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Molecular phylogeny of the actinorhizal Hamamelidae and relationships with host promiscuity towards *Frankia*

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Abstract

Several of the most studied actinorhizal symbioses involve associations between host plants in the subclass Hamamelidae of the dicots and actinomycetes of the genus *Frankia*. These actinorhizal plants comprise eight genera distributed among three families of 'higher' Hamamelidae, the Betulaceae, Myricaceae, and Casuarinaceae. Contrasting promiscuity towards *Frankia* is encountered among the different actinorhizal members of these families, and a better assessment of the evolutionary history of these actinorhizal taxa could help to understand the observed contrasts and their implications for the ecology and evolution of the actinorhizal symbiosis. Complete DNA sequences of the chloroplast gene coding for the large subunit of ribulose 1,5-bisphosphate carboxylase (*rbc*L) were obtained from taxa representative of these families and the Fagaceae. The phylogenetic relationships among and within these families were estimated using parsimony and distance-matrix approaches. All families appeared monophyletic. The Myricaceae appeared to derive first before the Betulaceae and the Casuarinaceae. In the Casuarinaceae, the genus *Gymnostoma* derived before the genera *Casuarina* and *Allocasuarina*, which were found closely related. The analysis of character-state changes in promiscuity along the consensus tree topology suggested a strong relationship between the evolutionary history of host plants and their promiscuity toward *Frankia*. Indeed, the actinorhizal taxa that diverged more recently in this group of plants were shown to be susceptible to a narrower spectrum of *Frankia* strains. The results also suggest that the ancestor of this group of plant was highly promiscuous, and that evolution has proceeded toward narrower promiscuity and greater specialization. These results imply that a tight relationship between the phylogenies of both symbiotic partners should not be expected, and that host promiscuity is likely to be a key determinant in the establishment of an effective symbiosis.

Keywords: actinorhizal plants, *Frankia*, Hamamelidae, molecular phylogeny, *rbc*L, symbiosis evolution

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Introduction

The actinorhizal symbiosis derives from an association between the filamentous soil bacteria *Frankia* (Actinomycetales) and an array of host woody dicots plants belonging to more than 20 genera in eight families (Bousquet & Lalonde 1990). The fixation of nitrogen resulting from this symbiosis is central to the dynamics of several ecosystems, many of the host plants being main components of early-successional communities established on poor and marginal sites (Benson & Silvester 1993). When considered all together, there is no close taxonomic affinity among the different actinorhizal plant families (Bousquet & Lalonde 1990). However, actinorhizal genera of the families Casuarinaceae, Myricaceae, and the single actinorhizal genus *Alnus* of the Betulaceae all involve hyphal penetration of deformed root hairs by *Frankia* followed by prenodule development and nodule lobe formation (Callaham *et al.* 1979, Benson & Silvester 1993). These genera are also classified in the same coherent group of 'higher' Hamamelidae (Cronquist 1981,

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1988). They are presumably closely linked to each other, together with the non-actinorhizal family Fagaceae (Takhtajan 1980; Cronquist 1988).

These various actinorhizal Hamamelidae taxa have been shown to differ drastically in their susceptibility to *Frankia*. The term 'promiscuity' has been coined to describe the plant's tolerance level to a range of genetically diverse *Frankia* strains (Baker 1987; Torrey & Racette 1989). For instance, within the Casuarinaceae, *Gymnostoma* has been shown to be susceptible to a wider array of *Frankia* strains (hence being more promiscuous) than *Allocasuarina* or *Casuarina* (Torrey & Racette 1989). *Myrica*, the largest genus of Myricaceae, has also been shown to be more promiscuous than either *Alnus* or *Casuarina* taxa (Baker 1987; Torrey & Racette 1989). Without a sound phylogenetic framework, it remains difficult to understand the ecological and evolutionary implications of these differences, which are central to a proper understanding of this symbiosis and its optimal utilization in field.

Recently, the chloroplast gene coding for the large subunit of the enzyme ribulose 1,5-bisphosphate carboxylase (*rbc*L) has been used to estimate the phylogeny of the family Betulaceae, and the results obtained were in complete agreement with the phylogeny estimated from morphological data (Bousquet *et al.* 1992b) and ribosomal DNA internal transcribed spacer sequences (Savard *et al.* 1993). *rbc*L gene sequences have also been used fruitfully to estimate phylogenetic relationships in various other groups of dicots (Soltis *et al.* 1990, 1993; Bousquet *et al.* 1992; Giannasi *et al.* 1992; Chase *et al.* 1993). Using *rbc*L gene sequences, the purpose of this study was to clarify the phylogenetic relationships among actinorhizal families of 'higher' Hamamelidae in order to help understand the ecological and evolutionary implications of promiscuity differences observed among these taxa.

Materials and methods

Plant materials

In addition to *rbc*L gene sequences already published (Table 1), the complete nucleotide sequence of the *rbc*L ORF was determined for five taxa: *Allocasuarina verticillata* (EMBL no. X69527), *Casuarina cunninghamiana* (EMBL no. X69528), *Gymnostoma webbianum* (EMBL no. X69531), *Myrica gale* (EMBL no. X69530), and *Comptonia peregrina* (EMBL no. X69529). Seeds from *A. verticillata*, *C. cunninghamiana*, and *G. webbianun* were germinated. After one month, a single branchlet from one individual of each species was used for DNA extraction. Lyophilized leaf tissues from *M. gale* and fresh leaves from *C. peregrina* were also used for DNA extraction. In all cases, DNA extractions were performed using a CTAB procedure (Bousquet *et al.* 1990).

DNA amplification and sequencing of rbcL

*rbc*L was amplified symmetrically following previously published procedures (Bousquet *et al.* 1992b). The primers used, including those upstream and downstream of *rbc*L, are described elsewhere (Frascaria *et al.* 1993). For each of the two DNA strands, a second asymmetrical amplification was conducted with a primer ratio of 1:50 where the primer in excess was at a concentration of 50 μM and the limited primer at 1 μM. The asymmetrically amplified fragments were purified by ultrafiltration with Centricon-30 (Amicon). Direct sequencing of the two DNA strains was performed by the dideoxy-nucleotide chain-termination procedure using the Sequenase Version 2.0 Kit (USB). Sequencing was conducted using 7% polyacrylamide gels according to the manufacturer's recommendations (LR). X-ray films were exposed for 24 h to several days.

Table 1 *rbc*L sequences used in this study. Actinorhizal species are indicated with an asterisk

Species	Family	Order	Dicot subclass	References
*Alnus incana**	Betulaceae	Fagales	Hamamelidae (higher)	Bousquet, Strauss and Li (1992)
Betula papyrifera	Betulaceae	Fagales	Hamamelidae (higher)	Bousquet, Strauss and Li (1992)
Corylus cornuta	Betulaceae	Fagales	Hamamelidae (higher)	Bousquet, Strauss and Li (1992)
Ostrya virginiana	Betulaceae	Fagales	Hamamelidae (higher)	Bousquet, Strauss and Li (1992)
Carpinus caroliana	Betulaceae	Fagales	Hamamelidae (higher)	Bousquet, Strauss and Li (1992)
Castanea sativa	Fagaceae	Fagales	Hamamelidae (higher)	Frascaria *et al.* (1993)
Quercus rubra	Fagaceae	Fagales	Hamamelidae (higher)	Bousquet, Strauss and Li (1992)
*Allocasuarina verticillata**	Casuarinaceae	Casuarinales	Hamamelidae (higher)	this paper
*Casuarina cunninghamiana**	Casuarinaceae	Casuarinales	Hamamelidae (higher)	this paper
*Gymnostoma webbianum**	Casuarinaceae	Casuarinales	Hamamelidae (higher)	this paper
*Myrica gale**	Myricaceae	Myricales	Hamamelidae (higher)	this paper
*Comptonia peregrina**	Myricaceae	Myricales	Hamamelidae (higher)	this paper
Liquidambar styraciflua	Hamamelidaceae	Hamamelidales	Hamamelidae (lower)	Bousquet, Strauss and Li (1992)
Magnolia macrophylla	Magnoliaceae	Magnoliales	Magnoliidae	Golenberg *et al.* (1990)

Phylogenetic analysis of rbcL sequences

Pairwise synonymous (K_S) and nonsynonymous (K_A) numbers of nucleotide substitutions corrected for multiple hits, and their standard errors, were calculated according to the two-parameter method of Li *et al.* (1985) modified by Li (1993). This method takes into account transition and transversion rates. Overall numbers of substitutions (K_O) were calculated as a weighted average of K_S and K_A. Substitution rates were also estimated using the one-parameter method of Jukes and Cantor (1969). One-parameter and K_O pairwise substitution rates were analysed with the neighbour-joining method of phylogenetic tree construction (Saitou & Nei 1987). In addition, parsimony analysis of nucleotide sequences were conducted using the Branch and Bound algorithm of PAUP 3.1 (Swofford 1993). Bootstrap confidence intervals (Felsenstein 1985) were calculated from 1000 replications for both types of analysis, parsimony (with PAUP), as well as for neighbour-joining using Jukes and Cantor's substitutions rates (software NJBOOT2 from T. S. Whittam and M. Nei, Institute of Molecular Evolutionary Genetics, Pennsylvania State University).

Evolutionary study of promiscuity differences

Information regarding the promiscuity of actinorhizal Hamamelidae genera towards *Frankia* were regrouped in Table 2.* Height groups of *Frankia* strains were delineated on the basis of their host genus of origin. Only the *Frankia* strains shown to reinfect their host of origin were considered. A host genus was considered nodulated by a particular *Frankia* strain if one nodulation was induced on at least one species within the genus. The genus *Comptonia* of Myricaceae was not considered because of insufficient data available on promiscuity. From Table 2, a cladistic character-state matrix was constructed where for each actinorhizal genus analysed, promiscuity towards each group of *Frankia* strains delineated was scored as positive (coded 1, all strains tested led to infection), or negative (coded 0, no infection), or polymorphic (coded 1/0, some strains tested led to infection). For each group of *Frankia* strains (the cladistic characters), character-state changes were mapped on the consensus phylogenetic tree derived from the analysis of *rbc*L sequences of actinorhizal Hamamelidae taxa, using MACCLADE 3.1 (Maddisson & Maddisson 1992). Changes were assumed irreversible and two different analyses were performed, whether promiscuity toward each group of *Frankia* strains was assumed derived or ancestral. Total number of steps and consistency index were monitored for each scenario.

*Table 2 is shown overleaf.

Results

rbc*L sequences*

The *rbc*L ORF sequence determined here for the three species of Casuarinaceae and the two species of Myricaceae was 1428 bp long. The degree of DNA homology was greater than 98% within families of 'higher' Hamamelidae (Table 3). Between the families, the DNA homology was about 97%, whether the families belonged to the same order or to different orders. The synonymous rate was 10–20 times larger than the nonsynonymous rate, except for the Myricaceae (Table 3). Within 'higher' Hamamelidae, Jukes and Cantor's one-parameter rates were almost identical to the overall two-parameter rates (K_O) calculated as weighted averages of K_S and K_A (Table 3).

Estimated phylogenies

Using two outgroups, *Magnolia macrophylla* and *Liquidambar styraciflua*, standard parsimony led to one most parsimonious tree with a total length of 273 steps (Fig. 1A). Of these, 153 were accounted for by the internal network linking the 'higher' Hamamelidae taxa. The consistency index (*CI*) excluding uninformative characters was 0.778 when considering the most parsimonious network linking the 'higher' Hamamelidae taxa. The topology obtained from neighbour-joining analysis of one-parameter substitution rates (Fig. 1B) was identical to the one obtained using two-parameter overall numbers of

Table 3 Mean ± SE diversity estimates derived from pairwise comparisons of *rbc*L sequences

Groups	*n*	Homology %	K_S	K_A	K_O	J.C.
Myricaceae	1	99.6	0.009 ± 0.005	0.003 ± 0.002	0.004 ± 0.002	0.004 ± 0.002
Casuarinaceae	3	98.7	0.044 ± 0.020	0.004 ± 0.001	0.013 ± 0.006	0.013 ± 0.005
Betulaceae	10	98.9	0.038 ± 0.015	0.004 ± 0.001	0.012 ± 0.004	0.012 ± 0.004
Fagaceae	1	99.2	0.028 ± 0.010	0.003 ± 0.002	0.009 ± 0.002	0.008 ± 0.002
Between families	51	96.9	0.115 ± 0.027	0.009 ± 0.003	0.033 ± 0.006	0.032 ± 0.005

Abbreviations used: *n*, number of pairwise sequence comparisons involved; K_S, K_A, and K_O, synonymous, nonsynonymous, and overall numbers of substitutions per site, respectively; J.C., Jukes and Cantor's number of substitutions per site.

Table 2 Promiscuity of actinorhizal Hamamelidae genera towards *Frankia*

Frankia strains*			Promiscuity of host genera†‡				
Host of origin	Name	Catalog no.§	*Allocasuarina*	*Casuarina*	*Gymnostoma*	*Alnus*	*Myrica*
Allocasuarina	AII1	HFP022801	+ (4,11,13)	+ (4,11,13,14)	+ (9,11)	– (14)	+ (11,14)
	TA	ORS022602	+ (13)	+ (13)			
	URU2	CFN022302	+ (13)	+ (13)			
	Dec	CFN022901	+ (13)	+ (13)			
Casuarina	CcI3	HFP020203	+ (11)	+ (3,5,11)	+ (9,11,12)	– (5)	+ (5,11)
	CeD	ORS020606		+ (5, 11)		– (5)	+ (5)
	CeF	ORS020607		+ (5, 11)		– (5)	
	Cj1–82	ORS021001	– (2)	+ (5,11,14)		– (5,14)	+ (5,14)
	CeI5	UFG026605	– (11)	+ (11)	+ (11,12)		+ (11)
	CgI1	UFG028501	– (11)	+ (11,12)	+ (11,12)		+ (11,12)
	Cc01	IAE02020001		+ (14)		– (14)	+ (14)
	Ce24	IAE020600234		+ (14)		– (14)	+ (14)
	1995	DAB020603		+ (14)		– (14)	+ (14)
	JCT287			+ (7,11,14)		– (14)	+ (14)
	1960	DAB021004		+ (14)		– (14)	+ (14)
	CgI4	HFP020604		+ (14)		– (14)	+ (14)
Gymnostoma	Gp1	HFP021801	– (11)	– (9,11)	+ (9,10,11,12)	– (9)	+ (9)
Alnus	AirI1	LLR01321				+ (1,5)	+ (5)
	ArI3	HFP013003			+ (9,12)	+ (8)	
	AvcI1	DDB01020110		– (5,14)		+ (5,14)	+ (5,14)
	AvsI3	DDB01360610		– (5,14)		+ (5,14)	+ (5,14)
	54012	DDB01362210		– (5)		+ (5)	+ (5)
	ACN1[ag]	ULQ0102001007				+ (6)	+ (6)
	Acc8207	IAE01438207		– (14)		+ (14)	+ (14)
	Af2	IMB01040002		– (14)		+ (14)	+ (14)
	Agc8204	IAE01078204		– (14)		+ (14)	+ (14)
	Ahc8201	IAE01098201		– (14)		+ (14)	+ (14)
Comptonia	CpI1	HFP070101		– (5)	+ (12)	+ (5,6)	+ (6)
	CpI3	DDB07010310		– (5)		+ (5)	+ (5)
Myrica	MpI3	DDB16201010		– (5)		+ (5)	+ (5)
	M+gI5	HFP161105		– (14)	+ (12)	+ (14)	+ (14)
	MGX35a					+ (6)	+ (6)
	MGX35b					+ (6)	+ (6)
	MGX39b					+ (6)	+ (6)
	MGX39c					+ (6)	+ (6)
	MGX40a					+ (6)	+ (6)
	MGX40o					+ (6)	+ (6)
	MGX31a					+ (6)	+ (6)
	MGX34a					+ (6)	+ (6)
	MGX34b					+ (6)	+ (6)
	MGX34c					+ (6)	+ (6)
	MGX34d					+ (6)	+ (6)
	MGX34e					+ (6)	+ (6)
	53051	DDB16060820		– (5)			+ (5)
	K2115	IMB16092115		– (14)		– (14)	+ (14)
	Mrc8302	IAE16248302		– (14)		+ (14)	+ (14)
Elaeagnaceae	TX31eHR	ULQ00231058				– (6)	+ (6)
	EUN1f	ULQ132500106				+ (6)	+ (6)
	Ea118	SIB13010118		– (14)		– (14)	+ (14)
	Egc107	IAE13310107		– (14)		– (14)	+ (14)
	K2061	IMB13312061		– (14)		– (14)	+ (14)
	K1510	IMB13271510		– (14)		– (14)	+ (14)
	Em273	SIB13320273		– (14)		– (14)	+ (14)
	Em373	SIB13320373		– (14)		– (14)	+ (14)
	Emoc1211	IAE13131211		– (14)		– (14)	+ (14)
	Eoc85	IAE13360085		– (14)		– (14)	+ (14)
	Eu131	SIB13250131		– (14)		– (14)	+ (14)
	Hr16	IAE14010016		– (14)		– (14)	+ (14)
	Hr21	IAE14010021		– (14)		– (14)	+ (14)
	Hr34	IAE14010034		– (14)		– (14)	+ (14)
	Hr37	IAE14010037		– (14)		– (14)	+ (14)
	Hr104	SIB14010104		– (14)		– (14)	+ (14)
	EaN1	ULQ130100144			+ (9)		
	E1	ULI13270210		– (15)		+ (15)	
	E2	ULI13270237		– (15)		+ (15)	
	E3	ULI13270250		– (15)		+ (15)	
	E4	ULI13270257		– (15)		+ (15)	
Colletia	WgCc1.17			– (14)		– (14)	+ (14)

* Excluding *Frankia* strains unable to reinfect their host of origin.
† Excluding *Comptonia* because of insufficient data.
‡ + = nodulation on at least one species tested within the genus; – = no nodulation. In parentheses, reference numbers as follow: (1): Lechevalier *e*
Sougoufara (1983); (3): Zhang *et al.* (1984); (4): Zhang & Torrey (1985); (5): Baker (1987); (6): St–Laurent & Lalonde (1987); (7): Rosbrook & Bov
St–Laurent *et al.* (1987); (9): Racette & Torrey (1989a); (10): Racette & Torrey (1989b); (11): Torrey & Racette (1989); (12): Racette *et al.* (1990); (1
(1990); 14: Du & Baker (1992); (15): Bosco *et al.* (1992).
§ Catalog numbers as defined by Lechevalier (1985).

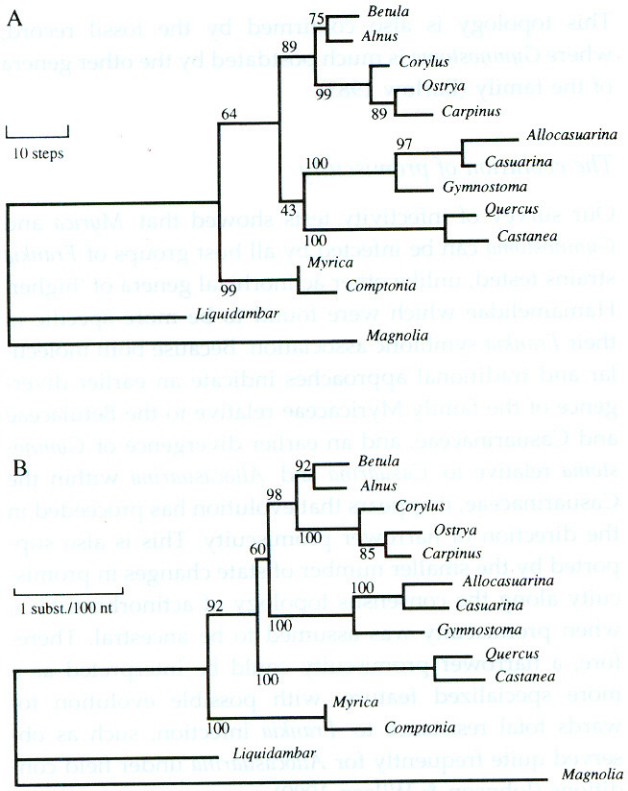

Fig. 1 (a) Tree obtained from parsimony analysis of *rbc*L nucleotide sequences; (b) tree obtained from neighbor-joining analysis of *rbc*L sequences using the one-parameter method. Numbers on nodes indicate bootstrap estimates from 1000 replications.

substitutions (K_O), and highly congruent with results from parsimony analysis.

In both parsimony and neighbour-joining trees, the different families were supported by high bootstraps (Fig. 1A,B). The order Fagales, which contains the families Betulaceae and Fagaceae, did not appear to form a coherent group from the different phylogenetic analyses conducted. The neighbour-joining analysis of substitution rates led to a regrouping of the families Casuarinaceae and Betulaceae, which was supported by a bootstrap value higher than 50% (Fig. 1B) (the alternative bootstrap for the Fagales using this method was only 5%). Standard parsimony analysis led to a regrouping of the families Casuarinaceae and Fagaceae with a bootstrap value (43%) higher than that obtained for the Fagales (15%). Overall, the divergence between the Fagaceae, the Betulaceae, and the Casuarinaceae appeared essentially as a trichotomy.

Neighbour-joining and parsimony analyses both indicated the Myricaceae to have diverged first among the families of 'higher' Hamamelidae analysed (Fig. 1A,B). Any other topologies placing the Fagaceae, Betulaceae, or Casuarinaceae as first group to diverge among the 'higher' Hamamelidae received bootstraps lower than 50%. In the family Casuarinaceae, both neighbour-joining and parsimony analyses indicated a subclade which contained *Allocasuarina* and *Casuarina* (the Casuarineae) (Fig. 1A,B). As previously reported, the Betulaceae were divided into two subclades, the tribe Betuleae containing the genera *Alnus* and *Betula* and the tribe Coryleae containing the remaining genera (Bousquet *et al.* 1992b).

Evolutionary analysis of promiscuity

The promiscuity of the each actinorhizal genus of the 'higher' Hamamelidae was scored successively for each group of *Frankia* strains (Fig. 2), based on available data presented in Table 2. Character-state changes, that is, the promiscuity differences among host genera relative to the various groups of *Frankia* strains, were then monitored over the *rbc*L consensus tree of actinorhizal plant taxa. *Comptonia* could not be included in the analysis because of insufficient data on promiscuity (see Table 2). A strong relationship could be observed between the phylogeny of actinorhizal Hamamelidae and their spectrum of promiscuity towards the various groups of *Frankia* strains (Fig. 2). *Myrica*, assumed to diverge first, is equally or more promiscuous than the other genera. Within the Casuarinaceae, *Gymnostoma*, assumed to diverge first, is more promiscuous than the other genera of the family.

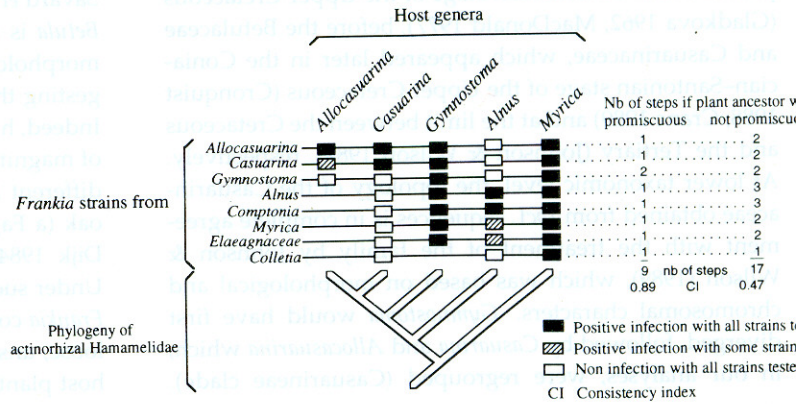

Fig. 2 Variation in promiscuity among actinorhizal genera of Hamamelidae (deduced from Table 2) and analysis of character-state changes in promiscuity of host genera along the *rbc*L consensus tree of actinorhizal genera. The host genus *Comptonia* was not included in the consensus tree because of insufficient data regarding its promiscuity. Names of host genera at the top of columns of the character-state matrix correspond to those at the tip of branches of the consensus tree. On the right, numbers of steps corresponds to the numbers of reversals in promiscuity along the branches of the tree, as produced by each group of *Frankia* strains on the left and the associated array of promiscuity differences.

Moreover, the Casuarinae group (*Allocasuarina* and *Casuarina*) appears to be restricted in its symbiotic association to a group of *Frankia* strains not infective on *Alnus* roots, while *Alnus* can only be infected by strains not infective on Casuarinae roots (Table 2 and Fig. 2). When promiscuity was assumed ancestral, the number of steps necessary to explain the observed character-state variation among actinorhizal Hamamelidae was much smaller than when promiscuity was assumed derived (Fig. 2).

Discussion

Molecular phylogeny of actinorhizal Hamamelidae taxa

The different families analysed appeared as natural entities through the different phylogenetic analyses conducted, but the order Fagales was not observed as a coherent group. This observation is congruent with those of Nixon (1989), who suggested a paraphyletic or polyphyletic origin of the order Fagales, based on morphological characters. However, the closer relationship we observed between the Fagales (Betulaceae and Fagaceae) and the Casuarinales (Casuarinaceae), than with the Myricales (Myricaceae), is in agreement with views proposed by Takhtajan (1980) and Conquist (1988). This is in contrast to other views considering the Casuarinaceae more primitive and classifying the family as evolutionary intermediate between the 'lower' Hamamelidae and other more specialized 'higher' Hamamelidae such as the Betulaceae, Fagaceae, and Myricaceae (Barabé *et al.* 1982). Part of the disagreement could be explained by the interpretation of morphological variation. As indicated by Cronquist (1988), Casuarinaceae flowers are reduced rather than primitively simple, and such a reduced aspect in morphological characters should not necessarily be linked to a primitive state, because of potential adaptation to environmental conditions.

The likely divergence of the Myricaceae before the Betulaceae (*Alnus*) and Casuarinaceae is in agreement with the fossil record where the family Myricaceae appeared in the Cenomanian stage of the Upper Cretaceous (Gladkova 1962; MacDonald 1977), before the Betulaceae and Casuarinaceae, which appeared later in the Coniacian–Santonian stage of the Upper Cretaceous (Cronquist 1988; Crane 1989) and at the limit between the Cretaceous and the Tertiary (Johnson & Wilson 1989), respectively. At lower taxonomic level, the topology of the Casuarinaceae obtained from *rbc*L sequences is in complete agreement with the treatment of the family by Johnson & Wilson (1989), which was based on morphological and chromosomal characters. *Gymnostoma* would have first diverged, followed by *Casuarina* and *Allocasuarina* which, in our analyses, were regrouped (Casuarineae clade). This topology is also confirmed by the fossil record, where *Gymnostoma* is much postdated by the other genera of the family (Barlow 1983).

The evolution of promiscuity

Our survey of infectivity tests showed that *Myrica* and *Gymnostoma* can be infected by all host groups of *Frankia* strains tested, unlike other actinorhizal genera of 'higher' Hamamelidae which were found to be more specific in their *Frankia* symbiotic association. Because both molecular and traditional approaches indicate an earlier divergence of the family Myricaceae relative to the Betulaceae and Casuarinaceae, and an earlier divergence of *Gymnostoma* relative to *Casuarina* and *Allocasuarina* within the Casuarinaceae, it appears that evolution has proceeded in the direction of narrower promiscuity. This is also supported by the smaller number of state changes in promiscuity along the consensus topology of actinorhizal taxa, when promiscuity was assumed to be ancestral. Therefore, a narrower promiscuity could be interpreted as a more specialized feature, with possible evolution towards total resistance to *Frankia* infection, such as observed quite frequently for *Allocasuarina* under field conditions (Johnson & Wilson 1989).

This evolutionary trend is also observed in the Betulaceae, even though it contains only a single actinorhizal genus, *Alnus*. The Betuleae clade, which contains the actinorhizal genus *Alnus* and the non-actinorhizal genus *Betula*, has been shown to be less morphologically advanced that the Coryleae clade (Crane 1989; Bousquet *et al.* 1992b), which contains only nonactinorhizal genera. This suggests the actinorhizal state typical of *Alnus* to be less advanced than the nonactinorhizal state. Furthermore, the most ancient Betulaceae fossils were pollen grains typical of the actinorhizal *Alnus* (Crane 1989), with pollen typical of other genera, particularly from the Coryleae clade, appearing later in the fossil record. Moreover, the non-actinorhizal genus *Betula* is found more closely related to *Alnus* than to other non-actinorhizal genera of the Coryleae clade (Bousquet *et al.* 1992b; Savard *et al.* 1993), and this is paralleled by evidence that *Betula* is more dependent on *Frankia* that taxa from the morphologically advanced Coryleae clade, again suggesting the association with *Frankia* to be less advanced. Indeed, higher densities of *Frankia* of more than one order of magnitude have been observed in soils under stands of different *Betula* species, as compared to stands of spruce, oak (a Fagaceae), or even *Corylus* (Coryleae clade) (Van Dijk 1984; Smolander 1990; Paschke & Dawson 1992). Under such *Betula* stands, it has further been shown that *Frankia* could survive as an associative nitrogen fixer in a loose, unspecific but beneficial relationship with the non-host plant *Betula* roots (Rönkkö *et al.* 1993).

Ecological and evolutionary implications for the actinorhizal symbiosis

This evolutionary trend toward a narrower promiscuity of the host plants, suggests that the host plant root system plays a key role in the specific relationships established with *Frankia*. Not only the array of *Frankia* strains capable of infecting a host root system seems to be controlled by the plant, but also other types of characters involved in the symbiosis such as vesicle formation and morphology (Lalonde 1979; Tjepkema *et al.* 1980; St-Laurent & Lalonde 1987; Benson & Silvester 1993), or the mechanism of *Frankia* penetration into host roots: in this case, *Frankia* strains able to infect both Elaeagnaceae (subclass Rosidae of the dicots) and some actinorhizal Hamamelidae species show differential type of root penetration depending of the host, either by root hair infection for the Hamamelidae taxa, or by intracellular penetration for the Elaeagnaceae (Miller & Baker 1986; Racette & Torrey 1989b).

Furthermore, variation in the efficiency of the symbiosis has been shown to be much more controlled by plant clonal effects than *Frankia* strain effects (Simon *et al.* 1985; Mackay *et al.* 1987; Prat 1989; Sougoufara *et al.* 1992). This might simply derives from differences in plant genotypes with respect to promiscuity towards compatible *Frankia* strains, which would result in efficiency differences. Such larger control of the symbiotic efficiency by the host plant has also been reported in the legumes (see Galiana *et al.* 1991) and in free nitrogen-fixation associations involving various bacteria and nonhost plants (Rönkkö *et al.* 1993). Therefore, for the practical use of the actinorhizal symbiosis in the field where an efficient system is required, selection of the optimal host genotype–*Frankia* combination should exploit knowledge of the promiscuity of the plant genotypes used. This would imply a larger emphasis on the number of host genotypes tested rather than the number of *Frankia* strains involved.

It is now generally agreed that taxonomic grouping of *Frankia* strains from root nodules is much more dictated by the host plant from which the strains were isolated, rather than by the spectrum of infectivity of the strains (Lalonde *et al.* 1988; Fernandez *et al.* 1989; Beyazova & Lechevalier 1992). While this observation should be considered as a direct effect of host promiscuity, it is also likely that *Frankia* strains naturally found on promiscuous plant taxa or genera would exhibit more genetic or taxonomic diversity than strains naturally found on less promiscuous plant taxa or genera. Indeed, for *Frankia* strains naturally found on actinorhizal Hamamelidae taxa and which could reinfect their host of origin, this relationship between host promiscuity and strain diversity seems to hold. It is supported by the apparently narrower genetic diversity of *Frankia* strains isolated from *Casuarina* and *Allocasuarina*, as compared to strains isolated from the more promiscuous *Alnus* (Fernandez *et al.* 1989; Nazaret *et al.* 1991). It is also supported by the apparently much larger biochemical and genetic diversity of *Frankia* strains isolated from the largely promiscuous genus *Myrica*, which usually fail to form coherent taxonomic groups (Gardes *et al.* 1987; St-Laurent *et al.* 1987; Bloom *et al.* 1989; Simon *et al.* 1989).

As a consequence of these promiscuity differences among actinorhizal plants, the stringency of coevolutionary relationships between *Frankia* and the plant host should vary extensively, and a tight relationship between the phylogenies of both partners involved in the symbiosis should not be expected. Such a scenario is also observed in the legume–*Rhizobium* symbiosis, where little correspondence is observed between the phylogenies of the symbiont and the host (Young & Johnston 1989). This is in contrast with other mutualistic or parasitic systems where much tighter relationships are often observed between the phylogenies of both partners (Mitter *et al.* 1991; Moran & Baumann 1994). As for the legume–*Rhizobium* symbiosis, differences in promiscuity among actinorhizal Hamamelidae and total resistance of closely related taxa to root invasion by *Frankia* should reflect various levels of long-term mutualistic and antagonistic interactions between the two partners (Young & Johnston 1989). Because *Frankia* is not an obligate symbiont, being able like *Rhizobium* to survive as a free-living organism in the soil, the lack of coevolutionary relationships should even be more apparent with rhizospheric *Frankia*, where the plant host effects on *Frankia* taxonomic and genetic diversity would be much relaxed, if not negligible.

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