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Chloroplast DNA restriction site polymorphism in *Genisteae* (*Leguminosae*) suggests a common origin for European and American lupines

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Abstract: Restriction site polymorphism in cpDNA of 35 legumes was studied in order to address natural relationships and geographic distribution within the tribe *Genisteae*. 386 sites were studied, 277 were polymorphic, 207 were informative. Phylogenetic inferences with distance and parsimony methods suggest that the American and Mediterranean *Lupinus* species belong to a monophyletic group which arose from a single center of diversification. The data furthermore indicate that *Lupinus* should not be included in the tribe *Genisteae* since at the level of cpDNA polymorphism *Anagyris foetida* (tribe *Thermopsideae*) appears more closely related to other *Genisteae* than *Lupinus* does.

Leguminosae, the third largest angiosperm family, encompasses about 650 genera with roughly 18,000 species (POLHILL & al. 1981) and has been the subject of several molecular investigations (for a review see DOYLE & al. 1992). Our attention within the legume family has focussed upon members and relatives of the tribe *Genisteae*, the taxonomy and evolution of which are controversial due to complex patterns of shared morphological traits among genera.

The taxonomic limits of the tribe have undergone numerous modifications (reviewed by POLHILL 1976). As defined by ROTHMALER (1944) and later adopted by GIBBS (1966), *Genisteae* comprises 20 genera and about 210 species of spiny or unarmed woody shrubs or perennial herbs. *Genisteae* sensu ROTHMALER corresponds to the tribes *Genisteae, Cytiseae*, and *Laburneae* recognized by HUTCHINSON (1964) and to parts of the subtribes *Spartiinae* and *Genistinae* described by TAUBERT (1891). The largest genera are *Genista* L. (76 species), *Chamaecytisus* LINK (42 species), and *Cytisus* L. (32 species). More recent morphological (POLHILL 1976, BISBY 1981) and serological (CRISTOFOLINI & FEOLI-CHIAPELLA 1984, CRISTOFOLINI 1989) studies have provided support for the inclusion of *Lupinus* L. (200 species, all of which are herbaceous) within the tribe, although this is still a matter of debate. In the present paper, *Genisteae* refers to the tribe as currently recognized by most authors (e.g., POLHILL 1976, BISBY 1981), i.e., including the genus *Lupinus*.

Members of the woody *Genisteae* occur today in Europe (about 140 native species) and the Mediterranean (about 70 species). Studies of general morphology (POLHILL 1976, TURNER 1981), phytochemistry (FAUGERAS & PARIS 1968, HARBORNE 1969, SALATINO & GOTTLIEB 1983) and seed protein serology (CRISTOFOLINI & FEOLI-CHIAPELLA 1984) suggest that the closest extant relatives of the tribe are the woody *Thermopsideae*. The region now corresponding to the Mediterranean is generally viewed as the center of *Genisteae* diversification (HOLUBOVA-KLASKOVA 1964), although Asia (CRISTOFOLINI 1989) has been suggested as its center of origin.

Contrary to the woody *Genisteae*, the genus *Lupinus* has amphiatlantic distribution with about 190 species native to the Americas and circa twelve species restricted to the Old World (GLADSTONES 1974, PLITMAN 1981). A number of conflicting views exist regarding the evolutionary history of *Lupinus* and its relationship to the woody *Genisteae* (see CRISTOFOLINI 1989 for an overview). For example PLITMAN (1981) and later PLITMAN & PAZY (1984) considered the European smooth-seeded and rough-seeded lupines as natural groups which arose through independent successive migrations during Miocene and Pliocene times, respectively, from a primary center of diversification in North America. The sharply contrasting view that *Lupinus* may be of Mediterranean origin (CRISTOFOLINI & FEOLI-CHIAPELLA 1984) was later modified (CRISTOFOLINI 1989) in favor of a Sinosindian (temperate-tropical E Asian) origin with independent migrations to both the Mediterranean and the Americas. TURNER (1981) suggested that *Thermopsideae* is ancestral to *Lupinus*, CRISTOFOLINI (1989) proposed that *Genisteae* (including the genera *Argyrolobium* and *Lupinus*) and *Thermopsideae* share a common woody ancestor which may have been similar to present day *Sophora*.

Here we report restriction site variation data from chloroplast DNA (cpDNA) from 21 species covering ten genera within the woody *Genisteae* and 12 species of *Lupinus* representing Mediterranean and American species. From 386 hexameric restriction sites studied, we identified 207 informative and 70 autapomorphic sites. The data indicate that *Lupinus* is monophyletic but, contrary to current views (POLHILL 1976, BISBY 1981, CRISTOFOLINI 1989), is evolutionarily quite distinct from the other genera within the tribe *Genisteae*, supporting the narrow definition of the tribe perceived by ROTHMALER (1944).

Material and methods

A list of the studied taxa and their source is given in Table 1. The majority of the woody *Genisteae* species were either collected as seed or fresh material from their natural habitats in Europe or were harvested from the Ecological Botanic Garden of Bayreuth University. Remaining accessions as well as most *Lupinus* spp. were obtained as seed material from botanic gardens or breeding institutes and were grown in a greenhouse. Young leaves were harvested for DNA extraction. All taxa were left to flower for reidentification.

Chloroplast DNA was extracted by the method of SANDBRINK & al. (1989) except that their buffer III was omitted and shorter alcohol precipitation times were used. This method gave satisfactory results for *Lupinus*, but was unsuitable for the other *Genisteae* due to large quantities of secondary metabolites. For these species, the amount of polyvinylpyrrolidone (PVP) in buffer I was increased to 10%, the ratio of buffer I to fresh weight was increased to 10 : 1 and plastids were pelleted by extended centrifugation to increase yield. For some species within *Genisteae*, this modification was still unsuitable and total DNA was thus extracted by the cetyltriethyl ammonium bromide (CTAB) method of DOYLE &

Table 1. Sources for *Leguminosae* material in this study. *B.G.* Botanical Garden, *F.A.L.* Forschungsanstalt für Landwirtschaft, *ILCA* International Livestock Centre for Africa, *Ö.B.G.* Ökologischer Botanischer Garten. For material collected from natural populations, the collector's name is given at the right in parentheses. For European species, the nomenclature of Flora Europaea (1968) has been used, otherwise Index Kewensis or the nomenclature of POLHILL (1976) was used with the exception of *Argyrocytisus battandieri* and *Lembotropis nigricans*, in which the assignments of HEYWOOD (1968) were used (see text)

Species	Source
Anagyris foetida L.	B.G., University of Cordoba, Spain
Argyrocytisus battandieri (MAIRE) RAYNAUD	ILCA, Addis Ababa, Ethiopia
Calicotome spinosa (L.) LINK	Levanto, NW Italy (A. BADR)
Chamaecytisus austriacus (L.) LINK	B.G., Universität Tübingen, Germany
C. blockianus (PAWL.) A. KLÁSKOVÁ	Ö.B.G., Universität Bayreuth, Germany
C. hirsutus (L.) LINK	Lake Idro, Italy (P. GERSTBERGER)
C. palmensis (CHRIST) BISBY & NICHOLLS	ILCA, Addis Ababa, Ethiopia
C. proliferus LINK	ILCA, Addis Ababa, Ethiopia
C. supinus (L.) LINK	B.G., Universität Düsseldorf, Germany
C. sagittale (L.) P. GIBBS	Ö.B.G., Universität Bayreuth, Germany
Cytisus emeriflorus REICHENB.	S Alps, Italy (U. JENSEN)
C. scoparius (L.) LINK	Ö.B.G., Universität Bayreuth, Germany
C. sessilifolius L.	Lake Idro, Italy (P. GERSTBERGER)
Genista falcata BROT.	B.G., University of Cordoba, Spain
G. germanica L.	Bayreuth, Germany (A. BADR)
G. pilosa L.	S Alps, Italy (U. JENSEN)
G. tinctoria L.	Ö.B.G., Universität Bayreuth, Germany
Laburnum alpinum (MILL.) BERCHT. & J. PRESL.	Ö.B.G., Universität Bayreuth, Germany
L. anagyroides MEDIK.	Ö.B.G., Universität Bayreuth, Germany
Lembotropis nigricans (L.) GRISEB.	Bayreuth, Germany (A. BADR)
Lupinus albus L.	F.A.L., Braunschweig, Germany
L. angustifolius L.	ILCA, Addis Ababa, Ethiopia
L. arboreus SIMS	Ö.B.G., Universtität Bayreuth, Germany
L. densiflorus BENTH.	F.A.L., Braunschweig, Germany
L. elegans H. B. K.	F.A.L., Braunschweig, Germany
L. hartwegii LINDL.	F.A.L., Braunschweig, Germany
L. luteus L.	ILCA, Addis Ababa, Ethiopia
L. micranthus GUSS.	F.A.L., Braunschweig, Germany
L. mutabilis SWEET	ILCA, Addis Ababa, Ethiopia
L. pilosus MURR.	F.A.L., Braunschweig, Germany
L. polyphyllus LINDL.	F.A.L., Braunschweig, Germany
L. varius L.	F.A.L., Braunschweig, Germany
Phaseolus vulgaris L.	A local German cultivar was used
Spartium junceum L.	Levanto, NW Italy (A. BADR)
Ulex europaeus L.	NW Italy (A. BADR)

DOYLE (1987). Final DNA pellets were dissolved to roughly 100 µg/ml and were digested with Apa I, Bam HI, Bst XI, Eco RI, Eco RV, Hind III, Kpn I, Pst I, Pvu I, Sal I, Sst I, and Xho I (BRL), separated on 0.7% agarose gels in 45 mM Tris-borate (pH 8.3), 1 mM EDTA (SAMBROOK & al. 1989) at 20 V and transferred to nylon membranes (Hybond N, Amersham). Filters were hybridized at low stringency to Pst I, Sal I, and Pst I/Sal I inserts of the mung bean cpDNA plasmid library described by PALMER & al. (1983, 1988) which was kindly provided by J. J. DOYLE. Fragments from the mung bean library which we used as hybridization probes cover about 80% of the chloroplast genome. Probes were labelled with digoxigenin, hybridized and visualized by chemiluminescence (Boehringer) on X-ray films (Amersham) according to manufacturers' protocols. Subsequent to exposition, filters were stripped with sodium hydroxide as recommended by the supplier, baked and rehybridized (up to six times).

Restriction sites were mapped as described by DOWLING & al. (1990), presence or absence of sites were scored as 1 or 0, respectively, for phylogenetic analyses. Cases of uncertainty in site mapping were removed from the data set, leaving 386 characters (sites) per species for phylogenetic analyses. Fractions of pairwise restriction site differences between haplotypes were obtained using the appropriate option of PAUP 3.0 (SWOFFORD 1991) and were used to construct a neighbor joining tree (SAITOU & NEI 1987). The 386 character matrix was then purged of invariant sites, leaving 277 polymorphic sites which were used as input for parsimony inference. Reliability of the neighbor joining topology was estimated by comparison to the results from Wagner parsimony analyses of resampled data using BOOT of PHYLIP 3.3 (FELSENSTEIN 1990) with global branch swapping as well as PAUP using the heuristic search option. Wagner parsimony results using HENNIG 86 (FARRIS 1988) were also obtained. *Anagyris foetida* (tribe *Thermopsideae*) and *Phaseolus vulgaris* (tribe *Phaseoleae*) were initially chosen as outgroups although *Anagyris* ultimately proved to be an ingroup (see discussion), leaving *Phaseolus* as the single outgroup for phylogenetic inference.

Results

A total number of 386 six base restriction sites were examined in the cpDNA of the taxa surveyed, corresponding to about 1.5% of the chloroplast genome. Of these, 109 sites were shared by all taxa and thus are phylogenetically uninformative. The other 277 sites included 207 polymorphisms shared by at least two taxa as well as 70 autapomorphic sites. The proportion of informative sites in our *Genisteae* data set is thus about 54%. Most polymorphisms were observed in the large single copy (LSC) region of the cpDNA. This region of the chloroplast genome has been shown to possess several structural mutations in *Leguminosae* including a 78 kb inversion which is shared by *Phaseolus* and mung bean, but is not found in *Lupinus* and non-*Phaseoleae* (PALMER & al. 1988, BRUNEAU & al. 1990). This inversion occurs very close to both ends of the LSC region and is therefore difficult to detect. We performed single and double digests of cpDNA from *Phaseolus vulgaris*, *Lupinus angustifolius*, and *Lupinus mutabilis*. Comparison of these results to the published mung bean cpDNA map (PALMER & al. 1983, 1988) revealed no informative rearrangements. We were thus able to map hybridizing fragments from all 35 taxa despite the fact that the 78 kb inversion is present in *Phaseolus*.

Polymorphisms at 277 sites were used for distance and parsimony phylogeny inference as described in Material and methods. Results of bootstrap parsimony analyses using a 207-site data set excluding the 70 autapomorphic characters did not differ in 50% consensus branching order from those based on 277 sites. The

binary data matrix of restriction sites as well as the distance matrix for proportions of restriction site differences are available upon request. The distance and parsimony methods produced very similar results for the taxa studied. The neighbor joining tree for proportions of restriction site differences is shown in Fig. 1. Wagner parsimony for bootstrap resampled data (BOOT of PHYLIP) with the global branch swapping option resulted in a consensus tree with very similar topology. No branches in the 50% majority rule Wagner parsimony bootstrap tree were found which are not present in the neighbor joining tree shown in Fig. 1. The parsimony bootstrap values for branches of the neighbor joining tree are shown in the Fig. 1. Phylogenetic analysis with HENNIG 86 produced two most parsimonious trees,

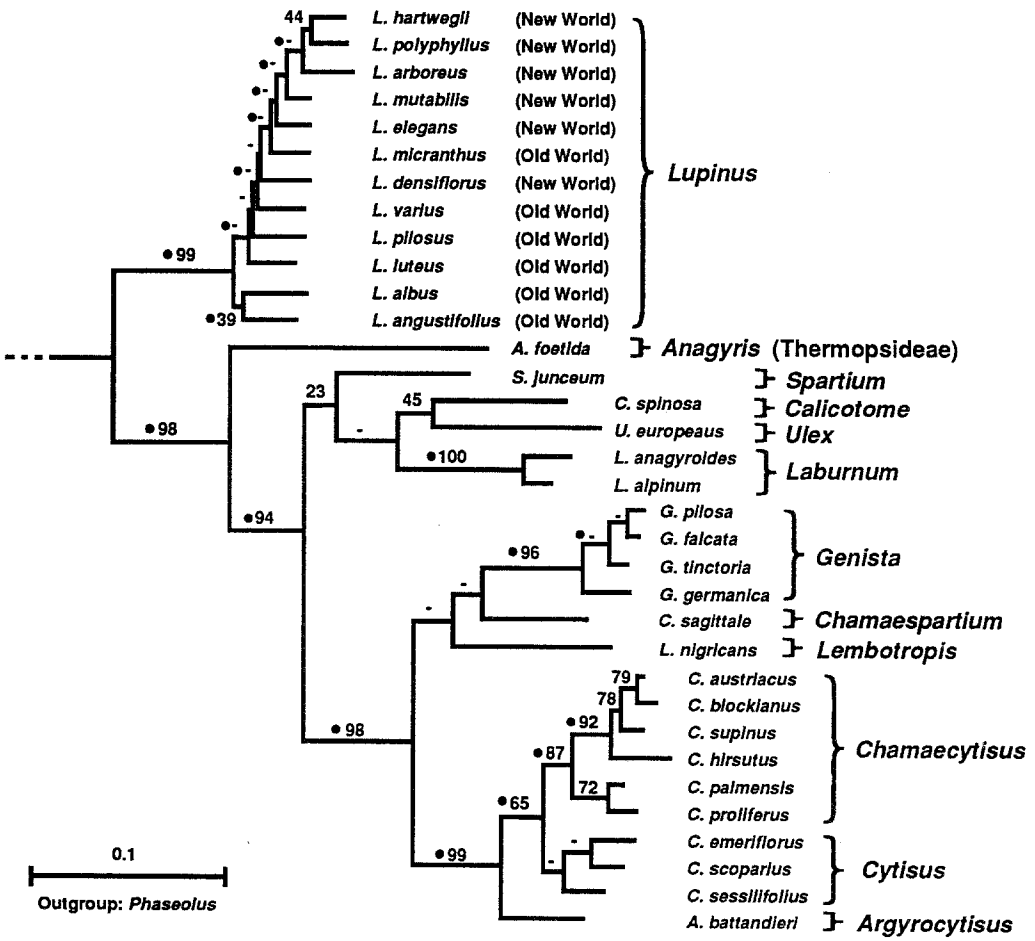

Fig. 1. Neighbor joining (SAITOU & NEI 1987) topology obtained for *Genisteae, Lupinus* spp., and *Anagyris foetida* on the basis of proportions of restriction site differences in cpDNA. The tree was constructed as described in Material and methods. Numbers above branches indicate the number of times out of 100 Wagner parsimony bootstrap replicas BOOT of PHYLIP 3.3 (FELSENSTEIN 1990) that the corresponding branch of the neighbor joining tree was detected, dashes indicate that the branch was detected in less than 20/100 replicas. The scale bar (lower left) indicates 0.1 polymorphisms per site surveyed (see Material and methods). Dots indicate branches which were detected in the two most parsimonious trees generated using HENNIG 86 (FARRIS 1988)

each with a length of 605 (mhennig option), a consistency index of 0.45 and a retention index of 0.79. The 50% majority consensus bootstrap parsimony topology for *Lupinus* spp. with either PAUP or PHYLIP is a rooted twelve point star (data not shown).

The deepest branch in Fig. 1 separates *Lupinus* from the other *Genisteae*, which shares a robust common branch with *Anagyris foetida* (tribe *Thermopsideae*). A second major dichotomy separates *A. foetida* from taxa belonging to the woody *Genisteae*, which appear to form a monophyletic group. Within this group the genera *Genista, Chamaespartium, Lembotropis, Chamaecytisus, Argyrocytisus*, and *Cytisus* seem to form a monophyletic group to the exclusion of *Spartium, Calicotome, Ulex*, and *Laburnum* (Fig. 1). However, common branch of the latter four genera in the neighbor joining tree is probably not significant since it is neither supported by the consensus tree of PHYLIP (bootstrap value = 23) nor the two most parsimonious trees produced by HENNIG 86. *Lembotropis* and *Chamaespartium*, associated in the neighbor joining tree with *Genista* species, are distinct from both *Genista* and the *Cytisus-Chamaecytisus* complex in the consensus Wagner parsimony of PHYLIP with bootstrap values of 96 and 99, respectively, as well as in the two most parsimonious trees of HENNIG 86.

A representative overview of the amount of restriction site polymorphism which we observed is given in Table 2. Not surprisingly, the degree of polymorphism observed between genera varies considerably across comparisons and correlates with the position of the respective taxa in the phylogenetic tree of Fig. 1. Congeneric species surveyed shared more than 92% of the 386 restriction sites studied in all cases. The greatest infrageneric polymorphism (30 restriction site differences) was observed within *Lupinus*, where the largest number of species were sampled. The smallest degree of polymorphism (only three restriction site differences) was observed between *Chamaecytisus austriacus* and *C. blockianus*.

These restriction site data do not provide resolution within the genera *Genista* and *Lupinus*. Within *Lupinus*, no significant subtopologies were found. Although the European species appear at the base of the *Lupinus* subtree in neighbor joining and HENNIG 86 results, bootstrap parsimony does not distinguish the American and European species. Robust subtopologies at the infrageneric level were only found within *Chamaecytisus*. Notable is the considerable separation of *Chamaecytisus palmensis* and *C. proliferus* from other *Chamaecytisus* species. The position of *Argyrocytisus battandieri* deserves attention since this species branches with the *Cytisus-Chamaecytisus* group, but is distinct from the investigated species of *Cytisus* and *Chamaecytisus*.

Discussion

cpDNA has become a major tool of modern plant systematics. Restriction site analyses of cpDNA have proved to be very useful both at the infrageneric level, e.g., *Clarkia* (SYTSMA & GOTTLIEB 1986 a, b), *Helianthus* (RIESEBERG & al. 1988), *Solanum* (HOSAKA 1986), *Pisum* (PALMER & al. 1985) and the familial and subfamilial levels, e.g., in *Rubiaceae* (BREMER & STRUWE 1992), *Solanaceae* (SPOONER & al. 1993, OLMSTEAD & PALMER 1992), and *Asteraceae* (JANSEN & al. 1990). Within the large and taxonomically difficult family *Leguminosae*, cpDNA polymorphism has been studied at the tribal (BRUNEAU & al. 1990, LAVIN & al. 1990, LAVIN &

Table 2. cpDNA restriction site polymorphism between selected taxa studied. *n* Number of individual pairwise comparisons, D_{av} average number of restriction site differences for the number of comparisons indicated, S_{av} average fraction of shared restriction sites for the number of comparisons indicated, equal to the maximum likelihood estimate of probability that two sequences share the same recognition sequence at a given site (NEI 1987), min and max = minimum and maximum values of infrageneric polymorphism for the genus indicated, respectively. For infrageneric comparisons, values in the columns D_{av} and S_{av} do not represent averages, but rather the values for the single comparisons indicated

Taxa compared		n	D_{av}	S_{av}
Intergeneric:	*Phaseolus*			
	Anagyris	1	116.0	0.699
	Lupinus	12	89.9	0.767
	Genisteae (excluding *Lupinus*)	21	132.5	0.658
	Lupinus			
	Anagyris	12	81.8	0.788
	Genisteae (excluding *Lupinus*)	252	99.2	0.743
	Anagyris			
	Genisteae (excluding *Lupinus*)	21	91.0	0.764
	Spartium/Calicotome/Ulex/Laburnum			
	Genista	20	69.9	0.819
	Chamaespartium	5	68.2	0.823
	Lembotropis	5	68.6	0.822
	Chamaecytisus	30	82.7	0.785
	Cytisus	15	82.1	0.787
	Argyrocytisus	5	80.2	0.792
	Genista			
	Chamaespartium	4	39.5	0.898
	Lembotropis	4	52.0	0.865
	Chamaecytisus	24	65.2	0.831
	Cytisus	12	51.2	0.867
	Argyrocytisus	4	53.0	0.863
	Chamaecytisus			
	Cytisus	18	24.6	0.936
	Argyrocytisus	3	73.0	0.811
Infrageneric:	*Lupinus*	66	–	–
	L. hartwegii-L. polyphyllus^{min}	1	10	0.974
	L. albus-L. arboreus^{max}	1	30	0.922
	Genista	6	–	–
	G. pilosa-G. falcata^{min}	1	5	0.987
	G. tinctoia-G. germanica^{max}	1	17	0.956
	Chamaecytisus	15	–	–
	C. austriacus-C. blockianus^{min}	1	3	0.992
	C. palmensis-C. hirsutus^{max}	1	23	0.940
	Cytisus	3	–	–
	C. emeriflorus-C. scoparius^{min}	1	11	0.971
	C. emeriflorus-C. sessiliflorus^{max}	1	17	0.956
	Laburnum	1	–	–
	L. anagyroides-L. alpinum	1	11	0.971

DOYLE 1991) and infrageneric levels (*Astragalus*: SANDERSON 1991, *Glycine*: DOYLE & al. 1990). *Genisteae* is particularly difficult in terms of natural systematics and is viewed by many as a "tribe of convenience", the relationship of *Lupinus* to *Genisteae* in particular has a long tradition of debate (POLHILL 1981).

In a thorough treatment of the *Genisteae*, BISBY (1981) maintained the genus *Lupinus* at the rank of subtribe *Lupininae* within *Genisteae*, a view which is widely accepted (e.g., DOYLE & al. 1992). Results from cpDNA restriction site analysis summarized in Fig. 1 clearly indicate that *Genisteae*, if defined to include *Lupinus* (i.e., *Genisteae* sensu lato), is not a natural group. Indeed, *Anagyris foetida*, a putative outgroup and woody member of the tribe *Thermopsideae*, is more closely related to the woody *Genisteae* than *Lupinus* is. Thus, although our results support the view that *Genisteae* and *Thermopsideae* share a common ancestor (BISBY 1981, POLHILL 1981, TURNER 1981, CRISTOFOLINI & FEOLI-CHIAPELLA 1984) they also indicate that *Lupinus* should be excluded from the *Genisteae*. However, *Anagyris* is known to be that member of the *Thermopsideae* which possesses greatest morphological similarity to the *Genisteae* (BISBY 1981). Since we did not sample a broader spectrum of representatives from *Thermopsideae*, the data presented here would not exclude the possibility that *Anagyris* is severely misplaced within *Thermopsidae* and that *Genisteae* could thus potentially be defined to include both *Lupinus* and *Anagyris*. Further studies should reveal whether intermediate branching between *Anagyris* and the remaining *Genisteae* also holds true for the other *Thermopsideae*.

The "*Cytisus-Chamaecytisus*" complex has been the subject of numerous revisions (see POLHILL 1976) and has encompassed, in various taxonomic treatments, four genera included in our sample: *Chamaecytisus, Argyrocytisus, Cytisus*, and *Lembotropis*. The results summarized in Fig. 1 are congruent with the strong morphological similarity observed between members of the genera *Chamaecytisus, Argyrocytisus*, and *Cytisus*, consistent with the serological similarity between the genera demonstrated by CRISTOFOLINI & FEOLI-CHIAPELLA (1977). Whereas *Argyrocytisus battandieri* is considered as a monospecific genus by some authors (HEYWOOD 1968, RAYNAUD 1975), it was assigned to *Cytisus* sect. *Petteria* as *Cytisus battandieri* by POLHILL (1976). At the cpDNA level, *A. battandieri* is distinct from *Cytisus* and *Chamaecytisus* species, consistent with its designation as *Argyrocytisus battandieri*. The species *Lembotropis nigricans, Cytisus emeriflorus*, and *C. sessilifolius* have undergone numerous reassignments at both the genus and section level in recent treatments of *Genisteae* (POLHILL 1976). The suggestion (BISBY 1981) to split the genus *Cytisus* by removing *Cytisus sessilifolius* as a monospecific genus *Cytisophyllum* LANG. is not supported by our data, nor is the suggestion by POLHILL (1976) to assign *C. emeriflorus* to *Lembotropis*. The subsequent suggestion (FEOLI-CHIAPELLA & CRISTOFOLINI 1980) to assign *Cytisus emeriflorus* to a monospecific section (*Emeroides*) within *Cytisus* also finds no molecular support.

The data indicate that the *Cytisus-Chamaecytisus-Argyrocytisus*-complex shares a common ancestor with three other genera within *Genisteae* – *Lembotropis, Chamaespartium*, and *Genista* – since these six genera share a very robust common branch. In the neighbor joining tree, *Chamaespartium* and *Lembotropis* are associated with *Genista*, but in 44/100 bootstrap parsimony replicates, *Lembotropis* and *Chamaespartium* shared a common branch and were ancestral to *Genista* and the

Cytisus complex. These results indicate that *Lembotropis* and *Chamaespartium* do not show specific affinities to other genera surveyed and thus support their respective status as monospecific genera.

We also find no support for the alternative and loose association of genera into a "*Genista*-group" (suggested to include, e.g., *Ulex* and *Chamaespartium*) and a "*Cytisus*-group" (suggested to include, i.e., *Laburnum* and *Lembotropis*) as sketched by BISBY (1981) since *Genista, Chamaespartium, Lembotropis, Chamaecytisus, Argyrocytisus*, and *Cytisus* clearly share a common ancestor to the exclusion of *Laburnum, Ulex, Calicotome*, and *Spartium* (Fig. 1). Our data thus support serological evidence that the latter four genera are distinct from *Genista* and *Cytisus* (CRISTOFOLINI & FEOLI-CHIAPELLA 1984). These four genera possess a number of morphological characters which are generally regarded as primitive within the tribe, but the data provide only weak evidence for the view that they may represent a line of common descent within the woody *Genisteae*.

The cpDNA data support CRISTOFOLINI's (1989) view regarding a common ancestor for *Thermopsideae* (*Anagyris*) and *Genisteae*, although the data require modification of his scenario with respect to the *Lupinus* lineage as discussed above. His suggestion that this separation may have occurred in tropical E Asia is easy to reconcile with the view (HOLUBOVA-KLASKOVA 1964, POLHILL 1981) that *Genisteae* may have undergone primary diversification in mountain forests of the Mediterranean, yet this region of primary diversification for the tribe need not necessarily be identical with that for diversification within the genus *Lupinus*. Various scenarios have been proposed to explain the transatlantic disjunction within *Lupinus*. DUNN (1971) suggested South American origins and migration of some species to the Euro-Mediterranean. An alternative scenario (PLITMAN 1981, PLITMAN & PAZY 1984) envisaged North American origins with two independent waves of migration to the Old World, which brought forth the Euro-Mediterranean smooth- and rough-seeded lupines, respectively. A more recent scenario (CRISTOFOLINI 1989) proposed E Asian origins for the genus with two independent routes of migration during the early Tertiary: one to America across the Bering land bridge and a second to the Mediterranean basin. The star phylogeny and low degree of infrageneric cpDNA divergence observed within *Lupinus* argue compellingly in favor of monophylesis for this genus and against two independent centers of infrageneric diversification, since the Euro-Mediterranean species are in no way significantly distinct from their American relatives. This same observation also argues against the view that the smooth- and rough-seeded Old World lupines are descendants of independent migration events.

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References

BISBY, F. A., 1981: *Genisteae*. – In POLHILL, R. M., RAVEN, P. H., (Eds): Advances in Legume systematics **1**, pp. 409–425. – Kew: Royal Botanic Gardens.

BREMER, B., STRUWE, L., 1992: Phylogeny of the *Rubiaceae* and the *Loganiaceae*: congruence or conflict between molecular and morphological data? – Amer. J. Bot. **79**: 1171–1184.

BRUNEAU, A., DOYLE, J. J., PALMER, J. D., 1990: A chloroplast DNA inversion as a subtribal character in the *Phaseoleae* (*Leguminosae*). – Syst. Bot. **15**: 378–386.

CRISTOFOLINI, G., 1989: A serological contribution to the systematics of the genus *Lupinus* (*Fabaceae*). – Pl. Syst. Evol. **166**: 265–278.

– FEOLI-CHIAPELLA, L., 1977: Serological systematics of the tribe *Genisteae* (*Fabaceae*). – Taxon **26**: 43–56.

– – 1984: Origin and diversification of the *Genisteae* (*Fabaceae*): a serosystematic purview. – Webbia **38**: 105–122.

DOWLING, T. E., MORITZ, C., PALMER, J. D., 1990: Nucleic acids II. Restriction site analysis. – In HILLIS, D. M., MORITZ, C., (Eds): Molecular systematics, pp. 250–317. – Sunderland, Mass.: Sinauer.

DOYLE, J. J., DOYLE, J. L., 1987: A rapid DNA isolation procedure from small quantities of fresh leaf tissue. – Phytochem. Bull. **19**: 11–15.

– – BROWN, A. H. D., 1990: A chloroplast DNA phylogeny of the wild perennial relatives of the soybean (*Glycine* subgenus *Glycine*): congruence with morphological and crossing groups. – Evolution **44**: 371–389.

– LAVIN, M., BRUNEAU, A., 1992: Contributions of molecular data to papilionoid legume systematics. – In SOLTIS, P. S., SOLTIS, D. E., DOYLE, J. J., (Eds): Molecular systematics of plants, pp. 223–251. – New York: Chapman & Hall.

DUNN, D. B., 1971: A case of long range dispersal and rapid speciation in *Lupinus*. – Trans. Missouri Acad. Sci. **5**: 26–38.

FARRIS, J. S., 1988: Hennig 86 reference version 1.5. Computer software and manual. Publ. by the author.

FAUGERAS, C., PARIS, R., 1968: Chemitaxinomie des Papilionacées-Genistées. – Bull. Soc. Bot. France, Mém. **1965**: 75–102.

FELSENSTEIN, J., 1990: PHYLIP manual, version 3.3. Univ. of Calif., Univ. Herbarium, Berkeley, Calif.

FEOLI-CHIAPELLA, L., CRISTOFOLINI, G., 1980: Sero-systematics of *Cytisus* sect. *Triantho-cytisus* (*Fabaceae*). – Pl. Syst. Evol. **136**: 209–216.

GIBBS, P. E., 1966: A revision of the genus *Genista*. – Notes Roy. Bot. Gard. Edinburgh **27**: 11–99.

GLADSTONES, J. S., 1974: Lupins of the Mediterranean region and Africa. – Dept. of Agricult. West Australia, Techn. Bull. **26**: 1–148.

HARBORNE, J. B., 1969: Chemosystematics of the *Leguminosae*: flavonoid and isoflavonoid patterns in the tribe *Genisteae*. – Phytochemistry **8**: 1449–1456.

HEYWOOD, V. H., 1968: A synopsis of the European species of *Cytisus* and allied genera. – Feddes Repert. **79**: 20–23.

HOLUBOVA-KLASKOVA, A., 1964: Bemerkungen zur Gliederung der Gattung *Cytisus* L. s.l. – Acta Univ. Carol. Praha, Biol., Suppl. **2**: 1–24.

HOSAKA, K., 1986: Who is the mother of the potato? – Restriction endonuclease analysis of chloroplast DNA of cultivated potatoes. – Theor. Appl. Genet. **72**: 606–618.

HUTCHINSON, J., 1964: The genera of flowering plants, **1**. – London: Oxford University Press.

JANSEN, R. K., HOLSINGER, K. E., MICHAELS, H. J., PALMER, J. D., 1990: Phylogenetic analysis of chloroplast DNA restriction site data at higher taxonomic levels: an example from the *Asteraceae*. – Evolution **44**: 2089–2105.

LAVIN, M., DOYLE, J. J., 1991: Tribal relationships of *Sphinctospermum* (*Leguminosae*); integration of traditional and chloroplast DNA data. – Syst. Bot. **16**: 162–172.

– – PALMER, J. D., 1990: Evolutionary significance of the loss of the chloroplast DNA inverted repeat in the *Leguminosae* subfamily *Papilionoideae*. – Evolution **44**: 390–402.

NEI, M., 1987: Molecular evolutionary genetics. – New York: Columbia University Press.
OLMSTEAD, R. G., PALMER, J. D., 1992: A chloroplast DNA phylogeny of the *Solanaceae*: subfamilial relationships and character evolution. – Ann. Missouri Bot. Gard. **79**: 346–360.
PALMER, J. D., SINGH, G. P., PILLAY, D. T. N., 1983: Structure and sequence evolution of three legume chloroplast DNAs. – Mol. Gen. Genet. **190**: 13–19.
– JORGENSEN, R. A., THOMPSON, W. F., 1985: Chloroplast DNA variation and evolution in *Pisum*: patterns of change and phylogenetic analysis. – Genetics **109**: 195–213.
– OSORIO, B., THOMPSON, W. F., 1988: Evolutionary significance of inversions in legume chloroplast DNA. – Curr. Genet. **14**: 65–74.
PLITMAN, U., 1981: Evolutionary history of Old World Lupines. – Taxon **30**: 430–437.
– PAZY, B., 1984: Cytogeographical distribution of Old World *Lupinus*. – Webbia **38**: 531–539.
POLHILL, R. M., 1976: *Genisteae* (ADANS.) BENTH. and related tribes (*Leguminosae*). – Bot. Syst. **1**: 143–368.
– 1981: *Papilionoideae*. – In POLHILL, R. M., RAVEN, P. H., (Eds): Advances in Legume systematics **1**: pp. 191–208. – Kew: Royal Botanical Gardens.
– RAVEN, P. H., STIRTON, C. H., 1981: Evolution and systematics of the *Leguminosae*. – In POLHILL, R. M., RAVEN, P. H., (Eds): Advances in Legume systematics **1**: pp. 1–26. – Kew: Royal Botanical Gardens.
RAYNAUD, C., 1975: Combinaisons nouvelles pour quatre espèces de la flore marocaine. – Bull. Soc. Bot. France **121**: 359–361.
RIESEBERG, L. H., SOLTIS, D. E., PALMER, J. D., 1988: A molecular reexamination of introgression between *Helianthus annuus* and *H. bolanderi* (*Compositae*). – Evolution **43**: 227–238.
ROTHMALER, W., 1944: Die Gliederung der Gattung *Cytisus* L. – Feddes Rep. **53**: 137–150.
SAITOU, N., NEI, M., 1987: The neighbor-joining method: a new method for the reconstruction of phylogenetic trees. – Mol. Biol. Evol. **4**: 406–425.
SALATINO, A., GOTTLIEB, O. R., 1983: Chemogeographical evolution of quinolizidines in *Papilionoideae*. – Pl. Syst. Evol. **143**: 167–174.
SAMBROOK, J., FRITSCH, E. F., MANIATIS, T., 1989: Molecular cloning: a laboratory manual, 2nd edn. – Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
SANDBRINK, J. M., VELLEKOOP, P., VAN HAM, R., VAN BREDERODE, J., 1989: A method for evolutionary studies on RFLP of chloroplast DNA applicable to a range of species. – Biochem. Syst. Ecol. **17**: 45–49.
SANDERSON, M. J., 1991: Phylogenetic relationships within North American *Astralagus* L. – Syst. Bot. **16**: 414–430.
SPOONER, D. M., ANDERSON, G. J., JANSEN, R. K., 1993: Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (*Solanaceae*). – Amer. J. Bot. **80**: 676–688.
SWOFFORD, D. L., 1991: PAUP: phylogenetic analysis using parsimony, version 3.0. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.
SYTSMA, K. J., GOTTLIEB, L. D., 1986 a: Chloroplast DNA evidence for the derivation of the genus *Heterogaura* from a species of *Clarkia* (*Onagraceae*). – Proc. Natl. Acad. Sci. USA **83**: 5554–5557.
– – 1986 b: Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* sect. *Peripetasma* (*Onagraceae*). – Evolution **40**: 1248–1261.
TAUBERT, P., 1891: *Leguminosae*. – In ENGLER, A., PRANTL, K., (Eds): Die natürlichen Pflanzenfamilien III, pp. 70–385. – Leipzig: Engelmann.
TURNER, B. L., 1981: *Thermopsideae*. – In POLHILL, R. M., RAVEN, P. H., (Eds): Advances in Legume systematics **1**, pp. 403–407. – Kew: Royal Botanical Gardens.

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