are arbuscular mycorrhizal (AM) or, in some cases, have both types. Tribe Cassieae has one nodulating genus, *Chamaecrista*. However, this has 330 species, a significant number of which have been recorded as nodulated and none as non-nodulated. This genus represents nearly half the species in the tribe. Further, sub-tribe Cassiinae, which contains *Chamaecrista*, appears to fall within the confines of tribe Caesalpinieae (Lewis, 2005a; Fig. 1.3), which contains all other known nodulating genera. Caesalpinieae has been divided into a number of groups, some of which contain only non-nodulating genera. Nodulated genera are scattered among several groups, with no apparent logic. In a more recent study, Bruneau et al. (2008) sampled all but one of the caesalpinioid genera, but with varying levels of rigour because of the availability and quality of DNA. This analysis clarified the relations among many of the genera. Unfortunately the nodulated genera remain scattered and the hope expressed by Haston et al. (2005) that, with the inclusion of more molecular characteristics, the nodulating genera may emerge as more closely related than generally thought has not yet been fulfilled. One generic change

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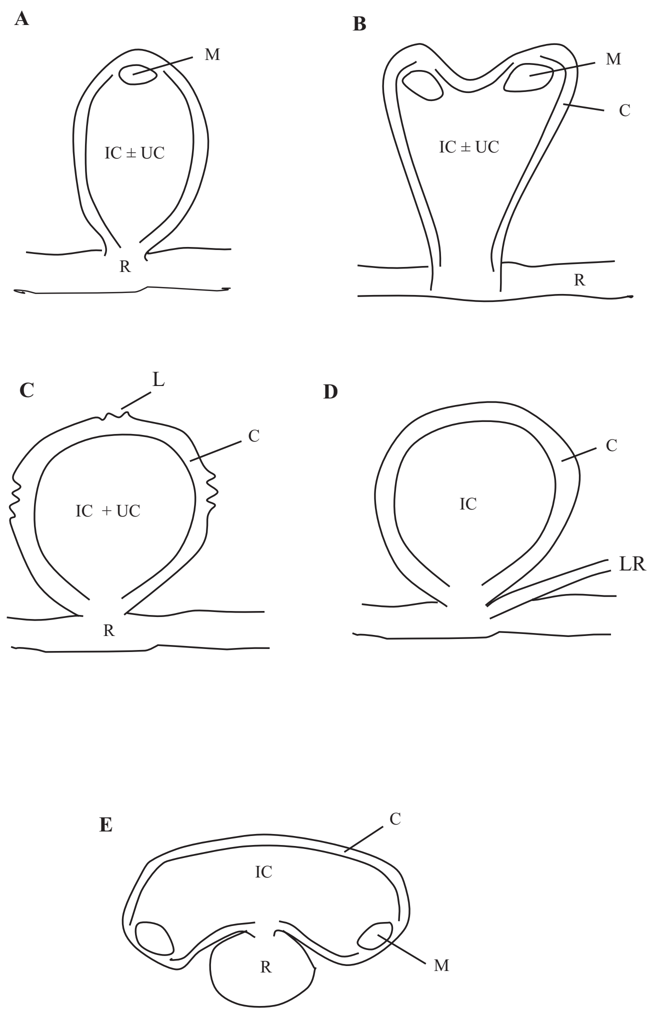


Figure 1.1 The arrangement of tissues in the major types of nodule. **A** and **B** are indeterminate forms (see also Fig. 1.2C), with a single or a branched apical meristem. Nodules that appear similar in morphology may have uniform infected tissue or a mixture of infected and uninfected cells, according to taxonomic position. **C**, desmodioid (determinate) nodule (Fig. 1.2A), with infected tissue always containing uninfected cells. **D**, aeschynomenoid nodule as found in legumes from the Dalbergioid clade. These are always associated with lateral or adventitious roots and have uniform infected tissue. **E**, a variant of an indeterminate nodule containing only infected cells in the infected region, but with two lateral meristems, resulting in a 'collar' or lupinoid nodule that encircles the subtending root (occasionally stem). Only known from a few Genistoid legumes. (M, meristem; C, nodule cortex, containing vascular tissue; R, subtending root; LR. lateral root; IC infected cells; UC uninfected cells.) (Modified from Sprent, 2007.)

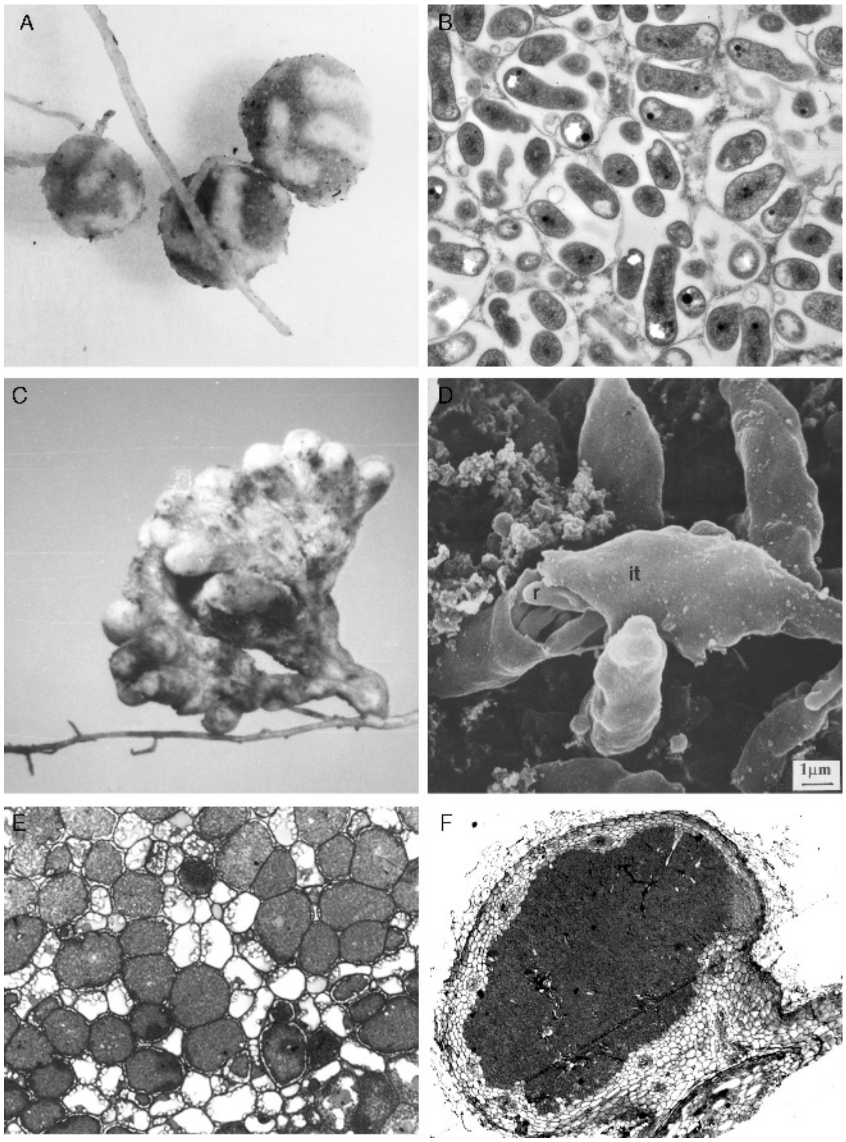
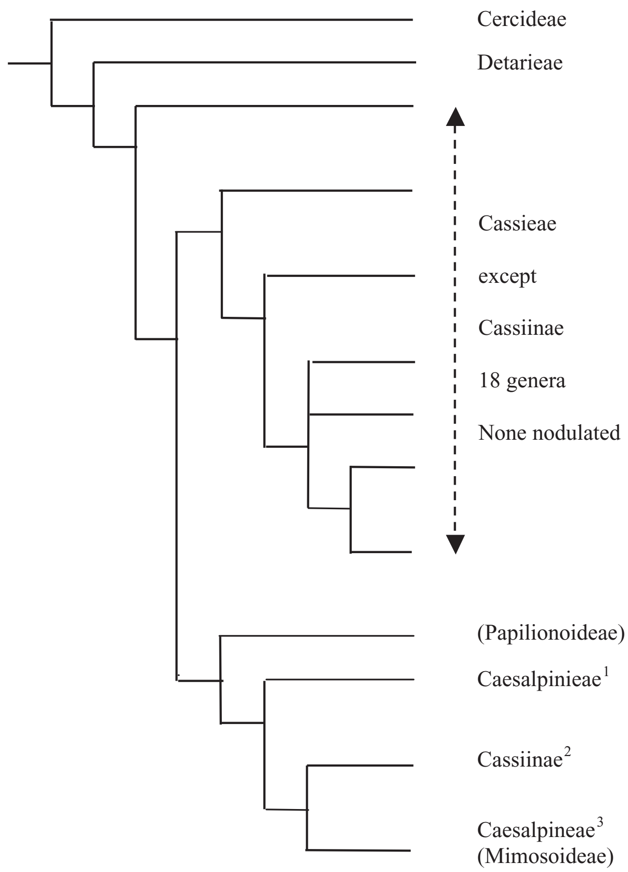


Figure 1.2 Major types of legume nodule. **A**, desmodioid nodule as found in the phaseoloid tribes (Table 1.15, and some members of tribe Loteae (Table 1.16). Lenticels are characteristic. Nodules vary from 2 to 5 mm in diameter and the infected tissue contains both infected and uninfected cells (Fig. 1.1C). **B**, symbiosomes; these membrane-bound structures may contain 1 to 8 bacteroids (the nitrogen-fixing form of rhizobia). **C**, a much-branched indeterminate nodule, as found in many species from all three subfamilies. Other indeterminate nodules may be much less branched or unbranched, and the infected tissue may contain only infected cells or a mixture of infected and uninfected cells (Fig. 1.1A, B), a taxonomic characteristic. Size varies from 3 mm to several cm in length. **D**, a broken modified infection thread (IT), often called a fixation thread, showing bacteroids. This is considered a primitive state in which bacteria are not released into symbiosomes and is found in caesalpinioid and some papilionoid nodules. **E**, infected tissue containing a mixture of infected (darkly stained) and uninfected cells (see Fig. 1.1). **F**, infected tissue containing only infected cells. Infected cells in either **E** or **F** arrangements may be highly vacuolate in some species. (From Sprent, 2007.)



[1] Pro parte; 7 genera, none nodulated

[2] 3 genera, including *Chamaecrista*

[3] Pro parte; 49 genera, including all 7 nodulated ones

Figure 1.3 Possible relationships between nodulated and non-nodulated groups in the Caesalpinioideae. The position of Papilionoideae in this diagram can be ignored. (After Lewis, 2005a.)

since Sprent (2001) is that *Sclerolobium* has been incorporated into *Tachigali*. Both can nodulate, although this was not one of the properties used in the reclassification. The seven genera known to nodulate (*Campsiandra, Chidlowia, Dimorphandra, Erythrophleum, Melanoxylon*, *Moldenhawera* and *Tachigali*) account for 171 of the 429 species in tribe Caesalpinieae. Again, this is a significant proportion, equal or exceeding that in some tribes of the Papilionoideae. All nodulating caesalpinioid genera, except *Chidlowia*, a monotypic genus from tropical Africa, and *Erythrophleum*, which is found in Africa and Australia, together with some herbaceous pan-tropical species of *Chamaecrista*,

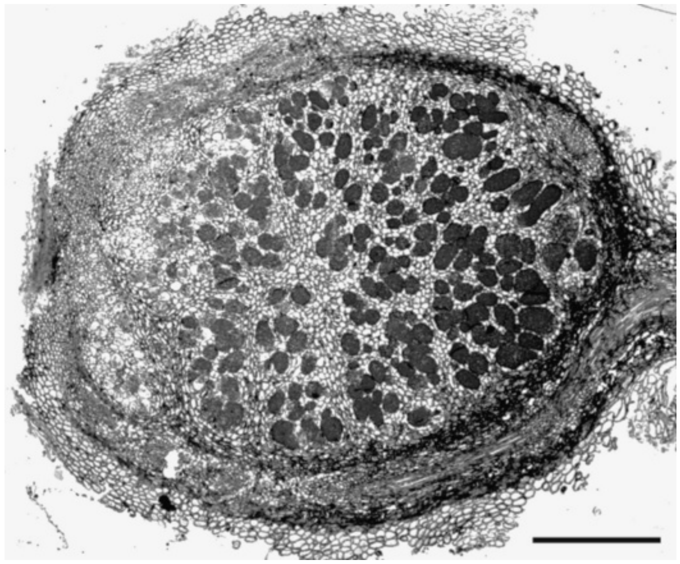


Figure 1.4 Longitudinal section of a typical caesalpinioid nodule, showing the characteristic blunt apex and infected tissue with a mixture of infected and uninfected cells. In some species nodules may branch and be lignified in the outer layers. Infected cells have bacteroids retained in fixation cells (Fig. 1.2D). Bar = 500 μm. (Courtesy of E.K. James.)

are from South America. All, excepting herbaceous species of *Chamaecrista*, have their nitrogen-fixing bacteria retained with modified infection threads, known as fixation threads (Fig. 1.2D). The evolutionary implications of these aspects of caesalpinioid legumes will be considered in Chapter 3. All nodules show indeterminate growth, are often branched and may be quite woody. The tips of nodules are usually flattened, and the infected tissue contains both infected and uninfected cells (Fig. 1.4).

Very little is known about the bacteria nodulating caesalpinioid legumes. The only species in commercial use, as a forage plant, is *Chamaecrista rotundifolia,* marketed in Australia as Wynn Cassia. The commercial inoculant for this is a broad-range strain of *Bradyrhizobium*, CB756 (A. McInnes, personal communication). *Ch. fasciculata* was the only caesalpinioid legume found to nodulate with the fast-growing, wide host range strain NGR234 (Pueppke & Broughton, 1999). Parker (2008) found that all 20 isolates from nodules of *Tachigali versicola* were bradyrhizobia and we have evidence that *Chamaecrista nictitans* nodules collected in the field in Brazil house a species of *Burkholderia* (E.K. James, personal communication).

1.2 Mimosoideae

In terms of genera, but not species, Mimosoideae is the smallest of the sub-families. (Table 1.1). Former tribes Parkieae and Mimozygantheae are now included in Mimoseae (in Lewis et al., 2005, Mimozygantheae is retained). All tribes are due for changes, the most comprehensive of which are in Acacieae, which contains the single genus *Acacia*.

Nodules in all mimosoid legumes studied are indeterminate, but vary considerably in morphology and whether or not they are branched. Structurally, none is known to have bacteroids retained within infection threads as they are in most Caesalpinioideae and some Papilionoideae. This is interesting in view of the possible position of Mimosoideae with respect to Caesalpinioideae in Fig. 1.3 and will be explored further in Chapter 3.

1.2.1 Acacieae

In Lewis (2005b), tribe Acacieae has one genus, *Acacia*. It has been known for decades that *Acacia* is not monophyletic, and for many years it has been divided into three sub-genera, *Acacia*, *Aculeiferum* and *Phyllodineae* (also called *Heterophyllum*). Generic names have been suggested for these, but not widely accepted. This may be just as well as it now appears that there should be at least five genera arising out of *Acacia*. These are listed in Table 1.3. In Lewis (2005b), the *A. coulteri* group is referred to as 'genus X'. This has now been named *Mariosousa* (Seigler et al., 2006). As well as the number of genera being in doubt (more may yet be added) the tribal affinities remain unclear. The suggestions in Table 1.3 may be altered when more taxa are sampled, but it is interesting that species of the seed-predating bruchid beetles support this tribal arrangement (Kergoat et al., 2007). If further evidence supports the divisions given in Table 1.3, then tribe Acacieae will cease to exist. Added to the scientific confusion are considerations of national pride. Many Australians regard acacias as part of their natural heritage, even though they usually refer to them as wattles, and wish to use the generic name *Acacia* for sub-genus *Phyllodineae*. Others, including some Australians, believe that the usual procedures should be followed, in which case sub-genus *Acacia* should retain that name. For this reason the old terms have been retained in Table 1.3. However, the subdivisions there have significance for symbiotic properties, which is why the controversy over terminology has been engaged, rather than opting for a quiet life!

Table 1.3 Possible sub-division of *Acacia* into five genera and re-assignment into tribes. Based on Maslin et al. (2003), Seigler et al. (2006) and Rico-Arce (2007)

Sub-genus	Species	Geographical range	Tribe?
Acacia	161	Mainly Africa and South America, some in Asia and Australia	Mimoseae
Aculeiferum	203	Section Aculeiferum. Africa, Asia, Section Monacanthea, pan-tropical	Ingeae
Acaciella	14	reinstatement of an old genus for *Aculeiferum*, section Filicinae New World	
Mariosousa	13	*A. coulteri* group, New World	
Phyllodineae	~960	Mainly Australia, some Asia	Ingeae

Sub-genus *Phyllodineae* is the only one that has members with both ecto- and arbuscular mycorrhizas, a feature that makes them very good for land reclamation and also for becoming invasive. When *Phyllodineae* is further subdivided, it may be that only certain sections have this attribute (Sprent, 1994a). Species can nodulate with a wide range of rhizobia (Lafay & Burdon, 2001, chapter 4), but *Phyllodineae* is the only sub-genus to nodulate effectively with the wide host range strain NGR234 (Pueppke & Broughton, 1999). Nodulation appears to be a generic characteristic. Leary et al. (2006a) cite two species that were reported not to nodulate, but these observations have not been confirmed and probably reflect conditions when samples were collected. Nodulation also appears to be generic for sub-genus *Acacia*, but not for *Aculeiferum*, which has two sections, Aculeiferum, found in Africa and Monacanthea, which is pan-tropical. So far 16 species of the latter section, from both Africa and America, have been found unable to nodulate (Sprent, 2001). Proving a negative is always difficult, but these species have been extensively studied and also form a very close group on molecular characteristics. It is likely that they have lost the ability to nodulate, rather than never having had it. The bacteria nodulating sub-genera *Acacia* and *Aculeiferum* vary greatly as does their effectiveness in fixing nitrogen. These aspects will be examined in more detail in Chapters 2 and 5.

1.2.2 Ingeae

Tribe Ingeae, apart from including at least sub-genus *Phyllodineae* from the Acacieae, currently consists of 36 genera, dominated in species by *Inga* (300) and *Calliandra* (135) (Table 1.4). The monospecific genus *Faidherbia* was for many years called *Acacia albida*. Because it is widespread in Africa and has some unusual properties, such as shedding its leaves in the wet season and producing them in the dry season, it has been the subject of much research. Most members of Ingeae occur in the New World, but with several in Africa (including Madagascar), Asia and Australia. Recent evidence suggests that the Australian and SE Asian genera are closely related (Brown et al., 2008). There are 10 genera (48 spp.) for which there is no information on nodulation status. These include *Viguieranthus*, many of whose species were formerly included in *Calliandra*. *Zapoteca*, on the basis both of a few field observations and of laboratory studies, has not yet been found to possess nodules. This is the only genus in the tribe with negative reports on nodulation and more information is urgently needed.

Members of Ingeae are nodulated by a wide range of rhizobia, including some *Burkholderia* spp. (Chapter 4). The most widely studied genera for their nitrogen-fixing properties are *Albizia, Calliandra* and *Inga. Albizia* spp. are important shade trees, especially for cocoa, in large parts of Africa. *Calliandra calothysus* is widely used in agroforestry in Africa, where it is preferred to other woody legumes such as *Leucaena* because it seeds less freely and therefore does not become a weed. Although native to Central America, it nodulates with a range of rhizobia wherever it is grown (Bala & Giller, 2001). *Inga* species are also used as nurse and shade crops, the latter often for coffee in South and Central America. All species have edible fruits and many, for example *Inga edulis*, are grown for this reason. These and many other uses of *Inga* species are described in the monograph by Pennington and Fernandez (1998). *Inga* spp. are very important in some ecosystems (Chapter 2).

Table 1.4 Known nodulation of genera (as in Lewis et al., 2005), in tribe Ingeae

Genus	Species	Nod.
Abarema Pittier	46	13
Albizia Durazz	120–140	46
Archidendron F. Muell.	94	6
Archidendropsis F. Muell.	14	2
Blanchetiodendron Barneby & Grimes	1	?
Calliandra Benth.	~135	25
Cathormium (Benth.) Hasske.	1	?
Cedrelinga Ducke	1	1
Chloroleucon (Benth.) Britton & Rose	10	4
Cojoba Britton & Rose	12	1
Ebenopsis Britton & Rose	3	2
Enterolobium Mart.	11	8
Faidherbia A. Chev.	1	1
Falcataria (Nielsen) Barneby & Grimes	3	1
Guinetia L. Rico & M. Sousa	1	?
Havardia Small	5	2
Hesperalbizia Barneby & Grimes	1	1
Hydrochorea Barneby & Grimes	3	3
Inga Mill.	~300	63
Leucochloron Barneby & Grimes	4–5	?
Lysiloma Benth.	8–9	4
Macrosamanea Britton & Rose	11	3
Marmaroxylon Killip	9–13	?
Painteria Britton & Rose	3	?
Pararchidendron I.C. Nielsen	1	?
Paraserianthes I.C. Nielsen[1]	1	1
Pithecellobium Mart.	18	6
Pseudosamanea Harms	2	1
Samanea Merr.	3	2
Serianthes Benth.	~18	2
Sphinga Barneby & Grimes	3	?
Thailentadopsis Kosterm	3	?
Viguieranthus Villiers	~23	?
Wallaceodendron Koord.	1	1
Zapoteca H.M. Hern.	20	0?
Zygia P. Browne	45–50	10

[1] The number of species listed in both ILDIS and GRIN is 3, but there is only one in Lewis et al. (2005).

1.2.3 Mimoseae

Tribe Mimoseae is very complex and has many interesting features with respect to nodulation. Of its 41 genera, 9 appear unable to nodulate, 22 can and there is no information for the remaining 10 (Table 1.5). One, *Piptadenia*, has recently been divided into three (Jobson & Luckow, 2007), but not all new combinations are yet available. However, enough are to know that at least one species of each genus can nodulate. An

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Table 1.5 Nodulation status of genera in tribe Mimosae, after Lewis et al. (2005) except that *Piptadenia* has been divided into three genera, after Jobson & Luckow (2007). One, formerly *Piptadenia viridiflora*, has yet to be assigned a generic name

Genus	Species	Nod.	Not-nod.
Adenanthera L.	13	0	5?
Adenopodia C. Presl.	~7	?	1
Alantsilodendron Villiers	10	?	?
Amblygonocarpus Harms	1	0	1
Anadenanthera Speg.	2	1	
Aubrevillea Pellegr.	2	?	?
Calliandropsis H.M. Hern. & P. Guinet	1	?	?
Calpocalyx Harms	11	0	1
Cylicodiscus Harms	1		1
Desmanthus Willd.	~24	4	0
Dichrostachys (DC) Wight & Arn	14	2	0
Dinizia Ducke	1	1	
Elephantorrhiza Benth.	9	4	0
Entada Adans.	~28	10	0
Fillaeopsis Harms	1	?	?
Gagnebina Neck ex DC.	8	?	?
Indopiptadenia Brenan	1	?	?
Kanaloa Lorence & K.R. Wood	1	?	?
Lemurodendron Villiers	1	?	?
Leucaena Benth.	22	17	0
Microlobius C. Presl.	1	1	
Mimosa L.	530+[1]	117	0
Mimozyganthus Burkart	1	1	
Neptunia Lour.	12	5	0
Newtonia Baill.	15	0	3
Parapiptadenia Brenan	6	3	0
Parkia R. Br.	~34	0	15
Pentaclethra Benth.	3	1	1
Piptadenia Benth.	23	6	0
Piptadeniastrum Brenan	1	1	
Piptadeniopsis Burkart	1	?	?
Pityrocarpa (Benth.) Britton & Rose	3	2	0
Plathymenia Benth.	1	1	
Prosopidastrum Burkart	~5	?	?
Prosopis L.	~44	19	0
Pseudopiptadenia Rauschert	11	2	0
Pseudoprosopis Harms	7	?	?
Schleinitzia Warb. ex Nevling & Niezgoda	4	2	0
Stryphnodendron Mart.	30	6	0
Tetrapleura Benth.	2	0	2
Xerocladia Harv.	1	1	
Xylia Benth.	9	0	4

[1] M. Simon, personal communication.

unusual feature is that *Pentaclethra*, which has three species, has at least one that can and one that cannot nodulate. There are many negative reports for *P. macrophylla* (African) and several positive reports for *P. macroloba* (South American). There are no reports for the third species, *P. eetveldeana* (African). Of the other non-nodulating mimosoid genera, most are African. As several of the genera whose nodulation status is unrecorded are grouped with known non-nodulating genera, it is likely that the proportion of non-nodulating genera will increase. Where they stand in possible evolutionary terms will be discussed in Chapter 3.

The largest genus in Mimoseae is *Mimosa*, with over 500 species, of which 115 are now known to nodulate (Elliott et al., 2008). Many of these nodulate with β-rhizobia (*Burkholderia* and *Cupriavidus*), rather than α-rhizobia, a topic that will be discussed further in Chapter 4. Elliott et al. (2007a) looked at the ability of genera related to *Mimosa* to nodulate with β-rhizobia and found no coherent pattern. The next largest genus, *Prosopis*, nodulates with a variety of α-rhizobia, but some species can also use β-rhizobia (James et al., unpublished data). Many of its species are highly drought and salinity tolerant and have interesting physiological properties such as bringing up water from deep in the soil (hydraulic lift). These properties are considered in Chapters 2 and 5. *Neptunia* has been quite widely studied because of the aquatic habitat of many of its species. Its species nodulate with a range of α-rhizobia (Chapter 4) from different genera.

1.3 Papilionoideae

Papilionoideae is the largest and most complex of the three sub-families. For the present purpose, it has been subdivided on characteristics related to nodulation, nodule structure and nodule physiology. Fig. 1.5 outlines the major groups.

1.3.1 Non-nodulation in the Papilionoideae

As with Mimosoideae, some genera appear never to have had the ability to nodulate, whilst in others this ability may have been lost (as in some species of *Acacia*, sub-genus *Aculeiferum*). This section will deal with genera thought to be in the first category.

Three tribes of papilionoid legumes contain genera that appear to be unable to nodulate, namely Dipterygeae, Sophoreae and Swartzieae (the non-nodulating branch coupled to the nodulating Swartzia group in Fig. 1.5). The last two of these tribes also contain nodulating genera, and these are listed in Table 1.6. Tribe Swartzieae currently consists of 17 genera, but this number is likely to change as it is known not to be monophyletic and was even once thought to belong in the Caesalpinioideae. Of the 13 genera not listed in Table 1.6, there is no information on four, one (*Bocoa*) has mixed reports, with the remaining eight (*Aldina, Amburana, Baphiopsis, Cordyla, Exostyles, Harleyodendron, Mildbraediodendron,* and *Zollernia*) having good evidence of non-nodulation (Sprent, 2001). The latter group has a total of 41 species. Ireland (2005) put the four nodulating genera, together with *Bocoa, Candolleodendron* and *Trischidium*, in one

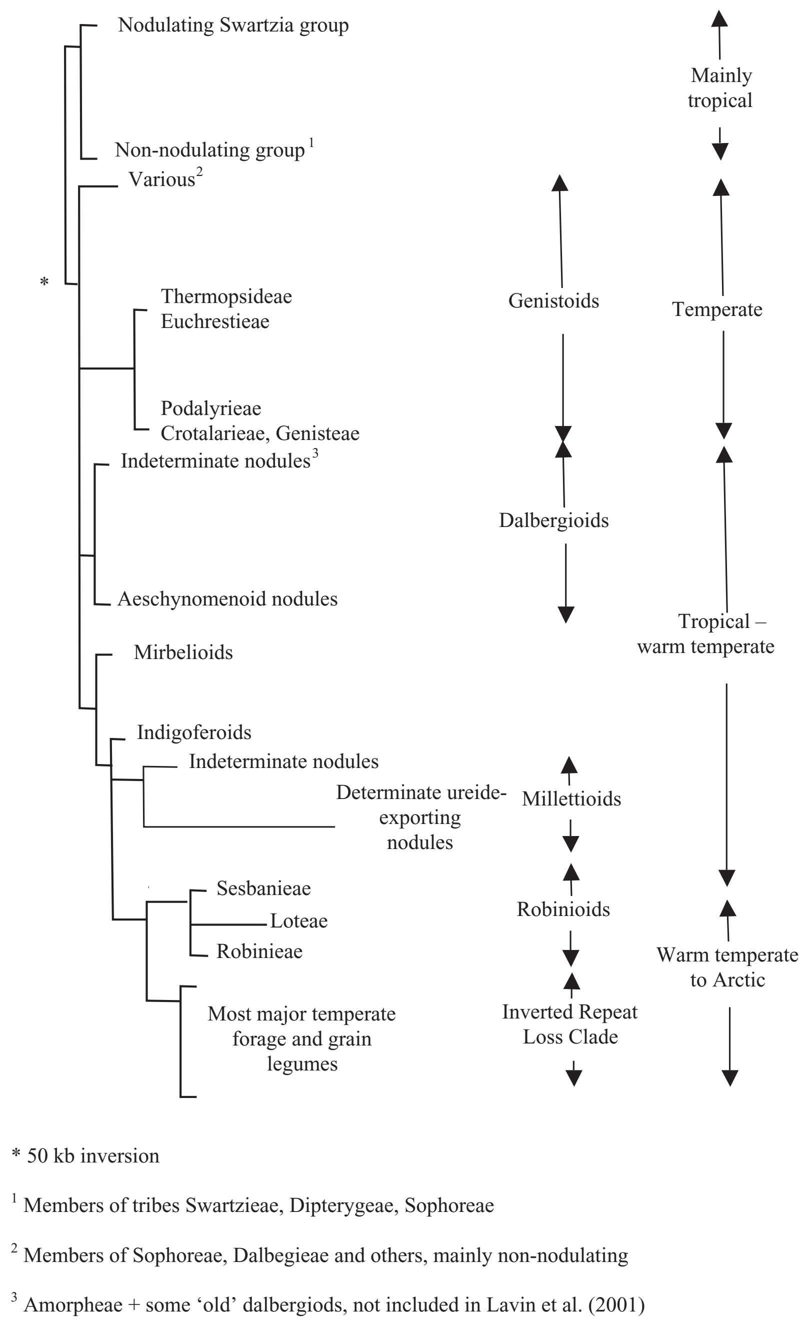

Figure 1.5 Positions of the major groups of nodulating legumes in the Papilionoideae. (Modified from Sprent, 2007.)

Table 1.6 Known nodulating members of tribes Swartzieae and Sophoreae

Tribe Genus	Species	Nod.
Swartzieae		
Ateleia (DC) Benth.	20	5
Bobgunnia J.H. Kirkbr. & Wiersema	2	1
Cyathostegia (Benth.) Schery	1	1
Swartzia Schreb.	∼180	27
Sophoreae		
Acosmium Schott.	17	3
Baphia Afzel. ex Lodd.	47	7
Bolusanthus Harms	1	1
Bowdichia Kunth.	2	2
Cadia Forssk.[1]	7	2
Calia Berland.	4	2
Clathrotropis (Benth.) Harms	6	3
Diplotropis Benth.	12	4
Maakia Rupr.	∼8	1
Ormosia G. Jacks.	∼130	26
Pericopsis Thwaites	4	3
Sophora L.	∼50	15

[1] Boatwright et al. (2008a) suggest that this genus might be better placed in tribe Podalyrieae.

branch of the tribe, which may eventually become the tribe in its entirety. Since the last two of these genera are segregates from *Swartzia*, and since evidence for *Bocoa* is equivocal (Sprent, 2001), more observations are urgently needed to see if this branch is a nodulating one, a suggestion that is strongly supported by the detailed analysis of Torke and Schaal (2008), who placed these genera, plus *Fairchildia panamensis* (formerly *Swartzia panamensis*, with no record of its nodulation) in a core Swartzioid clade. In Ireland (2005) the non-nodulating genera together with the unknown ones *Holocalyx* and *Lecointea* form three separate groups interspersed among parts of the Sophoreae. Tribe Sophoreae is also in a rather chaotic state and has been known for many years not to be monophyletic. In Pennington et al. (2005) it has 45 genera, 12 of which are known to nodulate (Table 1.6). Of the remainder, 12 (67 spp.) probably cannot nodulate and 21 (40 spp.) have no or unconfirmed reports.

The whole situation in tribes Sophoreae and Swartzieae is confused by the fact that both have genera with and without the 50kb inversion of the chloroplast genome. Considering the genera that do not have this inversion, there are four small groups of Sophoreae, a group of Swartzioid genera and tribe Dipterygeae, all of which appear unable to nodulate. Separate from these, but also lacking the inversion, is the group of nodulated Swartzioid genera mentioned above. The one anomaly from the nodulation perspective is the inclusion of *Pickeringia* from tribe Thermopsideae, a problem discussed in Sprent (2007). Genera that have the 50kb inversion include most of Sophoreae, a tribe that is divided into several groups. Although the evidence is far from complete, it cannot be excluded that all of these groups, except the one containing *Sweetia* and *Leutzelburgia*, contain only nodulating genera. *Sweetia* and *Leutzelburgia* are grouped with *Vatairia* and *Vatairiopsis*, two non-nodulating genera formerly in tribe Dalbergieae. Scattered among the groups of Sophoreae are some groups of non-nodulating Swartzioids (Pennington et al., 2005).

1.3.2 Nodulating papilionoids with primitive nodule structure

Five genera, (*Andira*, *Cyclolobium*, *Dahlstedtia*, *Poecilanthe*, and *Hymenolobium*), all with the 50kb inversion, are known to have bacteria retained within fixation threads (Fig. 1.2D), rather than released into symbiosomes (Fig. 1.2; Chapter 5). This feature is the norm in caesalpinioid legumes (see above) and has never been found in mimosoid legumes. It is also not known from nodules in tribe Swartzieae, generally considered to be basally branching within Papilionoideae. It is fair to say that the five genera listed above have been rather a headache for taxonomists, using a wide array of characteristics, but not including nodule structure. *Andira* and *Hymenolobium*, still formally placed in tribe Dalbergieae, are now included in a separate clade, close to the non-nodulating genera *Vatairia* and *Vatairiopsis* (Klitgaard & Lavin, 2005). The other three genera were earlier placed in tribe Millettieae, but *Cyclolobium* and *Poecilanthe* have recently been transferred to tribe Brongniartieae (Ross & Crisp, 2005), leaving *Dahlstedtia* in Millettieae. The present constitution of tribe Brongniartieae is given in Table 1.7. The large Australian component was formerly the *Templetonia* group of tribe Bossieaeeae (see section 1.3.5). Even within their new home, *Cyclolobium* and *Poecilanthe* are placed separate from the remainder of the tribe (Ross & Crisp, 2005). The only common features for the five genera with primitive nodule structure are that all are woody and all are found in South America, properties that are common to all but two of the nodulating caesalpinioid legumes (see section 1.1). Little is known of the rhizobia nodulating any of these genera or of other members of tribe Brongniartieae, with the exception of the Australian genera *Hovea* and *Templetonia*. The former was found to be nodulated only by bradyrhizobia in Southeast Australia (Lafay & Burdon, 1998) and both are nodulated by the fast-growing strain NGR 234 (Pueppke & Broughton, 1999).

Table 1.7 Nodulation in tribe Brongniartieae, genera as given in Ross & Crisp (2005)

Genus	Species	Nod.	Comment
Brongniartia Kunth.	~63	1	98% spp. in Mexico, none in Australia
Cristonia J.H. Ross	1	?	Ex *Templetonia*
Cyclolobium Benth	1	1	South America
Harpalyce Moçino & Sessé	24	2	Central America
Hovea R. Br. ex W.T. Aiton	37	8	Australia
Lamprolobium Benth.	2	1	Australia
Plagiocarpus Benth.	1	?	North Australia
Poecilanthe Benth	10–12	6	South America
Templetonia R. Br. ex W.T. Aiton.	10	2	Australia
Thinicola J.H. Ross	1	?	Ex *Templetonia*

In the overall phylogeny of legumes, as shown in Fig. 1 in Lewis et al. (2005), the tribe is placed near Sophoreae and far removed from the typical Australian tribes, discussed later.

1.3.3 Tribes with the 50kb inversion

All tribes discussed from this point on have the 50kb inversion. The first major branch, referred to as the Genistoid clade, is a rather heterogeneous group and includes some genera discussed earlier, i.e. those in tribe Brongniartieae, parts of Sophoreae, Swartzieae and anomalous members of old tribe Dalbergeae (the branch labelled 'various'). The remaining tribes included are Euchrestieae and Thermopsideae, which form a sub-clade with parts of Sophoreae and a group that includes Podalyrieae, Crotalarieae and Genisteae. On nodule characteristics, the last two fit well together, but the Podalyrieae have more in common with earlier tribes. They are so divided below.

Euchrestieae, Thermopsideae and Podalyrieae

Euchrestieae has only one genus, with four species found in Asia. It is likely that it will be included in a modified Sophoreae, possibly even included in genus *Sophora* (Ohashi, 2005). This is consistent with known nodulation characteristics. Thermopsideae are exclusively northern hemisphere and Podalyrieae exclusively South African, mainly from the Cape area. Their close relation thus seems a bit obscure. Table 1.8 lists their genera, but note that *Pickeringia* may be moved as discussed above. So far, genera in

Table 1.8 Genistoid tribes Thermopsideae and Podalyrieae

Tribe Genus	Species	Nod.
Thermopsideae		
Ammopiptanthus H.S. Cheng	1–2	1
Anagyris L.	2	2
Baptisia Vent.	15–17	5
Pickeringia Nutt.	1	1
Piptanthus Sweet	2	2
Thermopsis R. Br.	∼23	8
Podalyrieae		
Amphithalea Eckl. & Zeyh.	42	3
Calpurnia E. Mey.	7	2
Cyclopia Vent	23	12
Liparia L.	20	5
Podalyria Willd	14	10
Stirtonanthus B.-E. van Wyk A.L. Schutte	3	?
Virgilia Poir.	2	2
Xiphotheca Eckl. & Zeyh.	9	2

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Thermopsideae are only known to nodulate with α-rhizobia, but within that group, a range of fast- and slow-growing species. In Podalyrieae, five species of *Cyclopia* (Elliott et al., 2007b), three from *Podalyria* plus *Virgilia oroboides* (James & Sprent, unpublished data) can be nodulated by the β-rhizobium *Burkholderia tuberum*. In terms of nodule structure and morphology, both groups have indeterminate nodules, often branched and the infected tissue contains both infected and uninfected cells. Infection has not been widely studied, but in *Cyclopia* occurs through root hairs (Elliott et al., 2007b). Where information is available this is not true of tribes Crotalarieae and Genisteae (Sprent, 2007).

Crotalarieae and Genisteae

Genera in tribes Crotalarieae and Genisteae are given in Table 1.9. Both are likely to be revised. Whilst there appear to be similarities in nodule structure and possible infection mechanism, these two tribes have very different geographical ranges. Crotalarieae is largely African and this tribe will be considered first. Most species (about 510) of the largest genus, *Crotalaria*, are found in Africa, but there are significant numbers found in Asia and Australia (van Wyk, 2005). It was the first genus of legume where nodulation by non-classical rhizobia was reported. Sy et al. (2001) showed that a group of species of *Crotalaria* from Senegal could be split into two, depending on whether or not they could be nodulated by a methanol metabolising bacterium from the α-Proteobacteria, *Methylobacterium nodulans*. This division of species according to endophyte does not coincide with intraspecific groupings based on other characteristics (personal observations). Since then, similar results have been reported for species of *Lotononis* (Jafthe et al., 2002), a mainly South African genus, but extending into Mediterranean regions. Yates et al. (2007) showed that some, but not all species could be nodulated by a species of *Methylobacterium*, which in this case could not metabolise methanol. More details of these bacteria will be given in Chapter 4. *Aspalathus* is endemic to South Africa, with most species belonging to the Cape Floristic Region (CFR). Because of the importance of *A. linearis* for production of rooibos (red bush) tea, this species has been widely studied. However, the exact nature of its endophyte is still not clear. The genus as a whole nodulates with a variety of α-rhizobia (Deschodt & Strijdom, 1976), but a report of nodulation by *Burkholderia* (Moulin et al., 2001) has not been substantiated and Elliott et al. (2007b) found that several species could not be nodulated by *B. tuberum*, a species that nodulates several species of another CFR endemic, *Cyclopia* (tribe Podalyrieae). The genus *Lebeckia* as described by van Wyk (2005) has now been separated into three, *Calobota*, *Lebeckia* and *Wiborgiella* (Boatwright et al., 2008b). All are African, with most species in the CFR. The first two genera can be nodulated by α- and β- rhizobia (Phallane et al., 2008), but the endophyte of *Wiborgiella* spp. is not yet known. Nodules are indeterminate and can be extensively branched, a type of morphology described as crotalarioid by Corby (1988). Few detailed structural studies have been made, but those that have suggest that the infected tissue does not contain uninfected cells (summarised in Sprent, 2007). The recent study on *Lotononis angolensis* (Yates et al., 2007) clearly shows this and also that in this case nodule meristems may grow around the subtending root forming a type of nodule called

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Table 1.9 Tribes Crotalarieae and Genisteae

Tribe Genus	Species	Nod.
Crotalarieae		
Aspalathus L.	278	70
Bolusia Benth.	5	1
Calobota Eckl. & Zeyh.[1]	16	6
Crotalaria L.[2]	~600	145
Lebeckia Thunb.[1]	14	11
Lotononis (DC) Eckl. & Zeyh.[1]	~150	62
Pearsonia Dümmer	13	6
Rafnia Thunb.	19	14
Robynsiophyton R. Wilczek	1	1
Rothia Pers.	2	2
Wiborgia Thunb.	10	4
Wiborgiella Boatwr. & B-E van Wyk[2]	9	1
Genisteae		
Adenocarpus DC.	~15	3
Anarthrophyllum Benth.	15	?
Argyrocytisus (Maire) Raynaud	1	?
Argyrolobium Eckl. & Zeyh.	~80	30
Calicotome Link	2–3	2
Cytisophyllum O. Lang	1	1
Cytisus Desf.[3]	~65	18
Dichilus DC.	5	3
Echinospartum (Spach) Rothm.	5	?
Erinacea Adans.	1	?
Genista L.	~90	18
Gonocytisus Spach.	3	?
Hesperolaburnum Maire	1	1
Laburnum Fabr.	2	2
Lembotropis Griseb.	2	1
Lupinus L.	220–230	65
Melolobium Eckl. & Zeyh.	15	9
Petteria C. Presl.	1	1
Podocytisus Boiss. & Heldr.	1	?
Polhillia C.H. Stirt.	7	?
Retama Raf.	4	3
Sellocharis Taub.	1	?
Spartium L.	1	1
Stauracanthus Link.	3	1
Ulex L	10–20	2

[1] See revision of *Lebeckia* in Boatwright et al. (2008b).
[2] Subject to revision.
[3] Includes *Chamaectyisus*.

lupinoid by Corby (1988) because it is commonly found in lupins (Fig. 1.1). Similar nodule structure is found in all eight species of section Listii (Ardley et al., 2008), but no other sections of the genus sampled so far (Sprent & van Wyk, unpublished data), which is interesting in view of the likelihood that section Listii may form the basis of a separate genus (B.E. van Wyk, personal communication). Clearly this tribe is of considerable interest for its nodulation characteristics.

Tribe Genisteae is also interesting from a nodulation point of view, but its characteristics are different from those of the Crotalarieae. Most of the genera are woody shrubs from Mediterranean regions, extending into Africa and sometimes into mountainous regions, especially in the New World. The largest genus, *Lupinus*, is complex, but has been widely studied because of its ability to grow on poor acid soils and because some of its species, such as *L. albus*, are important grain legumes. There are regular international symposia for the lupin fraternity, held in very different locations, such as Iceland, New Zealand and Australia. Genera in Genisteae are nodulated by a variety of α-rhizobia, with none yet known to use bacteria outside this group. Lupin nodules are formed following a type of epidermal infection and infection threads are rarely seen. In *Cytisus* (under its previous name *Chamaecytisus*), infection threads are transient and are not part of the infection mechanism (Vega-Hernández et al., 2001). A detailed study of *Genista tinctoria* also found that the infected tissue contained no uninfected cells (Kalita et al., 2006). More details of nodule structure and its significance will be given in Chapters 3 and 5. Species of *Lupinus* are amongst those that can grow at high altitudes and their isolation in some such locations has enabled them rapidly to develop new species (Hughes & Eastwood, 2006). Legumes in mountain habitats will be considered in Chapter 2. Because of their ability to grow on poor soils and exposed locations, many genistoid legumes, especially species of *Cytisus* and *Ulex*, have invaded areas such as Australia and New Zealand where they are generally regarded as noxious weeds. The genus *Polhillia* is native to the CFR and its nodulation status is not known.

Amorpheae

Tribe Amorpheae is placed close to the Dalbergioid clade in Lewis et al. (2005). Its genera are listed in Table 1.10. All are from North or Central America, with some extending into South America. Most have xerophytic characteristics and can grow in desert areas.

Table 1.10 Tribe Amorpheae

Genus	Species	Nod.
Amorpha L.	15	7
Apoplanesia C. Presl.	1	?
Dalea L.	∼160	17
Errazurizia Phil.	4	?
Eysenhardtia Kunth.	∼10	1
Marina Liebm.	38	1
Parryella Torr. & A. Gray	1	1
Psorothamnus Rydb.	9	4

At least two genera (*Amorpha* and *Dalea*) have indeterminate nodules (Corby, 1988), making them distinct from those in the Dalbergioid clade. They can apparently be nodulated by various α-rhizobia, but little detailed work has been carried out. However, it is now being realised that some of the native plants of North America are being threatened by agriculture and should be studied. Mártir et al. (2007) looked at *Dalea purpurea* (purple prairie clover) and found it to be nodulated by several species of *Rhizobium*.

1.3.4 The Dalbergioid clade

The Dalbergioid clade is unique in the Leguminosae in that one of the characteristics used to define it is nodule morphology (Lavin et al., 2001). Table 1.11 lists its genera, excluding the anomalous ones discussed in section 1.3.2. There are three major sub-clades, corresponding to the tribes that have been amalgamated to form the new clade, except that the Dalbergia sub-clade also includes former tribe Aeschynomeneae. The Adesmia and Pterocarpus groups are largely Central and South American. The Dalbergia group has members from either side of the Atlantic and some that are pantropical. All that have been described have aeschynomenoid nodules (Fig. 1.1), which are located in the axils of lateral or adventitious roots and whose infected tissue does not contain uninfected cells. Infection, where known, is via cracks in the epidermis where roots emerge. *Brya* and *Cranocarpus*, which Corby (1988) noted had aeschynomenoid nodules, were formerly placed in tribe Desmodieae, but are now in the Pterocarpus group. Two closely related genera, *Nissolia* and *Chaetocalyx* (Adesmia group), appear to have lost the ability to nodulate. Within genus *Pterocarpus*, Brazilian species appear unable to nodulate, although species from elsewhere in South America and in Africa can (Faria et al., 1989). The genus *Aeschynomene*, which is currently being revised, has a number of species that can form nodules on stems. These are always associated with adventitious root initials and are true stem nodules, in that they are plumbed into the vascular system of the stem. There have been several reports of stem nodules on other legumes (e.g. *Neptunia* spp., Mimoseae), but these have all been shown to be plumbed into adventitious roots (Subba-Rao et al., 1995). It is common for trees and lianas in rainforest to produce adventitious roots on their trunks and these can be profusely nodulated (Fig. 1.2D in Sprent, 2001). Stem nodules are normally formed in wet conditions, especially when the root system is flooded. *Discolobium pulchellum*, a species from the large Pantanal freshwater region in Brazil, is unusual in that stem nodules only form under water (Loureiro et al., 1994). Nodules in the Dalbergioid clade may be formed in association with various α-rhizobia, usually slow-growing forms. The report that *Machaerium* can be nodulated by *Burkholderia* (Moulin et al., 2001) is almost certainly the result of incorrect identification of plant roots. Rasolomampianina et al. (2005) isolated seven different genera of nodulating bacteria, both α- and β-, from *Dalbergia* species in Madagascar, but these remain to be fully authenticated.

1.3.5 The Mirbelioid clade

In Lewis et al. (2005), the Mirbelioid clade includes three tribes, Hypocalypteae, Mirbelieae and Bossiaeeae. The first of these has only one genus, with three species and is

Table 1.11 Genera defined in the dalbergioid clade of Lavin et al. (2001). Where structure has been studied, nodules are aeschynomenoid (Sprent, 2001). Some genera apparently have lost the ability to nodulate

New sub-clade		
Genus	Species	Nod.
Adesmia		
Adesmia DC.	240	7
Amicia Kunth.	7	3
Chaetocalyx DC.	13	0
Nissolia Jacq.	13	0
Poiretia Vent.	11	1
Zornia J.F. Gmel.	75	15
Dalbergia		
Aeschynomene L.[1]	180	49
Bryaspis P.A. Duvign.	2	1
Cyclocarpa Afzel. ex Bak.	1	1
Dalbergia L.f.	250	51
Diphysa Jacq.	15	1
Geissapsis Wright & Arn.	2	2
Humularia P.A. Duvign.	35	1
Kotschya Endl.	31	8
Machaerium Pers.	130	25
Ormocarpopsis R. Vig.	6	?
Ormocarpum P. Beauv.	18	3
Peltiera Labat & Dupuy[2]	2	?
Pictetia DC.	8	?
Smithia Aiton	20	12
Soemmeringia Mart.	1	?
Weberauerella Ulbr.	2	2
Zygocarpum P. Beauv.	6	?
Pterocarpus		
Arachis L.	69	16
Brya P. Browne	4	2
Cascaronia Griseb.	1	?
Centrolobium Mart. ex Benth.	7	3
Chapmannia Torr. & A. Gray	7	1
Cranocarpus Benth.	3	1
Discolobium Benth.	8	3
Etaballia Benth.	1	1
Fiebrigiella Harms	1	?
Fissicalyx Benth.	1	?
Geoffroea Jacq.	2	1
Grazielodendron H.C. Lima	1	0
Inocarpus J.R. Forst. & G. Forst.	3	1
Maraniona Hughes et al., 2004	1	?
Paramachaerium Ducke	5	1

(*Continued*)

Table 1.11 (*Continued*)

Genus	Species	Nod.
Platymiscium Vogel	19	6
Platypodium Vogel	2	1
Pterocarpus Jacq.[3]	40	18
Ramorinoa Speg.	1	?
Riedeliella Harms	3	1
Stylosanthes Sw.	25	21
Tipuana (Benth.) Benth.	1	1

[1] About 16 of these are able to form nodules on stems as well as roots.
[2] This Madagascan genus is thought to be extinct.
[3] Brazilian species appear unable to nodulate.

endemic to the Cape region of South Africa. Nothing is known of its nodule structure. Tribes Mirbelieae and Bossiaeeae (Table 1.12) are Australian, with some Mirbelieae extending into south Papua New Guinea. Their taxonomy has been extensively studied, but their nodulation and nodule characteristics are only now beginning to be understood. Nodules in both tribes are indeterminate, often branched. A wide variety of fast and slow-growing α-rhizobia can nodulate them, the exact species often depending on soil pH (Lafay & Burdon, 1998; Thrall et al., 2000). Nodule structure in *Aotus ericifolia* shows infected tissue to contain both infected and uninfected cells and infection threads are seen (Lawrie, 1981) This does not necessarily mean that there is a root hair infection pathway (Sprent, 2007). Most genera are found in dry, often arid areas, in soils of very low fertility, a point which will be discussed in Chapter 2. *Viminaria* is unusual in that it is found in seasonally flooded soils and has negatively geotropic roots (pneumatophores) as well as cluster roots (Walker et al., 1983). These tribes have recently (∼5 Ma ago) diversified into arid areas (Chapters 2 and 3) and the characteristics that enable them to nodulate in environments that are generally regarded as unsuitable are urgently in need of study. Unfortunately, funds for studying nodulation in Australia (and in other countries) are targeted towards potential agricultural species. Members of these tribes may be toxic to grazing animals, with some species of *Gastrolobium*, for example, producing large quantities of fluoroacetate (Mead et al., 1985). Some populations of red kangaroos have co-evolved to cope with this.

1.3.6 The Millettioid clade

The Millettioid clade in Lewis et al. (2005) has three branches, first tribe Indigofereae, second Abreae + Millettieae, and third Desmodieae + Phaseoleae + Psoraleae. These branches will be considered in turn. Indigofereae has seven genera (Table 1.13), with the pantropical *Indigofera* being the third largest genus in Leguminosae. Although most species are African and Madagascan, considerable numbers are also found in Asia and Australia, with a few in the New World. Nodules are indeterminate and often branched, although *Indigofera linifolia* nodules when young appear desmodioid,

Table 1.12 Endemic Australian tribes

Tribe Genus	Species	Nod.
Bossiaeeae		
Aenictophyton A.T. Lee	1	?
Bossiaea Vent.	~60	16
Goodia Salisb.	2	2
Muelleranthus Hutch.	3	1
Platylobium Sm.	4	2
Ptychosema Benth.	2	?
Mirbelieae		
Almaleea Crisp & P.H. Weston.	5	?
Aotus Sm.	15–18	4
Callistachys Vent.	1	1
Chorizema Labill.	27	9
Daviesia Sm.	~135	20
Dillwynia Sm.	~40	12
Erichsenia Hemsl.	1	?
Euchilopsis F. Muell	1	1
Eutaxia R. Br. ex WT Alton	10	5
Gastrolobium R. Br.	109	24
Gompholobium Sm.	44	13
Isotropis Benth.	10	3
Jacksonia R. Br. ex Sm.	74	10
Latrobia Meisn.	6	1
Leptosema Benth.	13	1
Mirbelia Sm.	32	8
Otion Crisp & P.H. Weston	~8	?
Oxylobium Andrews	6	3
Phyllota (DC.) Benth.	11	3
Podolobium R. Br.	6	2
Pultenaea Sm.	104	27
Sphaerolobium Sm.	22	3
Stonesiella Crisp & P.H. Weston	1	?
Urodon Turcz.	4	?
Viminaria Sm.	1	1

Table 1.13 Tribe Indigofereae

Genus	Species	Nod.
Cyamopsis DC.	4	3
Indigastrum Jaub. & Spach.	~8	4
Indigofera L.	~700	200
Microcharis Benth.	36	3
Phylloxylon Baill.	7	?
Rhynchotropis Harms	2	?
Vaughania S. Moore	11	?

with lenticels, but when older become elongate, but retain the production of lenticels (H.S. Gehlot, personal communication). One species of *Indigastrum* is pan-tropical and genera *Phylloxylon* and *Vaughania* plus two species of *Microcharis* are from Madagascar, with the remaining species of all genera being African.

Abreae and Millettieae

Tribe Abreae has one pantropical genus, with seventeen species, four of which are known to nodulate. Nodules have been reported as desmodioid and (in other species) indeterminate, nodulating with slow-growing rhizobia (Sprent, 2001). In view of the fact that some phaseoloid genera are dimorphic for nodule morphology (see below), these data are consistent with placement of this genus near tribe Phaseoleae. Tribe Millettieae is complex, with two major groups being listed in Schrire (2005), with a much smaller third group (Table 1.14). The latter lacks the inverted repeat of the chloroplast genome and is included in the IRLC clade. (Fig. 1.5). The two main groups, referred to as the core and basal groups, span parts of the Phaseoleae. Somewhat depressingly, just over half the genera have not been examined for nodulation. However, all of those that have, from all three groups, possess indeterminate nodules, with the infected region containing both infected and uninfected cells. *Lonchocarpus muehlbergianus* lacks root hairs and is apparently infected between epidermal cells (Cordeiro et al., 1996). This feature may be of evolutionary significance (Sprent, 2007) as will be discussed in Chapter 3. As mentioned earlier, *Dahlstedtia* has primitive nodule structure. Where known, nodules are usually induced by slow-growing bacteria. In legume phylogenetic terms, understanding this largely tropical tribe is very important, and it is equally so for understanding nodule evolution. At present there is no reason on nodulation grounds to link any of the groups within the tribe to the remainder of the Millettioid clade, tribes Phaseoleae, Desmodieae and Psoraleae.

The Phaseoloid group; tribes Desmodieae, Phaseoleae and Psoraleae

Apart from some members of the temperate tribe Loteae (see section 1.3.7), tribes Desmodieae, Phaseoleae and Psoraleae (Table 1.15) are the only ones to have determinate (desmodioid) nodules (Figs. 1.1 and 1.2). There are occasional reports of genera with dimorphic nodules, for example in *Kennedia* and *Erythrina*. Also, although young nodules may be desmodioid, occasionally they may become lobed, or even branched when older (Sprent, 2001) All those tested from these tribes export ureides rather than amides as the products of nitrogen fixation (Sprent, 2001; Kanu et al., 2008, chapter 5). In current thinking, tribes Desmodieae and Psoraleae are nested within tribe Phaseoleae (Schrire, 2005), a fact entirely consistent with nodulation characteristics. However, there are still many genera that have not been sampled for either molecular or nodulation characteristics (42 out of 128 for the latter). Unlike many other legume tribes, there are no confirmed cases of phaseoloid legumes being unable to nodulate.

Two sub-tribes of Phaseoleae, Diocleinae (13 genera) and Ophrestiinae (3 genera), are separated from the rest of the group on molecular characteristics, being placed close to the core Millettieae. However, where known, their nodulation characteristics place them clearly in Phaseoleae (Sprent, 2001; Pueppke & Broughton, 1999). With some

Table 1.14 Tribe Millettieae. The three informal groups of Schrire (2005), core (C), basal (B) and IRLC groups are indicated

Genus	Species	Nod.	Group
Afgekia Craib	3	?	IRLC
Aganope Miq.	∼7	1	B
Antheroporum Gagnep.	∼4	?	IRLC?
Apurimacea Harms	∼2	?	C
Austrosteenisia Geesink	4	?	C
Behaimia Griseb.	1	?	C
Bergeronia M. Micheli	1	1	C
Burkilliodendron (Ridl.) Sastry	1	?	B
Callerya Endl.	∼20	2	IRLC
Chadsia Bojer	9	1	C
Craibia Harms & Dunn	10	1	B
Craspedolobium Harms	1	?	B
Dahlstedtia Malme.	2	1	C
Dalbergiella Baker f.	3	1	B
Deguelia Aubl.	∼17	5	C
Derris Lour.	55–60	2	C
Dewevrea Micheli	1–2	1	B
Disynstemon R. Vig.	1	?	B?
Endosamara Geesink	1–2	?	IRLC
Fordia Hemsl.	18	?	B
Hesperothamnus Brandegee	5	?	C
Kunstleria Prain	8	?	B
Leptoderris Dunn.	∼20	3	B?
Lonchocarpus Kunth.	∼120	13	C
Margaritilobium Harms	1	?	C
Millettia Wight & Arn.	∼150	16	C
Muellera L.f.	2	?	C
Mundulea (DC.) Benth.	12	1	C
Ostryocarpus Hook f.	1–2	?	B
Paraderris (Miq.) Geesink	∼13	?	C
Paratephrosia Domin.	1	?	C
Philenoptera Fenzl. ex A. Rich.	12	4	C
Piscidia L.	∼7	2	C
Platycyamus Benth.	2	1	B
Platysepalum Welw. ex Baker	7–8	?	B?
Pongamiopsis R. Vig.	3	?	C
Ptycholobium Harms	3	3	C
Pyranthus Du Puy & Labat.	6	?	C
Requiena DC.	3	2	C
Sarcodum Lour.	∼3	?	IRLC
Schlefflerodendron Harms	4	?	B
Sylvichadsia Labat & Du Puy	4	?	B?
Tephrosia Pers.	∼350	83	C
Wisteria Nutt.	5–6	6	IRLC
Xeroderris Roberty	1	1	B

Table 1.15 Tribes with determinate nodules and, where tested, exporting the ureides allantoin and allantoic acid as products of nitrogen fixation

Tribe Genus	Species	Nod.
Desmodieae		
Ackschlindlium H. Ohasi	1	?
Alysicarpus Desv.	25–30	14
Aphyllodium (DC) Gagnep.	7	1
Arthroclianthus Baill.	~30	?
Campylotropis Bunge	~37	2
Christia Moench	~10	2
Codariocalyx Hassk.	3	2
Dendrolobium (Wright & Arne)	18	3
Desmodiastrum (Prain) A. Pramanik & K. Thothathri	4	?
Desmodium Desv.	~275	74
Droogmansia De Wild	~5	2
Eleiotis DC.	2	?
Hanslia Schindl.	2	?
Hegnera Schindl.	1	?
Hylodesmum H. Ohashi & R.R. Mill.	14	5
Kummerowia Schlindl.	2	2
Leptodesmia (Benth.) Benth.	5	?
Lespedeza Michx.	~35	31
Mecopus Benn.	1	?
Melliniella Harms.	1	?
Monoarthrocarpus Merr.	1	?
Nephrodesmus Schindl.	6	?
Ougeinia Benth.	1	1
Ohwia H. Ohashi.	2	1
Phyllodium Desv.	8	2
Pseudarthria Wight & Arn.	3–4	2
Pycnospora R. Br. ex Wight & Arn.	1	1
Tadehagi H. Ohashi.	~6	1
Trifidacanthus Merr.	1	?
Uraria Desv.	~20	7
Phaseoleae		
Adenodolichos Harms.	15–20	1
Alistilus N.E. Br.	3	1
Amphicarpaea Elliot ex Nutt.	4–5	2
Apios Fabr.	~7	2
Austrodolichos Verdc.	1	?
Barbieria DC.	1	0?
Bolusafra Kuntze.	1	1
Butea Roxb. ex Willd.	2	1?
Cajanus DC.	34	7
Calopogonium Desv.	5–6	2
Camptosema Hook. & Arn.	10	3
Canavalia DC.	~60	7
Carrissoa Baker f.	1	?

Table 1.15 (*Continued*)

Tribe Genus	Species	Nod.
Phaseoleae		
Centrosema (DC.) Benth.	~36	14
Chrysoscias E. Mey	3–4	?
Cleobulia Mart. ex Benth.	3–5	1
Clitoria L.	~62	12
Clitoriopsis R. Wilczek.	1	?
Cochlianthus Benth.	2	?
Collaea DC.	7	1
Cologania Kunth.	~12	2
Cratylia Mart. ex Benth.	~7	3
Cruddasia Prain	~2	?
Cymbosema Benth.	1	?
Decorsea R. Vig.	6	1
Dioclea Kunth.	~40	9
Diphyllarium Gagnep.	1	?
Dipogon Liebm.	1	1
Dolichopsis Hassl.	1	1
Dolichos L.	~60	13
Dumasia DC.	~10	2
Dunbaria Wight & Arn.	20	4
Dysolobium (Benth.) Prain.	4	1
Eminia Taub.	~4	1
Eriosema (DC.) Rchb.	~150	29
Erythrina L.	~120	40
Flemingia Roxb. ex W.T. Aiton.	30–35	8
Galactia P. Browne	55–60	10
Glycine Willd.	19	17
Hardenbergia Benth.	3	2
Herpyza Sauvalle	1	?
Kennedia Vent.	~15	13
Lablab Adans.	1	1
Lackeya R.H. Fortunata, L.P. de Queiroz & G.P. Lewis.	1	?
Luzonia Elmer	1	?
Macropsychanthus Harms ex K. Schum. & Lauterb.	~2	?
Macroptilium (Benth.) Urb.	~17	7
Macrotyloma (Wight & Arn.) Verdc.	24	11
Mastersia Benth.	2	1
Meizotropis J. Voigt	2	?
Mucuna Adans.	~105	17
Mysanthus G.P. Lewis & A. Delgado	1	?
Neocollettia Hemsl.	1	?
Neonotonia Lackey	2	1
Neorautanenia Schinz.	5	3
Neorudolphia Britton	1	1
Nesphostylis Verdc.	4	?
Nogra Merr.	~3	?

(*Continued*)

Table 1.15 (*Continued*)

Tribe Genus	Species	Nod.
Phaseoleae		
Ophrestia H.M.J. Forbes.	~16	3
Oryxis A. Delgado & G.P. Lewis	1	?
Otoptera DC.	2	1
Oxyrhynchus Brandegee	4	1
Pachyrhizus Rich. ex DC.	5	4
Paracalyx Ali	6	?
Periandra Mart. ex Benth.	6	2
Phaseolus L.	60–65	11
Phylacium Benn.	2	?
Physostigma Balf.	~4	?
Pseudeminia Verdc.	4	1
Pseudoeriosema Hauman	~4	?
Pseudovigna (Harms) Verdc.	2	1
Psophocarpus DC.	~10	5
Pueraria DC.	~18	4
Ramirezella Rose.	7	1
Rhodopsis Urb.	2	?
Rhynchosia Lour.	~230	61
Shuteria Wight & Arn.	4–5	2
Sinodolichos Verdc.	2	?
Spathionema Taub.	1	?
Spatholobus Hassk.	29	2
Sphenostylis E. Mey.	7	4
Strongylodon Vogel.	12	3
Strophostyles Elliot	3	3
Teramnus P. Browne	9	4
Teyleria Backer	3	1
Vandasina Rauschert	1	1
Vatovaea Chiov.	1	?
Vigna Savi	~104	49
Wajira Thulin	5	1
Psoraleae		
Bituminaria Heist. ex Fabr.	2	2
Cullen Medik.	~34	11
Hoita Rydb.	3	2
Orbexilum Raf.	8	3
Otholobium C.H. Stirt.	61	10
Pediomelum Rydb.	21	3
Psoralea L.	~50	19
Psoralidium Rydb.	3	2
Rupertia J.W. Grimes	3	?

P1: SFK/UKS P2: SFK/UKS QC: SFK/UKS T1: SFK
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notable exceptions, such as tree species of *Erythrina*, most of the species in this group of tribes are small shrubs or herbaceous annuals and perennials. Apart from some species of *Lupinus* (tribe Genisteae), *Arachis* (peanut, groundnut, tribe Dalbergieae), *Vicia* and *Pisum* (tribe Fabeae), tribe Phaseoleae houses the world's most important grain legumes, as well as many forage, browse and tuber species These are described in many books, including Allen & Allen (1981) and Anon (1979), and some will be discussed further in Chapter 5. A few species, such as soybean (*Glycine max*) and dry bean (*Phaseolus vulgaris*), are industrial crops, although the latter is also extremely important for subsistence farmers in many developing countries, and thus their symbiosis with rhizobia has been extensively studied. This is particularly true for soybean. In the US, unlike Brazil, breeders have generally selected potentially high-yielding cultivars on fertilized plots and thus have unwittingly selected against nitrogen fixation. Denison and co-workers have been studying ways in which soybean plants might sanction against ineffective rhizobia and ensure that they only nodulate with effective strains. In their most recent study (Kiers et al., 2007), they have looked at a range of historic and modern cultivars and concluded that the latter were less able to sanction against inefficient rhizobia.

The ability to export ureides has been linked to the tropical and sub-tropical habitats of this group of legumes, for various reasons including their low solubility (Sprent, 1980). It would be interesting to know whether species occurring naturally in cooler areas – such as in the three genera *Strophostyles* (Phaseoleae), *Pediomelum* and *Rupertia* (Psoraleae) that are found in Southern Canada – export ureides. Because of their determinate nature, desmodioid nodules have a limited life span (usually a few weeks) and cannot regrow after environmental stress, unlike nodules with an apical meristem, which may be perennial. This again has been linked to living in warmer regions. The main environmental threat is drought and this may lead to nodule loss.

As might be expected of such a large group of genera, nodulation can be induced by a wide variety of bacteria. These include many genera of α-rhizobia, both fast- and slow-growing. The first reports of nodulation by β-rhizobia are now appearing. In their study of nodulation in *Cyclopia* (Podalyrieae), Elliot et al. (2007b) found that *Burkholderia tuberum* could effectively nodulate the promiscuous host plant *Macroptilium purpureum* (Desmodieae). More recently Garau et al. (2009) isolated a new species of *Burkholderia* from *Rhynchosia ferulifolia*, a phaseoloid species endemic to the Cape region of South Africa. This bacterium did not nodulate other species of *Rhynchosia* tested. Pueppke and Broughton (1999) tested a vast number of legumes for ability to nodulate with the wide host range bacterium NGR 234, isolated from Papua New Guinea, including five species of *Rhynchosia*. Three of these, all from either Africa or Asia, formed effective nodules, but the other two, from Central and South America, did not nodulate at all with this strain. Species of this very large genus appear to show quite a high level of specificity with widely differing bacteria. This contrasts, for example, with *Phaseolus vulgaris*, which nodulates with a wide range of rhizobia, but as yet none outside the major α-rhizobia.

1.3.7 The Robinioid clade

In Lewis et al. (2005) the Robinioid clade has three tribes, Loteae, Sesbanieae and Robinieae (Tables 1.1, 1.16), forming an interesting group from a nodulation point of

Table 1.16 Tribes Sesbanieae, Robineae and Loteae. The latter now includes Coronilleae, but the genera in these former tribes have been kept separate here, because of their different nodule morphologies

Tribe Genus	Genus	Nod.
Sesbanieae		
Sesbania Adans.	60	38
Robineae		
Coursetia DC.	~35	2
Genistidium I.M. Johnst.	1	?
Gliricidia Kunth.	5	1
Hebestigma Urb.	1	1
Lennea Klotzsch	3	?
Olneya A. Gray	1	1
Peteria A. Gray	4	?
Poissonia Baill.	4–5	?
Poitea Vent.	12	1
Robinia L.	4	2
Sphinctospermum Rose	1	?
Loteae Nodules determinate		
Acmispon Rafin.	8	2
Anthyllis L.	22	5
Cytisopsis Jaub. & Spach	2	?
Dorycnium Miller	8	4
Dorycnopsis Boiss.	2	?
Hosackia Benth. ex Lindl.	11	3
Hymenocarpos Savi.	1	1
Keberita Kramina & D.D. Sokoloff	1	1
Lotus L.	~125	53
Ottleya D.D. Sokoloff	11	2
Podolotus Benth.	1	1
Pseudolotus Rech. f.	1	?
Syrmatium Vogel	14	4
Tetragonolobus Scop.[1]	6	3
Tripodion Medik.	1	1
Former Coronilleae, indeterminate nodules, where known		
Antopetitia A. Rich.	1	1
Coronilla L.	9	4
Hammatolobium Fenzl	2	?
Hippocrepis L	34	6
Ornithopus L.	~5	5
Scorpiurus L.	2	2
Securigera DC.	13	4

[1] May be included in *Lotus* (Sokoloff & Lock, 2005).

view. The genus *Sesbania* has been taken out of tribe Robinieae and put in a tribe of its own (Lavin & Schrire, 2005). Some species are able to form nodules located on stems, but plumbed into adventitious root initials. *S. rostrata* has its own nodulating genus, *Azorhizobium caulinodans*, the only genus of the α-rhizobia so far shown to fix and assimilate nitrogen ex planta (Chapter 5). Stem nodules superficially appear aeschynomenoid, and have a crack infection, but later form infection threads and are structurally unlike aeschynomenoid nodules. Under certain conditions they become indeterminate and nodules on roots (these can be induced by rhizobia other than *A. caulinodans*) are infected via root hairs. These features are discussed in more detail in Chapter 5.

Tribe Robinieae (Table 1.16) is confined to the Americas and the Caribbean. Some species of *Coursetia* have been transferred to *Poissonia* and *Sphinctospermum* is now placed here rather than in Millettieae. The most widely studied species is *Robinia pseudoacacia*, which is widely grown as an ornamental, but which suckers badly and can become very invasive, for example in parts of Europe. *Gliricidia sepium* is widely used in agroforestry in Africa and elsewhere. It and other species in this tribe can nodulate with a wide variety of bacteria, with varying degrees of effectiveness (Bala & Giller, 2001). Nodules are generally indeterminate with no known unusual features.

Molecular and morphological data have now firmly placed former tribe Coronilleae in tribe Loteae, which is a pity from a nodulating point of view since genera in it have indeterminate and in the former Loteae determinate nodules (Corby, 1988; Sprent, 2001). In the revised Loteae, there are two main groups and these do not correspond to the former tribes (Sokoloff & Lock, 2005). The current tribe Loteae is thus an anomaly in its nodule characteristics. However, although some are determinate, they do not export ureides, as in the determinate nodules of the Phaseoloid group (see section 1.3.6). Some, such as *Lotus uliginosus*, can be found in the far north of Europe, where indeterminate nodules are the norm. These aspects will be discussed again in Chapters 2 and 5. The overall distribution of the tribe is north temperate, but extending down to South America and some species of *Lotus* are also found in Australia. *L. japonicus* is one of the 'model' legumes under intensive study (Chapter 3). Apart from this species, little is known of the rhizobia nodulating members of tribe Loteae, except that they are usually fast growing.

1.3.8 The inverted repeat lacking clade (IRLC)

The final group of tribes (together with some anomalous parts of other tribes) form the IRLC clade (Fig. 1.5), whose members have lost an inverted duplicated insertion in the chloroplast genome. This is a major genetic change and if it has only happened once, then those genera that have it should be segregated from those that do not. This would also apply to the third group of the Milletteae, discussed above. The tribes within it are Galegeae, Hedysareae, Cicereae, Trifoleae and Fabeae. Almost all genera are temperate or boreal, and all have indeterminate nodules, usually nodulated by fast growing rhizobia, with large variations in degrees of specificity (Chapters 4 and 5). There are no known genera that lack the ability to nodulate, suggesting that nodulation is particularly important in these geographical regions (Chapter 2). The genera are

Table 1.17 Tribes in the IRLC clade

Tribe Genus	Species	Nod.
Cicereae		
Cicer L.	43	3
Fabeae		
Lathyrus L.	~160	47
Lens Mill.	4–6	4
Pisum L.	2–3	1
Vavilovia Al. Fred	1	1
Vicia L.	~160	59
Galegeae		
Astragalus L.	2300+	~119
Barnebyella D. Podlech.	1	?
Biserrula L.	1	1
Carmichaelia R. Br.	23	13
Chesnya Lindl. ex Endl.	~30	1
Clianthus Sol. ex Lindl.	2	2
Colutea L.	~28	6
Eremosparton Fisch. & C. Mey	3	1
Erophaca Bioss.	1	1
Galega L.	6	3
Glycyrrhiza L	~20	8
Gueldenstaedtia Fisch.	~10	1
Lessertia DC.	~50	27
Montigena Heenan	1	1
Ophiocarpus (Bunge) Ikonn.	1	?
Oreophysa (Bunge ex Boiss.) Bornm.	1	?
Oxytropis DC.	300–400	22
Smirnowia Bunge	1	1
Sphaerophysa DC.	2	1
Spongiocarpella Yakovlev & N. Ulziykh	~7	?
Sutherlandia R. Br. ex W.T. Aiton	2	1
Swainsona Salisb.	24	21
Tibetia (Ali) Tsui.	4	1
Hedysareae		
Alhagi Gagneb.	~3	2
Calophaca Fisch. ex DC.	5–8	1
Caragana Fabr.	70–80	21
Corethrodendron Fisch. & Basin	4	2
Ebenus L.	~20	1
Eversmannia Bunge	4	?
Halimodendron Fisch. ex DC.	1	1
Hedysarum L.	140–180	11
Onobrychis Mill.	~130	15
Sartoria Boiss. & Heldr.	1	?
Sulla Medik	7	6
Taverniera DC.	15	2

Table 1.17 (*Continued*)

Tribe Genus	Species	Nod.
Trifolieae		
Medicago L.	83	43
Melilotus Mill.	~20	16
Ononis L.	~75	20
Parochetus Buch.-Ham. ex D. Don	2	1
Trifolium L.	~250	131
Trigonella L.	~55	23

listed in Table 1.17. There have been significant recent changes. The New Zealand tribe Carmichaelieae (*Carmichaelia, Montigena*) is incorporated into Galegeae. Some species of *Clianthus* (New Zealand) have been transferred to *Swainsona* (Australian). *Astragalus*, currently the largest genus in Leguminosae, has had some species transferred to new genera (*Erophaca, Ophiocarpus*) with more likely to follow in other new genera. The genus may eventually form the basis of a separate tribe. *Lessertia* and *Sutherlandia* are unusual in this group of tribes in being African endemics. Some species of *Oxytropis* grow within the Arctic circle. Many genera, including *Gueldenstaedtia*, *Chesnya* and *Tibetia* (Galegeae) have species that can be found high in the Himalayas. *Halimodendron* (Hedysareae) is a salt-tolerant plant of steppes. Many species of all tribes are important forage plants. *Cicer arietinum*, *Lens culinaris, Pisum sativum* and *Vicia faba* have a long history of agricultural use as major grain species and are often known collectively as cool season grain legumes, because they grow in Mediterranean areas with cool wet winters and warm, dry summers. Although being the largest fraction of Papilionoideae (and hence Leguminosae), this group presents few taxonomic challenges from a nodulation point of view: they are generally nodulated by α-rhizobia.