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Review article

The Interdependence between Taxonomy of Legumes and Specificity of Their Interaction with Rhizobia in Relation to Evolution of the Symbiosis

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Abstract

The specificity of legumes in relation to nodule formation after inoculation with rhizobial strains of different origins correlates with the taxonomic division of the family Leguminosae. The degree of similarity for this character in species of the same genus is higher than in species of different genera or tribes. At the same time, the specificity in formation of effective symbiosis is not related to plant taxonomy. The data indicate that the ability to nodulate and to form effective nitrogen-fixing symbiosis may have appeared at different stages of legume evolution.

Keywords: nodulation, nitrogen fixation, effective symbiosis, legume taxonomy, evolution of symbiosis, coevolution

1. Introduction

The ability to form a symbiosis with root nodule bacteria (rhizobia of the genera *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*) is a unique property of leguminous plants: it has been revealed in 92% of the legume species, however only one indoubtable example of symbiosis between rhizobia and a non-legume (*Parasponia*, family Ulmaceae) has been described (Trinick and Galbraith, 1980; Allen and Allen, 1981; Becking, 1992). The suitability of

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the legume-rhizobial system as a model for analysis of mechanisms of different beneficial plant-microbe interactions and of plant organogenesis as well as wide practical application of symbiotic nitrogen fixation, stimulated intensive study of biochemistry, cytology and molecular genetics of this system (Palacios et al., 1993b). At the same time, the taxonomic and evolutionary basis of the symbiotic activity in leguminous plants remains poorly understood.

Leguminosae is one of the most numerous families of angiosperms, which is widespread all over the world. It comprises three subfamilies (Papilionoidea DC, Caesalpinioidea Kunth and Mimosoideae Kunth) which contain more than 750 genera and 14000 species (Allen and Allen, 1981; Yakovlev, 1991). Analysis of symbiotic properties in different taxa leads to the following conclusions: (1) the nodulation ability is distributed non-randomly between subfamilies of Leguminosae: in the Papilionoideae and Mimosoideae more than 90% of the investigated species form nodules, while in Caesalpinioideae 70% of species lack nodules (Table 1); (2) legume species vary greatly in specificity of interaction with rhizobia. High symbiotic specificity is typical for the legumes in temperate regions: they usually form nodules only with restricted ranges of strains, belonging to the same species or even biovars. Some tropical legumes are also characterized by narrow symbiotic specialization, while for others high promiscuity of interaction with rhizobia was found (Norris, 1956; Provorov, 1985); (3) nodulation is most characteristic for specialized tribes

Table 1. Ability for symbiosis with nodule bacteria in different subfamilies of the Leguminosae (Allen and Allen, 1981)

Subfamily	Number of genera			Number of species		
	Total	In which ability to nodulate was*		Total	In which ability to nodulate was*	
		Studied	Discovered		Studied	Discovered
Papilionoideae	505	269	255 (95%)	12215–13792	2462	2416 (98%)
Mimosoidea	66	31	24 (77%)	2506–2920	388	351 (90%)
Caesalpinioideae	177	65	26 (40%)	2716–2816	258	78 (30%)
Total	748	365	305 (84%)	17437–19528	3108	2845 (92%)

* Percentage of genera (species) in which ability to nodulate was discovered, is indicated in brackets.

and genera in all subfamilies, while in "primitive" taxa this property is often absent (Young and Johnston, 1989). At the same time, attempts to find more clearly defined correlations between the symbiotic specificity and taxonomy of legumes were unsuccessful (Lieberman et al., 1985; Young and Johnston, 1989). The aim of this paper is to summarize data on specificity of nodulation and formation of effective symbiosis in legumes and to analyze the interdependence between this specificity and taxonomic division of the Leguminosae in relation to the evolution of the legume-rhizobial symbiosis.

2. Cross-Inoculation Groups of Leguminous Plants

The analysis of specificity of legume-rhizobial interactions began in 1888 when H. Hellriegel and H. Wilfarth said that the lupine microsymbiont could not nodulate pea and vice versa (Fred et al., 1932). Intensive accumulation of data on specificity of nodulation during the first three decades of this century led Fred et al. (1932) to the formation of the theory of "cross-inoculation groups" (CIG). According to that theory, all legume species could be divided into a number of groups which satisfy two simple rules: (1) each species from the given group is nodulated by every rhizobial strain isolated from nodules of each of the other species from the same group (i.e., all species from the same group are able to cross-inoculate); (2) cross-inoculation of species from different groups is not possible. According to these rules, Fred proposed 16 CIGs, including species from 42 genera. It appeared that only some of these CIGs were composed from taxonomically related species or genera. For example, clover CIG (group 2) consisted only of the *Trifolium* species and the alfalfa CIG (group 1) consisted of the species from three closely related genera—*Medicago, Melilotus* and *Trigonella.* At the same time, the cowpea CIG (group 7) included genera from all three subfamilies of legumes.

During the subsequent years, the theory of CIGs was an object of intensive discussions. In 1944, J.K. Wilson published numerous facts about cross-inoculation between species from different groups as well as about absence of cross-inoculation between species from the same groups. The proposition of the author to abandon the theory of CIG was not accepted by others, but the theory was critically revised.

First, the number of "genuine" CIGs was reduced to six (Table 2). The bacteria inoculating plants of these groups received the status of species. All other legumes (mostly, tropical) were assigned to the "cowpea miscellany" on the basis of their ability to cross-inoculate with cowpea (*Vigna sinensis* L.), a species with a very broad symbiotic specificity.

Table 2. Cross-inoculation groups (CIGs) of leguminous plants (Norris, 1956, modified)

Designation of CIG	Plants		Rhizobia which are able to form effective symbiosis with plants of the group
	Genera	Tribes	
Alfalfa CIG	*Medicago* L., *Melilotus* Mill., *Trigonella* L.	Trifolieae (Bronn) Benth.	*Rhizobium meliloti*
Clover CIG	*Trifolium* L.	Trifolieae	*R. leguminosarum* bv. *trifolii*
Pea CIG	*Pisum* L., *Vicia* L., *Lathyrus* L., *Lens* Mill.	Vicieae (Adans.) DC.	*R. leguminosarum* bv. *viciae*
Bean CIG	*Phaseolus* L.*	Phaseoleae DC.	*R. leguminosarum* bv. *phaseoli***, *R. tropici*
Soybean CIG	*Glycine* Willd.	Phaseoleae	*R. fredii*, *Bradyrhizobium japonicum*
Lupine CIG	*Lupinus* L., *Ornithopus* L.	Genisteae (Adans.) Benth., Hedysareae DC.	*R. loti*, *B.* sp. (*Lupinus*, *Ornithopus*)

* Only some species of *Phaseolus* (*P. vulgaris* L., *P. angustifolius* Roxb. *P. coccineus* L., *P. multiflorus* Lam.) are included into this CIG, while most tropical species of this genus belong to the cowpea miscellany (Barua and Bhaduri, 1967; Bal et al., 1982).

** The modern synonym is *R. etli* (Martinez et al., 1993).

Second, the general criterion for CIG differentiation was proposed to be the formation of effective symbiosis, resulting in intensive nitrogen fixation and sufficient rise of plant mass (Dorosinsky, 1970; Provorov, 1985). According to this criterion, the groups of bean, soybean and lupine (Table 2) can not be considered as "genuine" CIGs, because of the formation of effective symbioses upon cross-inoculation between *Glycine* and *Lupinus* (Dorosinsky and

Lazareva, 1968), *Glycine* and *Vigna* (Walker and Brown, 1935), *Phaseolus vulgaris* and various tropical legumes (Bal et al., 1982; Martinez et al., 1985). It is important to note that all three remaining CIGs which more or less fit the initial concept of Fred et al., consist of closely related species of the subfamily Papilionoideae belonging either to the same genus (clover CIG) or to several genera from the same tribe (alfalfa and pea CIGs). Therefore, at present the CIG theory can be used for the description of symbiotic specificity in only a small part of the Leguminosae.

3. The Structure of "Genuine" Cross-Inoculation Groups

The *Alfalfa group* includes three taxonomically related genera—*Medicago, Melilotus* and *Trigonella*, capable of effective symbiosis with well-defined species of fast-growing nodule bacteria—*Rhizobium meliloti*. Based on the ability to form symbiosis with different *R. meliloti* strains, the alfalfa CIG was divided into three subgroups (Table 3).

The first subgroup is most numerous: it consists of *Medicago* (including *M. sativa, M. falcata, M. truncatula*) and *Melilotus* (including *M. albus* and *M. officinalis*) species capable of effective symbiosis with a broad range of *R. meliloti* strains (Table 3). The second subgroup includes six species of *Medicago* and *Melilotus* with more specific *Rhizobium* affinities: they form effective symbiosis mostly with the strains isolated from their own nodules. Inoculation of species of the second subgroup with *R. meliloti* strains isolated from species of the first subgroup usually leads to the formation of ineffective (Fix^-) nodules, while a reciprocal inoculation often results in Fix^+ nodules. For example, strains from *M. sativa* are usually ineffective with *M. arabica*, while the strains from *M. arabica* often form Fix^+ nodules with both species (Provorov and Simarov, 1984). The third subgroup consists of a single alfalfa species, *M. laciniata* with very specific *Rhizobium* affinities. It can form Fix^+ nodules only with *R. meliloti* strains isolated from its own nodules, while the strains from species of the first and second subgroups are Fix^- or even Nod^- on *M. laciniata*. *R. meliloti* strains isolated from *M. laciniata* are usually Fix^- or Nod^- on the other alfalfa species.

To confirm the proposed division of the alfalfa CIG into subgroups, coefficients of similarity (S_j) were used to reflect the specificity of the effective symbiosis formation in different *Medicago* species (Table 4). It appeared that for species from the same subgroups S_j is close to 0.9 while for species from different subgroups it is less than 0.23 (Table 4).

The division of the alfalfa CIG into subgroups does not correlate with the taxonomic relationships between plant species. Indeed, the first and second

Table 3. Symbiotic activity in different species of alfalfa and sweet clover

Host species (subgenus)*	Number of *R. meliloti* strains used for inoculation of the hosts** CIG				Subgroup of alfalfa CIG
	Total	% of strains			
		Eff^+	Eff^- Nod^+	Nod^-	
Medicago borealis Grossh. (M)	47	95.7	4.3	0	1
M. cancellata Bieb. (M)	7	100	0	0	1
M. caerulea Less. ex Ledeb. (M)	47	97.9	2.1	0	1
M. falcata L. (M)	80	87.5	12.5	0	1
M. littoralis Rohde ex Loisel (S)	49	89.8	10.2	0	1
M. lupulina L. (L)	129	84.5	13.2	2.3	1
M. minima (L.) Bartalani (S)	89	96.6	3.4	0	1
M. orbicularis (L.) Bartalani (O)	120	77.5	20.0	2.5	1
M. praecox DC. (S)	80	89.9	6.3	3.8	1
M. quasifalcata Sinsk. (M)	7	100	0	0	1
M. rigidula (L.) All. (S)	80	96.2	0	3.8	1
M. sativa L. (M)	152	80.3	15.1	4.6	1
M. scutellata (L.) Mill (S)	49	85.7	14.3	0	1
M. truncatula Gaertn. (S)	80	82.5	17.5	0	1
Melilotus albus Medik.	216	79.6	19.9	0.5	1
M. officinalis (L.) Pall.	129	93.0	7.0	0	1
Medicago aculeata Gaertn. (S)	49	44.9	55.1	0	2
M. arabica (L.) Huds. (S)	160	23.2	75.0	1.8	2
M. hispida Bart. (S)	124	19.4	68.5	12.1	2
M. polymorpha L. (S)	80	23.7	76.3	0	2
M. rugosa Desr. (S)	49	20.4	79.6	0	2
Melilotus indicus (L.) All.	80	23.7	76.3	0	2
Medicago laciniata (L.) Mill. (S)	214	3.7	51.4	44.9	3

* For *Medicago* species, differentiation into subgenera *Medicago* Tutin (M), *Lupularia* (Ser.) Grossh. (L.), *Orbicularia* Grossh. (O) and *Spirocarpos* Grossh. (S) is presented according to Lesins and Lesins (1979)

** We used the data from Burton and Wilson (1939), Burton and Erdman (1940), Jensen (1942), Purchase et al. (1951), Vicent (1954, 1962), Brockwell and Hely (1961, 1966), Provorov and Simarov (1984, 1990), Provorov (1985)

subgroups include species from two different genera (Table 3) and mean value of S_j calculated for *Medicago* species belonging to the same subgenera is not higher than for the species from the different subgenera (Table 4). At the same time, all symbiotically specialized *Medicago* species (subgroups 2 and 3)

Table 4. Analysis for similarity of symbiotic specificity in the different species of genus *Medicago* (Provorov and Simarov, 1984; Provorov, 1985)

Compared species belong to:		Coefficient of similarity (S_j)
Same subgroups of alfalfa CIG		
	1st	0.92 ± 0.01
	2nd	0.86 ± 0.06
Different subgroups of alfalfa CIG		
	1–2	0.23 ± 0.01
	1–3	0.05 ± 0.01
	2–3	0.07 ± 0.01
Same subgenera of the genus *Medicago*		0.63 ± 0.08
Different subgenera of the genus *Medicago*		0.62 ± 0.05

S_j for the two compared species ("A" and "B") was calculated by the formula: $S_j = N_{AB}/N_A + N_B + N_{AB}$, where N_A is the number of rhizobia strains forming effective symbiosis with species "A", not with "B"; N_B is the number of strains effective with species "B", not with "A"; N_{AB} is the number of strains effective with both species, "A" and "B".

belong to the subgenus *Spirocarpus*, while unspecified species (subgroup 1) are distributed through all four subgenera (Table 3).

The *Clover group* consists of *Trifolium* species capable of symbiosis with a restricted group of *Rhizobium* strains (*R. leguminosarum* biovar *trifolii*). On the basis of symbiotic specificity, temperate *Trifolium* species were divided into three subgroups (Table 5). Cross-inoculation between the most numerous first and second subgroups usually leads to the formation of marginally-effective symbioses, while cross-inoculation inside these subgroups results in the formation of highly-effective symbioses (Jensen and Vincent, 1941; Purchase and

Table 5. Distribution of the European clover species among different subgenera of the genus *Trifolium* and subgroups of the clover CIG

Subgroups	Subgenera*	
1	*T. dubium* Subth., *T. hybridum* L., *T. procumbens* L., *T. repens* L.	*T. hirtum* All., *T. pratense* L.
2	*T.fragiferum* L., *T. glomeratum* L., *T. retusum* L.	*T. alexandrinum* L, *T. arvense* L., *T. incarnatum* L., *T. scabrum* L., *T. subterraneum* L.
3.	*T. ambiguum* Bieb.	–

* Differentiation of clover species into subgenera is presented according to Komarov, 1945.

Vincent, 1949; Burton and Briggeman, 1948; Vincent, 1954; Baird, 1955). The third subgroup is composed of a single species, *T. ambiguum*, which is nodulated effectively with *R. leguminosarum* bv. *trifolii* strains isolated from its own nodules from its natural habitat in the Caucasus and Turkey (Parker and Allen, 1952; Hely, 1963).

Symbiotic specialization in the temperate *Trifolium* species is not correlated with their taxonomic position (Table 5) as representatives of two subgenera (*Trifoliastrum* and *Lagopus*) are distributed almost randomly in the subgroups of the clover CIG.

The *Pea group* consists of four taxonomically related legume genera—*Pisum, Vicia, Lathyrus* and *Lens*. This group is not studied in as much detail as the alfalfa and clover CIGs. Nevertheless, *R. leguminosarum* bv. *viciae* strains isolated from the "European" *Pisum sativum* L. cultivars usually form effective symbioses with *Vicia* and *Lathyrus* species (Helz et al., 1927), but they cannot nodulate some pea genotypes originating from Afghanistan and Iran (Lie, 1984). Different cultivars of *V. sativa* L. vary in their symbiotic specificity not less than different vetch species—*V. sativa, V. villosa, V. grandiflora* (Saubert, 1958).

Therefore, analysis of the CIG structure demonstrates that taxonomic relatedness in legume species is correlated markedly with the specificity of nodulation, while the specificity for effective symbiosis inside the CIGs is not linked to the taxonomic position of legume species.

The taxonomy of analysed legumes is not correlated with the taxonomy of their microsymbionts (Table 2). For example, the plant genera from the same tribe may be inoculated effectively with non-related rhizobia species (*Medicago* and *Trifolium*—*R. meliloti* and *R. leguminosarum* bv. *trifolii*), while plant genera from different tribes may be inoculated effectively with closely related rhizobia (*Trifolium*, *Pisum* and *Phaseolus*/ – different biovars of *R. leguminosarum*). Recently, Eardly et al. (1990) distributed 232 *R. meliloti* strains (isolated from 13 alfalfa species representing three subgenera of the genus *Medicago* and two subgroups of the alfalfa CIG) into two divisions (A and B) on the basis of analysis of polymorphism for 14 chromosomally encoded enzymes. This classification was correlated markedly with the geographical origin of the bacteria but no significant correlations with the position of hosts in subgroups of the alfalfa CIG or with their assignment to different *Medicago* subgenera was noted.

4. Legumes Not Assigned to the Cross-Inoculation Groups

The legume species which are not assigned to CIGs vary enormously in their symbiotic specificity. Some of them (e.g., *Dalea* Lucanus., *Psoralea* L., *Astragalus* L., *Prosopis* L., *Acacia* Mill, *Galega* L.) form symbioses with a narrow range of strains isolated from their own nodules (Whiting et al., 1926; Appleman and Sears, 1942; Shen and Shu, 1944; Felker and Clark, 1980; Dreyfus and Dommergues, 1981; Lindström et al., 1983). The other legumes, mainly representing tribe Phaseoleae (*Vigna* Savi, *Macroptilium* (Benth.) Urban, *Lablab* Adans., *Phaseolus* L.) are able to form symbioses with a broad range of different strains (Allen and Allen, 1936; Walker and Brown, 1935; Barua and Bhaduri, 1967).

To summarize the data on the symbiotic specificity of legumes not assigned to CIGs, a notion of the "elementary test for cross-inoculation" (ETCI) is introduced here. If the rhizobial strain isolated from species "A" forms nodules on the roots of species "B", we have obtained one positive ETCI between the two plant species. This may lead to formation of either effective or non-effective symbiosis. If nodules are not formed, one negative ETCI is obtained. Using this approach, results of 66 papers published in 1914–1986 were analysed collecting data on 8680 ETCIs, performed with species from 132 genera, representing 31 tribes and all three legume subfamilies (Provorov, 1992). It appeared that for species from different tribes or subfamilies, the frequency of positive ETCIs (calculated from the total number of ETCIs analysed) is significantly lower than for species from different genera of the same tribe, which is in turn lower than for species from the same genus (Table 6). In contrast, the frequency of ETCIs, which lead to the formation of effective symbiosis (calculated from the total number of positive ETCIs) does not depend on the taxonomic relatedness of the studied species (Table 6).

The taxonomy of legumes not assigned to the CIGs is not correlated with the taxonomy of the respective microsymbionts: the distribution of these legumes into subfamilies and tribes is not related to their ability to form symbioses with fast- and slow-growing rhizobia (Young and Johnston, 1989).

5. Discussion

The analysis of data on the legume-rhizobial interactions reveals that the specificity of nodulation correlates well with the plant taxonomy: cross-inoculation groups (CIGs) are composed of a single genus or of several closely related genera. In the legumes not included into CIGs the ability to cross-inoculate is highest for the species from the same genus and lowest for those

Table 6. Ability for cross-inoculation in legume species as related to their taxonomic relationships (species not assigned to the cross-inoculation groups)

Taxonomic relatedness of the legumes: compared species belong to	Number of ETCIs		% of positive ETCIs	% of ETCIs in which effective symbiosis was formed
	Total	For which symbiotic efficiency was analyzed		
Same genus	1051	731	86.6 ± 0.87	76.2 ± 1.55
Different genera from the same tribe	1917	557	65.4 ± 1.09	72.0 ± 1.90
Different tribes from the same subfamily	3256	860	56.2 ± 0.86	75.0 ± 1.55
Different subfamilies	2456	722	58.6 ± 0.99	76.7 ± 1.47
For all legumes	8680	2870	62.5 ± 0.52	74.9 ± 0.81

ETCI—elementary test for cross-inoculation (see section 3)

from different tribes and subfamilies. In contrast, the ability to form an effective symbiosis resulting from the "positive" cross-inoculation (i.e., if nodulation has been already achieved) does not depend on the taxonomic relatedness between the plant species.

The presented data suggest that the ability of legumes to form nodules is defined genetically more strictly than the ability to form effective symbiosis. This suggestion is supported by data demonstrating that the ability to form nodules always dominates over non-nodulation while the ability to form effective symbiosis may be dominant, semidominant or even recessive plant features (Caetano-Anolles and Gresshoff, 1991; Phillips and Teuber, 1992).

Different stringencies of control of nodulation and nitrogen-fixing abilities in legumes may also be supported by the results of Al-Mallah et al. (1987) who putatively broke a host specificity barrier by treating the plant roots with pectinase and cellulase. They demonstrated that the inoculation of the treated clover plants with a "heterologous" *R. loti* strain (microsymbiont of *Lotus* spp.) resulted in the formation of only a few nodules on some plants, but specific nitrogenase activity (per g of nodule tissue) in the clover nodules containing *R. loti* strain was as high as the activity of nodules formed by "homologous" symbiont, *R. leguminosarum* bv. *trifolii*.

All these facts allow one to assume that the evolution of legume-rhizobial symbiosis proceeded in two stages. At first, legume progenitors may have acquired an ability to form nodule-like structures which initially had no specific symbiotic functions, but could serve as a trap for potentially beneficial

microorganisms. The ability to form such structures may be considered as a prerequisite ("preadaptation") of plants for "genuine" symbiosis. Such a preadaptation was suggested earlier for explanation of the rare occurence of nodulation in phylogenetically "primitive" legume taxa with respect to the "advanced" ones (Young and Johnston, 1989). A benefit for the plant from such a primitive symbiosis could be conditioned not only by the occasional N_2-fixing ability of nodule inhabitants, but also by the ability of the host to respond to some growth-promoting substances synthesized by bacteria in the nodules. The possible role of the nodule-like structures as organs for starch storage could also be considered (Caetano-Anolles et al., 1992). At least in some contemporary legumes (alfalfa), the proposed "preadaptation" for symbiosis may be displayed as an ability to form sterile "pseudonodules", which are similar in their organization to normal nodules and may be induced by non-virulent rhizobia mutants (Caetano-Anolles et al., 1992). The most suitable candidates for inhabiting the pseudonodules of the legume progenitors seem to be certain bacteria possessing the genes for invasion of plants and/or induction of proliferation of plant cells, such as *Agrobacterium*-like relatives of *Rhizobium*. The possibility to isolate the strains combining properties of rhizobia and agrobacteria from natural populations (Skotnicki and Rolfe, 1978) or to construct them via plasmid exchange (Strobel et al., 1985, 1986; Brom et al., 1988) support this hypothesis. The passage through the "parasitic" stage by the legume-rhizobial symbiosis in the course of its evolution was suggested by Sprent et al. (1993) on the basis of comparative analyses of nodule organization in primitive caesalpinoid legumes.

At the second stage of evolution, the occasional plant-bacterial associations were transformed to regular N_2-fixing symbiosis. This transformation may involve at least three main events: (1) origination of bacteria combining abilities to nodulate plants and fix N_2 inside nodules, possibly via lateral transfer of the genes for plant infection and for nitrogenase synthesis. Different pathogenic and diazotrophic organisms may have been involved; (2) elaboration by the hosts of the mechanisms of specific nodulation in response only to homologous microsymbionts (to prevent penetration of non-beneficial or deleterious organisms); and (3) evolution of molecular mechanisms which enable rhizobia to suppress (or escape) the "immune response" of the plant; in this the bacterial exopolysaccharides may be involved (Niehaus et al., 1993).

The data enabling discussion of the differences in possible mechanisms of legume-rhizobial coevolution at its two proposed stages are limited. Nevertheless, it is evident that the mechanisms of genetic complementation at early stages of partners' interaction may resemble a "gene-for-gene" interaction which is characteristic of the host-pathogen systems (Frank, 1992; Thompson

and Burdon, 1992). This has been demonstrated by analysis of "*sym*2-*nod*X" gene system in pea-*R. leguminosarum* bv. *viciae* symbiosis (Lie et al., 1987; Canter-Cremers et al., 1988) and "*rwt*1-*nod*M, *csu*1" system in subclover-*R. leguminosarum* bv. *trifolii* symbiosis (Lewis-Henderson and Djordjevic, 1991). There is no evidence for the operation of such gene systems during the functioning of the active nodules. Instead, data demonstrate that bacterial genes controlling all stages of symbiotic interaction may be subject to rather fast evolutionary changes. These may be caused either by intragenomic recombination which is typical, e.g. for such evolutionary young species as *R. etli* (Brom et al., 1991; Romero et al., 1991; Palacios et al., 1993a) or by the interstrain or interspecies transfer of plasmids harboring "symbiotic" genes which was demonstrated for many *Rhizobium* species (Jarvis et al., 1989; Vlades and Pinero, 1992; Sprent and Raven, 1992; Young, 1992).

Generally speaking, the mechanisms of plant-microbe coevolution in the legume-rhizobial and host-pathogen systems may be quite different. This is indicated, in particular, by the lack of correlation between the taxonomies of legumes and rhizobia. Such correlations are characteristic for many plant-pathogen systems (Hafner and Nadler, 1988).

For different species and genera of rhizobia, the divergence of 16S rRNA sequences correlates well with the divergence of *nif*-genes but does not correlate with that of the *nod*-genes, suggesting that for rhizobia the ability to fix nitrogen is a more ancient property than the ability to nodulate (Young, 1993). It seems that in rhizobia the interdependence of phylogeny with the main symbiotic properties (nodulation and nitrogen fixation) is opposite to that in the legumes in which nodulation specificity correlates well with plant taxonomy, while the specificity of effective symbiosis does not correlate. This difference may, to some extent, reflect the fact that for legumes the nitrogen-fixing activity of nodules is a much more advantageous property than the nodulation itself, while for rhizobia the nodulation ability is an apparently useful property, but the advantages of symbiotic nitrogen fixation are at least doubtful (Sprent et al., 1993).

Discussing the possible mechanisms of legume-rhizobial coevolution, it would be useful to mention the data indicating that the host plants may influence sufficiently the rate (and maybe, the direction) of bacterial evolution. For example, multiplication of the soil rhizobial populations in the presence of the host (Dorosinsky, 1975; Bottomley, 1992) may be caused by stimulation of bacteria growth via root exudates or by the release of rhizobia from dead nodules. Plasmids may be transferred between different rhizobia strains in the legume nodules (Chernova et al., 1986; Pretorius-Gütz et al., 1990) or in the host rhizosphere (Broughton et al., 1987). Genetic changes in rhizobia may be

induced by their passage through the legume nodules (Krasilnikov and Melkumova, 1963). Finally, the genetically controlled ability of the legume hosts for the "preferential selection" of particular bacterial genotypes from the soil and mixed inocula (Triplett and Sadowsky, 1992) may sufficiently enrich the soil populations with the strains effective for these hosts (Sherwood and Masterson, 1974). The other mechanism for such enrichment may be preferential multiplication of the effective strains in the N_2-fixing nodules (as compared with non-effective strains in Fix^- nodules) which ensure maintenance of *nif*-genes in the rhizobia populations via a mechanisms similar to the kin selection (Jimenes and Casadesus, 1989). The "altruistic model" of symbiosis evolution postulated by the latter authors demonstrated the possible mechanism of evolution in one symbiotic partner (rhizobia) of a property, useful primarily for the other partner (nitrogen-fixing activity exploited by the host).

The real inputs of the mentioned population- and molecular-genetic mechanisms into the partners' coevolution are to be clarified in the future. Nevertheless, even now it is evident that the leading role in the origin and subsequent evolution of the legume-rhizobial symbiosis was performed by the host. It is evident from the very fact that the plants capable of symbiosis with rhizobia compose a taxonomically defined group (family Leguminosae), while their microsymbionts are of independent origin. Processes involved in the nodule morphogenesis in legumes are apparently plant-controlled, and the function of rhizobia is mainly to switch on the "nodulation program" of the genome in specific host (Caetano-Anolles and Gresshoff, 1991). It seems logical to suppose that in the course of partners' coevolution the legume plants were the major factor responsible for selection of bacteria which combined nodulating and N_2-fixing abilities and thus made their hosts independent of the presence of nitrogenous compounds in the environment.

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